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Total numbers of glomeruli and individual glomerular cell types in the normal rat kidney

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Summary. Alterations in numbers of glomeruli and glomerular cells occur in various renal disorders. Although values for these parameters have previously been reported for several species, the estimates have often been biased due to assumptions regarding glomerular and/or nuclear size and shape. Other studies have used tedious serial-section reconstruction methods. In the present study, unbiased stereological methods were used to estimate total numbers of glomeruli and individual glomerular cell types in normal rats. The kidneys of seven adult Sprague-Dawley rats were perfused with 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer and embedded in either glycolmethacrylate (for light microscopy, LM) or Epon/Araldite (for transmission electron microscopy, TEM). Total glomerular number was estimated using an LM physical disector/fractionator combination; the total number of cells per average glomerulus was estimated using an LM optical disector/Cavalieri combination; and TEM physical disectors were used to count individual cell types. The normal rat kidney was found to contain 31764 ± 3667 (mean ± SD) glomeruli. An average glomerulus contained 674 ± 129 cells, of which 181 ± 53 were epithelial cells (podocytes), 248 ± 53 were endothelial cells, and 245 ± 45 were mesangial cells. An average renal corpuscle contained 117 ± 27 parietal epithelial cells. Following sectioning and staining, less than 6.5 h was needed to obtain the above estimates for a single animal, with coefficients of variation (SD as a percent of the mean) ranging from 10% to 25%. The unbiased stereological methods used in the present study constitute an unbiased, precise and cost-efficient set of quantitative tools for assessing glomerular morphology in health and disease.

Key words: Kidney – Glomerulus – Stereology – Morphometry – Disector – Quantitative methods, structural – Rat (Sprague Dawley)

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The number of glomeruli in a kidney and the number of different cell types in a glomerulus can change as a result of disease and aging (Heptinstall 1983). Although morphometric methods, including stereological techniques have been frequently used to estimate values for these glomerular parameters, the results have often been biased, either due to technical artefacts or to the inherent bias of the counting method employed. For example, traditional stereological methods require assumptions of particle (e.g. glomerulus, nucleus) shape and size in order to estimate number (Weibel 1979). In other studies, serial-section reconstruction techniques were used (see Bendtsen and Nyengaard 1989). No assumptions were required, but the time required was prohibitive.

Compared with counting whole cell nuclei, and thereby estimating cell number, a more common practice in glomerular cell morphometry is to count nuclear profiles (bits and pieces of nuclei in sections) and relate this number to either a unit area of section or average glomerular cross-sectional area. While such strategies have undoubtedly been successful in identifying and monitoring changes in cell populations, it is important to be aware of the limitations of this approach. Firstly, the number of nuclear profiles in a section, or the frequencies of different types of nuclear profiles in a section, do not equate with the number of nuclei in the original three-dimensional tissue, since the likelihood of a nucleus being sectioned depends not only on frequency, but also size, shape and orientation (Weibel 1979). Secondly, such estimates can be severely influenced by technical artefacts such as the dimensional changes (shrinkage/swelling) associated with the processing of tissue for microscopy (Bahr et al. 1957; Bertram et al. 1986; Zhengwei et al. 1990). And thirdly, these parameters provide no information about the total numbers of cells in a glomerulus.

The past 10 years have witnessed the development of a new generation of stereological counting methods (Gundersen et al. 1988a; Cruz-Orive and Weibel 1990; Bertram and Nurcombe 1992; Coggeshall 1992). These

methods are said to be unbiased because they do not require assumptions of particle shape, size or orientation. Moreover, these methods can provide estimates of total number. The effects of shrinkage/swelling artefacts, for example, become irrelevant because number is not referenced to a unit area of section or a unit volume of tissue.

In the present study, new unbiased stereological counting methods were used to estimate the total number of glomeruli in the normal rat kidney, as well as the total number of cells in an average glomerulus, and the total number of individual glomerular cell types in an average glomerulus. A physical disector/fractionator combination was used at the light-microscopic (LM) level to estimate glomerular number, an optical disector/Cavalieri combination was used at the LM level to estimate the total number of cells in an average glomerulus, and physical disectors were used with transmission electron microscopy (TEM) to estimate numbers of individual cell types.

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. The rats were placed in metabolic cages and allowed unrestricted access to food and water. After a minimum of 2 days of acclimatization in the cages, 24-h urine samples were collected on the next 2 days. Urine volume and protein concentrations (Bio Rad assay, Bradford 1976) were determined, allowing calculation of urinary protein excretion rate. Urinary protein excretion rates were within normal limits for this strain of animals, never exceeding 10 mg/day.

Rats were anaesthetized with sodium pentobarbitone (5 mg/100 g body weight; Nembutal, Abbott Laboratories, Australia) given intraperitoneally, and a midline abdominal incision was made and the superior mesenteric artery tied. The aorta was then ligated below the level of the renal arteries and a cannula was inserted in a retrograde direction above the level of the ligature. The cannula was tied in place, the aorta tied above the level of the renal arteries, and the kidneys perfused for about 30 s at 180 mm Hg pressure with Hanks' Balanced Salt Solution, followed by a mixture of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 5 min. An incision in the inferior vena cava served as an outlet for the perfusate.

Tissue sampling and processing

Following perfusion, the left kidneys were weighed and cut into 1 mm-thick slices in the horizontal plane using a razor blade fractionator (Baddeley et al. 1986). Every second slice was taken for LM, with the first being chosen at random. The cortex on the remaining slices was diced, and about 20 blocks per animal were randomly selected for TEM.

For LM, the 1 mm-thick slices were immersion-fixed in 10% buffered formalin for 1 to 2 days following perfusion. Prior to embedding, the slices were dehydrated through a graded series of ethyl alcohol solutions (70%, 80%, 90% for 2 h each, and 95% for approximately 18 h). Infiltration and embedding were done with 2-hydroxyethyl methacrylate (glycolmethacrylate) (Polaron Embedding Medium, Bio Rad). Kidney slices were infiltrated for 5 to 6 h with glycolmethacrylate without hardener prior to embedding.

Following perfusion, blocks for TEM were immersion-fixed for a further hour in the same fixative. The blocks were then washed in a 5% w/v sucrose solution in 0.1 M phosphate buffer (3 × 15 min) and postfixed in 2% aqueous osmium tetroxide for 1 h. After routine washes in buffer and dehydration steps, the blocks were embedded in Araldite/Epon.

Estimating the total number of glomeruli in a rat kidney

Sectioning and staining. Each glycolmethacrylate block containing either one or two kidney slices was mounted in a microtome chuck and the distance from the base of the chuck to the block face recorded with a digital micrometer (Mitutoyo, Japan) with a precision of 1 μm. The slices were then exhaustively sectioned at a nominal thickness of 20 μm using a Reichert-Jung 1150/Autocut fitted with glass knives. The total number of sections cut was recorded, and the distance from the base of the chuck to the block face remeasured. Average section thickness (t) was calculated for the block. It is important to note that knowledge of t was not required to estimate glomerular number, but to estimate glomerular cell number. During serial-sectioning, every 10th (sample section, see below) and 11th ("look-up section") sections were mounted, with the first chosen using a random number table.

Sections were floated on a cold water bath, mounted on gelatinized glass slides and dried at 60° C for 2 days. They were stained with haematoxylin (Mayer's) for 30 min and 0.1% eosin for 15 min.

Sampling of mounted sections. When the kidney was cut into slices before embedding, the razor blades produced artificial surfaces. When the embedded tissue slices were sectioned, the glass knives produced further artificial surfaces. These artificial surfaces interfere with glomerular counting (Nyengaard and Bendtsen 1990). To overcome this potential problem, only those sections between the first and last sections of a block containing the complete circumference of the kidney were used for counting glomeruli. To estimate the fraction of the sampled section area used to count glomeruli, the sampled sections were projected on a Fuji Minicopy Reader at a magnification of 19.2 ×. An orthogonal grid (area per grid point a(p) equal to approximately 1.8 mm^2) was attached to the viewing screen and the number of grid points overlying all kidney sections (P_s) as well as complete kidney sections (P_f) determined. The fraction of the sectioned tissue used for counting was designated by P_f/P_s.

Counting glomeruli. To estimate the total number of glomeruli in a kidney, glomeruli were counted using physical disectors (Sterio 1984) in a known fraction of the kidney. This method can be termed a physical disector/fractionator combination.

Two light microscopes (Olympus BH-2) adapted for projection were used. A sampled histological section was placed in the first microscope and the corresponding "look-up section" in the second microscope. The fields of vision were projected side by side onto a table in a semi-darkened room at a final magnification of 153 ×. An unbiased counting frame (Gundersen 1977) was applied to the field of vision of the first microscope.

To count glomeruli, corresponding regions of the two sections were found and those glomeruli sampled by the unbiased counting frame of the first microscope that were not present in the look-up section were counted (Q^-; see Fig. 1). To double the efficiency, those glomeruli in the look-up section that were sampled by a counting frame, but not present in the first section, were then counted.

Glomeruli were counted in fields that were selected using a systematic uniform random scheme. An automated stage fitted to the first microscope was used for this purpose. An orthogonal grid (a(p) of approximately 0.019 mm^2) within the unbiased counting frame was used to count points overlying kidney sections, and this allowed calculation of the fraction of the section area (f_a) used to count glomeruli. The formula for estimation of the total

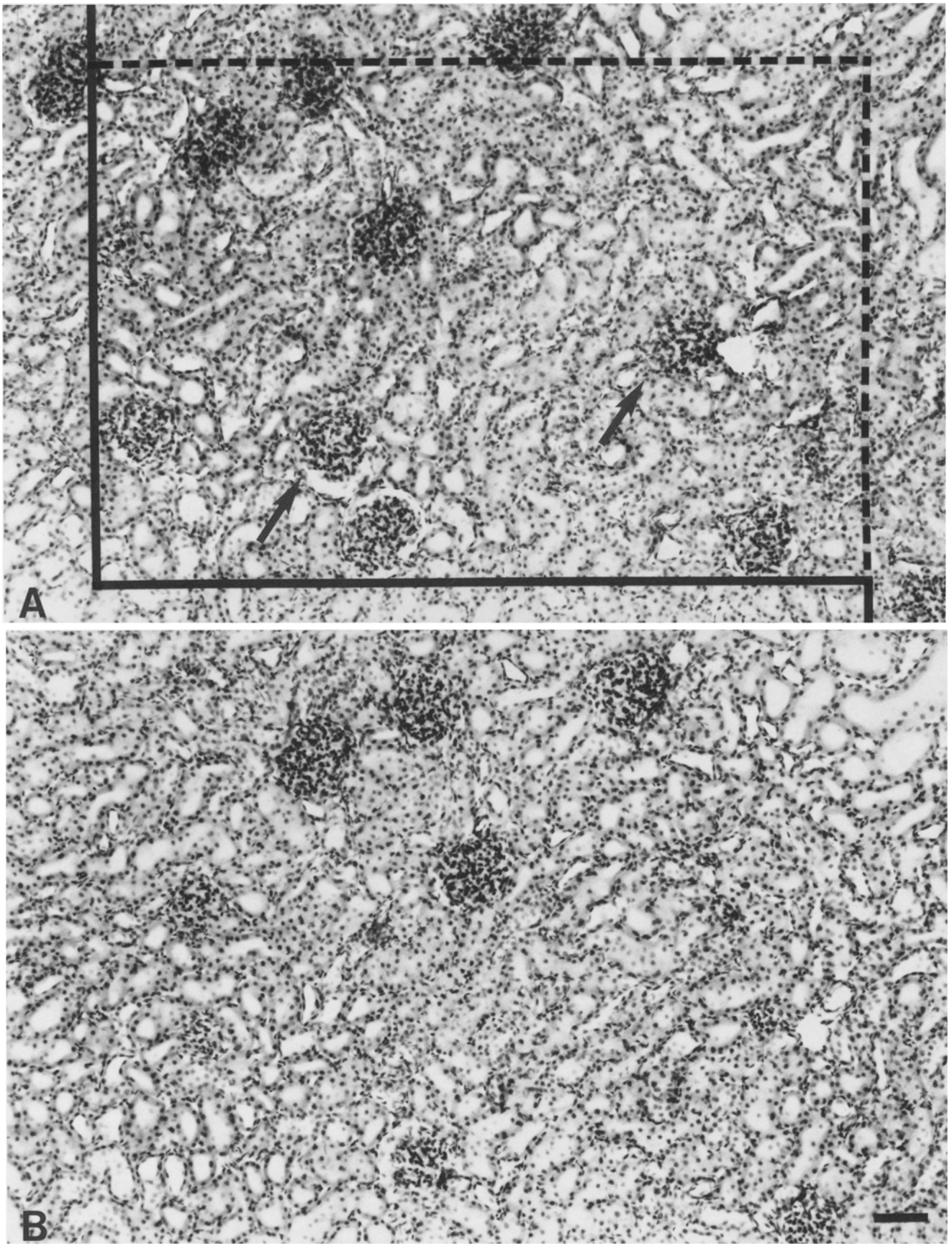


Fig. 1. Photomicrographs showing a visual field from a sampled kidney section (**A**) and the corresponding visual field on the "look-up" section (**B**). An unbiased counting frame is superimposed on the visual field hitting the sampled section. Glomerular profiles are said to be sampled if they are contained completely or partly in the frame, and are not hit by the exclusion line (*solid line*). Sampled glomeruli are counted if they are not present in the look-up section. In this case, the two glomeruli indicated by *arrows* are counted; $Q^- = 2$. $\times 150$; *bar*: 100 μm

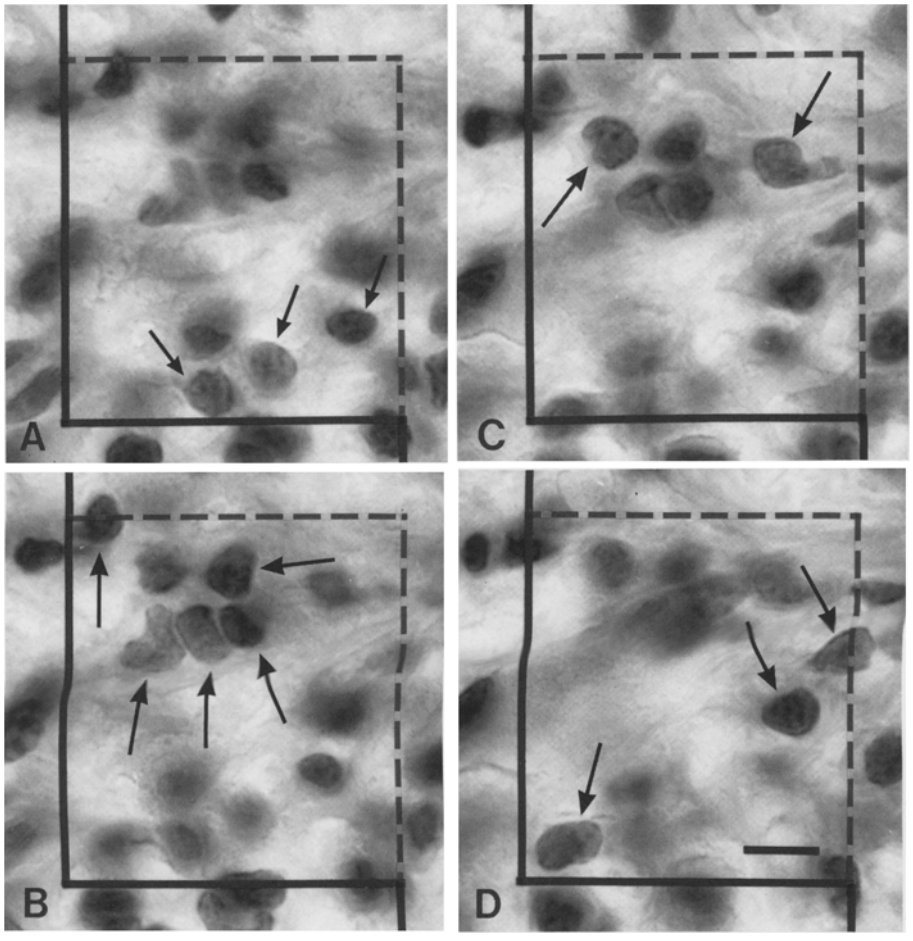


Fig. 2A–D. Photomicrographs showing a series of optical sections (separated by 2 μm) through a glomerulus. Unbiased counting frames are superimposed on the optical sections. The nuclei that come into focus within the frame are counted. Nuclear profiles in contact with the broken edges of the frame are considered to be inside the frame, and those touching the solid lines are defined as being outside the frame. Starting at the first section, **A**, the three nuclei in focus (*small arrows*) are not counted because they did not come into focus. Five nuclei come into focus at **B** (*large arrows*). None of them touch the solid "forbidden" line and therefore they are all counted. In **C**, two new nuclei (*large arrows*) come into focus and are counted. At the last section, **D**, three new nuclei come into focus and all are counted (*large arrows*). Ten nuclei are counted between levels A and D, a distance of 6 μm ($Q^- = 10$). It is important to note that the division of the total disector height of 6 μm into 2 μm is arbitrary and only for the purpose of illustration. In practice, one focuses down through the section and counts nuclei as they come into focus. ×1000; *bar*: 10 μm

number of glomeruli in a kidney ($N_{glom,kid}$) was

$$N_{glom,kid} = 2 \times 10 \cdot (P_s/P_f) \cdot [1/(2f_a)] \cdot Q^-, \quad (1)$$

where 2 is the inverse of the slice sampling fraction, 10 is the inverse of the section sampling fraction, P_s/P_f and $1/(2f_a)$ give the fraction of the total section area used to count glomeruli, and Q^- is the actual number of glomeruli counted. Approximately 123 ± 23 glomeruli were counted in each kidney in order to estimate total glomerular number. The time required to estimate the total number of glomeruli in a kidney was approximately 2.5 h.

Estimating the total volume of an embedded rat kidney. Kidney volume (V_{kid}) was estimated using the Cavalieri principle (Gundersen and Jensen 1987; Gundersen et al. 1988b), through

$$V_{kid} = 2 \times 10 \cdot t \cdot a(p) \cdot P_s. \quad (2)$$

Approximately 20 min were required to estimate the volume of a kidney, with about 40 sections analysed per kidney.

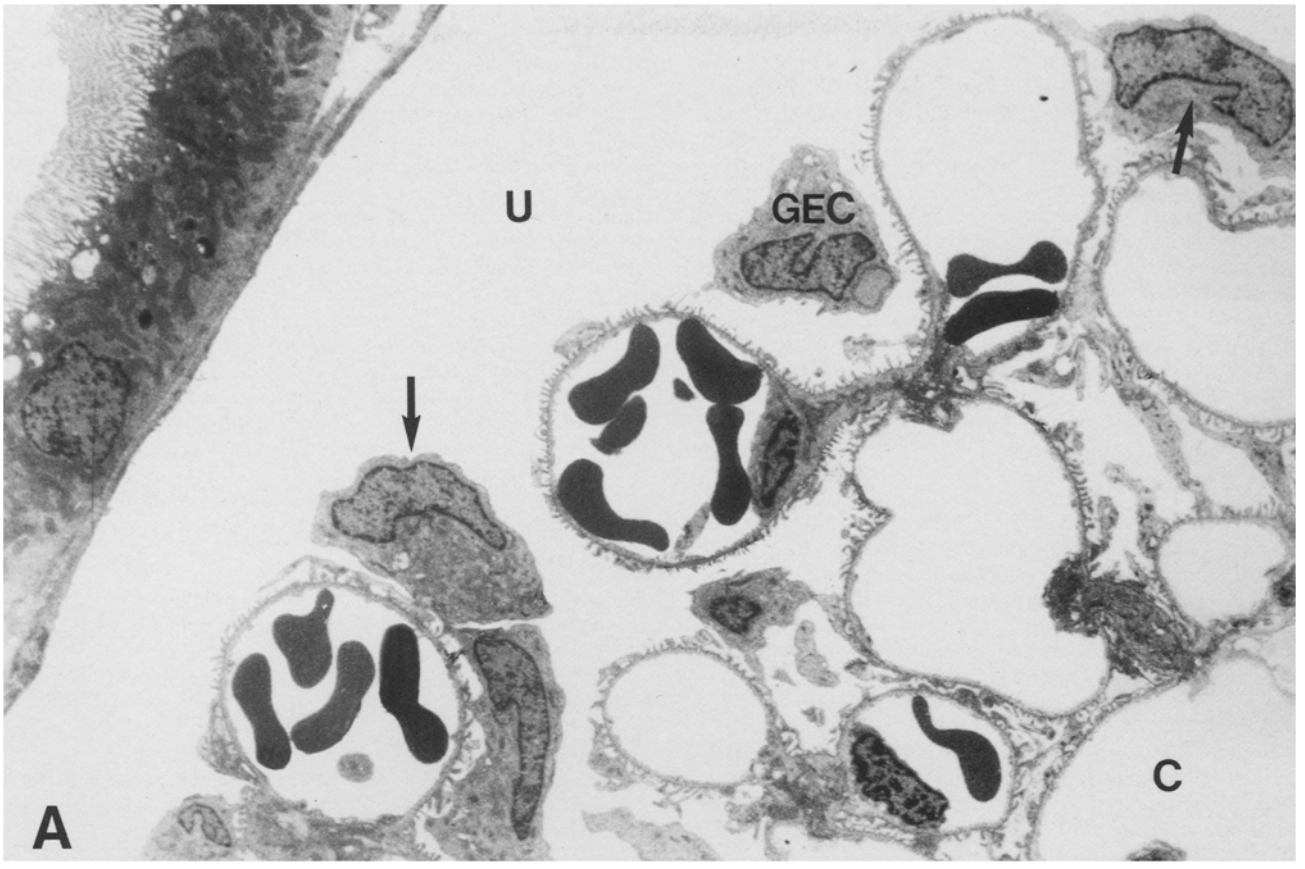
Estimating mean glomerular volume. Mean glomerular volume (V_{glom}) was estimated using

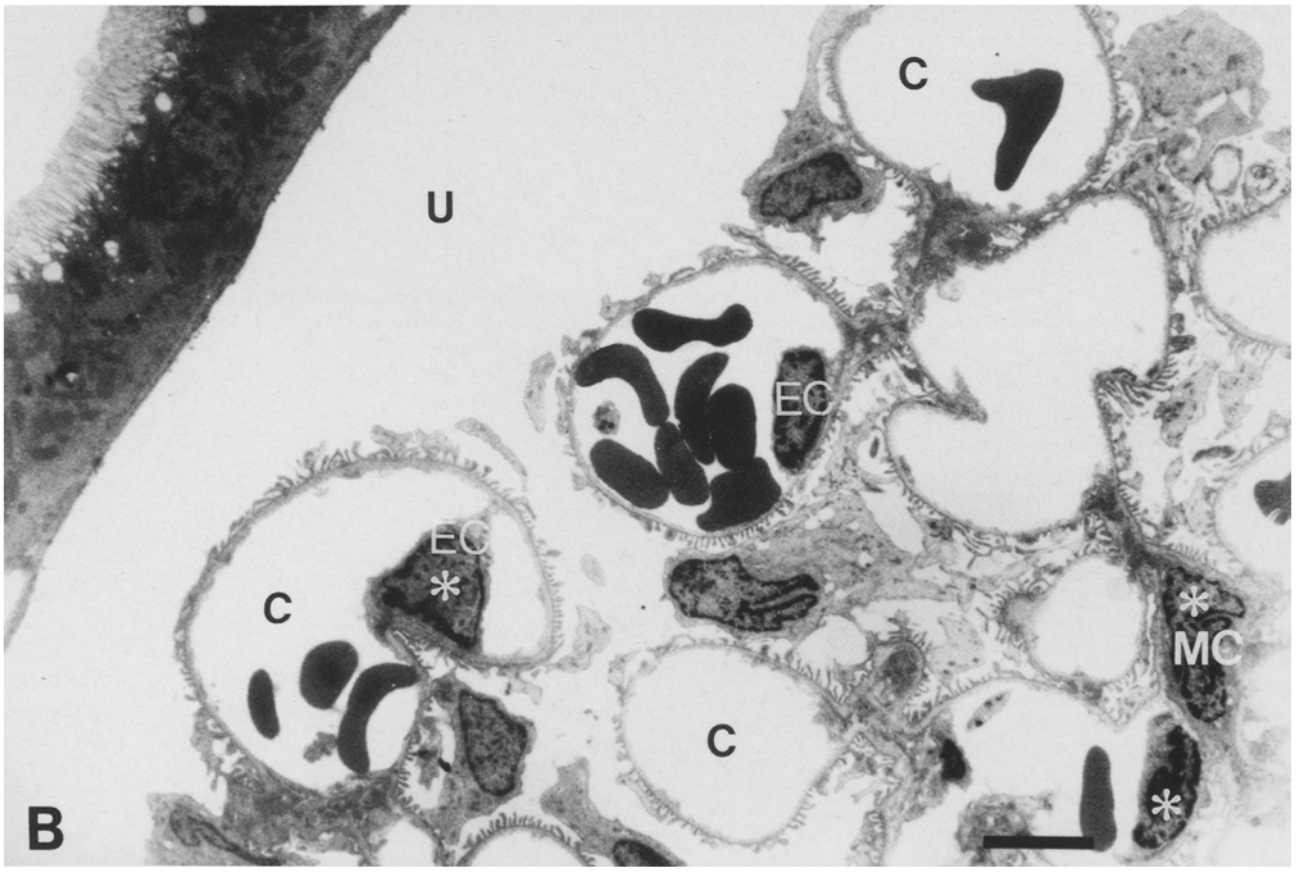
$$V_{glom} = V_{V(glom,kid)}/N_{V(glom,kid)}, \quad (3)$$

where $V_{V(glom,kid)}$ was estimated by dividing the number of points overlying glomerular profiles by the number of points overlying kidney sections. Points on glomeruli were counted at the same time that points on kidney were counted when counting glomeruli with physical disectors. $N_{V(glom,kid)}$ was calculated by dividing $N_{glom,kid}$ by V_{kid}.

Estimating the total number of cells in an average glomerulus. The total number of cells in an average glomerulus was estimated using optical disectors (Gundersen et al. 1988a). Before counting cells, it was important to choose unbiasedly those glomeruli in which cells would be counted. Accordingly, the sections used previously to count glomeruli were placed on a 1 mm × 1 mm grid in order to select a set of fields in which to choose glomeruli. The first field was selected using a random number between 1 and 9, and thereafter every 9th field was selected, giving about two fields per section. The fields were outlined with a pen on the underside of the slides, which were then placed on a microscope (Olympus BH-2) equipped with a 63× oil immersion lens (NA = 1.4) and an electronic microcator (Heidenhain VRZ401) giving the position of the Z-axis (with a precision better than 0.5 μm). Observation of fields was via a colour video camera (JVC Model TK-870E) and the image was displayed on a monitor (Digital Model VR 241-BY) at a final magnification of 1280×. The projection lens to the video camera was equipped with a square graticule (Graticules Pty Ltd, U.K.).

Fig. 3A, B. A pair of TEM micrographs used for counting individual glomerular cell types. The micrographs come from two sections about 1.5 μm apart. Nuclei were counted using the disector principle; they were counted when they were present in one section but not the other section. The two GECs indicated by *arrows* in **A** are counted because they are not present in section **B**. Those nuclei present in **B** but not present in **A** were also counted. These nuclei are indicated by *asterisks*. Counting frames were not used when counting cells at the TEM level because the entire glomerular cross-section was analysed. *U* Urinary space; *C* capillary lumen; *GEC* glomerular epithelial cell; *MC* mesangial cell; *EC* endothelial cell. ×3000; *bar:* 5 μm


U
GEC
C
A


U
C
EC
EC
C
C
MC
B

One glomerulus in each field was sampled at low magnification, this being the glomerulus whose centre was closest to the centre of the field. Optical disectors were then sampled in the glomerulus by choosing every 14th disector with a random start between 1 and 14. About 50 disectors were sampled in approximately 25 glomeruli per animal.

An optical disector for measurement was defined by focusing onto the top of the section, zeroing the microcator, and then moving 3 μm into the section. At this time, the number of grid points overlying the glomerulus, urinary space and parietal epithelium were counted. The microcator was re-zeroed, the microscope was focused through the section for 10 μm, and cells were counted when the nuclear edge and associated chromatin came into focus. For more details on counting cells with optical disectors, see Fig. 2, and also Braendgaard et al. (1990), West et al. (1990) and Nurcombe et al. (1991).

The numerical density of cells in glomeruli was calculated using the equation

$$N_{V(cells,glom)} = Q^- / [10 \cdot P_{glom} \cdot a(p)], \quad (4)$$

where Q^- is the total number of glomerular cells counted per kidney, 10 μm is the thickness of the optical disector, P_{glom} is the number of points lying on the sampled disectors, and a(p) is the area associated with each grid point (approximately 1.6×10^{-4} mm^2).

An unbiased estimate of the total number of cells (nuclei) in an average glomerulus ($N_{cells,glom}$) was obtained through

$$N_{cells,glom} = N_{V(cells,glom)} \times V_{glom}, \quad (5)$$

where estimation of V_{glom} was as described above. Approximately 171 ± 30 cells were counted in each kidney in order to estimate $N_{cells,glom}$. Approximately 2 h were needed to count this number of cells per kidney using optical disectors.

Estimating the total number of each cell type in an average glomerulus. Physical disectors of unknown thickness were used at the electron-microscopic level to estimate the relative frequencies (proportions) of glomerular epithelial cells (GECs), mesangial cells, endothelial cells, and parietal epithelial cells (PECs) in renal corpuscles. It is important to note that although the disector is often thought of and described as a counting method, it is more correctly defined as a three-dimensional probe that samples particles with uniform probability, irrespective of their size or shape (Sterio 1984). When estimating cell or nuclear numerical density (number per volume) with the disector (physical or optical), the height or thickness of the disector must be known. However, when estimating relative frequencies of cells, knowledge of disector thickness is not required. In the present study, these frequencies were multiplied by the total number of cells per glomerulus ($N_{cells,glom}$) to obtain the total number of each cell type per average glomerulus.

Thick sections were cut from six randomly selected TEM blocks per animal and stained with methylene blue. The glomerulus whose centre was closest to the centre of the section was chosen for TEM analysis. Two thin sections about 1.5 μm apart were cut from these sampled glomeruli, mounted on a single slot grid coated with a film of 0.6% Butvar, and stained with uranyl acetate for 30 min and lead citrate for 5 min. Sections were viewed in a Philips EM 400, and the entire cross-section of the renal corpuscle photographed. Montages, each consisting of about ten micrographs, were constructed at a final magnification of approximately 3900×. Those nuclei present in the top section of the disector pair but not in the bottom section were counted, and vice versa (see Fig. 3). Unbiased counting frames were not used to count cells at the TEM level because the entire cross-section of the renal corpuscle was analysed; the renal corpuscle was not sub-sampled. An average of about 250 nuclei was counted at the TEM level from each kidney. This took about 1.5 h per kidney.

The following equation was used to estimate, for example, the total number of GECs in an average glomerulus:

$$N_{GEC} = (Q^-_{GEC} / Q^-_{all\ glom\ cells}) \times N_{cells,glom}, \quad (6)$$

where Q^-_{GEC} was the number of GECs counted, $Q^-_{all\ glom\ cells}$ was the total number of all glomerular cells counted, and $N_{cells,glom}$ was the total number of cells in an average glomerulus.

The total number of PECs in an average renal corpuscle was estimated using,

$$N_{PEC} = (Q^-_{PEC} / Q^-_{all\ corp\ cells}) \times N_{cells,corp}, \quad (7)$$

where $Q^-_{all\ corp\ cells}$ was the sum of the glomerular cells and PECs counted, and $N_{cells,\ corp}$ was the total number of cells in an average renal corpuscle.

Estimating the total number of each glomerular cell type in a normal kidney. To estimate the total number of each glomerular cell type as well as the total number of PECs in a normal rat kidney, $N_{glom,kid}$ was multiplied by the total number of that cell type in an average glomerulus (from Equation 6 or 7).

Statistics

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

Table 1. Body weights and light-microscopic stereological estimates for 7 normal rats. Values of Q^- (the actual number of glomeruli or glomerular cells counted) are given in parentheses

Animal	Body weight	Total volume of kidney	Average glomerular volume	Total no. of glomeruli per kidney	Total no. of cells per glomerulus
	g	V_{kid} mm^3	V_{glom} mm$^3 \times 10^{-4}$	$N_{glom,kid}$	$N_{cells,glom}$
1	218	649.822	7.140	31912 (134)	646 (145)
2	234	819.525	6.341	32440 (127)	609 (145)
3	201	942.017	5.249	35920 (132)	655 (202)
4	189	822.191	5.542	31445 (87)	536 (173)
5	210	819.046	7.882	25960 (98)	913 (218)
6	228	1032.646	6.604	36109 (126)	583 (140)
7	227	854.656	7.636	28563 (157)	775 (172)
Mean	215	848.558	6.628	31764	674
SD	16	118.719	1.001	3667	129
CV (%)	7.4	14.0	15.0	11.5	19.1

Table 2. Estimates of the total number of each cell type in an average renal corpuscle of the normal rat kidney. Values of Q^- (the actual number of each cell type counted) for the TEM counting are given in parentheses

Animal	Glomerular epithelial cells	Endothelial cells	Mesangial cells	Parietal epithelial cells
1	158 (50)	238 (75)	250 (79)	85 (30)
2	175 (54)	249 (77)	185 (57)	118 (34)
3	133 (44)	288 (95)	234 (77)	157 (39)
4	139 (55)	200 (79)	197 (78)	95 (31)
5	291 (72)	315 (78)	307 (76)	150 (34)
6	173 (66)	165 (63)	245 (93)	107 (40)
7	197 (57)	284 (82)	294 (85)	104 (32)
Mean	181	248	245	117
SD	53	53	45	27
CV (%)	29.3	21.4	18.3	23.1

Results

The results are presented in Tables 1 and 2. The normal rat kidney was found to contain 31764 ± 3667 glomeruli, with a coefficient of variation (CV) of 11.5%. The absolute number of cells per average glomerulus was 674 ± 129 (CV = 19.1%). There were 181 glomerular epithelial cells per average glomerulus, as well as 248 endothelial cells and 245 mesangial cells. The capsule surrounding a glomerulus on average contained 117 parietal epithelial cells. The normal rat kidney contained a total of $5.614 \pm 1.060 \times 10^6$ glomerular epithelial cells (CV = 18.9%), $7.793 \pm 1.444 \times 10^6$ endothelial cells (CV = 18.5%), $7.685 \pm 1.125 \times 10^6$ mesangial cells (CV = 14.6%), and $3.699 \pm 0.988 \times 10^6$ parietal epithelial cells (CV = 26.7%). Glomerular volume was $6.628 \pm 1.001 \times 10^{-4}$ mm^3.

Discussion

In this study we used some new unbiased stereological methods to count glomeruli and individual glomerular cell types, and to determine glomerular volume in normal rats. Our estimates are of the same order as those obtained previously by other workers, most of whom used either inherently biased stereological methods, slow serial-section reconstruction techniques, or maceration methods (see below). The cost efficiency and precision of the present methods are remarkable: on average, following sectioning and staining, less than 6.5 h was needed to estimate values for all parameters for a single animal. The mean inter-individual CVs generally ranged from 10% to 25%. We believe the LM physical disector/fractionator combination, the LM optical disector/Cavalieri combination, and the TEM physical disector of unknown thickness as used in the present study constitute an unbiased, precise and cost-efficient set of quantitative methods for assessing glomerular morphology in health and disease.

Glomerular number

The present estimate for the total number of glomeruli in the normal rat kidney (31764 ± 3667; 7 rats) is similar to previous estimates obtained by Kittelson (1917), Arataki (1926), Vimtrup (1928), Kunkel (1930), Rytand (1938), and Nyengaard and Bendtsen (1990) whose estimates were 28863 (1 rat), 30000 (1 rat), 33826 (1 rat), 30922 ± 1170 (2 rats), 30800 (1 rat) and 23000 ± 2365 (4 rats), respectively. Kittelson (1917) and Arataki (1926) employed serial-section reconstruction techniques, whereas Vimtrup (1928), Kunkel (1930) and Rytand (1938) used maceration techniques. The agreement between the present estimate and these previous estimates is satisfying, given the well-known shortcomings of serial-section reconstruction (including sampling procedure, tissue deformation and edge effects) and maceration procedures (Moore 1931; Larsson et al. 1980). Nyengaard and Bendtsen (1990) used a physical disector/fractionator combination similar to the present method.

It is important to note the advantages of the physical disector/fractionator combination as a method for counting glomeruli. With this method, glomeruli are counted using the disector principle in a known and predetermined fraction of the kidney. No assumptions of glomerular shape, size, uniformity of size, or orientation are required. Furthermore, the estimates are not influenced by shrinkage/swelling artefacts associated with tissue processing. This is an especially important consideration when diseased glomeruli for example are being studied, since their shrinkage/swelling characteristics may well be different from normal glomeruli. In addition, section thickness need not be determined and the estimates are not influenced by section compression. The only risk of bias with this technique is ambiguous identification of glomeruli.

Total glomerular cell number

The present estimate for the total number of cells in a normal rat glomerulus (674 ± 129) is of the same order

as that reported by Helmchen et al. (1977) and Marcussen (1992), whose estimates were 553 (1 rat) and 576 (1 rat) cells, respectively. Helmchen et al. (1977) used serial electron micrographs to reconstruct the cellular structure of a single rat glomerulus. About 2500 sections were used. Although this method is unbiased, because no assumptions of nuclear shape, size or orientation are required, the time needed to cut sections and count nuclei is prohibitive. Marcussen (1992) used a double-disector approach to count glomerular cells. Physical disectors were used to count glomeruli (and thereby estimate mean glomerular volume) and optical disectors were used to count glomerular cells. Although this appears to be a faster method to count glomerular cells than the method used in the present study, the double-disector of Marcussen (1992) does not provide estimates of total glomerular number.

The present study confirms the optical disector as a cost-efficient method for counting cells. Only about 2 h were needed to count 150 to 200 glomerular cells per kidney and thereby estimate the total number of cells in an average glomerulus. In previous studies in which optical disectors were used to count cells, Braendgaard et al. (1990) took less than 7 h to estimate the total number of neurons in the human neocortex, West and Gundersen (1990) took less than 4 h to estimate the total number of neurons in the five subdivisions of the human hippocampus, and Nurcombe et al. (1991) took just 30 min to estimate the total number of neurons in the lateral motor column of the developing chick embryo.

It is important to emphasize that the estimates of cell numerical density (number per volume) obtained using optical disectors in the present study are almost certainly influenced by shrinkage and swelling artefacts produced when the tissue was processed for microscopy. For this reason, cell numerical densities are not presented. However, when cell numerical densities are multiplied by mean glomerular volume (Equation 5) to provide estimates of total cell number, the effects of shrinkage and swelling artefacts cancel.

Numbers of individual glomerular cell types

To our knowledge, there have only been a few previous reports of the total number of individual cell types in the normal rat glomerulus. In their TEM serial-section reconstruction of a single glomerulus, Helmchen et al. (1977) found the glomerulus to consist of 282 endothelial cells, 181 mesangial cells and 90 GECs. Fries et al. (1989) used a traditional stereological method (Weibel and Gomez 1962) to estimate the mean number of GECs in the glomeruli of Munich-Wistar rats. Despite the necessary assumptions of nuclear shape and size, their estimate of 142 GECs per glomerulus is quite close to the present estimate (181 GECs).

The advantages of counting cells with disectors have already been discussed. However, the physical disector as used in the present study at the TEM level to count individual glomerular cell types, has the added advantage that section thickness (or the distance between the pair of sections) need not be known. The disector was simply used to give the relative frequencies of the different cell types, and these frequencies were then multiplied by the estimates of total cell number per average glomerulus previously obtained at the LM level (Equations 6 and 7). We suggest that this combined LM/TEM approach utilizing TEM disectors of unknown thickness is an ideal method to count cells that cannot be identified or counted with LM. Again, the method is reasonably cost-efficient. Only about 1.5 h were needed to count about 250 nuclei per kidney at the TEM level. Obviously, considerable time is needed to cut the pairs of thin sections and to prepare electron micrographs.

Glomerular volume

It must be remembered that the present estimates of glomerular volume are almost certainly influenced by shrinkage/swelling effects. Although the dimensional changes in tissues embedded in glycolmethacrylate are considerably less than in tissues embedded in paraffin, dimensional changes do occur. The estimates of volume are simply included to illustrate that the present set of stereological methods also provides estimates of glomerular volume, a parameter that is known to alter in a variety of glomerular diseases.

Limitations/requirements of the methods

As alluded to above, the major drawback of the methods used in this study is the need for TEM to identify individual glomerular cell types. Obviously, cells should always be counted using LM if at all possible.

Although not a limitation as such, the importance of sampling when using these new methods cannot be overstated. Typically, only 100–200 glomeruli, or cells need to be counted per animal. Unless the approaches for obtaining a representative sample of sections and fields on sections are strictly adhered to, the final estimates may be inaccurate.

Concluding remarks

The stereological methods used in the present study provide unbiased estimates of glomerular number and glomerular cell number. In addition, estimates of mean glomerular volume are obtained. These methods are remarkably cost-efficient and seem ideally suited to studies on glomerular morphology in health and disease.

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References

Arataki M (1926) On the postnatal growth of the kidney, with special reference to the number and size of glomeruli (albino rat). Am J Anat 36:399–436

Baddeley AJ, Gundersen HJG, Cruz-Orive L-M (1986) Estimation of surface area from vertical sections. J Microsc 142:259–276

Bahr GF, Bloom G, Friberg U (1957) Volume changes of tissues in physiological fluids during fixation in osmium tetroxide or formaldehyde and during subsequent treatment. Exp Cell Res 12:342–355

Bendtsen TF, Nyengaard JR (1989) Unbiased estimation of particle number using sections – an historical perspective with special reference to the stereology of glomeruli. J Microsc 153:93–102

Bertram JF, Nurcombe V (1992) Counting cells with the new stereology. Trends Cell Biol 2:177–180

Bertram JF, Sampson P, Bolender RP (1986) Influence of tissue composition on the final volume of rat liver blocks prepared for electron microscopy. J Electron Microsc Tech 4:303–314

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Braendgaard H, Evans SM, Howard CV, Gundersen HJG (1990) The total number of neurons in the human neocortex unbiasedly estimated using optical disectors. J Microsc 157:285–304

Coggeshall RE (1992) A consideration of neural counting methods. TINS 15:9–13

Cruz-Orive L-M, Weibel ER (1990) Recent stereological methods for cell biology: a brief survey. Am J Physiol 258:L148–L156

Fries JWU, Sandstrom DJ, Meyer TW, Rennke HG (1989) Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. Lab Invest 60:205–218

Gundersen HJG (1977) Notes on the estimation of the numerical density of arbitrary profiles: the edge effect. J Microsc 111:219–223

Gundersen HJG, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. J Microsc 147:229–263

Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ (1988a) The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. APMIS 96:857–881

Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ (1988b) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96:379–394

Helmchen U, Kneissler U, Helmchen U, Bohle A (1977) Three-dimensional electron microscopy of the structure of the normal mesangium of the rat glomerulus. Kidney Int 11:215–216

Heptinstall RH (1983) Pathology of the Kidney, 3rd edn. Little Brown, Boston

Kittelson JA (1917) The postnatal growth of the kidney of the albino rat, with observations of an adult human kidney. Anat Rec 13:385–397

Kunkel PA (1930) The number and size of the glomeruli in the kidney of several mammals. Bull Johns Hopkins Hosp 47:285–291

Larsson L, Aperia A, Wilton P (1980) Effect of normal development on compensatory renal growth. Kidney Int 18:29–35

Marcussen N (1992) The double disector: unbiased stereological estimation of the number of particles inside other particles. J Microsc 165:417–426

Moore RA (1931) The total number of glomeruli in the normal human kidney. Anat Rec 48:153–168

Nurcombe V, Wreford NG, Bertram JF (1991) The use of the optical disector to estimate the total number of neurons in the developing chick lateral motor column: effects of purified growth factors. Anat Rec 231:416–424

Nyengaard JR, Bendtsen TF (1990) A practical method to count the number of glomeruli in the kidney as exemplified in various animal species. Acta Stereol 9:243–258

Rytand DA (1938) The number and size of mammalian glomeruli as related to kidney and body weight, with methods for their enumeration and measurement. Am J Anat 62:507–520

Sterio DC (1984) The unbiased estimation of number and sizes of arbitrary particles using the disector. J Microsc 134:127–136

Vimtrup BJ (1928) On the number, shape, structure and surface area of the glomeruli in the kidneys of man and mammals. Am J Anat 41:123–151

Weibel ER (1979) Stereological Methods. Vol 1. Practical Methods for Biological Morphometry. Academic Press, London

Weibel ER, Gomez DM (1962) A principle for counting tissue structures on random sections. J Appl Physiol 17:343–348

West MJ, Gundersen HJG (1990) Unbiased stereological estimation of the number of neurons in the human hippocampus. J Comp Neurol 296:1–22

Zhengwei Y, Wreford NG, DeKretser DM (1990) A quantitative study of spermatogenesis in the developing rat testis. Biol Reprod 43:629–635