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A Stereological Study of Key Histological Structures in the Kidneys of Rats from Young to Old Age

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Abstract: Quantitative (morphometric) study of the total amounts (per organ) and mean sizes of histological structures in individuals from young to old are essential for the understanding of age-related organ changes; stereological techniques are essential, reliable tools to obtain the quantitative data. Stereological study of all renal components, especially renal tubules, was lacking in age-related kidney studies and few studies used rats older than 24 months of age for a stereological analysis of the aging kidney. In the present study isotropic uniform random renal sections (methacrylate-embedded) were obtained from male Sprague-Dawley rats (8–9 per age-group) randomly sampled from a single cohort of normal animals at the ages of 3, 6, 12, 24 and 36 months, respectively. The sections were measured using various stereological methods to estimate the total amounts (per kidney) or mean sizes of key renal structures. The results demonstrated that the volume of kidney and the total volume or length of renal tubules increased continually from 3 to 24 months of age and then plateaued between 24 and 36 months of age. The total volume of renal corpuscles, glomeruli, Bowman's space or interstitial tissue and the mean volume of renal corpuscles or glomeruli increased continually from 3 to 24 months and further until 36 months of age. The total number of glomeruli remained essentially constant and the relative volume of the cortex or medulla and the relative length of different segments of the renal tubules remained basically stable throughout the ages. Less than 5% of the renal corpuscular or tubular profiles were apparently atrophied at 24 or 36 months of age. The age-related results suggested that the rat renal tissues continued to develop adaptively or work actively, from young to old, to maintain normal physiological functions and the aging change in the kidney was primarily a compensatory or hypertrophic histological change.

Keywords: Rats, Kidney, Renal Corpuscles, Renal Tubules, Development, Aging, Histology, Stereology

1. Introduction

Aging, or cell senescence, is natural and inevitable in the long run, and histological structure and function of organs, especially kidneys, are prone to changes with aging and age-related changes may be risk factors for organ injuries or diseases; understanding, and therefore possible manipulation or intervention, of the aging process may help in deferring or preventing, to some degree, the process of aging or the development of age-related diseases, or providing better or individualized care for the elderly [1-6]. Quantitative (morphometric) study of the total amounts (e.g. volume or length) and mean sizes (e.g. volume or diameter) of histological structures (e.g. renal glomeruli and tubules) in individuals from young to old are essential for the understanding of age-related organ changes; stereological techniques are essential, reliable tools to obtain the quantitative data [7-10]. Stereological study of all renal components, especially renal tubules, was lacking in age-related kidney studies and few studies used rats older than 24 months of age for a stereological analysis of the aging kidney. This study aimed to provide a reliable and comprehensive understanding of the aging process in the rat kidney by quantitatively studying all key renal structures, including

acquisition of the total number and mean volume of renal glomeruli and the total length and mean diameter of renal tubules, with (a) sophisticated stereological techniques including the use of isotropic random renal sections, (b) methacrylate resin-embedded renal sections that are better than paraffin sections for stereological studies [9, 11], and (c) groups of rats that were randomly sampled from a single cohort of normal animals at the ages of 3, 6, 12, 24 and 36 months – ages corresponding roughly to sexually mature and 18, 30, 60 and 90 years of age in humans, respectively [10, 12, 13].

2. Materials and Methods

2.1. Animals and Kidneys

The animals used in the present study were the same normal male Sprague-Dawley rats in the corresponding age groups reported in our recent study [10]. Briefly, a cohort of 216 male pups were obtained from 46 litters born from 46 pairs of parents. They were kept in cages (each housing 4 rats at most) under the same condition throughout the experiment: *ad libitum* access to the same rat chow and water, controlled light (12-hr light and 12-hr dark) and air-conditioning (temperature set at 18°C). At 3, 6, 12, 24 and 36 months of age respectively, 8–9 rats were randomly sampled out of the cohort from different litters. The animal experiment was approved by the Science and Technology Department of North Sichuan Medical College and ethical guidelines constituted by the College were followed during the experiment.

Both kidneys were removed from each rat and immersion fixed in Bouin's solution for 2 days before storage in 70% ethanol at 4°C. One kidney from each rat was randomly sampled, from which tissue blocks and sections were obtained for histology and stereology (below). After removal of the perirenal fat, the volume of the kidney was obtained by measurement of the renal weight and density as previously described [10]. The average renal densities were 0.968–0.981 and 0.931–0.938 g/cm^3 in the age-groups of 3–12M and 24–36M, respectively. A couple of remaining kidneys in each age-group were additionally utilized to cut some larger sections through the whole kidneys (Figure 1) and learn the general histological features (especially compartmentation) of the kidneys, for better observation of the sections for histology and stereology.

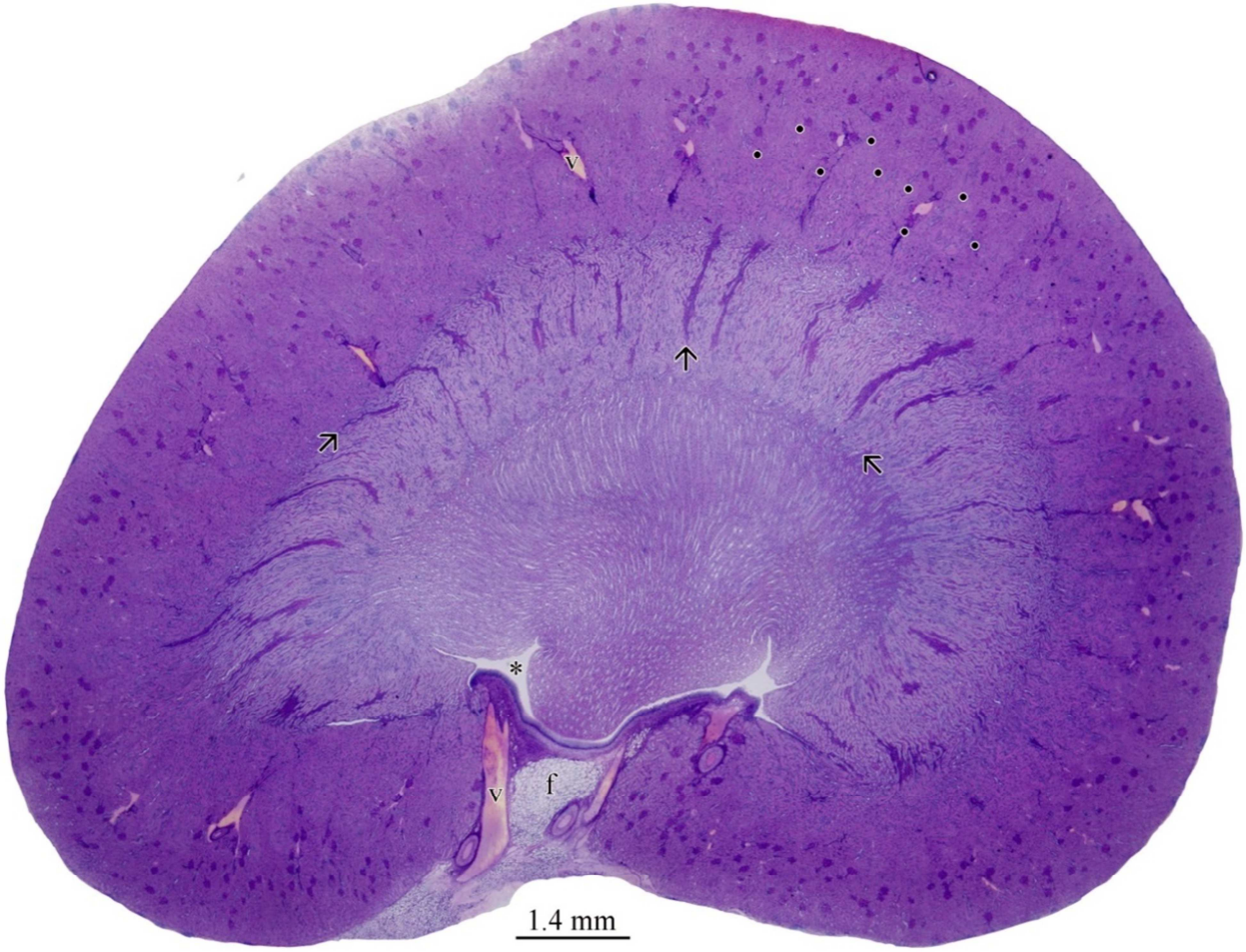

Figure 1. *Micrograph of a section (embedded in methacrylate resin and stained with PAS –periodic acid-Schiff's reagent and hematoxylin) cut along the convex and concave border of a kidney, from a normal male Sprague-Dawley rat aged 3 months. •, uneven border between the cortex and the outer stripe; ↗, border between the outer and inner stripes; ↖, border between the inner stripe and the inner medulla; ↑, connective tissue bundle; v, interlobular vein in the cortex (up) and small renal vein in the renal sinus (below); * and f, renal calyx lumen and fat tissue in the renal sinus.*

2.2. Tissue Blocks and Sections

Stereologically, uniform random (in terms of positions within the organ) sections are essentially required for all stereological estimations and isotropic random (in terms of 3-dimensinal orientations within the organ) measurement required for orientation-based stereological estimations such as tubular length estimation with profile counting and glomerular mean volume estimation with point sampled intercepts in the present study [7-9]. Isotropic uniform random sections, useful for all stereological estimations, were therefore obtained as follows in the present study, with the tissue blocks being uniformly sampled from the kidney [8, 9] and isotropic directions of the sections (i.e. directions of cutting the tissue blocks) being determined with the orientator principle [7, 9, 14, 15], see Figure 2.

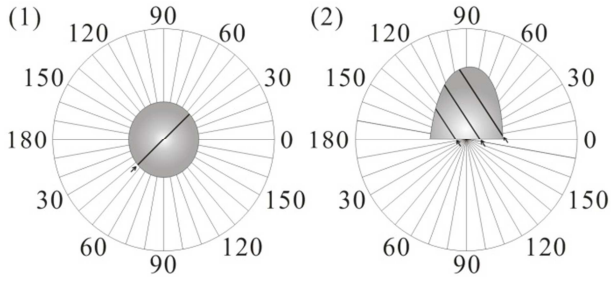

Figure 2. *Schematic illustration showing the method (orientator) of obtaining an isotropic renal section. (1) A half kidney, with the cut (first cut) surface (an arbitrary cut-surface dividing the kidney into 2 approximate halves) being placed on a flat board of uniform angles (0°–180°), is cut (second cut) into 2 smaller halves along a random angle (arrow, 45° in the figure) and perpendicular to the board. The random angle is obtained with the Excel software using the equation "=RAND()*180". (2) A quarter kidney (a smaller half obtained after the second cut), with the line of intersection between the first and second cuts being aligned along the 0°–180° line, is cut (third cut) into 4 tissue blocks along another random angle (arrows, 123° in the figure) and perpendicular to the board. This random angle is obtained with the Excel software using the equation "=acos(1-2*RAND())*180/PI()". This third cut results in isotropic random cut-surfaces (arrows) and any tissue section finally obtained along the cut-surfaces (i.e. parallel to the third cut) is an isotropic section through the kidney.*

Each kidney was cut (first cut) into 2 haves, through the middle and perpendicular to the long axis (approximately between the cranial and caudal poles) of the kidney. (a) One half was arbitrarily placed in the center of a flat board of directions with uniformly distributed angles 0°–180° (Figure 2). Along a uniform random angle (between 0° and 180°) and perpendicular to the flat board, the half kidney was cut (second cut) into 2 smaller halves through the center. One smaller half (quarter kidney) was chosen randomly (in a blinded way) and placed on the board, with the line of intersection between the first and second cuts being aligned along the 0°–180° line. Along another random angle (between 0° and 180°) and perpendicular to the board, the quarter kidney was cut (third cut) into 4 consecutive pieces (tissue blocks) with approximately equal thickness. (This random angle, kind of angle-weighted random, is the arccosine of a random number (1–2r), where r is a uniform random number between 0 and 1 [14]. This random angle and the first uniform random angle were pre-determined with the Excel software [9, 15], see the legend of Figure 2.) Two alternate tissue blocks (the 1st and 3rd blocks or the 2nd and 4th blocks) were randomly sampled. In the age-group of 3M, the third cut resulted in only 3 tissue blocks given the small size of the quarter kidney and 2 blocks were randomly sampled. (b) From the other half kidney (after the first cut), another 2 tissue blocks were independently obtained in the same way. Thus 4 tissue blocks were uniformly sampled from each kidney. Care was taken in the later embedding and sectioning (below) of each tissue block so that the section finally cut and stained from each block was parallel to the direction of the third cut, which was isotropic random through the kidney (Figure 2).

Each tissue block was embedded in glycol methacrylate (2-hydroxyethyl methacrylate, Historesin by Leica Microsystems Nussloch GmbH, Germany) and one intact section was cut from each block at 5 μm (thickness) with a microtome (RM2235, Leica Biosystems, Germany). Each section, after heating on a hotplate (H17.5D, Ingenieurbüro, M. Zipperer GmbH, Germany) at 90°C for 30 minutes for prevention of section detachment from glass slide, was stained with periodic acid-Schiff's reagent (PAS) and hematoxylin by the same procedures, which included 30 minutes in 1% periodic acid, Schiff's reagent and hematoxylin, respectively [15, 16]. The average area of each section finally cut and stained (Figure 3) was 33.8, 37.5, 46.3, 72.7 and 68.8 mm^2 in the age-groups of 3, 6, 12, 24 and 36 months, respectively.

2.3. Histological Compartments and Structures

The rat renal tissue was divided into the cortex, medulla and sinus (Figures 1 and 3). The medulla (one renal pyramid), surrounded by the renal calyx around the sinus, was composed of the outer medulla (consisting of the outer and inner stripes) and inner medulla. Different segments of tubules –uriniferous tubules (excluding the renal corpuscles) collectively referred to as renal tubules in the present study – were densely packed in different compartments of the cortex and medulla. Between the renal tubules (in the cortex and medulla) and corpuscles (in the cortex) was the interstitial tissue. The renal corpuscle consisted of the wall (the parietal layer of the Bowman's capsule), glomerulus (capillary tuft) and Bowman's space (capsular cavity between the wall and glomerulus). The renal tubules were mainly (a) the convoluted segments of proximal and distal tubules in the cortex; (b) the straight segments of proximal and distal tubules in the outer stripe; (c) the straight segments of distal tubules, a short length of collecting ducts (or tubules), and a short length of the thin segments of Henle's loops in the inner stripe; and (d) collecting ducts and many thin segments of the Henle's loops in the inner medulla [15, 17].

For clear definition and easy identification, and therefore reliable quantitation, of key structures in the present study, as we previously practiced and described [15], (a) the renal tubules in the cortex or outer stripe were classified as proximal tubules and distal tubules, the distal tubules being readily distinguished from the proximal tubules by the absence of brush border and light

staining of the tubular epithelium [Figures 4(1) and (2) and Table 1]. (b) The renal tubules in the inner stripe were classified as distal tubules (Figures 4(2) and (3)). Collecting ducts in the inner stripe, and even in the outer stripe, were classified into distal tubules because of their morphological similarity and relatively short length. (c) The thin segments of Henle's loops were not studied because they were not easily distinguishable from the surrounding blood vessels. The area of interstitial tissue that was intermingled with apparent thin segments of the Henle's loops, especially in the inner medulla, was separately measured in terms of volume (Figures 4(2) and (3) and Table 1). (d) Apparent boundary area of the outer stripe projecting into the cortex in the form of papillae (like dermal papillae, see Figure 1) or medullary rays (Figure 3) still belonged to the outer stripe. Occasionally, some single or scattered tubules (straight segments of proximal and distal tubules) from the outer stripe were seen in apparently cortical areas (Figure 4(1)). In this case, the tubules (in term of both volume and length) were regarded as belonging to the cortex. (e) The interstitial tissue included the basement membrane of the renal corpuscles and tubules.

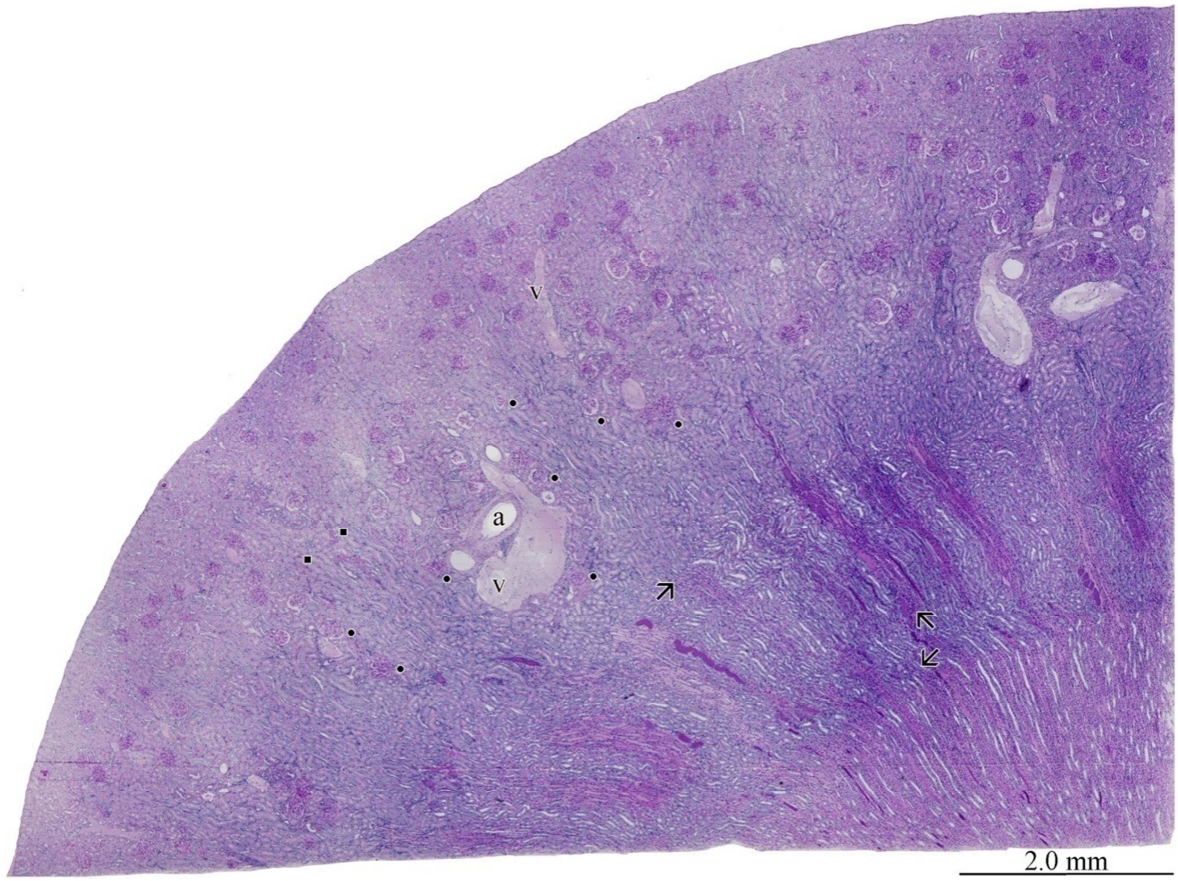

Figure 3. *Micrograph of an isotropic renal section (methacrylate resin-embedded, stained with PAS and hematoxylin) obtained from a normal male Sprague-Dawley rat aged 36 months and used for stereological estimation. • (near renal corpuscles) and ▪, uneven border between the cortex and the outer stripe; ↗, border between the outer and inner stripes; ↙, border between the inner stripe and the inner medulla; ↖, connective tissue bundle filled with small blood vessels and some renal tubules; a, arcuate artery; v, interlobular vein (up) and arcuate vein (below).*

2.4. Stereological Measurements

Each section was observed on a computer screen (final magnification of full screen image × 510) through a × 20 objective lens (Uplan FL N, numerical aperture 0.50) of an Olympus BX53 light microscope (Japan) equipped with a stereology system (NewCAST, Visiopharm, Hørsholm, Denmark). 6×6 densely arranged test points (green cross-shaped), 3×3 loosely arranged test points (9/36 of the green crosses, each encircled by a blue arc line) and 2 rectangular unbiased counting frames (or forbidden-line frames) were generated and superimposed on the histological image (on screen) as shown in Figure 4. Fields of view were sampled on the whole section in a uniform (equally-spaced) random manner by means of a computer-assisted motorized stage (ProScan III, Prior Scientific Inc., Rockland, MA, USA).

All sections were first observed and measured (First Measurement) for parameters of the renal tubules, interstitial tissue and sinus and for the diameters of renal corpuscular profiles. Then all sections were again observed and measured (Second Measurement) for other parameters of the renal corpuscles, including the volume of the basement membrane surrounding the corpuscles. In either of the Measurements, sections from different age-groups were measured in turns: first the 4 sections from an animal at 3M (3 months of age) were measured, then the 4 sections from an animal at 6M were measured, the 4 sections from an animal at 12M were measured, and then 8 sections randomly sampled from the 68 sections, which were pooled from all animals at 24M and 36M and blinded by random numbering, were measured to prevent

the influence of possible subjective factors on the difference in aging that we supposed most likely occurred between 24M and 36M. Then the 4 sections from another animal at 3M were measured… The distances between fields of view on section along the X-axis or Y-axis, based on experience and sectional areas, were set at 1.10 mm (for sections from rats aged 3M), 1.30 mm (6M), 1.50 mm (12M) and 1.70 mm (24M or 36M) in the First Measurement and at 0.90 mm (3M), 1.05 mm (6M), 1.20 mm (12M) and 1.35 mm (24M or 36M) in the Second Measurement, respectively, so that roughly similar and sufficient amount of measurements were performed per kidney in either Measurement.

While the fields on the section were observed and measured, the whole section at a low magnification was also observed at the same time on another computer screen, with real-time display of the fields (positions) on the section, thus helping to better identify structures being measured on the other screen.

To obtain morphometric data (below), the following stereological measurements were performed on each of the fields sampled on each section: (a) the numbers of test points (centers of the crosses) hitting (or in) different structures were separately counted/recorded (in both of the First and Second Measurements). For efficient point counting [7, 9], the loosely arranged test points (fewer) were used for counting of renal tubules, which occupy most of the kidney volume, while the densely arranged test points (4 times the number of loosely arranged test points) were used for counting of other structures. For final estimation of volume fractions of different structures in the kidney (below), the number of loosely arranged test points counted was converted to the number of densely arranged test points, by multiplying the number of loosely arranged test points by 4. (b) The numbers of the lumens of different tubular profiles "within" the inner smaller counting frame (Figure 4) were counted/recorded according to the unbiased counting rule (First Measurement), see the "Total length of renal tubules per kidney" part below for the rule. (c) The (short axial) diameter of each round (or elliptical) tubular profile that was counted (above) and also single luminal was measured as shown in Figure 4(3) and the histological features of these profiles were recorded to calculate the percentage of tubular profiles with certain histological features (First Measurement). (d) The profiles of renal corpuscles, which should be counted "within" the outer larger counting frame (Figure 4) according to the unbiased counting rule, were sampled (First and Second Measurements), and the long and short axial diameters of the profiles were measured as shown in Figure 4(1) (First Measurement) and the histological features of the profiles were recorded to calculate the percentage of corpuscular profiles with certain histological features (Second Measurement). (e) A point-sampled intercept was measured through each test point (densely arranged) hitting any of the renal corpuscular or glomerular profiles on the field, within the corpuscular or glomerular profile and along a pre-determined direction (X-axis on screen) as shown in Figure 4(1) (Second Measurement). In most cases, a point-sampled intercept through the corpuscle and a point-sampled intercept through the glomerulus were both measured through the same test point hitting the corpuscle or glomerulus and, in almost all cases on sections from animals aged 3M or 6M (Table 1) and in many cases on sections from other animals, both of the intercepts measured through the same test point were of essentially the same length because the corpuscle or glomerulus had the same boundary visible along the intercepts [Figure 4(1)].

2.4.1. Total Volume of Structure Per Kidney

The total number of (densely arranged) test points counted on the 4 tissue sections from each kidney was 2992 on average in the First Measurement and 4992 on average in the Second Measurement. Of this total number, the fractions of (densely arranged) test points hitting different structures were direct estimates of the volume fractions (percentages) of different structures in the kidney; multiplying the fractions by the kidney volume, we obtained the total volumes of different structures per kidney [7, 9, 10].

2.4.2. Total Length of Renal Tubules Per Kidney

The tubular profile with lumen that should be counted "within" the counting frame was either completely or partially inside the frame. In the latter case, the tubular profile could only intersect the counting lines of the frame (the upper and right green sides shown in Figure 4), not in any way intersecting the forbidden-lines of the frame – the red lines shown in Figure 4: (a) the left side and its (infinite) extension upward, (b) the lower side, and (c) the (infinite) extension of the right side downward. For each tubular profile counted in this way, we in fact counted the number of the tubular lumens. So we defined in the present study, as we previously described [15], that a tubular profile was encircled by the basement membrane (of the tubular epithelium) or the interstitial boundary, and a tubular lumen was encircled by the apical or luminal boundary (and its underlying cytoplasm) of the tubular epithelium that did not include the brush border of proximal tubules. In other words, the interstitial/luminal boundary formed a continuous and closed line around each tubular profile/lumen; a tubular lumen might be of an empty tubular cavity or filled with a cast or some brush border, and a tubular profile might have one or more tubular lumens (Figure 4).

Multiplying the total number of tubular lumens (in each segment of renal tubular profiles counted per kidney) by 2 divided by the total area of frames (used for the counting), we directly obtained the length density of the tubules in the kidney; multiplying the density by the kidney volume, we obtained the total length of the tubules per kidney [7, 9, 15]. (Note, the total area of frames, which was the area of each frame times the total number of frames within the renal sections, was unbiasedly estimated by multiplying the area of each frame by the numerical ratio between the total number of densely arranged test points counted and the number (36) of densely arranged test points superimposed on the field [7, 9].)

The total number of tubular lumens counted per kidney was 241 on average. Of this total number, an average of 8.7, 0.7 and 0.1 lumens per kidney were counted in 2-lumen (each tubular profile had 2 lumens), 3-lumen and 4-lumen tubular profiles respectively, mostly proximal tubular profiles in the

cortex and outer stripe (Figure 4). Other lumens (96.1%) were counted in single luminal tubular profiles.

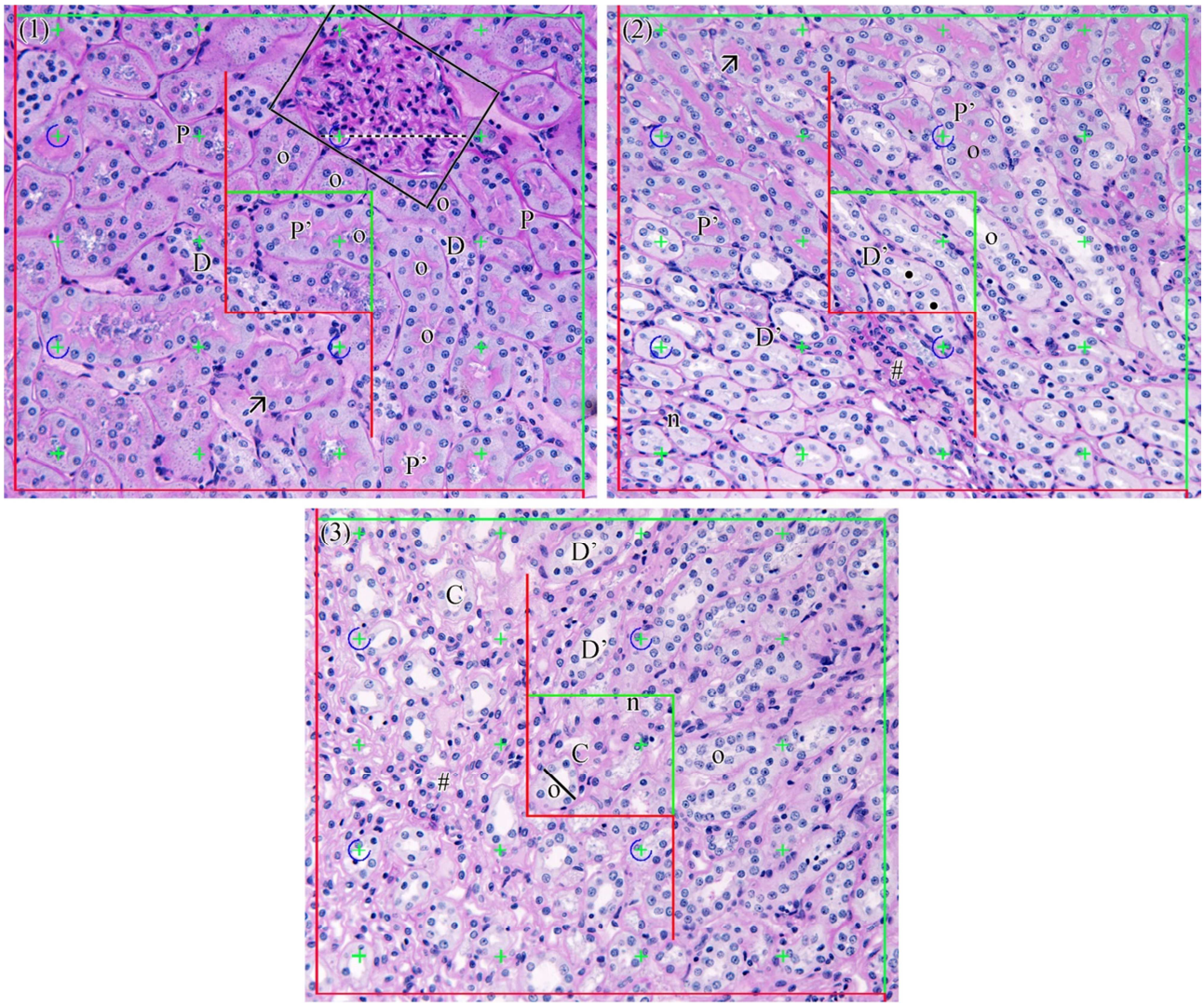

Figure 4. *Three microscopic fields (each a part of the field sampled for stereological measurement) taken on the same renal section (methacrylate-embedded, stained with PAS and hematoxylin) that was obtained from a normal male Sprague-Dawley rat aged 12 months, with test points (+) and 2 rectangular unbiased counting frames (inner smaller one 120 × 100 μm and outer larger one 470 × 390μm) superimposed on the field. P and P', convoluted and straight segments of proximal tubules; D and D', convoluted and straight segments of distal tubules; C, collecting duct; ↗, brush border of proximal tubules; o, tubular lumen in the tubular profiles that should be counted with the inner unbiased counting frame; n, tubular profile without a lumen; #, area of the interstitial tissue filled with thin segments of the Henle's loops. Note, one tubular profile [upper P' in (1)] has 3 tubular lumens (o) and another tubular profile [upper D' in (2)] has 2 tubular lumens (•).The position of the fields sampled in the kidney: (1) mostly in the cortex, partly (lower right boundary) in the outer stripe (lower P'); (2) upper field in the outer stripe and lower field in the inner stripe; (3) upper right field in the inner stripe and lower left field in the inner medulla. With respect to length measurements, the length and width of the oblique rectangle around the renal corpuscle profile (1) are the long and short axial diameters of the corpuscle profile. The length of the dotted line (1) is a point sampled intercept within the renal corpuscle/glomerulus, measured though the test point (+) hitting the corpuscle/glomerulus. The length of the solid line (3) is the short axial diameter of the tubular profile (lower C).*

2.4.3. Size and Histology of Renal Tubules

Of the tubular profiles counted (above), the round or elliptical, single luminal profiles were further measured for their diameters (for round profiles) or short axial diameters (elliptical profiles). These diameters were regarded as the approximate diameters of the 3-dimensional renal tubules in the present study [15, 18]. (Assuming the renal tubule is a cylinder-shaped structure, which is reasonable even though it may not be true [15], a section through the tubule is a circle or ellipse and the diameter of the circle or short diameter of the ellipse is the diameter of the tubule.) The histological (morphological) features of the tubular profiles were recorded after the diameter measurement.

2.4.4. Size, Histology and Number of Renal Corpuscles

The average of the long and short axial diameters of each renal corpuscular profile sampled and measured in the First Measurement was regarded as an approximate estimate for the diameter of the corpuscle. (The diameter is a useful, intuitive parameter, but it is a diameter of a 2-dimensional corpuscular profile, not representing the true size of the 3-dimensional corpuscle [19]). The average number of corpuscular profiles measured in this way was 49.7 per kidney.

In the Second Measurement, an average of 79.3 corpuscular profiles were sampled per kidney and their histological features were recorded.

The average numbers of point-sampled intercepts measured for the renal corpuscles and glomeruli were 116 and 103 per kidney, respectively. Calculating the mean of the intercepts cubed and then multiplying the mean by (π/3), we directly obtained the volume-weighted mean volume of the corpuscles or glomeruli, an unbiased estimate of the mean volume of the 3-dimensional corpuscles or glomeruli in the volume distribution [8, 9, 19].

As (a) the total number of particles (such as Sertoli cell nuclei or renal glomeruli) is equal to the total volume of particles divided by the arithmetic mean volume (i.e. the number-weighted mean volume or the mean volume in the number distribution) of particles and (b) the volume-weighted mean volume of particles approximates the number-weighted mean volume of particles (see the "Number of renal corpuscles or glomeruli" part in the Discussion for more detail), we obtained, as previously done for Sertoli cell nuclear number estimation [19], an approximate estimate of the total corpuscular/glomerular number (per kidney) by dividing the total volume of the corpuscles/glomeruli (obtained in the First Measurement) by the volume-weighted mean volume of the corpuscles/glomeruli (Second Measurement).

2.5. Statistics

The data in Tables 1–3 and some in the text are shown as $\bar{x}$±SEM (standard error of the mean). Comparison of data between the 5 age-groups (Tables 1–3) was performed using the one-way analysis of variance (in conjunction with the Student-Newman-Keuls method for all pairwise multiple comparisons) and the t-test was used for comparison of data between 2 age-groups (Table 3). Within-organ comparison between diameters of different renal tubules in each age-group was performed using the one-way repeated-measures analysis of variance (in conjunction with the Student-Newman-Keuls method for all pairwise multiple comparisons). The significance of difference was set at $p<0.05$.

3. Results

3.1. Histology

In general (Figure 5), (a) from 3–12M (3–12 months of age) to 24–36M, the staining of structures, especially the brush border and cytoplasm of proximal tubular epithelium, on sections became lighter and the lumen of renal tubules larger. (b) From young to old, the renal corpuscles grew larger, with the Bowman's space becoming more evident and the corpuscular basement membrane appearing slightly thicker at 24–36M. (c) The proximal tubules were much larger than other renal tubules. (d) The renal corpuscles and tubules were densely packed in the kidney, interspersed with bundles of connective tissue that was more evident in the outer medulla (Figures 1 and 3). (e) In older animals, the lumens of small arteries and arterioles in the renal connective tissue appeared larger and the internal elastic membrane was less clear and its folding less apparent, but the vessels had no vascular signs of arterionephrosclerosis such as thickening of the tunica intima (intimal fibrosis) or hyaline deposits in the vascular wall (hyalinosis) [20].

Apparently atrophied renal corpuscles or tubules began to appear at 24M, seen in less than 5% of the corpuscular or tubular profiles on sections between 24M and 36M (Table 3). They often had thickened basement membrane and focal inflammatory cells (mainly lymphocytes) infiltration around or near them (Figure 6).

Renal corpuscles were generally larger at 24-36M, usually associated with larger capsular cavity and capillary lumen (Figures 5 and 6). Apparently dilated corpuscles, as we judged mainly by the apparently larger renal capsular cavity and relatively smaller renal glomerulus that often had fewer or narrower capillary lumen, were present in 5% of the corpuscular profiles at 24M, and more (10%) at 36M (Figure 6).

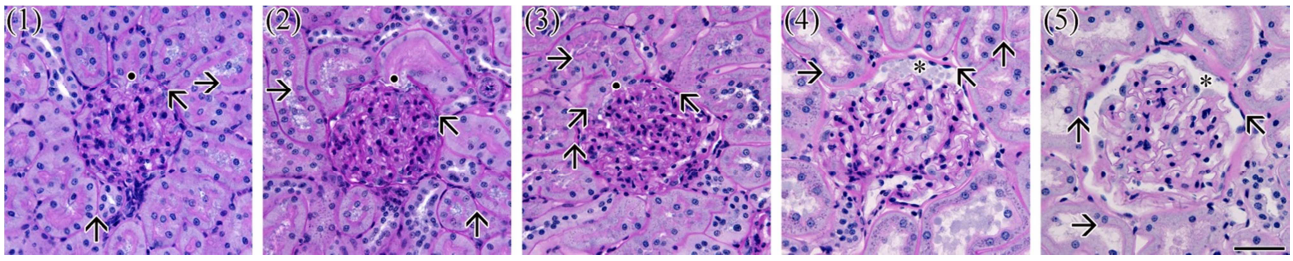

Figure 5. *Micrographs taken on renal sections (methacrylate-embedded, stained with PAS and hematoxylin) that were obtained from normal male Sprague-Dawley rats aged 3 months (1), 6 months (2), 12 months (3), 24 months (4) and 36 months (5), showing mainly the general changes with age of histological structures, especially the renal corpuscles. *, Bowman's space (between the glomerulus and the parietal layer of Bowman's capsule) in the renal corpuscle; •, where the Bowman's space is continuous with the proximal tubular lumen; ↑ and →, basement membrane and brush border of the proximal tubule; ↖, basement membrane of the renal corpuscle; ↗, simple cuboidal epithelium (part of the parietal layer of the Bowman's capsule). Scale bar (lower-right)=50 μm.*

Apparently dilated renal tubules, often with thinner lining epithelium, were seen in less than 4% of the tubular profiles on sections between 24M and 36M (Table 3), with 60% of the dilated profiles seen in the inner stripe and 90% of the dilated profiles being associated with hyaline casts (Figure 6) – homogenous PAS-positive substance filled in the tubular lumens [20-22].

The parietal layer of the Bowman's capsule was usually lined by a simple squamous epithelium. But a part of the epithelium occasionally became simple cuboidal (Figures 5 and 6) starting

from 3M. This so-called cuboidal metaplasia [23] was seen, at 24–36M, in 13%–19% of the corpuscular profiles (Table 3), with only 5%–6% of the metaplastic profiles being apparently atrophied or dilated. The cuboidal epithelium often resembled the lining epithelium of the neighboring proximal tubules, with its length on section mostly constituting less than half of the parietal layer, and occasionally it was seen continuous with the proximal tubular epithelium (Figures 5 and 6).

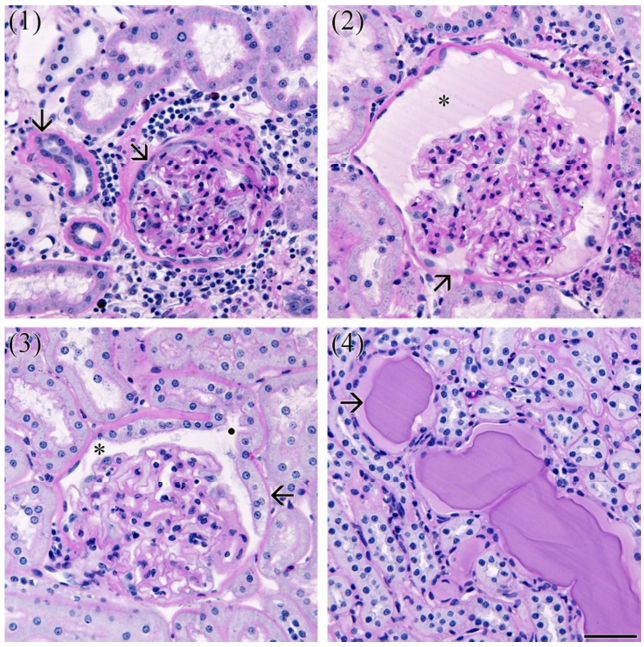

Figure 6. *Micrographs showing some histological features of the renal corpuscles and tubules, taken on sections (methacrylate-embedded, stained with PAS and hematoxylin) that were obtained from normal male Sprague-Dawley rats aged 24 months (1 and 2) and 36 months (3 and 4). *, Bowman's space (between the glomerulus and the parietal layer of Bowman's capsule) in the renal corpuscle; •, where the Bowman's space is continuous with the proximal tubular lumen; ↘, atrophied renal corpuscle; ↗, dilated renal corpuscle with a dilated Bowman's space; ←, simple cuboidal epithelium (part of the parietal layer of the Bowman's capsule); ↓, atrophied renal tubule; →, hyaline cast in a dilated renal tubule. Scale bar (lower-right)=50 μm.*

3.2. Total Quantities

From 3M (3 months of age) to 24M, the mean body weight of the rats increased in a curved line: the increase with age was an average of 38 g (from 3M to 6M), 22 g (6M–12M) and 11g (12M–24M) per month, respectively; thereafter (24M–36M), the body weight decreased significantly by 7 g per month [10]. In contrast, the mean volume of kidneys increased almost linearly with age (in a straight line) from 3M to 24M: the monthly increments were 79 mm^3 (3M–6M), 72 mm^3 (6M–12M) and 91 mm^3 (12M–24M), respectively, which plateaued afterwards without a decrease from 24M to 36M (Table 1). This trend of age-related change in the volume of kidney was basically similar to that in the total volume of the renal compartments – cortex, outer stripe, inner stripe or inner medulla (Table 1). The volume fraction (relative volume) of each of these compartments in the kidney remained basically stable from 3M to 36M: the mean of the 5 mean volume fractions for the 5 age-groups was 60.4%±1.1% for the cortex, 22.8%±0.5% for the outer stripe, 12.4%±0.6% for the inner stripe and 3.4%±0.3% for the inner medulla, respectively, with no significant difference between ages being detected in the volume fraction of any compartment.

A similar trend of linear increasing from 3M to 24M was also observed in the total volume of most histological structures (Table 1). From 24M to 36M, the total volume of renal tubules or tubular wall plateaued, but the total volume of renal corpuscles (or their components and the surrounding basement membrane) or interstitial tissue kept increasing linearly. In addition, the total volume of the tubular brush border peaked at 12M and that of the tubular lumen (not including the brush border) increased sharply at 24M from 3–12M and then decreased significantly at 36M (Table 1). Relatively, the volume fraction of (a) the capsular cavity in the renal corpuscle or (b) the tubular lumen in the renal tubule was small at 3–12M (volume fractions: a, 0.8%–1.4%; b, 2.3%–4.3%), which increased sharply at 24M (a, 10.4%±1.6%; b, 19.6%±1.5%) or 36M (a, 14.9%±2.8%; b, 17.5%±1.3%).

With respect to blood vessels (capillaries, arteries and veins) in the interstitium not shown in Table 1, the total volume (including the vascular lumen, endothelium and tunica media which could be clearly seen) was 22.9–23.3 mm^3 at 3–6M, which increased sharply to 68.0±9.1 mm^3 at 12M, 89.1±10.1 mm^3 at 24M and 95.8±14.6 mm^3 at 36M, without statistical difference being detected between the latter 3 age-groups. The volume fractions of the blood vessels in the interstitium were 14.4%–21.0%, without significant difference between the 5 age-groups.

From 3–6M to 12M, there was a sharp increase (by 38%–48%) in the total length of all renal tubules measured, which did not change significantly afterwards (Table 2). The relative length (percentage of the total length per kidney) of the proximal tubules (36.0%–41.5%) or distal tubules (11.2%–15.1%) in the cortex, the proximal tubules (17.7%–19.5%) or distal tubules (9.2%–10.3%) in the outer stripe, or the tubules (15.2%–23.0%) in the inner stripe and medulla remained basically stable, without significant differences between the age-groups.

The total numbers of renal glomeruli per kidney were 28.9–33.4 thousands in the 5 age-groups (Table 2), without significant difference between groups (analysis of variance: p=0.46). The total numbers of renal corpuscles (27.4–34.8 thousands in the 5 age-groups) independently estimated were similar, without a significant age-related change (analysis of variance: p=0.09), either.

3.3. Mean Sizes

There was a linear increase in the volume-weighted mean volume of renal glomeruli, by 93% from 3M to 12M and by 96% from 12M to 24M (Table 2). Similar trend of increase was also observed in the volume-weighted mean volume of renal corpuscles from 3M to 12M, but a sharp increase (by 148%) occurred from 12M to 24M. Further increase by approximately 20% was seen from 24M to 36M in the volume-weighted mean volume of renal glomeruli or corpuscles. In contrast, the age-related increase in the mean

diameter of renal corpuscular profiles was small, by 22% from 3M to 12M and by 32% from 12M to 24M and further increase (by 4%) from 24M to 36M was not significant (Table 2).

Basically, there was a linear increase (approximately by 35%–45%) in the mean diameters of all renal tubules, proximal tubules or distal tubules from 3M to 24M, which then declined (by approximately 10%) from 24M to 36M (Table 2). As to the diameters of collecting ducts, it appeared that the linear increase was from 3M to 12M, which then plateaued from 12M to 36M (Table 2).

The diameters (about 40–50 μm) of the proximal tubules in the cortex, approximating those in the outer tripe, were considerably larger (by roughly 50%–60%) than the diameters of the distal tubules in the cortex or medulla (Table 2). The diameters of the distal tubules in the outer stripe (a) were about 20% smaller than those of the distal tubules in the cortex or inner stripe, or collecting ducts in the inner medulla at 3–6M, but (b) became similar to the diameters of other tubules (excluding the proximal tubules) at 12–36M (Table 2).

4. Discussion

The aging change in the kidney is a research of concern which numerous studies addressed, but few studies quantitatively analyzed all major structures in the kidney of rats from young to old age. This is (a) a stereological (quantitative) study which measured large area of good-quality (methacrylate-resin embedded) renal sections and comprehensively analyzed all major renal components (renal tubules, corpuscles and interstitium) in a cohort of rats from 3 months (sexually mature) to 36 months (very old) of age, (b) one of only several studies [e.g. 15, 24] which used isotropic renal sections to estimate renal tubular length, and (c) the first study which estimated the volume-weighted mean volume of renal corpuscles/glomeruli. So the present study provides both methods and baseline data for future experiments involving the renal stereology or histology and helps to summarize and reach a clear and reliable conclusion on the kidney aging by comparison with other rat and human studies.

4.1. Renal Volume

The present study demonstrated a 3.3-fold linear increase in the rat kidney volume, larger than the 2.2-fold increase in the body weight, from 3M (3 months of age) to 24M, when the kidney volume plateaued (until 36M) while the body weight began to decrease (by 12% at 36M). Similar trend of age-related change was also observed in the volume of spleens, brains or livers (data unpublished) obtained from the same cohort of rats as used in the present study, but the basically linear volume increase from 3M to 24M was only 1.8-fold (spleen), 1.2-fold (brain) or 2.0-fold (liver). Moreover, the epididymides or seminal vesicles obtained from the same groups of rats had a 1.5-fold (epididymis) or 2.7-fold (seminal vesicle) volume increase from 3M to 12M, when the volumes basically peaked [10]. This suggests that there is a greater need of continuous kidney (compared with other organs) growth or development in older rats for maintaining the renal function, which depends, to a greater degree, on the amount of renal structures.

In other rat studies, variable age-related changes in the kidney weight were reported (a) in male Wistar/Lou M rats: unchanged between 12M and 24M and 29% increase from 24M to 36M [25]; (b) in Munich Wistar rats: 53% increase from 3M to 1.5 years and unchanged between 1.5 and 2.5 years [26], or 13% increase from 7–9M to 18–20M [27]; and (c) in male Sprague-Dawley rats: 57% increase from 3–4M to 16–17M [28]. In humans, it was often assumed that the renal mass increases progressively from birth to the fourth decade of life and then decreases thereafter at a 10% reduction rate per decade [29]. But the results in some actual human studies were also variable: (a) there was a 9% increase of kidney weight from 16–39 (average 30) to 40–58 (50) years and then a 35% decrease from 40–58 to 76–87 (81) years of age [30], (b) there was a 16% decrease in the volume of kidney cortex (measured with *in vivo* computed tomography) between 18–29 and 70–75 years of age [31], and (c) there did not seem to be a trend of kidney weight decrease between 46–55 and 86–98 years of age [32]. So a conclusion on the age-related change of renal mass could hardly be drawn from previous studies and more studies will be needed to verify the trend of change found in the present study.

The present study also demonstrated that, with the increase of the overall renal volume with age, the relative volume of the renal compartments (cortex or others) that constituted the renal mass, or the relative length of the different renal tubules that constituted the bulk of the renal mass and played different roles in the urine formation and transport, remained basically constant throughout the ages. That is, the growth or development of different key renal structures with age was uniform or proportional. So the implication is that the age-related renal volume change is generally a physiological compensatory development or hypertrophy of key structures in the kidney and, considering the continuous increase of the total or mean glomerular volume until 36 months of age, the compensation of glomeruli is still active at a very old age.

4.2. Glomerular Sclerosis

Glomerulosclerosis, primarily defined as accumulation or increase of glomerular matrix and obliteration or decrease of glomerular capillaries [20], is often regarded as a key abnormal or pathological effect of kidney aging. The percentage of sclerotic glomeruli was previously reported to be 7.9% in male Munich-Wistar rats aged 2.5 years [26], 9% in male Fisher 344 (F344) rats aged 24 months [33] and 25.2% in male Sprague-Dawley rats aged 24 months [34]. The present study did not focus on the classification of sclerotic glomeruli. But we presume that the atrophied corpuscles and some of the dilated corpuscles observed at 24–36M (Table 3) might be regarded as sclerotic. So the sclerotic glomeruli would be less than 8% (24M) or 15% (36M) in the present study. It is worth noting that the judgment of sclerosis from single glomerular profiles on single sections, which is often performed in renal studies, is subjective and the percentage of

sclerotic glomeruli may mislead us into thinking that it represents the percentage of glomerular number or volume degenerated or lost. No matter how many glomeruli were actually sclerotic, the present study demonstrated that there was no decrease in the total or mean glomerular volume, nor loss of glomeruli in the aging kidney. This highlights the importance of glomerular sclerotic study combining with study of the mean and total glomerular volume.

Table 1. *Volumes (mm^3) of kidneys and total volumes (mm^3) of key renal structures per kidney.*

	3M (n=9)	6M (n=9)	12M (n=9)	24M (n=9)	36M (n=8)
Volume of kidney (unilateral)	753±42	992±46[a]	1426±63[a,b]	2520±79[a,b,c]	2491±142[a,b,c]
Cortex	425±24	595±31[a]	883±40[a,b]	1520±77[a,b,c]	1570±89[a,b,c]
Outer stripe in the outer medulla	185±18	229±14	328±33[a,b]	573±25[a,b,c]	529±44[a,b,c]
Inner stripe in the outer medulla	109±12	113±11	155±13	331±25[a,b,c]	302±34[a,b,c]
Inner medulla	20±8	42±6	49±9	63±20	86±14[a]
Renal sinus	13±6	13±3	10±5	34±14	5±2
Renal corpuscles	22.0±1.4	30.3±0.9	46.9±3.1[a,b]	88.7±7.1[a,b,c]	124.4±8.8[a,b,c,d]
Glomeruli in the corpuscles	21.0±1.3	28.8±1.0	44.2±2.9[a,b]	76.0±6.2[a,b,c]	98.9±4.8[a,b,c,d]
Bowman's space in the corpuscles	0.2±0.1	0.4±0.1	0.7±0.3	9.3±1.6[a,b,c]	20.1±4.8[a,b,c,d]
Parietal layer of the Bowman's capsule	0.9±0.2	1.1±0.1	1.9±0.4	3.4±0.8[a,b,c]	5.4±0.8[a,b,c,d]
Basement membrane of the corpuscles [*]	0.8±0.1	2.1±0.2	4.2±0.5[a]	11.1±1.3[a,b,c]	18.2±1.1[a,b,c,d]
Renal tubules[*]	575±34	748±39[a]	999±43[a,b]	1743±69[a,b,c]	1588±91[a,b,c]
Wall of renal tubules	446±25	567±31[a]	762±35[a,b]	1220±41[a,b,c]	1155±58[a,b,c]
Brush border of renal tubules	117±10	158±12	194±12[a]	179±15[a]	152±19
Lumen of renal tubules	13±2	23±3	42±5	344±35[a,b,c]	281±28[a,b,c,d]
Area of thin segments of Henle's loops[*]	21±5	39±4	55±9	127±22[a,b,c]	159±18[a,b,c]
Interstitial tissue[*]	122±8	160±9	313±24[a,b]	522±15[a,b,c]	609±43[a,b,c,d]

Data are shown as $\bar{x}$±SEM. 3M–36M: normal male Sprague-Dawley rats aged 3–36 months; n: sample size. [*]The renal tubules included the collecting ducts, not the thin segments of Henle's loops; the area of thin segments of Henle's loops included the inter-segmental connective tissue; the interstitial tissue was connective tissue between the corpuscles, tubules and thin segmental area; the basement membrane of the renal corpuscles and tubules was part of the interstitial tissue. $p<0.05$: in comparison with the group of 3M[a], 6M[b], 12M[c] or 24M[d].

Table 2. *Sizes, lengths or numbers of renal corpuscles and tubules in the kidney.*

	3M (n=9)	6M (n=9)	12M (n=9)	24M (n=9)	36M (n=8)
Mean D of profiles of renal corpuscles (μm)	93±2	103±3	113±1[a]	149±6[a,b,c]	155±4[a,b,c]
$\bar{v}_v$ of renal corpuscles (10^6×μm^3)	0.69±0.04	1.02±0.07	1.36±0.08	3.37±0.32[a,b,c]	4.02±0.36[a,b,c,d]
$\bar{v}_v$ of renal glomeruli (10^6×μm^3)	0.69±0.04	1.02±0.07	1.33±0.08[a]	2.61±0.24[a,b,c]	3.18±0.23[a,b,c,d]
Total number of renal glomeruli per kidney (10^3)	30.6±1.2	28.9±1.4	33.4±1.9	29.9±2.5	31.9±2.2
D of all tubules (μm)	31.8±0.5	34.5±0.6 [a]	35.5±0.5[a]	43.5±1.1[a,b,c]	40.0±1.0[a,b,c,d]
D of proximal tubules in the cortex (μm)	38.6±0.5	41.0±0.9	41.5±0.8	51.4±1.4[a,b,c]	48.6±0.9[a,b,c]
D of distal tubules in the cortex (μm)	26.7±1.1	27.4±0.7	29.1±0.9	36.4±1.0[a,b,c]	31.8±0.8[a,b,c,d]
D of proximal tubules in the outer stripe (μm)	38.5±1.3	42.5±0.9	42.9±1.0	55.0±1.7[a,b,c]	49.9±1.8[a,b,c,d]
D of distal tubules in the outer stripe (μm)	21.1±0.5	22.8±0.9	27.1±1.3[a,b]	32.0±1.8[a,b,c]	29.2±1.5[a,b]
D of distal tubules in the inner stripe (μm)	25.7±0.6	26.8±0.8	28.5±1.0	36.1±2.3[a,b,c]	33.1±1.3[a,b,c]
D of collecting ducts in the inner medulla (μm)	24.1±2.3	28.3±1.4	31.2±1.0	32.0±4.4	31.5±1.7
L of all tubules (m)	502±22	536±20	742±42[a,b]	846±41[a,b]	811±30[a,b]
L of proximal tubules in the cortex (m)	179±10	210±10	306±23[a,b]	330±21[a,b]	325±13[a,b]
L of distal tubules in the cortex (m)	56±5	80±5[a]	105±10[a]	102±12[a]	105±6[a]
L of proximal tubules in the outer stripe (m)	99±6	98±7	143±17[a,b]	153±12[a,b]	145±13[a,b]
L of distal tubules in the outer stripe (m)	53±5	52±3	76±15	81±13	83±15
L of distal tubules in the inner stripe (m)	108±15	83±10	93±8	170±18[a,b,c]	140±14[b,c]
L of collecting ducts in the inner medulla (m)	8±3	13±1	19±5	10±5	13±4

Data are shown as $\bar{x}$±SEM. 3M–36M: normal male Sprague-Dawley rats aged 3–36 months; n: sample size. Mean D, mean of the long and short axial diameters of the corpuscle profile on sections; $\bar{v}_v$, volume-weighted mean volume of the corpuscles or glomeruli; D, renal tubular diameter; L, total length of renal tubules (excluding the thin segments of Henle's loops) per kidney; the collecting ducts (if any) in the compartments other than the inner medulla were classified into the distal tubules. $p<0.05$: in comparison with the group of 3M[a], 6M[b], 12M[c] or 24M[d].

Table 3. *Percentage of the profiles (on sections) of renal corpuscles and tubules with certain histological features in the kidney.*

	3M (n=9)	6M (n=9)	12M (n=9)	24M (n=9)	36M (n=8)
Renal corpuscles atrophied	0	0	0	3.6%±1.2%	4.9%±1.2%
Renal corpuscles dilated	0	0	0	4.9%±1.6%	10.3%±1.9%[d]
Renal corpuscles with cuboidal parietal epithelium	2.1%±0.8%	5.3%±1.3%	9.1%±2.3%	18.6%±4.8%[a,b]	13.4%±3.0%[a]
Renal tubules atrophied	0	0	0	0.81%±0.36%	1.91%±0.79%
Renal tubules dilated	0	0	0	3.80%±2.07%	2.47%±0.98%

Data are shown as $\bar{x}$±SEM. 3M–36M: normal male Sprague-Dawley rats aged 3–36 months; n: sample size. Atrophied renal corpuscle or tubule, often with surrounding lymphocytes infiltration; dilated renal corpuscle, with a dilated Bowman's space and a relatively smaller glomerulus; dilated tubule, mostly with a proteinaceous cast in the tubular lumen; cuboidal parietal epithelium, with part of the parietal epithelium of the Bowman's capsule being simple cuboidal. $p<0.05$: in comparison with the group of 3M[a], 6M[b] or 24M[d].

4.3. Size of Renal Corpuscles

In the present study, both the mean diameter of renal corpuscular profiles and the volume-weighted mean volume of renal corpuscles or glomeruli showed a trend of continual age-related increase until the age of 36 months, although the diameters did not show the change as clearly and markedly as the volumes. The increase in the mean corpuscular/glomerular sizes was associated with the age-related increase in the total corpuscular/glomerular volumes.

Age-related increase of glomerular size was also reported in previous rat studies [26-28, 33, 35, 36] although the increase was minimal in a study with male Sprague-Dawley rats aged 12, 24 and 36 months of age [25]. In humans, however, there was a ∧-shaped change of mean glomerular volumes from 30 to 65–68 years of age, with the glomerular volume peaking at 40 years of age [35], or there was a negative correlation between glomerular volume and age, with a decrease of 9% (at an average of 50 years of age), 22% (67 years) or 22% (81 years) from the mean volume at 30 years of age [30]. This suggests that there is a difference in the time course of age-related renal histological adaptation between rats and humans. And the species difference may be related to this striking species difference: proteinuria or albuminuria is abnormal (normally absent) in humans [37] but normal in rats, which may be present from 3 months of age with a sharp increase around 24 months of age in rats [21, 25-27, 33, 34, 38, 39]. We speculate that due to the loss of protein through the kidney at early ages, the rat kidney may work more actively to prevent it from more loss. As a result, the rat renal structures, especially renal corpuscles, are more functional and therefore "healthier"– less likely atrophied or lost (see also the part below).

4.4. Number of Renal Corpuscles or Glomeruli

The number estimation of renal corpuscles/glomeruli was a by-product of the present study, extra information extrapolated from the estimated total volume and volume-weighted mean volume of the renal corpuscles/glomeruli. The number is an underestimate of the true number because the volume-weighted mean volume is larger than the number-weighted mean volume, and the degree of underestimation (bias) depends on the coefficient of variation of the corpuscular/glomerular volumes. When the within-organ coefficient of variation of corpuscular/glomerular volumes is 33%, the number estimated with the volume-weighted mean volume will be 10% smaller than the true number per kidney [8, 9, 19]. The great advantage of the number estimation in the present study is its simplicity: only point counting and length measuring on single renal sections were necessary. Serial renal sections would be needed for unbiased estimation of glomerular number with the disector method [8, 28, 30, 36, 39], which would be much more time-consuming.

Interestingly, the total glomerular numbers (per kidney) approximately estimated in the present study, $29–33\times10^3$ for the 5 age-groups, were comparable to the total glomerular numbers ($24–32\times10^3$) previously estimated in Sprague-Dawley or Wistar rats (aged from 10 days to 24 months) using unbiased stereological methods [28, 36, 39], without studies showing a decrease of glomerular number with age. In contrast, age-related decrease in the glomerular numbers or loss of glomeruli in the aging kidney was consistently reported in humans [30, 31, 40, 41]. These results suggest that there may be indeed another species difference in the age-related change of glomerular numbers between rats and humans. The age-related glomerular numerical change in humans might be explained by the above-mentioned mean glomerular volume change; more studies will be needed for further clarification of the scenario.

4.5. Parietal Layer of the Bowman's Capsule

It was previously shown that part of the parietal epithelium of the Bowman's capsule became cuboidal in a 30-week-old male Sprague-Dawley rat with minimal chronic progressive nephropathy [23]. We found in the present study that (a) the cuboidal metaplasia occurred early, increasing with age and mostly in normal renal corpuscles, and (b) the metaplastic epithelium was either continuous with or resembled the lining epithelium of the proximal tubules. Therefore we speculate that the so-called metaplastic epithelium [23] was not transformed from the parietal layer of the Bowman's capsule, but an extension of the proximal tubular epithelium (continuous with the Bowman's space) due to the rapid development and enlargement of renal corpuscles with age.

4.6. Hyaline Casts in the Renal Tubules

As shown previously [21] and in the present study, hyaline casts – proteinaceous casts [21, 22], were often seen in dilated renal tubules in old rats. So tubular dilation may be an aging effect in the kidney [21]. We speculate that it might indicate an obstruction or stasis of the ultrafiltrate or urine in some uriniferous tubules, especially in the inner stripe where the casts were mostly seen, due to proteinuria commonly seen in old rats (above). Dilation of the Bowman's space observed in old rats in the present study might be a result of the stasis, which might also result in a pressure-mediated glomerular hypertrophy and/or degeneration. That is, hyaline casts in the renal tubules might be another factor other than the proteinuria (see above) that contributed to the kidney aging effect in the rat. If this were proved to be true, diuresis might be a potential choice for deferment or prevention of kidney aging.

4.7. Length and Diameter of Renal Tubules

Age-related change in the length and diameter of renal tubules was seldom studied. The present study found that the lengths or diameters of different segments of renal tubules generally increased with age in proportion, indicating a uniform, physiological compensation or hypertrophy with age. From 24 to 36 months of age, some reduction in the diameter of renal tubules or the total volume of tubular lumen was observed while the total length of tubules plateaued between 24 and 36 months of age (Table 2). This might indicate a

reduction in the amount of glomerular ultrafiltrate from 24 to 36 months of age.

Length of linear tissue structures such as renal tubules are best estimated with stereology by counting the (number of) sections (profiles) of the linear structures on tissue sections [7, 9, 15]. For unbiased estimation, however, the linear structure must be (assumed to be) isotropic, or the tissue section must be isotropic (i.e. random in terms of orientation in 3-dimensioanl space). Apparently, renal tubules in the kidney are radially distributed from the renal pyramid's papilla to the outer border (Figures 1 and 3), without a random orientation. This was why we went out of the way to obtain isotropic renal sections using the orientator in the present study. And to optimize sampling of tissue sections, we cut tissue blocks along 2 independent random orientations and obtained 4 blocks per kidney.

Tubular profiles can be counted in two ways. We can count all tubular profiles or the tubular profiles with tubular lumens [15, 42]. Since a tubular profile with a lumen can be better identified (than a tubular profile without a lumen) and such a profile can be better observed and measured, we performed the latter counting in the present study: we counted the lumens of tubular profiles as we previously did [15].

4.8. Interstitial Tissue

Interstitial fibrosis is often regarded as an aging effect in the human kidney [1, 5, 6, 43]. In the present rat study, we showed an increase of the interstitial tissue with age and the increase was associated with larger volume of blood vessels. But the interstitium observed was basically normal except for some focal inflammatory infiltration, and renal tubules and corpuscles were still densely packed in the kidney without much connective tissue between them. In our previous study of the epididymides and seminal vesicles from the same groups of animals as used in the present study, interstitial inflammatory infiltration (in the connective tissue of the gonads) was not evident except that inflammatory cells were observed in the seminal vesicle (mainly in the glandular lumen near the lining epithelium) of one animal (aged 36 months of age) only [10].

4.9. Gender Difference

The present investigation studied only male rats from our previous study in which no female rats were included in the experiment [10], without addressing the gender difference. It was recognized that age-related changes such as glomerular sclerosis in the male were often more apparent or severer than the female [21, 27, 28, 38]. So the aging effects shown in the present study might represent the worse scenario in the Sprague-Dawley rat population living under the same condition.

4.10. Summary

For a reliable, quantitative study of the morphological changes in the aging kidney, isotropic uniform random sections, ideal for unbiased stereological (quantitative) estimations, were obtained in the present study from the kidneys of male rats (8–9 per age-group) randomly sampled from a single cohort of normal animals at the ages of 3, 6, 12, 24 and 36 months, respectively. The renal sections, after embedding in methacrylate resin with negligible tissue shrinkage or expansion [9, 44] and staining with PAS and hematoxylin for better showing the boundary of structures, were measured on a computer screen using various, sophisticated stereological methods to estimate the total amounts or mean sizes of key renal structures. The results indicated that the volume of kidney and the total (per kidney) volume or length of renal tubules increased continually from 3 to 24 months of age and then plateaued between 24 and 36 months of age. The total volume of renal corpuscles, glomeruli, Bowman's space or interstitial tissue and the mean volume of renal corpuscles or glomeruli increased continually from 3 to 24 months and further until 36 months of age. The mean diameter of renal tubules or the total volume of renal tubular lumen increased continually from 3 months and peaked at 24 months of age. Throughout the ages, the total number of renal glomeruli remained essentially constant, and the relative volume of the cortex or medulla and the relative length of different segments of the renal tubules remained basically stable. Apparently atrophied or dilated (dilation of Bowman's space with relatively smaller glomerulus) renal corpuscles began to appear at 24 months of age, accounting for 8% (at 24 months of age) and 15% (36 months) of the corpuscular profiles. Apparently atrophied or dilated (mostly associated with hyaline proteinaceous casts) renal tubules accounted for approximately 5% of the tubular profiles at 24 or 36 months of age. In conclusion, the present study demonstrates a comprehensive age-related histological change of key renal structures which suggests that the renal tissues continued to develop adaptively or work actively from young to old to maintain normal physiological functions, with only a small part of the tissues beginning to degenerate after 24 months of age. So the aging change in the rat kidney was primarily a compensatory or hypertrophic histological change, without marked pathological changes prevalent in the aging kidney. Besides, the present study also discussed about the species difference in the age-related renal histological adaptation between rats and humans.

5. Conclusion

The present study quantitatively analyzed key histological structures in the kidneys from a cohort of male Sprague-Dawley rats aged 3 to 36 months with sophisticated stereological methods. The results demonstrated that the total (per kidney) volume or length of the renal tubules increased continually from 3 to 24 months of age and the total volume of glomeruli increased continually from 3 to 36 months of age while the total number of glomeruli remained essentially constant throughout the ages, with only a small part of the tissues beginning to degenerate after 24 months of age. The conclusion is therefore that the renal tissues (including renal glomeruli and tubules) continue to develop adaptively or work

actively from young to old age to maintain normal physiological functions and the aging change in the kidneys is primarily a compensatory or hypertrophic histological change.

Competing Interests

The authors declare that they have no competing interests.

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