invited review

The use of microcomputed tomography to study microvasculature in small rodents

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Bentley, Michael D., Maria C. Ortiz, Erik L. Ritman, and J. Carlos Romero. The use of microcomputed tomography to study microvasculature in small rodents. Am J Physiol Regulatory Integrative Comp Physiol 282: R1267–R1279, 2002; 10.1152/ajpregu.00560.2001.—Appropriate nephron function is dependent on the intrarenal arrangement of blood vessels. The preferred and primary means to study the architecture of intrarenal circulation has been by filling it with opaque substances such as India ink, radio-opaque contrast material, or various polymers for study by light or scanning electron microscopy. With such methodologies, superficial vessels may obscure deep vessels and little quantitative information may be obtained. Serial-section microtomy has not been practical because of problems relating to alignment and registration of adjacent sections, lost sections, and preparation time and effort. Microcomputed tomography (micro-CT) overcomes such limitations and provides a means to study the three-dimensional architecture of filled vessels within an intact rodent kidney and to obtain more quantitative information. As an example of micro-CT's capabilities, we review the use of micro-CT to study the alterations in renal microvasculature caused by the development of liver cirrhosis after chronic bile duct ligation. In this example, micro-CT evidence shows a selective decrease in cortical vascular filling in the kidney, with a maintenance of medullary vascular filling. These changes may contribute to the salt and water retention that accompanies cirrhosis. These results indicate that micro-CT is a promising method to evaluate renal vascular architecture in the intact rodent kidney relative to physiological and pathological function.

kidney; imaging; microcirculation; vasculature

APPROPRIATE NEPHRON FUNCTION appears to be dependent on the detailed three-dimensional interrelationship of blood vessels with the tubular components. Many techniques and/or methods have been developed to examine the microanatomic arrangement of pre- and postglomerular vasculature (4, 29, 36, 40, 54, 55, 59, 71, 98). In this respect, such efforts were prompted by early studies showing that urinary excretion varied in relation to the intrarenal environment and was dependent on the coupling of renal microcirculation and tubular components. In 1947, Trueta and colleagues (96), studying the crush syndrome in rabbits, observed that there was a shift of blood flow from the renal cortex to the medulla during hemorrhage. They suggested this shift to be the major explanation for the lack of urine flow, despite a preservation of renal blood flow (RBF). The shift of blood flow within the renal cortex from superficial short nephrons to deep long nephrons was later observed in sodium-retaining states such as cardiac insufficiency, hepatic cirrhosis, or hypovolemia (1, 18, 53, 104). Selkurt and colleagues (87–89) showed that changes in renal perfusion pressure, within a range (75–130 mmHg) in which RBF remains unchanged (RBF autoregulation), were followed by dramatic changes in sodium excretion. This situation is comparable to that seen in the early stages...
of hepatic cirrhosis, where increased sodium reabsorption occurs without a change in RBF (2, 12, 79, 104).

These findings suggest that the anatomical relationship between the nephrons and their surrounding peritubular capillaries could explain the disparity between blood pressure and sodium excretion in a number of pathological situations (1, 17, 18, 44, 53, 96). However, because of methodological limitations, the three-dimensional anatomical complexity of the renal vasculature and its geometrical relationship with specific tubular segments along the nephron has been difficult to study in a quantitative manner. In this paper, we review the methods that have been used to study renal vasculature and the advantages of using microcomputed tomography (micro-CT) to study changes of renal vasculature during experimental pathophysiological conditions. As an example, we review the use of micro-CT to study the alterations in renal microvasculature after chronic bile duct ligation (CBDL).

METHODS TO STUDY RENAL VASCULAR ARCHITECTURE

Vascular Filling Techniques

Among the early investigators to study renovascular architecture was Bowman (14), who in 1842 injected wax into the vasculature and corroded the tissue away to expose the resultant cast or injected various precipitates into the vasculature to study the finer vessels of the kidney. Since that time, the preferred and primary means to study the architecture of intrarenal circulation has been by filling it with opaque substances such as India ink, radio-opaque contrast material, or various polymers. Subsequently, the tissue is either cleared, X-rayed, or corroded so that the filled vasculature may be studied by light or scanning electron microscopy. Most notable among early investigations was that of Trueta and colleagues (96) who used a variety of these filling techniques to study the vasculature of the rabbit kidney as well as other species.

**Light microscopy.** To study the renal vasculature by light microscopy, an opaque substance such as India ink or an opaque polymer is infused into the renal circulation (5, 7, 31, 54, 65, 75). The tissue is then treated with a clearing agent such as methyl salicylate to render the tissue transparent and expose the filled blood vessels for examination after modified procedures introduced by Hirsch and Spalteholz (43).

Because of its inert properties, silicone rubber has been extensively used as a filling agent to study microvasculature. It has a low viscosity that allows it to fill completely the vascular compartments with little resistance (24, 92). It polymerizes at room temperature with minimal shrinkage, allowing quantitative measurements. The hydrophobic properties of silicone rubber keep it contained within the vascular compartment, and extravasation has only been reported in situations such as inflammation where physical leaks are present (85).

With the use of silicone rubber, Beeuwkes and colleagues (5–7) described the vascular-tubular relationships in dog kidneys. Silicone rubber was microinjected into the vasculature and tubules of perfusion-fixed kidneys, and the kidney tissue was cleared with methyl salicylate so that 3-mm-thick slices could be studied by light microscopy. With this methodology, Beeuwkes showed that a variety of efferent vascular patterns were present in the dog that varied according to the cortical and medullary regions of the kidney and showed that each nephron was functionally dependent on efferent blood from glomeruli of other nephrons. Evan and Dail (31) using silicone rubber showed that similar vascular-tubular relationships existed in the rat. The specific peritubular vascular pattern was dependent on the cortical location of the glomerulus and its efferent arteriole.

**Microangiography.** To study renal tissue by microangiography, a radio-opaque substance is infused into the renal vasculature (20, 42, 61, 91). X-ray images of thick slices of the infused tissue are exposed on high-resolution X-ray film and subsequently examined by light microscopy. This methodology has been used to examine vascular changes that occur in experimental disease conditions such as acute renal failure (21, 41), diabetic nephropathy (63), papillary necrosis (25), and allograft rejection (22).

**Scanning electron microscopy.** To study renal tissue by scanning electron microscopy, a low-viscosity plastic resin is infused into the renal vasculature (11, 19, 27, 28, 102, 103). After the resin has polymerized, the tissue is treated with a corrosive agent such as KOH to remove the tissue from the vascular cast for examination. Because the specimen stage of a scanning electron microscope can be rotated and tilted, the microvasculature may be examined from different viewpoints, giving some three-dimensional analysis capability. Furthermore, the images obtained with scanning electron microscopy represent surfaces that have a three-dimensional appearance due to the angle-specific interaction of the electron beam with the specimen surface. Among all of the methods to examine renal vasculature, scanning electron microscopy has the widest range of magnifications and the highest resolution, allowing fine details to be seen, such as endothelial cell indentations on the surface of the polymer filling the blood vessels. Furthermore, scanning electron microscopy provides a means to measure dimensions of small blood vessels. For example, Denton et al. (27) measured dose-related reductions in afferent and efferent arteriole diameters after angiotensin II infusion. However, beyond measuring linear dimensions such as vessel diameters, the quantitative capabilities of scanning electron microscopy are limited. In addition, only the superficial vessels exposed to the electron beam may be examined by scanning electron microscopy and the deeper vessels are obscured.

Three-Dimensional Analysis of Serial Sections

Most of the understanding of renal vascular microarchitecture has come from vascular filling methods. Generally, “slabs” of tissue (i.e., very thick section, >1
membranes in thickness) containing filled blood vessels have been studied by vascular filling techniques so that superficial vessels are superimposed over deeper vessels within the slab. Consequently, with the vascular filling methodologies, the deeper vessels may be obscured or, because of the superimposition, may appear to be connected. Thus issues such as the presence of shunts bypassing glomeruli have been controversial (19). Furthermore, vascular filling methodologies have been limited in providing quantitative information regarding the vasculature in the different regions of the kidney, which could change under pathophysiological conditions.

The superimposition problem could be eliminated by serial-section microtomy and analysis of the sections (100). The complex three-dimensional structure of the glomerular capillary bed has been studied using serial section microtomy (16, 50, 76, 90). However, the method generally has not been practical for studying the overall renal vascular architecture because of physical problems relating to the alignment and registration of adjacent sections, lost or distorted sections, and time and effort involved in the preparation and analysis of the sections. Furthermore, once the tissue is sectioned, the intact volume is lost for further analysis by other methods.

Advancements in imaging technology have provided means for analysis of virtual or computerized serial sections, alleviating many of the problems associated with mechanical serial section microtomy and allowing the vasculature to be viewed in three dimensions. These advancements include confocal microscopy, magnetic resonance microscopy, and X-ray microcomputed tomography (micro-CT).

Confocal microscopy. With confocal microscopy information is derived from the plane of focus without the blurred information from contiguous sections that are out of the plane of focus (23). Thus serial “optical sections” may be derived by adjusting the depth of the focal plane without physically sectioning the specimen. Confocal microscopy is a useful tool to study microvessels at the cellular and tissue level. For instance, Itoh et al. (46) were able to obtain three-dimensional reconstructed images of cells in the pituitary gland and their microvascular environment using confocal laser microscopy. Yasumura et al. (105) were able to determine by confocal laser microscopy microvessel densities and permeabilities in three-dimensional reconstructed images of rat mammary tumor. In the kidney, the capillary network of the glomerulus (50) and its circulation (70) have been studied. Boyde et al. (15) were able to visualize individual red blood cells and white blood cells as they flowed through the peritubular capillary circulation near the surface of living kidneys. However, the maximum depth of focus for all of these studies is <1 mm and, consequently, in a whole kidney that has greater tissue depth, continuous measurement from the arterial trunk to the downstream capillaries has not been possible.

Magnetic resonance microscopy. Another microimaging modality to visualize serial sections is magnetic resonance microscopy (26, 45, 94). The resolution of magnetic resonance microscopy depends directly on the magnetic field strength (38). In the rat kidney, Hedlund et al. (39) were able to detect damage to the outer medulla after exposure to bromo-ethanolamine. The damage was presumably related to changes in renal water content. Oliverio et al. (68) used magnetic resonance microscopy to measure kidney parenchyma from mice lacking AT1A receptors with a voxel resolution of 51 μm and were able to detect small changes in the corticopapillary dimensions of the mice. Recently, Tang and colleagues (95) used high-resolution magnetic resonance imaging to measure organ and tissue composition, including that of kidneys in a longitudinal study of living rats. The investigators found high correlations to postmortem organ weights and tissue composition. The study illustrates the utility of high-resolution magnetic resonance imaging to study tissues of rodents noninvasively and without exposure to radiation. However, although information concerning the renal tissues, per se, has been obtained with magnetic resonance imaging, there is currently no information about the three-dimensional microvasculature of the tissues.

X-ray microcomputed tomography. Micro-CT provides a means to study the three-dimensional vasculature architecture of rodent organs. In 1984, Feldkamp et al. (32) developed the cone-beam reconstruction algorithm, which was initially used to investigate the three-dimensional microstructure of bone (33, 58). Since that time, micro-CT has been used extensively to study the three-dimensional microstructure of bone (3, 13, 57) and its microvasculature (77). Furthermore, the capabilities of micro-CT make it an ideal tool to study the three-dimensional architecture of basic functional units of small organs such as those found in rodents (48). In addition to bone tissue, micro-CT has been used to study renal microvasculature (37), the microvasculature of liver (99), the biliary system of liver (62), the microvasculature of lung (47), myocardial microvasculature (51, 82), and the vasa vaso rum of coronary arteries (56, 60). The images obtained with micro-CT represent three-dimensional arrays of voxels having values representing X-ray opacities. With this information, quantitative information may be derived from the images.

MICRO-CT OF THE KIDNEY

Micro-CT Scanners

X-ray microtomographic scanners generally consist of an X-ray source, a rotatable specimen stage, an X-ray-to-light converting scintillator, a light imaging device [usually a charge-coupled device (CCD)-based video camera], and a controlling computer. One such system is described in detail elsewhere (48). Briefly, scanning is performed by rotating the specimen in specified angular increments in an X-ray beam and acquiring an X-ray transmission image at each angle of view. A schematic diagram of a microtomographic system is shown in Fig. 1. Three-dimensional images are
reconstructed from multiple angular views using a tomographic reconstruction algorithm such as a modified Feldkamp’s filtered backprojection algorithm (32). The reconstructed images are comprised of cubic voxels (typically ranging from 1 to 20 μm on a side, size depending on the optical magnification of the micro-CT setup), each voxel having a gray scale value that represents the X-ray attenuation at its corresponding locus in the specimen.

Paulus et al. (72) reviewed the issues that must be considered in the design of a micro-CT system. These issues include the scanner geometry, the X-ray source, and the detector components. All three components influence the resolution and quality of the image. Geometry considerations involve the focal spot size of the X-ray source, the resolution of the detector array, and the position of the specimen with respect to the source and the detector. In addition, the magnification factor is a trade-off between desired resolution and the imaged volume of the object. Finally, vibration must be minimal and rotation must be precise to minimize geometric artifacts in the image reconstruction. The X-ray source must emit an X-ray energy in which attenuation by the object provides optimal contrast. Because X-ray attenuation is strongly dependent on the X-ray energy spectrum, prefiltration of the X-ray beam, to make the beam more monochromatic, reduces problems related to beam hardening. In the detector components, the scintillator must have adequate resolution at the selected energy level and adequate quantum efficiency. The magnifying optics must be distortion free, and the CCD must have adequate dynamic range, pixel size, and field uniformity.

Currently, several micro-CT systems are commercially available. Manufacturers include Bioimaging Research (www.bioimaging.com), Enhanced Visions Systems (www.evscorp.com), Imtek (www.imtekinc.com), Skyscan (www.skyscan.com), and Stratec Medizintechnik (Norland Medical Systems, www.norland.com). Typically, manufacturers claim resolutions <50 μm and usually in the 10-μm range. For the most part, these systems have been used to evaluate hard tissues such as bone and teeth, and the utility of these systems for evaluating renal microvasculature has not been established.
Two micro-CT systems are used by our group to study the microvasculature in rat kidneys (37). One system uses a synchrotron X-ray source located at Brookhaven National Laboratory’s Synchrotron Light Source (beam line ×2B). This system was developed by Flannery et al. (34) to study the microstructure of porous rock and was subsequently used to envision basic function units of biological tissue (78). The synchrotron source has many advantages over other sources, especially the intense collimated beam from which tuned monochromatic X-rays can be selected. These capabilities are necessary for high-resolution three-dimensional images (e.g., voxel dimensions of 1 μm) with signal linearity and high signal-to-noise ratio. However, the major limitation with the synchrotron source of X-ray is the small sample size exposed by the X-ray beam. In addition, availability and access to synchrotron sources is limited, although such access is rapidly being facilitated at several sites (e.g., U.S. Argonne National Laboratory and European Synchrotron Radiation Facility). Our second micro-CT system is a “benchtop” version (48) that has a configuration that is similar to the synchrotron scanner. The main difference between systems is the X-ray source. The X-ray source in the benchtop scanner is a Phillips spectroscopy long, fine focus X-ray tube with a molybdenum. The effective focal spot size is 0.6 × 0.4 mm. The tube is operated at 35-Kev peak and 50 ma, providing molybdenum kα emission that when filtered with zirconium foil to remove unwanted higher energy components of the beam’s spectrum, provides predominantly a 17.5-Kev beam. The specimen stage is positioned 1 m from the X-ray focal spot, minimizing the source’s cone-beam angle and the penumbral blurring. The specimen stage provides precise rotation to within 0.001 degree and translation to within 1 μm. The specimen is rotated at angular increments of 0.499 degrees between views, providing 721 views around 360 degrees. The exposure time for each view is typically 85 s. An image of each view is acquired on a clear cesium iodide crystal doped with thallium. A 50-mm f2.8 enlarger lens is positioned between the scintillator and the CCD camera to provide variable magnification. The CCD camera is cooled to −30°C and records an image (1,024 × 1,024 pixels) at each view. The output images from the CCD are digitized to 16 bits and transferred to the controlling computer. Scans are made so that each CCD pixel sampled a square area of 24 μm on a side within the specimen. Three-dimensional volume images are reconstructed from the angular views using a modified Feldkamp’s filtered back-projection algorithm (32). Routinely, the reconstructed images are generated from cubic voxels, 21 mm on a side. However, with this system an entire (0.5 × 1 × 2 cm) intact rat kidney may be studied with images having typical cubic voxel dimensions as small as 5–10 μm. The opacity of each voxel is represented by a 16-bit gray scale value. Recently, a variation of this benchtop scanner was developed to scan fresh-frozen specimens (e.g., a rat kidney) so that molecular analyses requiring fresh specimens can subsequently be performed and then related to the three-dimensional micro-CT image data (49).

**Preparation of Tissue for Micro-CT**

To prepare rat kidneys for micro-CT, the blood is flushed from renal vasculature at physiological pressure with an infusion of heparinized 0.9% saline (37, 69). The infusion is continued with a formalin solution to fix the tissue, and the free formalin is flushed from the kidneys with a saline solution. Radiopaque silicone rubber (Microfil, MV-122, Flow Tech, Carver, MA) containing a suspension of lead chromate is then infused into the vasculature. When filling is complete, the kidneys have a uniform coloration and the microfil flows freely from the renal veins. Throughout the entire procedure, the perfusion pressure is maintained at physiological pressure by adjusting the flow rate of the...
infusion pump. After the infusion, the renal vessels are ligated and the silicone rubber is allowed to polymerize. The kidneys are then embedded in plastic or paraffin for micro-CT scanning.

Analysis of Images

Qualitative analysis. For analysis of the images, the ANALYZE (80) software package provides methods to compute, display, and analyze orthogonal and oblique sections from the reconstructed volume images. In addition, the software provides volume-rendering methods to envision the three-dimensional architecture of the renal vasculature. This architecture may be explored and viewed by a number of computer techniques, ranging from digital sections to volume rendered displays of the entire vasculature.

Quantitative analysis of images. In addition to the three-dimensional capabilities of micro-CT, perhaps the greatest strength is the capability to derive quantitative information from the images. Linear dimensions of blood vessels are measured by the Pythagorean theorem in the “calipers” application of the software while glomerular diameters are determined by counting the number of sections through individual glomeruli and multiplying the number of sections by section thickness. The number of glomeruli is determined by using the method of Sterio (93). With this method, the number of glomeruli are counted in small sample volumes systematically taken throughout the entire cortex. To determine the volume of an entire kidney and its cortical and medullary regions, a stereologic application is employed that places a three-dimensional array of points spaced orthogonally throughout the entire scanned volume (101). The fraction of the volume array that is the tissue of interest is determined by counting the number of points within the boundary of the tissue and dividing by the total number of points.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue Volume, μl</th>
<th>Vascular Volume Fraction, %</th>
<th>Vascular Volume, μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>976.7 ± 179.4</td>
<td>27.6 ± 4.0</td>
<td>272.5 ± 73.9</td>
</tr>
<tr>
<td>OSOM</td>
<td>299.3 ± 69.8</td>
<td>18.6 ± 2.8</td>
<td>51.9 ± 7.2</td>
</tr>
<tr>
<td>ISOM</td>
<td>200.0 ± 41.6</td>
<td>30.0 ± 1.1</td>
<td>59.9 ± 12.8</td>
</tr>
<tr>
<td>IM</td>
<td>115.2 ± 33.4</td>
<td>18.2 ± 2.1</td>
<td>20.4 ± 6.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. OSOM, outerstrip of outer medulla; ISOM, inner stripe outer medulla; IM, inner medulla.

Fig. 4. Orthogonal computer rendered views of renal volume images. Top: transparent views; middle, surface views; bottom, surface views rendered to illustrate large blood vessels. The images are from the benchtop scanner, and the reconstructed voxel size was 21 μm (37).
The tissue volume is then determined by multiplying that fraction by the total scanned volume of the image (8).

One of the most useful measurements has been the determination of the vascular volume fraction in the renal tissue (i.e., the fraction of vasculature in tissue). The vascular volume fractions are determined following the method used by Hillman et al. (41). The determinations are made from midtransverse slices that included the cortex, the outer stripe of the outer medulla (OSOM), the inner stripe of the outer medulla (ISOM), and the inner medulla (IM). The opacity is measured from regions of interest within a large interlobar artery (i.e., concentrated microfil) in the vascular bed of a tissue that represents a mixture of the vasculature with tissue components and in the background matrix outside the kidney (Fig. 2). This method assumes that the lead chromate is mixed homogeneously with the silicone rubber of the microfil, that the entire vasculature is completely filled with microfil, and that the microfil has not “leaked” into the surrounding tissue. Because the opacity of tissue represents the microfil within the vessels of a vascular bed “diluted” by the opacity of nonvascular tissue, the method is independent of voxel size and resolution of the scanner.

Micro-CT Images of Rat Kidneys

The images are represented by three-dimensional arrays of cubic voxels having opacities representing the amount of microfil in blood vessels. In sections taken from the arrays, Garcia-Sanz et al. (37) observed that the regions of the kidney are easily identified by their characteristic vascular features (Fig. 3). The cortex is characterized by glomeruli and the medulla by parallel bundles of vasa recta. The OSOM, the ISOM, and the IM may be identified by the density of the vasa recta bundles. From these characteristics the regional volumes of the kidney were determined (Table 1) and found to be similar to those reported by Pfaller (74). The arteries and their corresponding veins were opacified by the microfil, indicating that the entire renal circulation had been filled (Fig. 4). The percentage of renal mass filled with microfil (i.e., the vascular volume fraction) varied with the regions of the kidney (Table 1) and was similar to that reported by Hillman et al. (41).

In general, with the exception of the capillaries, the morphological features of the blood vessels were distinct (37). In low-magnification images, because the voxel size (10–20 μm) was larger than the diameter of a capillary, the morphological details of the capillaries were blurred and not distinguishable. However, in the high-magnification images with voxel sizes that were <6 μm, the capillaries (i.e., the finest details) were clearly evident (Fig. 5). When microfil was microinjected into the tubule and vessel lumens of intact kidneys, the complex interrelationship of the capillaries surrounding the tubules could be visualized (Fig. 6).

Of importance in the study by Garcia-Sanz et al. (37) was that not only could the distribution and three-dimensional architecture of renal vasculature be observed, but it could also be quantitated by micro-CT. Although vascular filling techniques had previously been used extensively (see above) to provide a fundamental understanding of renal microvascular architecture and tubular vascular relationships, quantitative information had been difficult to obtain. Furthermore, with micro-CT, not only is it possible to obtain quantitative information throughout the kidney, the organ remains intact.

USE OF MICRO-CT TO INVESTIGATE DISEASE CONDITIONS IN THE KIDNEY

The micro-CT provides a means to explore alterations of microvasculature during experimental dis-
ease conditions. An instructive example is the alteration in renal microvasculature during the development of liver cirrhosis (69). The progression of this disease in experimental models and in humans is associated with a spectrum of circulatory and renal functional alterations (30, 35, 66, 67, 86). There is reduced systemic peripheral vascular resistance and arterial blood pressure and an increased cardiac output (86); thus a hyperdynamic circulatory state is present. Despite this hyperdynamic circulation, there is increased renal vascular resistance, renal hypoperfusion (35), and retention of water and salt, which precede the development of ascites (30, 67). The increase in renal vascular resistance in patients with cirrhosis appears to mainly affect the superficial cortex, sparing medullary blood flow, as suggested by studies using either ultrasound (83, 84) or xenon-washout techniques (52). Thus, because more of the RBF is shunted toward the deeper nephrons, which have an enhanced sodium reabsorbing capability, it is possible that this redistribution is contributing to the enhanced sodium and water retention that is typically seen in cirrhosis (104). In addition, changes in intrarenal blood flow may be implicated in the progression of human (79) and experimental cirrhosis (2, 12).

Ortiz et al. (69) used micro-CT to investigate the abnormalities that are associated with the altered intrarenal distribution of blood flow after chronic bile duct ligation (CBDL). Volumetric measurements of images obtained by micro-CT indicated that the total and the volumes of the cortical and medullary tissues in the CBDL kidneys were similar to the corresponding tissues in the controls. However, the CBDL animals had a marked reduction in the vascular volume of the cortex that was accompanied by a relatively small reduction in total RBF; the medullary vascular volume

Fig. 6. Volume rendered images of nephrons microinjected with silicone polymer. A: proximal convoluted tubule (PCT), the descending limb (DL) and ascending limb (AL) of the renal loop, and the distal convoluted tubule (DCT). B: details of the PCT and DCT as well as a peritubular capillary bed microinjected with the polymer around the nephron on the right. In A, the nephrons were scanned with a $\times 4$ objective, and the voxel size of the reconstructed images is 6 $\mu$m. In B, the nephrons were scanned with a $\times 10$ objective, and the voxel size of the reconstructed images is 2.5 $\mu$m.

Fig. 7. Midcoronal 42- $\mu$m-thick slices selected from a reconstructed kidney from a chronic bile duct-ligated (CBDL) rat (left) and a control rat (right). The contrast range of both images is the same (69).
was maintained (Figs. 7 and 8). The reduction in the cortical vascular volume fraction from CBDL kidneys suggested that there was either a reduction in the number of vessels in the cortex or a decrease in the diameter of the vessels. Because there was a similar number of glomeruli in both groups of kidneys (Table 2) but a lower cortical vascular volume fraction in both intra- and interglomerular areas in the CBDL kidneys, it was likely that the predominant mechanism for the reduced vascular volume was a decrease in the diameter of the vessels in the renal cortex, rather than a reduction in the number of vessels.

Despite the marked decrease in cortical vascular filling, the decrease in RBF was smaller than expected (69). In kidneys filled with opaque silicone polymer, the opacity of the medulla represents both the pre- and postglomerular vasculature of juxtamedullary glomeruli, whereas the opacity of the cortex represents the pre- and postglomerular vasculature of the superficial cortex (5–7, 31) that is open to filling. In this respect, it is important to note that the vascular volume is not a direct measure of blood flow. This is because blood flow velocity may be increased through the smaller diameters, which would tend to sustain blood flow (as occurs during autoregulation). However, in the experiments of Ortiz et al. (69), blood flow velocity was unlikely increased because blood pressure was decreased. Thus it is probable that the decreased cortical vascular volume represented a fall in cortical blood flow. Indeed, previous studies (64) have also suggested decreased cortical blood flow. Moreover, despite the fact that vascular volume is not a measure of blood flow it does tend to be a marker of blood flow under many physiological circumstances. Fast computerized tomography studies in the dog have shown that as RBF is reduced, there is a corresponding decrease in cortical blood flow, which is more dependent on reduced vascular volume than transit time (9). A second possible explanation for the apparent discrepancy between the preserved RBF in the presence of decreased cortical vascular volume is suggested by the preserved medullary vascular filling. This preservation of medullary vascular filling in the presence of decreased cortical vascular filling suggests that the medullary blood flow may be enhanced (thus tending to preserve total RBF). The redistribution of intrarenal blood flow has been described in a variety of conditions such as reduction of renal artery perfusion pressure (1), hemorrhagic hypotension (18), ureteral occlusion (17), sodium restriction (44), and renal venous pressure elevation (53).

The activation of vasoconstrictor and antinatriuretic systems (e.g., renin-angiotensin, sympathetic nervous system, arginine vasopressin) as a consequence of systemic arterial vasodilation could play a critical role in the pathogenesis of the renal vasoconstriction. The cortical vasoconstriction may be due to agents that either act on or are located predominantly in the cortex (35). Indeed, Ubeda et al. (97) showed an increased renin-mRNA levels in kidneys from rats without ascites 3 wk after bile duct ligation (i.e., at the same stage as the rats studied by micro-CT). Because there is a transcortical gradient of renin, with greater quantities present in the superficial glomeruli than in the juxtamedullary glomeruli (81), it is possible that the increased levels of renin (and angiotensin II) present in the cortex cause a predominant local cortical vasoconstriction in CBDL, thus contributing to the vasoconstriction. Furthermore, the medullary circulation might be preserved due to specific activation of medullary vasodilators such as PGE₂ and/or nitric oxide (35, 84).

### DISCUSSION

Micro-CT is a promising method to visualize the architecture of renal vasculature and, importantly, to derive quantitative information from the images. The method allows the entire kidney to be studied intact, without physically sectioning or otherwise disrupting the tissue. However, a limitation of the method is that to visualize optimally the vasculature, the kidneys must be removed and prepared for examination. Although Paulus and colleagues (73) showed low-magnification micro-CT images of kidneys in living mice, the microvasculature was not apparent in the images. To envision the microvasculature, long scan times and a large radiation exposure without the interference of

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**Table 2. Glomerular data of kidneys from rats subjected to CBDL (n = 8) or sham surgery (controls; n = 6)**

<table>
<thead>
<tr>
<th>Diameter, μm</th>
<th>Number per μl Cortex</th>
<th>Number per Kidney</th>
<th>Intraglomerular Vascular Volume, %</th>
<th>Interglomerular Vascular Volume, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>130 ± 5</td>
<td>47.5 ± 3.1</td>
<td>36,609 ± 3,167</td>
<td>27.6 ± 2.0</td>
</tr>
<tr>
<td>CBDL</td>
<td>133 ± 5</td>
<td>48.9 ± 2.8</td>
<td>36,430 ± 1,908</td>
<td>20.4 ± 0.9*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. CBDL, chronic bile duct ligation. *Significantly different from control, P < 0.05.
tissue outside imaged field are necessary to obtain a sufficient signal (48).

Vascular filling techniques only provide information about the material filling the lumens of the blood vessels. Features of vessel walls and the nephron components also may be important in certain experimental situations. With appropriate preparation and embedding techniques, corresponding histological sections may be prepared after the micro-CT scans. Alternatively, use of en bloc heavy metal stains may provide adequate X-ray attenuation to envision the tissue components with micro-CT. Furthermore, with the use of a cryostatic stage (49), the tissue preparation does not require fixation and chemical analysis may be carried out after the scans.

To date, radio-opaque silicone rubber has been used to opacify the renal vasculature. An important assumption for quantitative determinations is that the microfil must completely fill the vasculature and not leak into the surrounding tissue. This assumption is important regardless of the contrast material used to fill the vessels and regardless of the imaging method. Iodinated contrast agents used for angiography have been injected intravenously and intraperitoneally to opacify organs of mice (73). With the use of cryostage (49), it may be possible to obtain images of angiographic agents snap frozen in transit through microvasculature.

Resolution of vessels imaged with benchtop scanners is in the range of 10–20 μm (i.e., the effective voxel size). The diameters of capillaries in the kidney are generally <10 μm and cannot be resolved reliably because of partial volume effects. Although the capillaries cannot be visualized with benchtop scanners, they contribute to the overall tissue opacity and vascular volume fraction (the fraction of vasculature in tissue) may be derived from the tissue opacity using the method of Hillman et al. (41). To visualize the actual capillaries, a synchrotron radiation source must be used, where submicron resolutions may be obtained. However, with finer resolution and smaller voxels, the practical size of the imaged volume is smaller. Furthermore, because there are currently only a few synchrotrons, availability and access is limited. For our kidney studies, we used the synchrotron source primarily as a means to confirm our observations with the benchtop scanner. Alternatively, scanning electron microscopy could be employed to confirm vasculature architecture.

One of the advantages of micro-CT is that an entire rodent kidney may be sampled without physically disrupting its structure by microtomy. However, with that advantage, practical sampling strategies are a fundamental concern. As in any morphometric study, sampling must be representative and be statistically reliable. Because the entire kidney is available for analysis, it is possible to develop a sampling strategy without physically disrupting the tissue in the process. To measure renal tissue volumes we found that a stereological approach (8, 101) is expedient compared with other tissue segmentation methods. Likewise, a sampling strategy (93) rather than a complete census is an efficient means to count glomeruli in a given kidney. Because the imaged tissue is represented as a digital array, computerized algorithms may eventually be developed for automatic recognition, measurement, and counting of objects in the entire tissue.

At this time, micro-CT images of renal microvasculature have been obtained mostly in rats. However, micro-CT also has utility in the study of kidneys of genetically modified mice (72, 73). Furthermore, it is possible to study portions of a kidney from large animals such as the pig. Recently, micro-CT was used to measure and count the number of different sized vessels in the renal cortex of pigs fed normal and high-cholesterol diets (10). The results indicated that the cholesterolemic pigs had increased numbers of vessels. Because entire pig kidneys could not be imaged by micro-CT, lobes of the kidneys were filled with opacified polymer and removed for analysis. Eventually, with appropriate heavy metal staining, micro-CT may have utility in the study and analysis of renal biopsies.

CONCLUSION

Numerous studies in the past used various vascular filling techniques to demonstrate the qualitative microvascular architecture of the kidney under normal and pathophysiological conditions. However, in the past, the tissues were usually physically cut into sections to visualize the vasculature. Furthermore, quantitative information has been limited. With micro-CT, the microvasculature of the kidney may be both seen and quantified while the tissue remains intact for further analysis. This capability provides a means to evaluate changes in renal vascular architecture relative to physiological and pathological functional alterations, such as those seen in the kidney during the development of hepatic cirrhosis after chronic bile duct ligation.

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