Efferent vascular patterns and early vascular-tubular relations in the dog kidney

REINIER BEEUWKES III
Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115

Efferent vascular patterns and early vascular-tubular relations in the dog kidney. Am. J. Physiol. 221(5): 1361-1374. 1971.—Glomerular efferent vascular patterns were studied in cleared dog kidneys injected with silicone rubber. In addition, to show vascular-tubular relations, kidneys were perfusion-fixed in vivo and their vasculature was filled with silicone rubber. Proximal tubules were then injected by means of micropipets inserted into the urinary spaces of glomeruli in cleared slices of the fixed kidneys. Ten efferent vascular patterns including two subcapsular, four midcortical, and four inner cortical patterns are described. Close association between the efferent capillary network and proximal tubule of the same glomerulus was found only in the subcapsular cortex. In the bulk of the cortex, proximal tubules were found to be dissociated from the efferent vessels of the same glomerulus. Hence, it appears that the proximal tubules accessible to micropuncture have vascular-tubular relations different from proximal tubules in the bulk of the cortex. Furthermore, close association between the proximal tubule and efferent vascular network of the same glomerulus should not be assumed in proposed mechanisms of glomerular-tubular balance.

METHODS

Efferent vessels. The postglomerular vascular pathways were studied in dog kidneys injected with silicone rubber (2). Mongrel dogs of both sexes were sacrificed by rapid intravenous overdose of pentobarbital. The kidneys were rapidly removed through a flank or abdominal incision and the renal artery was cannulated with polyethylene tubing of appropriate size. The silicone material consisted of equal parts of Microfil MV-112 (Canton Biomedical Products, Boulder, Colo.) and MV diluent to which 3% by volume of dibutyltin dilaurate catalyst was added just before infusion. The vascular cannula was connected to a constant-pressure reservoir containing the rubber compound and the infusion maintained at a pressure of 75 or 150 mm Hg for at least 4 min. The renal pedicle was then clamped and the organ was suspended by this clamp at room temperature until the rubber had cured, usually less than an hour. The kidney was then refrigerated overnight and cut into slices perpendicular to its long axis. These slices were dehydrated in increasing concentrations of alcohol, usually remaining in each concentration for 24 hr. They were transferred from absolute alcohol to methyl salicylate (oil of wintergreen) for clearing and storage.

Vascular-tubular relations. Vascular-tubular relations were demonstrated by in vivo fixation of the kidney, followed by arterial injection of silicone material. The kidneys were then dehydrated, cleared, and sliced. Under direct observation micropipets were maneuvered into the urinary spaces of glomeruli in each part of the cortex, and proximal tubules injected via the glomerulus with silicone rubber of contrasting color.

In vivo fixation was accomplished in three kidneys by arterial perfusion with glutaraldehyde in a modified Tyrode solution (Maunsbach’s solution no. 4 (32)). Dogs were anesthetized with intravenous pentobarbital (30 mg/kg), and the left kidney was exposed by flank incision. A polyethylene cannula (PE-100) was maneuvered via the femoral artery and aorta into the left renal artery without interruption of renal blood flow distribution; silicone rubber; subcapsular; midcortical; inner cortical patterns; proximal tubule; glomerular-tubular balance; renal blood flow distribution; silicone rubber.
renal blood flow. A loose tie was placed around the renal artery. The operating table was then placed before an open fume hood having vigorous airflow. Using a peristaltic pump, a rapid washing infusion of warm (37°C) solution was begun through the transfemoral renal artery catheter. The arterial tie was tightened so as to prevent blood flow to the kidney. The renal vein was tied to prevent bleeding from the cava and the vein opened close to the kidney to allow free drainage from the organ. The pressure of the perfusion was maintained at 150 mm Hg for 1 min before switching the pump intake to the fixative solution. This was identical to the previous perfusion fluid but with the addition of 1% glutaraldehyde.

Perfusion of the kidney with glutaraldehyde was maintained for 15 min. The animal was then sacrificed. Silicone rubber mixture, consisting of equal parts Microfil MV-117 (red color) and MV diluent, with 3% catalyst, was then injected into the kidney via the femoral catheter. Hand pressure and a 30-ml syringe were used, and the injection was halted when the silicone rubber first appeared on the kidney surface, so as to fill only the early parts of the glomerular efferent structures. The catheter was then clamped. The kidney was left in situ for 30 min to permit the rubber to cure before it was removed, dehydrated, and cleared by standard procedures.

The tubular microinjections were photographed using an apparatus built in the laboratory. A lathe bed was mounted vertically on a large, vibration-isolated, steel plate. An Olympus model X-1r stereomicroscope was mounted to the lathe trolley. The camera, a motor driven Bolex H-16, was mounted on a three axis compound which permitted alignment with the photographic tube of the microscope without direct mechanical connection. The specimen was mounted on an inverted milling table which provided two-axis motion and rotation about the optical axis. The microscope, mounted to the lathe carriage, was focused by a reversible motor driving the lead screw and controlled by a two-way footswitch. A second footswitch operated the camera.

The slice of kidney to be injected was secured in a brass dish by the pressure of soft copper wires on its edges. The dish was ball mounted so that wedge shaped slices could be tilted to make the upper face horizontal. To carry away escaping injection material, the dish was provided with an overflow drain connected to an aspirator. Fresh clearing medium was continuously supplied from a hanging drip bottle and inlet tube. Illumination was provided through a quartz-inch fibercopic bundle from a 150-w projection lamp. The micropipets used were drawn from Aloe V48302 capillary tubing and had tip diameters of approximately 20 μ. They were attached to 27-gauge disposable hypodermic needles by a small amount of rapid setting epoxy cement. A male Luer adapter was mounted on the micro-manipulator and connected to a micrometer syringe by a short length of heavy-wall polyethylene tubing. The syringe, tube, and adapter were filled with water; the micropipet was filled via the needle hub with silicone rubber (uncatalyzed) before mounting on the Luer adapter. The rate of injection could be varied by adjustment of the micrometer syringe. Red Microfil was used for the vascular injections; white compound was used for the tubular injections. High speed Ektachrome type B, 16 mm, color film was used in the camera. Still pictures were made by enlarging single frames onto Polaroid type 58 color material.

RESULTS

Efferent vessels. The glomerular efferent vessels were found to have configurations which differed in each region of the cortex. In all, 10 efferent types were distinguished on morphological grounds. In general, all of these correspond to types observed by earlier authors in other species. For ease in reference a shorthand nomenclature has been devised in which efferent vessels located in the subcapsular, middle, and inner zones of the cortex are denoted by Roman numerals I, II, and III, respectively. The form of an efferent may be long, immediately convoluted, or short, designated by the suffixes L, C, or S, respectively. A long efferent with a major convoluted branch is designated LC. If it has a twig whose disposition is unknown, the uncertainty is indicated by adding a prime to the descriptors, as II L′ for a midcortical long efferent with a twig. While these descriptors could be combined in many different ways, only 10 labels seem to apply. Through use in the laboratory these originally generic descriptions have tended to become "brand names" and here, generally, refer only to a particular configuration of efferent structures.

The most prominent subcapsular efferent is the I L (Fig. 1A). Its glomerulus is usually derived from the terminal portion of an interlobular or superficial cortical artery. This efferent vessel rises directly upward for about a millimeter through the agglomerular subcapsular zone, and divides at or near the capular surface into a profuse and voluminous peritubular capillary network. As it rises it increases progressively in diameter before dividing, usually doubling its 8- to 10-μ initial diameter at its end. Those that branch just beneath the capsule constitute the "stellate vessels" or "welling points" described in micropuncture studies.

The type I C efferent is difficult to identify because it is obscured by its own capillary meshes. Its glomerulus lies at approximately the same level as that of the I L and is derived from an interlobular terminal afferent (Fig. 1B).

The characteristic midcortical long efferent is the II L. Its glomerulus lies beside the trunk of the interlobular artery, to which it is connected by an afferent arteriole which is short compared with those of the subcapsular zone. The II L efferent makes a loop toward the medulla before reversing direction and rising above the glomerulus (Fig. 2A). It increases in diameter along its course, much as the type I L does, and divides at a long distance from the glomerulus, typically 500-800 μ away. Unlike the convoluted form of the I L, its capillary network is simple and long meshed and lies in the medullary ray, a region classically associated with collecting ducts, not convoluted tubules.

Occasional type II L′ efferents have been found with a small twig branching off from the bottom of the characteristic loop (Fig. 2B). This suggests a possible duality of efferent pathways.

Also frequently found in midcortex are efferents which supply the medullary ray directly through a relatively short, straight, constant-diameter pathway. These are designated II S. They are more common in the inner part of the mid-
EFFERENT VESSELS AND VASCULAR-TUBULAR RELATIONS

A large proportion of the midcortical efferents are tightly convoluted. These II C efferents make compact bundles, often enclosing their parent glomerulus, which lies close to the interlobular vessel (Fig. 2D). When well filled, they merge with adjacent efferents and distinct regions of influence become impossible to define. The dense capillary network thus formed effectively fills the space between the medullary ray and the interlobular artery. Thus, proceeding outward from that artery, one passes first into the capillary network supplied by II C efferents and then into the region supplied by II L and II S efferents.

In the inner cortex the most prominent efferent is the III L, which, with the III S, supplies the vasa recta of the outer medulla (Fig. 3A). The afferent arterioles to the parent glomerulus are long, and typically arise in a retrograde direction from the lowest parts of the interlobular arteries, or occasionally from the terminal arcuate vessel. The glomeruli are slightly larger than those of the outer cortex, though the difference is only apparent upon measurement. A striking feature of the III L efferent vessel is that it has a larger diameter than the afferent, a property which makes this family unique. The efferent arteriole, which may extend toward the medulla for as much as a millimeter, branches abruptly into a sheaf of vasa recta which enters the medulla as a bundle. It is not uncommon to find small twigs or branches emerging from this long single efferent arteriole. Sometimes these vessels can be seen to form a convoluted capillary network lying beside the main efferent path. In such cases the name III LC is applied to designate a primarily long type structure with a secondary convoluted characteristic.

The III S is similar to the III L, but subdivides into vasa recta almost immediately upon leaving the glomerulus (Fig. 3B). Thus, it is located lower in the cortex, right at the corticomedullary border. It may be centered directly atop the bundle which it supplies, or may be located between two or more bundles, and give off vessels to each of them. In this context it is like the III L, which often supplies more than one bundle. The III S efferent arterioles typically have twigs or small branches supplying a loose meshed network between the tops of the bundles in the outer medulla.

In the region between the glomeruli giving rise to III L and III S efferents, glomeruli are found having diffusely convoluted efferent structures which do not envelop the glomerulus (Fig. 3C). These III C efferents are less commonly seen than the other inner cortical types.

No aglomerular pathways from the arterial system to the peritubular capillary network were found.

These 10 characteristic efferent blood pathways, the subcapsular long (I L) and convoluted (I C), midcortical long (II L), short (II S), convoluted (II C), and branched (II L'), and inner cortical long (III L), short (III S), long with convoluted branch (III LC), and convoluted (III C), seem sufficient to describe all the efferents which we have identified in the dog kidney. Since the distinction between types was made entirely on the basis of efferent vascular system configuration, these constitute vascular families. It may be that all the convoluted types constitute but one family in terms of function or that the II L and II S forms are not physiologically different. But until functional information becomes available, it seems worthwhile to retain the more detailed classification.

The pathway from the efferent capillary network to the renal veins is also characteristic for each region of the kidney. In general, however, the outer and midcortical efferent networks connect with veins within their respective regions. Hence it appears that no single efferent can perfuse the whole length of its respective nephron. The subcapsular capillary network derived from I L and I C efferents empties directly into the large superficial cortical veins. Generally, these small capillaries do not converge successively, but
FIG. 2. Photomicrographs of mideortical glomeruli and efferent vessels injected with silicone rubber. (Scale: bars = 0.2 mm.) A: a glomerulus with a long efferent forming a simple, long-meshed network about collecting ducts in medullary ray (type II L). B: a similar structure with a small side branch indicated by arrow (type II L').

C: a glomerulus with a short efferent supplying long-meshed network of medullary ray (type II S). D: a glomerulus with a highly convoluted efferent structure close to interlobular artery and vein (type II C).

directly enter a vein perhaps 100 times as large (Fig. 4A), or together with others converge suddenly like the shoots of a dense bush joining its trunk just at the ground. This lack of successive convergence explains why the renal veins literally bristle with tiny vessels when injected in a retrograde direction (Fig. 4B). The veins in midcortex run parallel with and close to the interlobular arteries. The convoluted capillary network derived from type II C efferents also lies close along this same vascular axis, and makes frequent connection to the vein. The long-meshed network supplied by II L efferents is located at some distance from the veins but interconnects on all sides with the convoluted network, and presumably reaches veins through it. The long-meshed network also connects with veins at its inner end, in the deep zones of the cortex. Thus, two paths are available, and relative venous pressure relations would be expected to control the distribution of flow between them. The inner cortical convoluted network derived
from type III C efferents appears to join veins in the inner cortex, possibly together with the inner parts of the long meshes.

The pathway between the III L and III S efferent arterioles and the vein is quite different from the cortical pattern. These efferents supply the outer and inner medulla by way of the vasa recta bundles, which contain both arterial and venous vessels. There is, however, no junction

**FIG. 3.** Photomicrographs of inner cortical glomeruli and efferent vessels injected with silicone rubber. (Scale: bars = 0.5 mm.) A: two glomeruli (arrows) with long efferents dividing to form a vasa recta bundle which enters the medulla (type III L). B: a glomerulus with an efferent dividing immediately to form a vasa recta bundle (type III S). C: a glomerulus (arrow) with an efferent vessel forming a diffusely convoluted network (type III C).

**FIG. 4.** Photomicrographs of cortical venous structures injected with silicone rubber. A: a typically abrupt junction between subcapsular peritubular capillary network and a superficial cortical vein. (Scale: bar = 0.5 mm.) B: at lower magnification, cortical veins have a brushlike appearance due to absence of successive convergence. (Scale: bar = 5 mm.)
between these vessels within the bundle, which is an array of parallel vessels, not a grouping of tight loops. A single bundle typically contains 50–100 arterial vessels derived from several type III L or III S efferents. A given bundle may contain vessels from both long and short efferents. The capillary network at the junction of the outer and inner medulla ("frizzled" zone) is formed from vessels in the periphery of the bundle which swing away and abruptly divide into a series of hooks or loops. The fine network breaks off from these and fills the space between the distal parts of the bundles (Fig. 5A). In low-pressure injections only the bundle tips are frizzled; with increasing filling the zone extends toward the cortex. These capillaries coalesce abruptly into venous channels which have a characteristic "wavy" appearance as they course upward between the bundles. The wavy vessels generally run some distance into the bundles, that is, in the wavy vessels, but in direct approaches these vessels become the venous components of the vascular bundles. It must be emphasized that the venous channels of inner and outer medulla are supplied from the periphery of the bundles and drains via wavy vessels distinct from the venous channels originating near the papilla of Bellini. From this network the venous capillaries of the inner medulla emerge, and rise without branching or converging. Below the bundles they become grouped, and these vessels become the venous components of the vascular bundles. It must be emphasized that the venous channels from the inner medulla do not return to the cortex between the bundles, that is, in the wavy vessels, but in direct opposition to the arterial vessels in the vasa recta bundles.

Thus, the medulla is seen to have three distinct capillary networks, each with its own mode of supply and drainage; the outermost is supplied by III C, and twigs from III LC and III S efferents. The frizzled zone network at the junction of inner and outer medulla is supplied from the periphery of the bundles and drains via wavy vessels distinct from the vasa recta. The inner network is located near the papilla tip. Its supply and drainage run in countercurrent opposition within the cores of the bundles.

The pelvic vasculature, while apparently not directly concerned with urine formation, is of interest because it is highly structured. Two networks, the outermost is supplied by III C, and twigs from III LC and III S efferents. The frizzled zone network at the junction of inner and outer medulla is supplied from the periphery of the bundles and drains via wavy vessels distinct from the vasa recta. The inner network is located near the papilla tip. Its supply and drainage run in countercurrent opposition within the cores of the bundles.

**Vascular-tubular relations.** The relations between early efferent vascular structures and the early proximal tubule have been defined for 39 glomeruli in the dog. Due to imperfect fixation and problems with tubules emerging at the cut surface, only two complete nephrons have been injected in dog kidneys, although this has been readily achieved in the rat using the same technique. The 39 glomeruli in which unambiguous identification and good filling were obtained include nearly the whole range of efferent types previously described. Ten I L, seven II L, four II C, ten III L and seven III S are included among these, along with a single I C. No distinction has been drawn in this group between III L and III LC, since small efferent twigs are often difficult to recognize in the photographs. The III L efferent vessels runs almost directly toward the kidney surface from a glomerulus in the outer millimeter of the cortex. At the surface it breaks up into a vigorously convoluted network of peritubular vessels. In all 10 successful injections of type I L units the proximal tubule paralleled the efferent vessel, albeit taking an irregular course, and formed its initial convolutions within the capillary network derived from its own glomerulus (Fig. 6A). The tubule and efferent arteriole were generally not in close contact until the capillary network proper began. There the proximal convolutions formed a compact bundle within the area defined by the efferent vascular structure. The tubule was often seen to run along one side of a capillary, then turn and come back along the other side. The single I C proximal tubule injected formed a compact mass around its parent glomerulus, and was thus in close association with the periglomerular efferent capillary network.

The II L efferent vessel generally makes a short loop toward the medulla before rising to enter the long-meshed capillary network of the medullary ray. Of the seven units of this type successfully injected, all proximal tubules tended to remain close to the interlobular axis and none entered the medullary ray. The proximal tubules of three units lay along the fringe of the long-meshed network supplied by the related efferent. They lay in the region where the venous return from the part of the ray supplied by the parent glomerulus might pass on its way to the vein. Thus these might have been supplied by efferent blood from the same glomerulus, but after the blood had left the ray network. The remaining four units had convolutions well away from the region which might have been supplied by venous return derived from the same glomerulus (Fig. 6B).

Type II C efferent arterioles derive from midcortical glomeruli and break up almost immediately into complex capillary networks. Two proximal tubules derived from glomeruli having II C efferents were completely dissociated and these lay in regions well away from the capillary network. Two other tubules lay on the fringes of the injected pillaecary network.

The type III L efferent vessel derives from a juxamedullary glomerulus. It is a long large vessel which branches in the outer medulla to form vasa recta. Often twigs emerge from its side to supply a diffuse network in the corticomedullary zone. Ten type III L units were injected. Their early proximal tubules generally formed convolutions near and above the glomerulus, although a few extended well up into the cortex (Fig. 6C). In two instances the tubules were possibly supplied in part by twigs from the efferent arteriole, but the general pattern placed the bulk of the convolution diametrically opposite the efferent vessel, or well out to one side. Thus, given the long straight III L efferent structure, there was essentially complete dissociation between this efferent and the corresponding proximal tubule.
Fig. 5. Photomicrographs of medullary vascular structures injected with silicone rubber. A: at junction of outer and inner medulla a dense capillary network between bundles is seen to arise by branching of vasa recta. (Scale: bar = 0.5 mm.) B: in this low power view, vasa recta of inner medulla are seen to pass through outer medullary capillary network (between arrows). These inner medullary vessels generally do not branch until papilla tip. (Scale: bar = 5 mm.) C: straight and unbranched character of papillary blood supply is clearly seen at right. Complex vasculature of renal pelvis (to left) merges into ureteral vessels at bottom of picture. (Scale: bar = 0.5 mm.)
Seven successful injections were made of tubules from glomeruli having type III S efferent vessels. These glomeruli are located immediately above vasa recta bundles, and their efferents break up into a sheaf of vessels which enter bundles. One of these tubules formed a knot close by the glomerulus and efferent where it might have been supplied by a small twig. Three tubules lay away to one side of their respective glomeruli and occupied a space between and over the bundle tops. The three remaining proximal tubules had no convoluted part, but turned almost immediately downward to run alongside the bundle in the tubules had no convoluted part, but turned almost immediately downward to run alongside the bundle in the outer medulla. Thus the III S units had more varied proximal tubular configuration than the other types investigated.

These preliminary results permit some preliminary generalization. In the superficial cortex there seems to be constant association between the efferent arteriole and the proximal convolution of the same glomerulus. In midcortex the tubule and efferent are usually dissociated, but the pattern is not fixed. Rather, the proximal convolution lies close to, or along, the interlobular axis and is perfused either by blood returning from the medullary ray or by the efferent capillary network of another glomerulus. In the inner cortex dissociation is usually complete. The proximal tubules of glomeruli having long efferents (III L) appear entirely dependent on glomeruli of the midcortex for their perfusion. The tubules from short efferent units (III S) may well be supplied by III C efferent and the offshoots of type III LC and III S efferent vessels. Thus, except for the most superficial cortex, the proximal tubule and efferent vessels of the same glomerulus appear to be generally dissociated.

**DISCUSSION**

**Methods.** The advantage of injection methods for the study of vascular and tubular architecture is the unequivocal nature of the evidence. If a structure fills, then it is demonstrated to exist; it need not be inferred from some supposed functional correlate. India ink, collodion, and neoprene latex have formerly been used, with the filled pathways revealed by transillumination of thin slices, or by corrosion of the supporting tissue. The use of inert silicone materials combined with tissue clearing as introduced by Sobin et al. (44), and applied by Barger and co-workers (3) to renal vascular studies, has proved a great advance over these older techniques in that structures may be observed in situ with the natural relations of their parts maintained by the surrounding tissue.

The technique applied here for demonstrating vascular-tubular relations is apparently new. To have reconstructed 39 proximal tubules by the classical serial-section methods of Huber (23) would have required enormous expenditure of time and effort. To have traced the peritubular capillary network in the same reconstructions would have been almost impossible. Huber did not attempt it. Bowman (8) and Trueta et al. (50) observed the course of proximal tubules filled by vascular injectate through ruptured glomerular capillaries. Bialestock (6) observed the relation of microdissected tubules to blood vessels filled with pigmented debris resulting from transfusion with incompatible blood. Morison (35) microdissected human kidneys after vascular injections of collodion. Steinhausen and his colleagues (46) have related superficial cortical vascular and tubular structures in the rat by filling the vessels with latex via the artery, and single proximal tubules with latex by micropuncture. The flexible double cast was then studied in acid-macerated tissue. This technique is suitable only for study of the outermost cortex. Any microdissection method depends, of course, on removing the blood vessel or tubule from its natural surroundings, with a near certainty that its natural configuration will be altered. In none of these methods may glomerular units be preselected on the basis of vascular pattern before defining the tubular arrangement. In these respects, the present methods may give a more accurate description of renal vascular and tubular structures than has previously been available.

**Vascular structure.** The pattern of major arterial and venous vessels observed in these injections is not different from that so excellently described by Kügelgen and coworkers (25) in 1959. In addition, the present studies confirm the observations of Bowman (8), Huber (23, 24), Morison (35), and others that characteristic efferent vascular patterns are found in the various zones of the kidney. Bowman observed in 1842 that the juxtamedullary glomeruli were larger than those in the outer cortex. He noted that their efferent arterioles were large and directed toward the medulla, in contrast to the small convoluted efferent vessels of the cortex. In addition to the juxtamedullary and cortical types of Bowman, Huber (24), in 1906, described characteristic outer cortical efferent vessels which passed far out into the agglomerular subcapsular region before forming capillary plexuses. The efferents described by Bowman and Huber thus correspond to the types here designated as III I, I C and I L, respectively. Hinman et al. (22), Morison (35), and Lee-Brown (26) developed improved methods of collodion and dye injection which permitted more detailed classification of glomeruli by efferent structure. In human material, Lee-Brown and, later, Morison described four glomerular families characteristic of different regions. These were: subcapsular, corresponding to type I L; cortical, corresponding to types I C and II C; medullary and corticomedullary, which correspond to types III L and III LC.

An additional type of efferent vessel was described by Gansslen (20), in 1932, which ran directly to the long-meshed capillary network of the medullary rays, thus corresponding to type II L. Gansslen showed that the drainage of these straight capillaries was via the convoluted network, thus explaining the difficulty of filling long meshes of the medullary ray by retrograde venous injections. Daniel, Peabody, and Pritchard (11) pointed out, in 1992, that glomeruli of the cortical type, in particular those with short efferents breaking up immediately into a peritubular network, work, could be found in the juxtamedullary region. Edwards (13) has estimated that these, corresponding to type III C efferents, exist in a one-to-four ratio to the characteristic medullary types in the human kidney.

To these seven efferent types from human kidneys, the present study adds three new forms (II L', II S, and III S), making a total of 10 types in the dog. Many of these types of efferent structure appear in photographs of rat kidneys made by Rollhauser, Kriz, and Hejnicke (43). That human,
**FIG. 6.** Photomicrographs showing early proximal vascular-tubular relations in dog renal cortex. Vascular structures were injected with colored silicone rubber in kidneys fixed in vivo. Kidney slices were then dehydrated and cleared, and tubules were injected with white silicone rubber under direct vision by means of micropipets inserted into glomerular urinary space. Glomeruli and their efferent vascular structures are indicated in corresponding tracings. (Enlarged from part of a 16-mm motion picture frame.) 

A: subcapsular cortex. Early proximal tubule and efferent vessel (type I L) of this glomerulus are closely associated. 

B: midcortex. Efferent network (type II C) lies below this glomerulus. Filled part of proximal tubule is above glomerulus and is completely dissociated from efferent capillary network. 

C: inner cortex. Efferent vessel of this glomerulus forms a vascular bundle which enters medulla (type III L). Proximal tubule extends above glomerulus and is entirely dissociated from efferent vessels.
EFFERENT VESSELS AND VASCULAR-TUBULAR RELATIONS

rat, and dog kidneys show the same configurations argues strongly for the generality of such vascular families.

Of particular interest is the possibility of directing the efferent blood from a given glomerulus to one of two or more possible pathways. In the midcortex the type II efferent has a branch which may supply a path quite different from the main route to the collecting ducts in the medullary ray. Near the medulla, the type III efferent offers pathways to either peritubular capillaries or the vasa recta bundle of the outer medulla. Within the outer zone of the medulla, blood may possibly be sent straight through to the papilla tip, or diverted to the local capillary network. These controls do not seem to be all-or-none in action. For example, the frizzled zone capillaries do not fill in inverse proportion to the filling of the long vessels of the inner medulla when both are supplied by the same glomerulus. The relation between these variations in flow pattern and renal functional state is presently under intensive study.

The marked vascular zonation of the canine medulla shown in this study confirms and extends the findings of many previous investigators. Golubow (21) noted, in 1893, that the outer medullary zone of the rat kidney had a distinctive capillary network quite unlike that of the inner medulla. Huber (24) observed this frizzled zone at the junction of outer and inner medulla in the rat and other species. Moffat and Fourman (19, 33) have described this capillary plexus in detail, and excellent photographs have been recently published by Prong et al. (41). Plaekke and Pfeiffer (39) have shown that the heavier, which has little concentrating ability, lacks both zonation of the medulla and the fuzzy zone of capillaries. They suggested, as had Fourman and Moffat, that this indicated a special role of the vascular system of the outer medulla in the concentrating mechanism. This is supported by the recent observation of Fisher (49) that the rhesus monkey can produce quite hypertonic urine with little or no characteristic inner medullary structure.

The generally accepted role of the vasa recta bundles is to serve as countercurrent isolators protecting the hypertonicity of the medulla, a role extensively discussed by Berliner and co-workers (5) in 1958. However, Pomeranz, Birch and Barger (40) have pointed out (and the present study confirms the observation) that the drainage of the outer medullary frizzled zone supplied by the bundles is via veins which return to the cortex outside the vasa recta bundles; hence these structures would not seem to be efficient countercurrent isolators of the outer medulla. On the contrary, the frizzled zone network seems specialized to remove substances from the outer medullary area, or to provide a nutrient supply to tubular transport mechanisms located in the region (1). The characteristic tubular pattern of the outer medulla described long ago by Peter (37), and given recent quantitative support by Munkacsi and Palkovits (36), is the region of overlap between thick descending and thick ascending tubular segments. At the present stage of the tubular-injection technique, no new information is available concerning the vascular-tubular relations in this outer medullary region of the dog kidney.

Unlike the outer medulla, the inner medulla is both supplied and drained by vessels of the vasa recta bundle. In the core of the bundle these arterial and venous vessels arc so packed together as to form a true rete mirabile. Indeed, Longley, Banfield and Brindley (29) found that cross sections of rat vascular bundles were essentially identical to the swim bladder rete as described by Fawcett and Wittenberg (16). The arterial vessels in the bundle were shown to have an unperforated endothelium whose cells overlapped at their borders. The venous capillaries were much simpler, having only a "carnose fenestrated structure." Both of these vessel types have since been found in the inner medulla by Thoenes (47, 48) and by Bulger and Trump (10). It is striking that the vascular network supplied and drained by these vessels is almost completely localized to the papilla tip, so that blood committed to the inner medulla must always take the longest path.

Since, within the bundle, arterial pathways tend to be surrounded by venous channels and vice versa, the structure seems optimized for diffusion exchange. However, the difference between the fine structure of the descending and ascending vessels is not required for such exchange, and the thick wall of the arterial capillary would even tend to hinder it. Longley and co-workers (29) briefly noted that the thick-walled vessels commonly contained red cells and a dense granular plasma precipitate while the thin venous vessels appeared empty. This difference in plasma concentration was also seen in swim bladder rete by Fawcett (15), who thought it might indicate "a considerable flux of water from one set of capillaries to the other." Such water transfer has been proposed as the source of inner medullary hypertonicity by Lever (77). This mechanism has been strongly attacked by those who view the vascular bundles as passive countercurrent isolation systems. It must be noted, however, that in a countercurrent isolater system the blood returning from the concentrated region at the papilla tip must be more concentrated than the blood supplied, not less concentrated, as Longley's observation seems to imply. Clearly, the water reabsorbed from collecting ducts in a concentrating kidney must be removed from the papilla somehow, if not in the tubules, then in the blood vessels.

Vascular-tubular relations. A striking finding of the present work is that a difference in vascular-tubular relations exists between the nephrons of the subcapsular cortex and those in the bulk of the organ. There is little in either classical studies or current texts that might have suggested such a finding. Bowinan (c) noted that the efferent vascular network was "freely anastomotic," so that no fixed association between vasculature and tubules was necessary. But in his illustrations efferents are shown coursing about the tubules of their parent glomeruli. Well known textbooks, old and new, speak of freely anastomosing networks, yet their diagrams consistently show the efferent network and tube of the same glomerulus in absolute association. Despite this apparent unanimity, I have found only four statements in the renal literature which bear directly on the relations between the blood vessels and tube arising from a given glomerulus, and they are conflicting. Morison (35) concluded from his microdissection experiments that: "it seems probable that the proximal and distal convoluted portions are supplied directly by the efferent vessel and that the remaining portions receive their blood supply from a
common capillary bed derived from the free anastomoses of the efferent branches of other glomeruli."

Trueta and his colleagues (30) observed one instance where neoprene from a vascular injection broke through a glomerulus into a proximal tubule. He noted: "The capillaries filled from the efferent vessel do not surround either the glomerulus or the proximal convoluted tubule, but are situated away from these in the region of a medullary ray."

Bialestock (6) microdissected tubules from a kidney whose capillaries were filled with pigmented debris. She commented: "One noteworthy point in passing was that the capillaries arising from any one efferent arteriole did not, as a rule, supply the tubule of the parent glomerulus, but more often tubules from adjacent glomeruli."

Steinhausen, Eisenbach, and Galaske (4b) have recently filled rat superficial cortical tubules by micropuncture in vivo and the blood vessels via the artery. They report: "As a rule, we found that (i) each convolution is supplied by its own vas efferens and represents, therefore, an independent unit, and (ii) each vas efferens passes to the loop of the proximal convolution furthest from the glomerulus."

Hence, the most important consequences of dissociation between the 'vascular units'. That is, single loops of a nephron may be supplied by peritubular capillaries from other welling-points, even under physiological conditions."

In the present study it has been shown that in the superficial cortex, where Steinhausen’s observations were made, the association between efferent arteriole and proximal tubule is nearly absolute. In the mid- and deep cortex, as described by Trueta et al. (50) and Bialestock (6), the converse is true and dissociation is the rule.

A possible consequence of vascular-tubular dissociation relates to the extraction of PAH and other compounds secreted by the proximal tubules. Reubi (42) has proposed that medullary flow is represented by that fraction of renal blood not cleared of PAH. Pilkington and co-workers (38) have extended and applied this proposal, taking care to define medullary flow as including inert tissue. If there is no association between glomerular filtration and tubular perfusion, then it is possible to have cortical areas in which blood perfuses tubule segments having no urine flow. Without filtrate flow to carry away accumulated PAH, the extraction process might become gradient limited or completely blocked. Thus, it would be possible to have a variable amount of inert tissue within the cortex, with resulting overestimation of apparent medullary flow. Of course, such considerations would appear to contradict in general, and suggest that a filtration-perfusion ratio for each part of the cortex might be a relevant parameter. This would be analogous to the ventilation-perfusion ratio which applies to gas exchange in the lungs, with tubular flow taking the role of ventilation. Thus exchange would be limited in any region having good perfusion but little tubular ventilation or having large tubular flow with inadequate capillary perfusion. This limitation might reasonably apply not only to secretion, but to tubular reabsorptive processes as well. Hence, the most important consequences of dissociation may relate to the balance of filtration and reabsorption.

Martino and Earley (30, 31), Windhager et al. (51), Lewy and Windhager (28), Spitzer and Windhager (45), and Falchuk and co-workers (14) have reported that proximal tubular reabsorption changes in association with changes in renal venous pressure, or, more directly, changes in the hydrostatic and oncotic pressure of the peritubular capillary perfusate. This relationship has been suggested as a possible mechanism for filtration-reabsorption balance. Increased filtration is seen as raising peritubular oncotic pressure, which in turn enhances proximal reabsorption. To operate on a single-nephron basis, such a mechanism requires that the proximal tubule and efferent capillary network from the same glomerulus be closely associated, otherwise the tubule will not be affected by the oncotic pressure of blood in the efferent capillary. The structural relations here presented indicate that such association does not generally exist in the dog kidney, and thus lend no support to a single-nephron hydrostatic-oncotic pressure mechanism of glomerular tubular balance throughout the kidney. The micropuncture experiments consistent with the theory are, perhaps, explained by the fact that proximal tubules are accessible to micropuncture only in the superficial cortex, a zone in which the proximal vascular tubular relations of each glomerulus are very much closer than in any other part of the kidney.

A regional balance mechanism might be consistent with single nephron dissociation, although this lacks the elegant simplicity of the single-unit proposal. Such regional hydrostatic-oncotic balance would seem to require three demonstrations. First, that there physically exist groups of glomeruli whose proximal tubules and efferent vessels form a functional unit. Second, that all the glomeruli within a group are controlled as a single unit so as to maintain the regional filtration-perfusion balance. Third, that the operative balance mechanism is, in fact, based on hydrostatic-oncotic parameters, at least, that the tubules involved are sensitive to these parameters. Given the present knowledge of vascular-tubular relations, it is not yet possible to confirm or deny the physical existence of such functional units. The requirement for unit control of glomerular and efferent perfusion receives indirect support from the observation that, in certain diuretic and hormonal states, in which regional balance might be assumed to be abnormal, the perfusion within certain regions varies markedly among nearby glomeruli (7, 17, 18). For example, in dogs undergoing ethacrynic acid diuresis, those midcortical glomeruli whose efferents directly supply collecting ducts in the medullary ray (types II L and II S) are much better perfused than neighboring glomeruli which have highly convoluted efferents. One might speculate that the direct supply of efferent blood to the cortical collecting system might result in dilution of any efferent hyperosmolarity before the blood could perfuse proximal tubules on its way to the veins. Given an oncotic effect, such dilution would inhibit proximal reabsorption of sodium and water. Antidiuretic hormone has been shown to bind to cortical collecting structures (12) and could act to modulate the degree of dilution. If the cortical collecting ducts retain some water permeability in the absence of ADH, then the flow distribution induced by ethacrynic acid would tend to enhance removal of water from them even in diabetes insipidus. Such an effect might be related to the paradoxical antidiuretic action of ethacrynic acid and other diuretics observed in this disorder. These and most other specula-
The kidney is inaccessible to micropuncture, hence such measurements must await new experimental methods.

The author thanks Professor A. C. Barger for his advice and encouragement and Drs. A. B. Barnes, F. D. Gutmann, and F. Spinelli for their assistance. He is indebted to Mr. F. W. Smith for the preparation of drawings and photographs and to Mrs. A. Swanson for secretarial assistance.

This study was supported by Public Health Service Grants GM 00919 and HE 04943.

The author was a Public Health Service Trainee in Physiology during the performance of this work.

This study constitutes part of a PhD thesis submitted to Harvard University in 1970 and was presented, in part, to the American Physiological Society, Bloomington, Ind., in August 1970.

Received for publication 14 April 1971.

REFERENCES


43. SIEB, S. S., W. G. FEASER, JR., AND H. M. TONER. Vasa


