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VASCULAR ANATOMY OF THE PIG KIDNEY GLOMERULUS: A QUALITATIVE STUDY OF CORROSION CASTS

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Abstract

Pig kidney glomerular vascular anatomy was studied by scanning electron microscopy of vascular corrosion casts. A generalized vascular architecture is presented to describe the pig kidney glomerulus based upon the observation of 3,800 vascular cast glomeruli. The relative simplicity of the pig glomerular vascular architecture has allowed the characterization of different vascular segments more completely than has been possible in other mammals. Based upon relationships to the afferent arteriole, a nomenclature and definition of primary, secondary, tertiary and anastomotic vessels is proposed for the distributing vessels comprising the glomerular tuft. The existence and formation of a large central hemispheric vessel deep within the confines of a glomerular hemisphere is micrographically documented. Micrographic evidence is presented supporting the formation of the single efferent arteriole by the merging of two central hemispheric vessels within the confines of the glomerular tuft. Failure of the merging of these two vessels may result in multiple efferent arterioles.

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Introduction

A review of the literature reveals a generalized vascular pattern for the kidney glomerulus based upon observations in the rat (Moffat and Fourman 1963; Murakami *et al.*, 1971; Winkler *et al.*, 1991), man (Smith, 1956), the cat (Marais, 1987), the dog (Barger and Herd, 1971; Beeuwkes and Bonventure, 1975; and Anderson and Anderson, 1976), and in rhesus monkey (Unehira, 1989). A quantitative morphological analyses of several glomerular networks have also been performed in the rat kidney (Shea, 1979; Shea and Raskova, 1984; Yang and Morrison, 1980). Blood enters the glomerulus via the afferent arteriole, a branch of an intralobular artery in the kidney cortex. Blood then passes into a tuft of glomerular capillaries formed from branches of the afferent arteriole. It is within these capillaries that filtration of blood occurs. Blood leaves the glomerulus via the efferent arteriole.

Studies by Hall (1955) in rabbit, rat, dog, and man; Murakami *et al.* (1971) and Winkler *et al.* (1991) in rats; Elias (1957) and Elias *et al.* (1960) in man; Anderson and Anderson (1976) in dog; and Unehira (1989) in rhesus monkey, suggest a basic pattern of afferent arteriole branching. The pattern appears to be one in which the afferent arteriole generally branches to form two vessels of nearly equal diameter. These initial branches are often referred to as "primary vessels" (Winkler *et al.*, 1991), although Hall (1955) called them "basal branches" and Unehira (1989) referred to them as "afferent rootlets" or "lobular branches".

The literature also reveals a wide discrepancy noted in the number of "primary vessels" for a single glomerulus in different species. For example, Wilmer (1941) claimed 2-10 primary vessels in humans, while there are 2 in the rat and 2-6 in the rhesus monkey as reported by Winkler *et al.* (1991), and Unehira (1989), respectively.

Within the glomerulus, the "primary vessels" divide further to form a ball of capillaries commonly termed the glomerular tuft (Brown, 1981). To our knowledge however, few attempts have been made to clearly

describe or classify subsequent branching patterns of the "primary" (or "basal", "afferent rootlets" or "lobular") branches. Although Wilmer (1941) found "secondary" vessels arising from the primary branches, the distinction between primary and secondary branching patterns remains unclear and ill defined.

We began to examine corrosion casts of pig kidney glomeruli in light of published observations of other species. The purpose of this paper is to describe the basic pattern and notable variations observed in our studies of the pig and to propose the establishment of a defining criteria and to offer a nomenclature for glomerular vessels that may be used in comparing the vascular organization of glomeruli of different species. It is relevant to note that Dobyan and Bulger (1988) suggested that the minipig kidney would be a good model for study because of similarities to both dog and human. The present report should help to reduce the confusion which occurs as a consequence of the numerous terms often used to identify (reviewed by Ditrich and Splechtna, 1990) the vascular structures which comprise the kidney glomerulus.

Materials and Methods

Four female pigs, approximately 90 kg (6-months of age) were euthanized with a captive bolt gun. The abdominal cavity was quickly opened and both kidneys removed while cutting the renal artery and vein close to the aorta and vena cava, respectively. A cannula was inserted and secured into the stump of the renal artery of each kidney, and the vasculature was purged with Hank's modified solution containing 4% mannitol (w/v) using a peristaltic pump (Cole Parmer Model No. 7520-25; Cole Parmer Instrument Co., Chicago, IL) following the basic method of Casellas *et al.* (1982). Good perfusion was indicated when the kidney became uniformly blanched in appearance and blood no longer appeared in the effluent exiting the stump of the renal vein.

After clearing the kidney of blood, approximately 35 cc of 5% neutral formalin was hand-injected with syringe into the arterial cannula. Blue methylmethacrylate plastic (Mercox Cl-2B) was quickly prepared for an approximate 4-minute curing time, drawn into a 60 cc syringe and hand-injected into the renal arterial cannula. Vascular filling was evidenced by observing the uniform change in color of the kidney to a purplish-blue and by the appearance of Mercox at the renal vein stump. The Mercox was allowed to flow out of the kidney for a short time before the renal vein was clamped. A slow rate of injection of the plastic was carefully continued until the kidney expanded approximately to it's normal *in vivo* size. Then, the arterial cannula was also clamped while hand-pressure was maintained. The kidneys were wrapped in plastic wrap while the polymerization of the Mercox proceeded to completion at room temperature for 30 minutes. The kidneys were then refrigerated until needed.

Tissue pieces were cut from the kidney cortex using an acetone cleaned razor blade, measured in three dimensions, and marked for later identification and orientation. These pieces were subsequently corroded for 48-72 hours in daily changes of a solution of warm (50° C) mixture of saturated potassium hydroxide (80%) and methanol (20%). After corrosion, the vascular casts were carefully cleaned by rinsing in fresh distilled water (daily changes) for three to four days. The casts were then frozen in distilled water at -20° C and freeze dried in preparation for scanning electron microscope (SEM) viewing. Freeze-drying was performed because less shrinkage of casts has been reported with this technique than with the critical point drying (Kikuta and Murakami, 1989; Lametschwandtner *et al.*, 1990) or air drying (Lametschwandtner *et al.*, 1989). The dried vascular casts were mounted on 1-1/8 inch (2.85 cm) aluminum stubs (Ted Pella, Inc.), gold-palladium sputter coated (SPI sputter-coater) and viewed with either the ISI DS-130 or the ISI-40 SEM operated at 5 kV.

Only glomeruli fitting the criteria of a good casting indicated by the presence of nuclear impressions on both the afferent and efferent arterioles (Hodde *et al.*, 1977; Shah-Yukich and Nelson, 1988; Ditrich and Splechtna, 1987; and Lametschwandtner *et al.*, 1990) were used in this investigation. Glomerular capillaries also had to present well rounded filled contours with smooth surfaces (Smith, 1956; Murakami *et al.*, 1971) as seen at low magnifications (under 700X). Glomeruli containing round ended tips of blind ending vessels which indicated inadequate or poor vascular filling (Ditrich and Splectna, 1987), were not used. Afferent and efferent arterioles were identified and/or distinguished using criteria based upon profiles of nuclear impressions established by Hodde *et al.* (1977), Hodde and Nowell (1980), Hodde *et al.* (1990), and Miodonski *et al.* (1978), and reviewed by Lamentschwandtner *et al.* (1990).

Results and Observations

Results reported here summarize the observations made on approximately 3,800 glomeruli of the pig kidney. In general the capillaries forming each glomerulus are organized and arranged in such a way that the glomerulus is essentially spheroidal in shape. All glomeruli in the pig kidney were seen to have two distinct hemispheres with a cleft separating the two hemispheres.

Afferent Arteriole

A single afferent arteriole gives rise to all of the

vessels which contribute to the formation of a single glomerulus. In approximately 50% of the glomeruli studied, upon approaching the glomerular tuft, the diameter of the afferent arteriole appeared to increase just prior to bifurcating to form the primary branches (Figures 1 and 2).

Primary Branches

Two primary vessels of generally equal diameter, were formed by the bifurcation of the afferent arteriole. Each primary vessel was found to distribute branches to only one of the two hemispheres which together comprise the total glomerular tuft.

The formation of the two primary vessels occurred external to, but usually within 50 μm of the glomerulus. However, on rare occasions (e.g., Fig. 3), the bifurcation occurred at a greater distance from the glomerular tuft.

Secondary Branches

Each primary branch may give rise to secondary branches which generally show a slight constriction at their origin. The number of secondary vessels arising from one primary vessel in a given glomerulus is highly variable, ranging from zero to three for either hemisphere of the glomerulus. One or two secondary branches per hemisphere are most common, while a third branch is occasionally distinguishable. In the same glomerulus, one hemisphere may have one or more secondary vessels while the other hemisphere may have none. Each secondary branch is usually relatively short (approximately 10μm), but occasionally a longer (25-30μm) secondary branch was seen (Fig. 1).

Tertiary Vessels

Tertiary vessels are defined as branches of secondary vessels. Each secondary branch consistently divided into two tertiary vessels having nearly equal diameters (Figure 1 and 2). However, in some instances where secondary vessels were not distinguishable by the criteria as stated above, branches arising directly from the primary vessels were considered to be tertiary vessels based upon their observed distribution (Fig. 4).

Tertiary vessels in pig glomeruli were found to distribute in a contorted fashion over the outer surface of the glomerulus from the vascular pole towards the urinary pole. Along this contorted pathway, numerous branches having differing diameters are given off. The diameters of some of these branches appeared to be as large as the parent tertiary vessel while others were smaller. Despite its contorted configuration and numerous branches, most tertiary vessels retained their initial diameter throughout their length (as could be observed).

As many as five tertiary branches (Fig. 4) may distribute to one hemisphere. Of these five, a final tertiary vessel was seen to continue over the glomerular surface on the edge of the cleft separating the two hemispheres (Fig. 5).

In some instances, a tertiary vessel may communicate with other tertiary vessels via anastomotic branches. These branches were usually observed leaving the tertiary vessel at a right angle.

Anastomotic Branches

Anastomotic branches were observed on the surface as well as beneath the surface of the glomerulus as revealed in fractured casts. Compared to the anastomotic branches on the surface, those beneath the surface generally appeared to be smaller in diameter. Anastomotic branches were observed: **a**) to join directly with other branches from the same parent tertiary vessel; **b**) to join with branches from another tertiary vessel, or **c**) to rejoin its parent tertiary vessel farther downstream (Fig. 1). Most anastomotic vessels were seen to combine with others in an anastomotic network. This network eventually rejoined the parent tertiary vessel at some point more distally along the tertiary vessel as it appeared to traverse over the hemisphere.

Some anastomotic branches of the tertiary vessel appeared to have much reduced diameters at the site of branching. This localized restriction is suggestive of a sphincter (Figures 2, 8 and 10). Distal to this suggestive sphincter region, the anastomotic branch consistently increased in diameter and in some instances became as large as that of the parent tertiary vessel.

Efferent Arteriole

The efferent arteriole exits the glomerulus at the vascular pole. In the pig it passes between the two primary branches of the afferent arteriole at or near the site of origin of these branches. The efferent arteriole typically takes a tortuous course to a nearby capillary bed.

That portion of the efferent arteriole which was located within the confines of the glomerulus, appeared to be larger in diameter than the portion of the vessel which lay outside the tuft. The efferent arteriole showed little or no change in its diameter throughout its course outside the confines of the glomerulus.

Fractured glomerular casts revealed much information about the highly variable vascular architecture associated with the formation of the efferent arteriole deep within the glomerular tuft (Figures 6, 7 and 8). Figure 6 shows the best view of the arrangement by which the glomerular capillaries form the efferent vessel in the pig kidney. The efferent vessel is formed within the glomerular tuft and it may extend across nearly the entire diameter of the glomerular tuft from the urinary pole to the vascular pole. We have termed this vessel the "central" or "central hemispheric" vessel. The central vessel typically possesses a rather large diameter throughout its

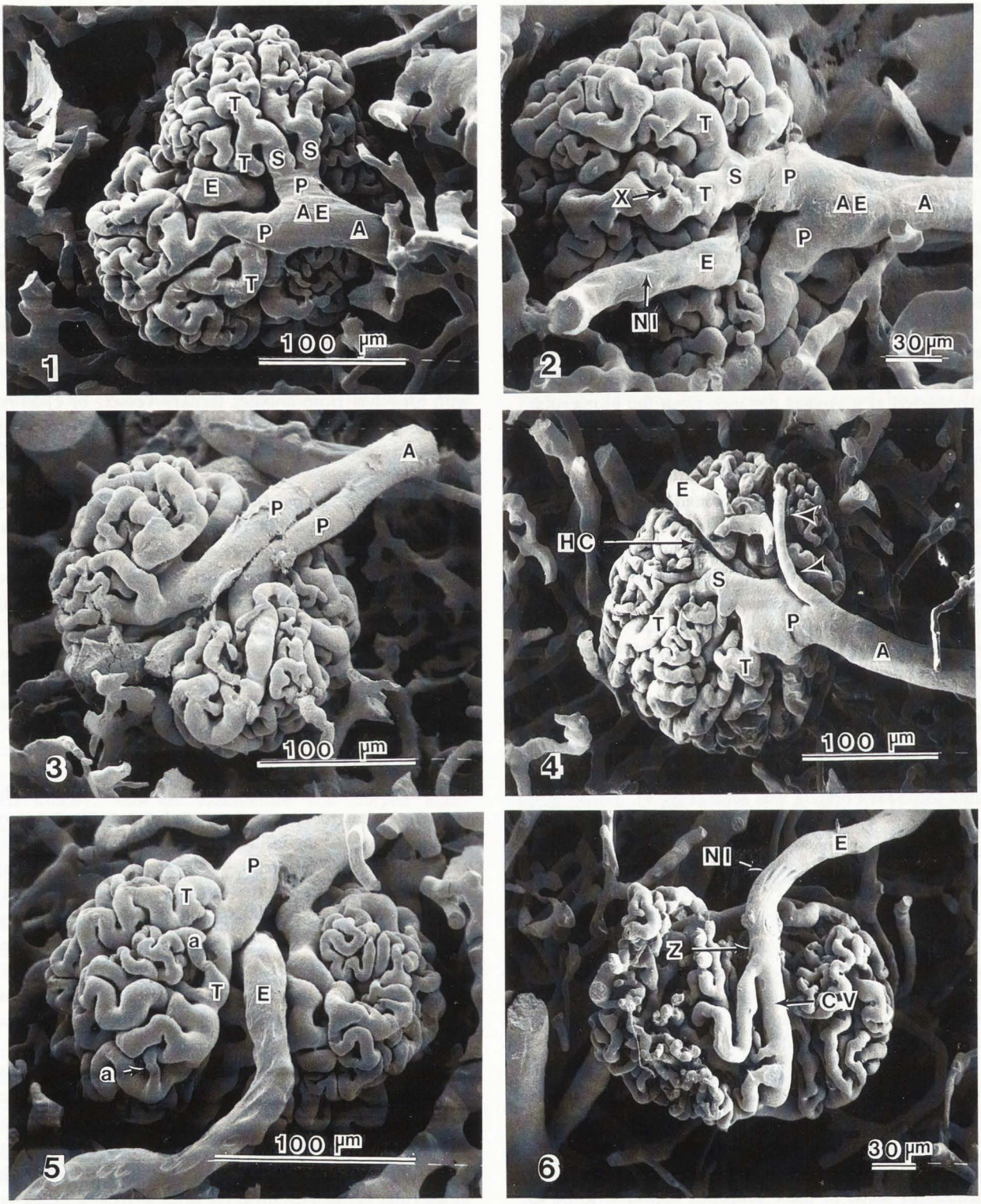


Figure 1. Afferent enlargement (AE) of afferent arteriole (A) plus primary (P), secondary (S) and tertiary (T) branching pattern. Secondary vessels longer than usual are seen here.

Figure 2. Short, secondary vessels (S) are more typical. Efferent arteriole (E) is typically seen emerging between the two primary vessels (P) of the afferent arteriole (A). (X = suggestive sphincter; NI = endothelial nuclear impressions into cast surface).

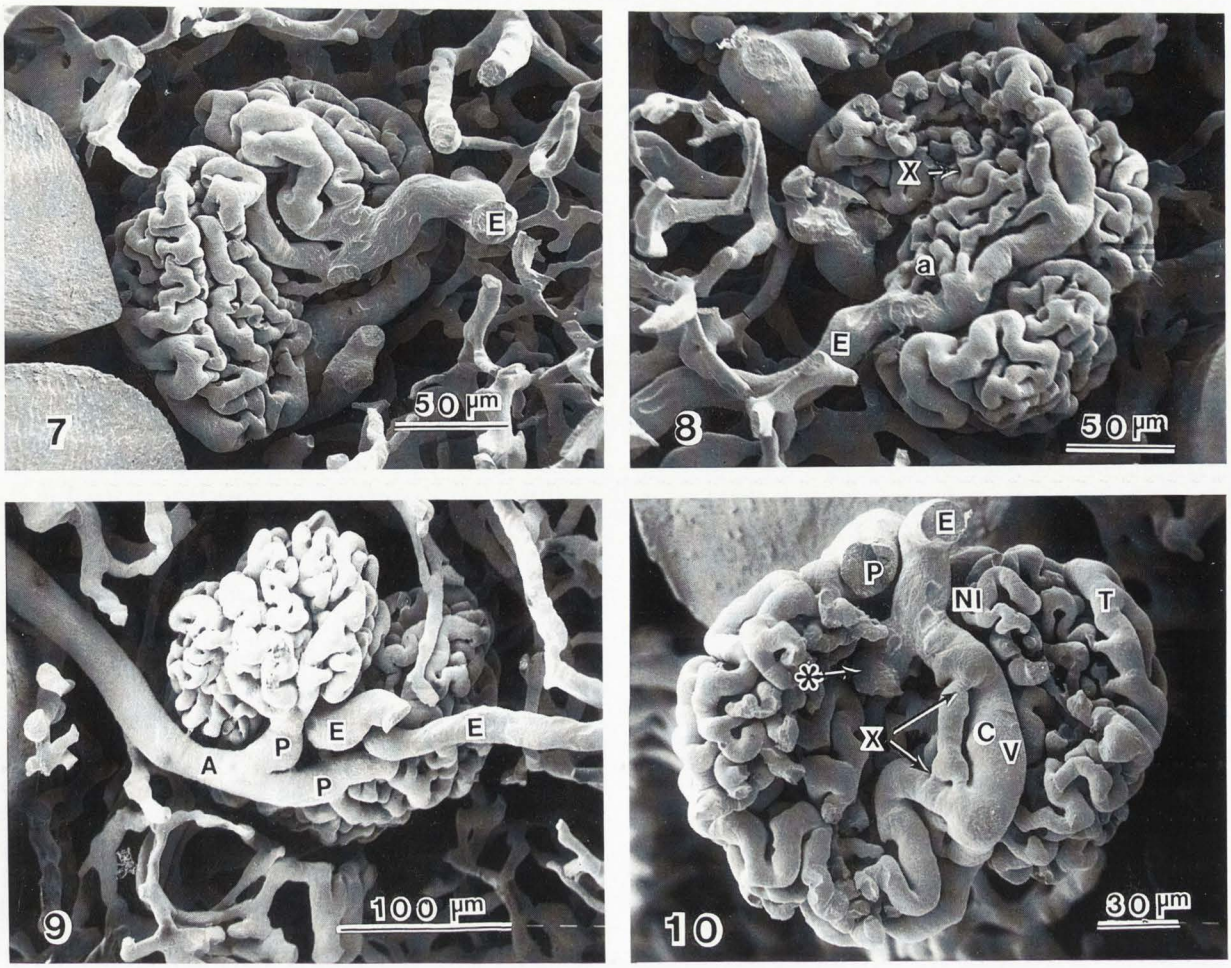


Figure 3. Unusually long (approximately 100 μm) primary branches (P) of the afferent arteriole (A).

Figure 4. This unusual branch (arrowheads) from the afferent arteriole (A) may represent a developing shunt to bypass an affected glomerular hemisphere as described by Loomis (1936) and MacCullum (1939). Note the reduced size of the affected upper hemisphere and the lack of large vascular branches to this hemisphere. (HC = hemispheric cleft.)

Figure 5. Glomerular hemispheres with the efferent arteriole (E) emerging between them. (P = primary branch; a = anastomotic vessel).

Figure 6. Fractured glomerulus allows a view of the central vessel (CV) leading to the exiting efferent arteriole (E). Near the vascular pole, the stumps of two large vessels broken off from the efferent arteriole. (NI = endothelial nuclear impressions into cast; Z = zone of endothelial transition from capillary type to efferent arteriole type).

Figure 7. In the formation of the efferent arteriole, some vessels are seen to come from the surface of the glomerular tuft.

Figure 8. A surface vessel is seen to penetrate the tuft to join the efferent vessel (E). Anastomotic vessels (a) and the suggestion of vessel sphincters (X) are seen within the tuft of capillaries.

Figure 9. A possible example of a double efferent arteriole (E) emerging from the glomerular tuft.

Figure 10. An unusual anastomotic pattern is seen to be involved in the formation of the central vessel (CV) of one hemisphere. The (*) indicates what is suggested to be the stump of the central vessel of the opposite hemisphere. (X = two of three sphincters are indicated on one anastomotic vessel; NI = endothelial nuclear impressions; P = stump of primary vessel; E = stump of efferent arteriole).

length and does not appear to increase in diameter when other vessels join with it.

Figure 6 also shows three large vessels and one smaller vessel joining the central vessel. The orientation of the smaller vessel suggests that it may have come from the outer surface of the glomerulus and as such may be the continuation of a surface tertiary vessel. Other vessels come from within the depths of the glomerular tuft to join the central vessel. As seen in Figure 6, impressions suggestive of cell nuclei are found in the cast material of the central vessel near the point where it emerges from the glomerular tuft and becomes the efferent arteriole. In addition, the smooth endothelial surface of the central vessel changes to the roughened, irregular surface that is characteristic of the efferent arteriole. Only rarely were nuclear impressions observed on the cast of either the tuft capillaries or the central hemispheric vessel from a capsular viewpoint.

In all but one instance in the present study, the efferent arteriole exited the glomerulus as a single vessel. The only exception occurred with an apparently double efferent arteriole emerging from one glomerulus (Fig. 9). However, in this case, these vessels could not be traced in their distribution to confirm their being efferent arterioles.

Discussion

Afferent Arteriole

The findings of the present study of pig glomeruli suggest that blood is delivered to each kidney glomeruli via a single afferent arteriole. This conclusion is consistent with that of Murakami *et al.* (1971) who reported that in the rat, a given afferent arteriole supplies blood to a single glomerulus. Wilmer (1941) described a somewhat different architecture in the human kidney wherein the afferent arteriole occasionally splits into two vessels, each of which in turn supply a discrete glomerulus.

Observations made of the afferent arteriole in the cat indicate the presence of a sphincter located just proximal to the formation of the primary vessels (Marais, 1987). Although Murakami *et al.* (1971) reported a similar structure in the rat, Gattone *et al.* (1983a,b) could not find such a structure. In the study of glomeruli of macaque monkey, Nopanitaya (1980) described "...a unique endothelial arrangement consisting of a single row of ovoid endothelial impressions located at its connection with the capillary rootlets". Nopanitaya suggested this to be evidence of a sphincter. In the present study, there were no markings or impressions in the vessel wall which would be suggestive of a sphincter in this location on the afferent arteriole supplying the pig kidney glomerulus.

Endothelial fenestrations in afferent arterioles were first described in transmission electron microscopic studies of several species by Rosivall and Taugner (1986). Casellas (1986) reported the presence of endothelial fenestrations at the bifurcation of the afferent arteriole facing the extraglomerular mesangial area as seen by SEM. Anderson *et al.* (1988) in their SEM study of the afferent arteriole of the black bear reported leakage of casting material out of the afferent arteriole at this location. A similarly placed leakage of casting materials could not be found in human (Murakami *et al.*, 1985) or macaque monkey (Nopanitaya, 1980) kidney preparations. Murakami *et al.* (1971) found leakage in this vessel in the rat kidney, but others (Gattone *et al.*, 1983a,b; and Shah-Yukich and Nelson, 1988) have not. Unehira (1989) did report leakage of casting material in rhesus monkey kidney but the leakage occurred within the tuft of capillaries and not from the afferent arteriole. Based upon the casting technique used in the present study, no evidence of casting material leakage was observed in any segment or region of the glomerular vasculature in the pig.

It was common to find enlargements of the afferent arteriole proximal to the bifurcation which gives rise to the primary branches. An enlargement in this region of the vessel has not been reported in the dog (Barger and Herd, 1971), the macaque (Nopanitaya, 1980), or rhesus monkey (Unehira, 1989), or in human (Murakami *et al.*, 1985) kidney, but it is found in the kidneys of sheep (Bovee, 1983), the cat (Marais, 1987) and the black bear (Anderson *et al.*, 1988).

Primary Branches

In the process of forming a glomerulus, Brown (1981) reports that in mammals in general, the afferent arteriole divides into four to six branches. Hall (1955) reported that from two to four "basal" branches are normally seen to leave the afferent arteriole in the kidney of the rabbit, rat, dog and human. Aeikens *et al.* (1979) reported only one or two capillaries formed from the afferent arteriole in the rat. Further, Wilmer (1941) stated that the vascular branching pattern of the afferent arteriole in the pig closely resembles that reported for the human where there may be two to ten primary branches at the vascular pole. Winkler *et al.* (1991) reports only 2 primary branches in the rat while Unehira (1989) suggests 2-6 afferent rootlets are present in the rhesus monkey.

In our study of the pig kidney, the afferent arteriole as defined, bifurcates to form only two primary vessels as it approaches the glomerulus. It is suggested that in some earlier reports, branches which we have defined as occurring subsequent to the formation of the primary vessels (secondary and tertiary branches) were counted

as primary vessels. This would account for the large variation in the number of afferent branches reported in the literature. We suggest that there are only two primary branches of the afferent arteriole, one to each hemisphere of the glomerulus in the pig. Then, secondary and tertiary branching occurs.

Secondary Branches

When present, these vessels arise directly from the primary vessels and divide to form two usually equal in diameter tertiary vessels. These vessels are very short and usually possess a slight constriction at their origin.

Tertiary Vessel

Each tertiary vessel gives rise to numerous branches along its path. The pattern of anastomoses and rejoining of branches is complex but occurs without an apparent change in the parent tertiary vessel diameter throughout its contorted pathway over the surface of the glomerulus.

Anastomotic Branches

Anastomotic branches arising from tertiary vessels show considerable variability in anastomotic patterns and diameter. In some instances, a sphincter-like structure was present on these vessels at the location of its branching from the tertiary vessel. Branches from adjacent tertiary vessels are shown to anastomose in the pig. This fact can explain the inability to separate individual lobules of the glomerulus which Murakami *et al.* (1971) and Kikuta and Murakami (1989) attempted to do in the rat. Unehira (1989) also reports anastomotic branches connecting afferent rootlets which would not allow the physical separation of lobules.

Efferent Arteriole

In the human kidney, the efferent arteriole dilates within a short distance after leaving the glomerular tuft (Barger and Herd, 1971). This was not observed in the present study of the pig glomerulus, nor has it been seen in a study of the black bear kidney (Anderson *et al.*, 1988).

In studying human glomerular serial sections, Elias (1957) and Elias *et al.* (1960), illustrated in stereograms that the efferent vessel arose at the vascular pole as a small sac where small vessels empty into it. This sac was positioned at the vascular pole and did not arise from within the glomerular tuft. Murakami *et al.* (1971) described the efferent arteriole formation in the rat as a "confluence" of several rootlets or the ends of capillaries of glomerular lobules.

In our present study, the tuft capillaries (branches of the tertiary vessel) were often found to empty into a central vessel (Fig. 6), which appears as an elongated sac-like structure. This central vessel extends through the central region of the glomerulus from near the urinary pole region to the opposing vascular pole of the glomerulus where it is continued out of the glomerular tuft as the efferent arteriole.

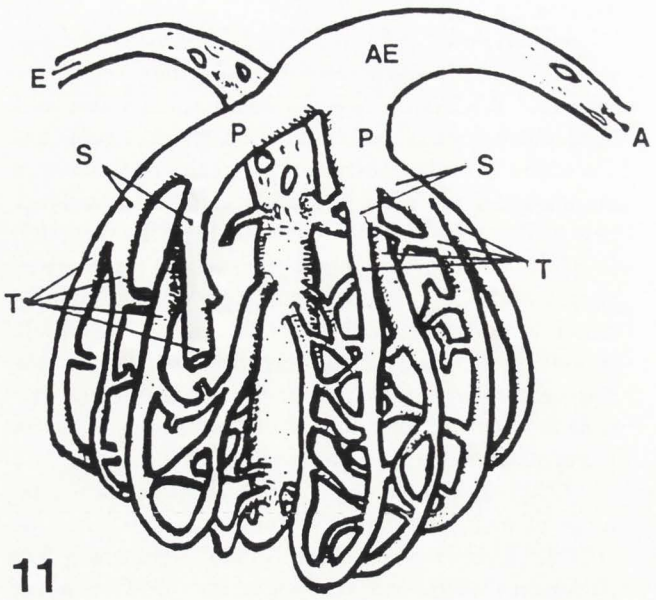
Our view of the central vessel has been obtained from glomeruli which were fractured into two hemispheres. We cannot rule out the possibility that each hemisphere contains a central hemispheric vessel, and that central vessels from both hemispheres may converge at some point within the glomerular tuft to eventually appear at the vascular pole as the single efferent arteriole (Fig. 10). We further suggest that should these central vessels not join within the glomerular tuft, multiple efferent arterioles exiting the tuft can be the result. Winkler *et al.* (1991) in one case, demonstrated the confluence of two vessels to form the single efferent arteriole as occurring just outside the boundaries of the rat glomerular tuft (serial section reconstructions).

Other investigators have also reported the occurrence of multiple efferent arterioles. Murakami *et al.* (1971), Shanyo and Mann (1944), Anderson and Anderson (1976), and Winkler *et al.* (1991) observed multiple efferent arterioles in the rat. Moffat and Fourman (1963) also reported their presence in the rat but added that this anatomy was seen mostly in the juxtamedullary region. Smith (1956) reported that triple efferents are often seen in the human kidney. Our evidence indicates multiple efferent arterioles are very uncommon in the pig kidney. Only 1 of 3,800 glomeruli observed provided evidence which suggested the possibility of a multiple efferent arteriole.

From scanning electron microscopy observations of cast glomeruli made prior to corrosion (unpublished observations), no tertiary branches or tuft capillaries were seen to join the efferent arteriole either outside of Bowman's Capsule or outside the confines of the cast pig glomerular tuft. This observation has been made by others in the human (Smith, 1956) and the rat (Murakami *et al.*, 1971) kidney. These findings lead to the conclusion that all of the vessels which join and help to form the efferent arteriole in the pig kidney are enclosed within Bowman's Capsule. As shown in Figure 6, vessels joining the central hemispheric vessel may join at any point along the extent of the central vessel. Ditrich and Splechtna (1990) have described the generalized, simplest mammalian form of glomeruli typical of rat, mice, and cat as lobular vessels reuniting near the vascular pole for the formation of the efferent arteriole. Winkler *et al.* (1991) has presented similar evidence in the rat by stating that the last two branches joining to form the efferent arteriole occurs about 10-22 μm from exiting the glomerulus.

Vascular Diagram

Elias (1957) and Elias *et al.* (1960) presented a stereogram of the human renal glomerulus based upon



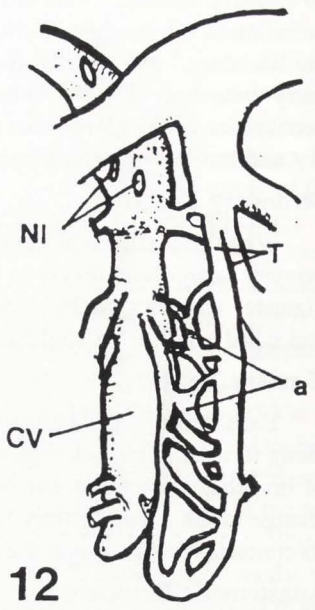


Figure 11 (at left). Diagram of proposed basic vascular organization of pig kidney glomerulus. View is of one side of one hemisphere where one tertiary vessel (T) and its branches have been removed to reveal the central vessel (CV) of the hemisphere which is continuous with the efferent arteriole (E). Roughened surface of efferent arteriole cast indicates a change in endothelium from that of capillary type to that more typical of efferent arteriole.

Figure 12 (at right). Diagram detailing the configuration of one tertiary vessel (T) and its anastomotics (a) of Figure 11. The diagram illustrates the hypothesis that an individual lobule may consist of a tertiary vessel and its anastomotic branches. The tertiary vessel has its origin at the vascular pole and terminates as a vessel joining in the formation of the central vessel (CV) of the hemisphere which in turn leads to the formation of the efferent arteriole (E).

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studies of serial sections. Figures 11 and 12 show similar diagrams which summarize our concept of the vascular anatomy in pig kidney glomerulus. This is presented with special reference to the formation of the efferent arteriole. Some features of the tertiary vessels formed in the pig glomeruli agree with those reported by Elias (1957) and Elias *et al.* (1960) for the human kidney glomerulus. Our simplified representation of the glomerulus shows that the tertiary vessels join with, and help to form, the central hemispheric vessel. The central vessel extends through approximately the middle of the glomerulus, and at some point within the confines of the glomerular tuft, transforms into the efferent arteriole as illustrated in Figures 7, 8 and 10. Figure 12 is a conceptualization of the branching pattern of a single tertiary vessel; the vessel is shown from its origin to a proposed termination, and includes some possible anastomotic branches. As reflected in our findings, anastomoses connect various segments of the loop formed by the larger tertiary vessel. Our data support the suggestion that this loop of the tertiary vessel, with its varied arrangements of anastomotic interconnections, may constitute a single lobule of the glomerulus, and that each glomerulus is made up of several lobules equal at least to the number of tertiary vessels. This interpretation in the pig is consistent with that of the rat by Winkler *et al.* (1991); Kikuta and Murakami (1989) and Murakami (1972).

The diameter of the tertiary vessel appears to remain nearly constant even after many branches are given off. This finding, illustrated in Fig. 12, supports the suggestion that there may be little change in overall blood volume throughout this vascular segment.

Richards and Schmidt (1924) observed the *in vivo* flow of red blood cells through the glomeruli of the frog kidney. Their observations were facilitated due to the fact that unlike mammalian kidneys, frog glomeruli are very near the surface of the kidney and are readily seen with the aid of a dissecting microscope and proper lighting. They reported that within a single glomerulus, the number of capillaries with flow of red blood cells is variable moment to moment and that glomeruli contain different sizes of capillaries since some capillaries showed red blood cells in single file while others contained pools

of slowly flowing red blood cells.

Also, the pathway of flowing red blood cells can vary from moment to moment with some capillaries showing only plasma coursing through them. Richards and Schmidt (1924) further suggest that the size of the opening into each capillary determined the flow of red blood cells and it may not be that the capillaries are of different sizes.

Hall (1955) suggested that the larger surface vessels (tertiary vessels) may be a direct pathway for red blood cells to bypass the smaller anastomotic vessels and continue directly to the efferent vessel. He also postulated, that skimming off of plasma into the smaller anastomotic channels occurred at constricted sphincter-like entrances. This organizational pattern of vessels has also been described by Chambers and Zweifach (1944) for rat mesoappendix and dog omentum capillary networks.

Steinhausen, *et al.* (1983) were able to visualize microscopically in vivo, individual glomerular loops within split hydronephrotic kidneys of rats and to trace fluorescent labeled erythrocytes through different capillary pathways. They also reported Angiotensin II affecting the pattern and rate of glomerular blood flow through different capillary routes of the glomerulus. The reader is referred to Steinhausen, *et al.*, (1990) for a review of glomerular blood flow and the effect of various agents upon blood flow through the glomerulus.

In the pig, the observed pattern of vascular branching suggests that blood or blood plasma, flowing within the internal or surface anastomotic vessels, may eventually return to its parent tertiary vessel at a point farther downstream prior to joining the central hemispheric vessel or in some cases into the central hemispheric vessel directly. In the pig glomerulus, the central hemispheric vessel(s) is in direct continuity with the efferent arteriole. What we have termed the central hemispheric vessel may be what Winkler *et al.* (1991) has referred to as the intraglomerular segment of the efferent arteriole. However, Winkler's group present evidence that for the rat, only one intraglomerular efferent segment was present and it lies within the mesangial stalk.

Unpublished observations (in preparation) of the glomerular tuft also indicate a size difference in the diameter of vessel types described in this study of the pig.

Conclusions

A rather predictable pattern of vascular anatomy exists for pig kidney glomeruli. As the afferent arteriole approaches the glomerulus, it divides into two primary branches. Each primary branch distributes to one of two hemispheres of vessels which are arranged to form the glomerulus. In each hemisphere, a primary branch may split into secondary vessels or directly into tertiary vessels. Secondary vessels, when present, generally quickly divide to form two tertiary vessels. Tertiary branches serve as the distributing vessels of the glomerular tuft and give off numerous branches along their highly contorted pathway.

The tertiary vessels may serve as the most direct route for the flow of blood especially blood cells, through the glomerular tuft. The small sphincter-like diameter of some side branches of the tertiary vessels suggests that they may prevent blood cells from passing through while allowing the flow of plasma through them. Some tertiary vessels make a direct connection to a central hemispheric vessel whereas others make their connection to this vessel through anastomotic branches. The anastomotic branches of a tertiary vessel often rejoins the parent vessel at some point more distal along its path. In some instances, anastomotics join with those of another tertiary vessel which in turn eventually joins the central hemispheric vessel.

There is evidence for the presence of one central vessel for each hemisphere of a given glomerulus. Tertiary and/or anastomotic branches were found to join the central hemispheric vessel at any point along its length without producing a discernable increase in the diameter of this central vessel. The central vessel for each hemisphere subsequently join together to form the single efferent vessel near the vascular pole of the glomerular tuft. It is suggested that failure of central hemispheric vessels to join prior to exiting the glomerular tuft, result in multiple efferent arterioles.

The relative simplicity of the pig glomerular vascular architecture has allowed characterization of different vascular segments that we suggest, has not been possible in other mammals. In this regard, the pig may serve as the standard for comparison with other mammals.

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Discussion with Reviewers

H. Ditrich: The authors propose a classification of glomerular vessels into secondary and tertiary branches as well as anastomoses (quartery branches?). However, which criteria are to be applied if: **a**) secondary branches can be indistinguishable or absent, and **b**) anastomoses and tertiary branches can have the same diameter and thus are not different in the SEM?

Authors: The pattern described is generalized and only occasionally were secondary branches not present in both glomerular hemispheres. The pattern of distribution of these vessels must be considered, i.e., the tertiary vessels are the main distributing vessels for the glomerular tuft. It is true that because of similarity in diameter of some anastomotic and tertiary vessels, when neither origin nor termination can be established, one cannot say with certainty that a specific vessel is one or the other.

H. Ditrich: The term "sphincter" is used to describe narrowings of vascular lumina in cast glomerular vessels. Accordingly, this structure should be able to contract (presence of muscle cells). In my opinion the term "tapering" or "narrowing" should be used unless it is clear that such structures are either muscular sphincters (unlikely), endothelial nuclear bulges (compare to Ditrich and Splechtna, 1991), or endothelial cushions (compare to Moffat and Creasey, 1971; Casellas *et al.*, 1982).

Authors: We agree with the distinction concerning classifications of sphincters, etc. Nevertheless, lacking other anatomical evidence in the present study, we feel that the features we observed relating to this morphology are best described by the phrases "suggestive of" and "sphincter-like" because of the abrupt narrowing of a restricted portion of the vessel lumen which resembles a narrowing that a sphincter would produce.

H. Ditrich: Might the variations in the number of branches of the afferent vessel (primary branches) reported from different authors (Discussion) be also the result of natural variability (i.e., different branching patterns in different mammals)?

Authors: It is reasonable to expect that there will be a variability among mammals. Of importance here is that there has been apparent inconsistency in the definition of primary vessels contributing, in part, to variations reported. The relative simplicity of the pig glomerular vascular architecture has allowed us to visualize the extent of each vascular segment with the result that we have been able to more completely characterize these vessels than may have been possible in other mammals. In this regard, we suggest that the pig may serve as the

standard for comparison with other mammals.

H. Ditrich: What are the advantages of prefixation with 5% formalin before injecting, apparently this procedure is not sufficient to stabilize the overall size of the organ.
Authors: The kidney was infused with 5% formalin for the purpose of stabilizing the endothelium and vascular muscle tissue so as to minimize or prevent constriction of vessels at the time the resin was injected. It was not our purpose to fix the entire organ.

D. Casellas: There are some basic, well-known morphological differences between superficial, midcortical and juxtamedullary glomeruli (i.e. glomerular size, afferent arteriolar length, efferent vascular pattern etc.). Do the authors' conclusions about glomerular vascular pattern apply to all these nephron populations; in other words, were glomeruli sampled randomly throughout the renal cortex?
Authors: Our purpose here has been to describe the qualitative aspects of the vascular architecture. Although glomeruli were sampled from all depths within the cortex, we did not detect a systematic difference in the vascular pattern that was related to depth. We are confident that the vascular pattern that we have described applies to pig glomeruli in general. We are presently quantifying glomerular vessel diameters and also overall glomerulus size relative to depth.

D. Casellas: Did the authors observe any narrowing of the outflow segment of the efferent arterioles? Such sphincter-like arrangement was recently described in the rat and is characterized by the bulging of endothelial cells within the lumen of the efferent arteriole (Schnabel E. *et al.*, Renal Physiol. **10**:318-326, 1987).
Authors: Qualitatively, the diameter of the central vessel was consistently larger than its continuation as the efferent arteriole. We did observe the bulging of endothelial cell within the lumen at the transition zone (see Figures 6 and 10). We are hesitant to describe this as sphincter-like structure, but rather the transition to the endothelial morphology typical of the efferent arteriole.

A. Kikuta: Are there any positional differences in the glomerular vascular arrangements? It is known that in the rat cortical, subcortical and juxtamedullary glomeruli show different morphology.
Authors: As noted above, we believe that we have described a general qualitative pattern in pig glomeruli. A subsequent publication will quantify any differences we have observed relating to position within the cortex in the pig.

A. Kikuta: Have you observed direct connections or preferential pathways connecting afferent and efferent arterioles?
Authors: We never observed a direct connection between afferent and efferent arteriole. We cannot comment on the matter of a preferential pathway.

A. Kikuta: In Figures 7 and 8, the central vessel in not seen. Is the central vessel a constant structure of the glomerular vascular tuft?
Authors: Our observations support our conclusion that a central vessel is a consistent feature of the pig glomerulus. Most fractures revealed all of this vessel. Other fractures revealed portions of the vessel, or anatomy which strongly suggested connections to a central vessel.

V.H. Gattone II: Is the "central vessel" lined by a fenestrated endothelium, e.g., is it utilized in the filtration process?
Authors: Under high magnification, the surface of the resin of the central vessel was very smooth. Also, there was never any leakage of the resin at any point on the vessel. We realize that this is not conclusive evidence of fenestrations present or absent but the lack of extravasated resin suggests the absence of fenestrations. The nature of our data do not permit us to speculate on the contribution of the central vessel on filtration.

V.H. Gattone II: How often did you see the linear array of nuclear impression in the proximal efferent arteriole (NI in Figure 6)? Were they more prominent in inner cortical glomeruli? Gattone *et al.* (Am. J. Physiol. **247**, F219-F228, 1984) described prominent endothelial cells only in rabbit inner cortical efferent arterioles.
Authors: These impressions were frequently observed, but quantitation of their distribution was not made.

A. Kikuta: I think that Mercox is not good casting medium for studying conglomerated vascular tufts such as renal glomeruli. Once polymerized, Mercox become so fragile and it is hard to soften it, although it is not impossible. You can heat corroded casts on a hot pan (150-200 °C) and make them soft and press or stretch the casts to extend conglomerated networks. Then you can observe the inside view of the tufts. If you prepare a half-polymerized methyl methacrylate resin and make corrosion casts according Murakami's method (1971, 1972), you can more easily soften corroded casts.
Authors: Thank you for your comments. We had attempted softening by alcohol per Murakami (1972) [and Murakami *et al.* Arch. Histol. Jpn. **33**, 179-198, (1971)], but were not successful in pulling them apart apparently due to intralobular anastomotics. We hope to follow up on your suggestions in the future.