Blood Vessels of the Mammalian Renal Medulla

Abstract. *Sections and macerated specimens of kidneys from several species of wild and domestic mammals injected with India ink or vinyl acetate, or both, show that the specialized blood vessels of the renal medulla (vasa recta) consist of parallel, relatively unbranched vessels which break up into plexuses at different levels of the medulla. This concept differs from that expressed in many current textbooks. In some species there is a distinct zonation of the medullary blood supply, and in certain other species no zonation was observed. Functional differences exist between these two groups, and it is suggested that these differences may be due to the vasa recta.*

The specialized blood vessels of the mammalian renal medulla (vasa recta) are known to be important components of the countercurrent exchange mechanism of urine concentration. Gottschalk states (*1*): "The efficiency of the countercurrent exchange in the vasa recta is critical." Despite the importance of these structures, there is little accurate information on their anatomy; this has resulted in confusion in the literature about the precise form of these vessels. A recent edition of a widely used histology text (*2*) illustrates the renal medullary blood supply as a conventional capillary network, and a new renal physiology text (*3*) shows the vasa recta as specialized, parallel, looped structures. This latter concept is now widely accepted by physiologists and is supported by the studies of Trueta (*4*), which are among the most accurate and complete descriptions of renal blood vessels that have been available.

However, this concept has recently been disputed in England by Moffat and Fourman (*5*) who have published descriptions of the renal blood vessels of several laboratory mammals and of man. They studied ink-injected, macerated, and sectioned material and neoprene casts of the renal vascular tree and point out that the study of only sectioned material can lead to misinterpretations. Moffat and Fourman found no looped vasa recta in any species and describe vasa recta that end in capillary plexuses at various levels of the medulla with many unbranched vasa recta continuing to a plexus at the tip of the papilla. They believe that loops have been described because these fine vessels have been observed mainly in sections.

We have studied the renal blood vessels of several species of wild and domestic mammals possessing widely varying abilities to concentrate urine. Our purpose in this report is to summarize some of our observations (*6*) and to suggest some correlations between the form of the renal medullary blood supply, the arrangement of nephron components in the medulla, and renal function. We hope that this report may help to end the current confusion about the form of these important blood vessels.

We have studied the blood vessels of the kidneys of seven kangaroo rats (*Dipodomys*), three gerbils (*Meriones*), seven opossums (*Didelphis*), two domestic cats, one domestic pig, and three beavers (*Castor*), using methods described in Pfeiffer *et al.* (*7*). In our study we also injected papaverine hydrochloride (13 to 20 mg/kg of body weight) about 15 minutes prior to injecting India ink. Both arterial and venous ink injections were made in all species except the pig, in which only the arterial route was used.

In addition, we injected the kidneys of four opossums with vinyl acetate; one pair was injected via the arterial route and one pair via the venous route, after which they were completely macerated in KOH to obtain casts of the arterial and venous trees up to the glomeruli. The other two pairs of kidneys were injected with India ink followed by vinyl acetate via the renal artery and were then partially macerated in HCl so that we could observe the relationships of the renal

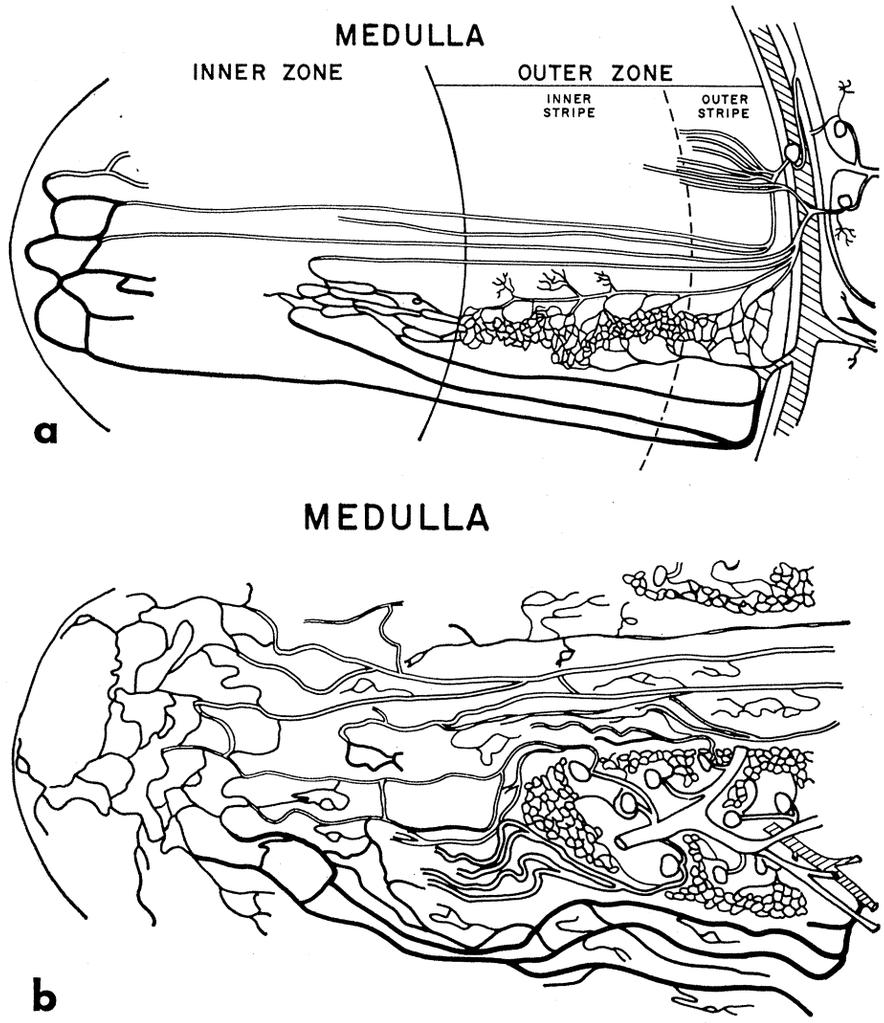

Fig. 1. Diagrammatic representations of the vascular bed of the kidney of a mammal (*a*) showing zonation of the medulla (opossum), and (*b*) showing no zonation of the medulla (beaver). Diagrammatic form adapted from Moffat and Fourman (*5*).

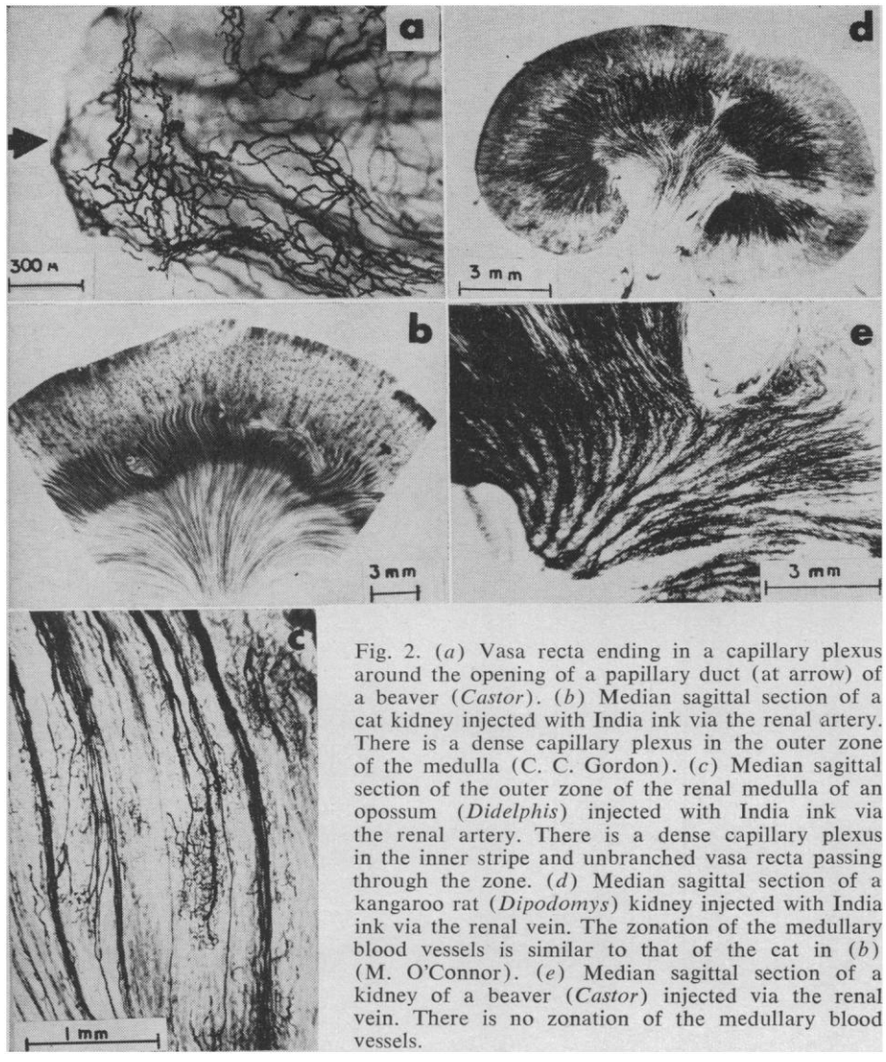

Fig. 2. (*a*) Vasa recta ending in a capillary plexus around the opening of a papillary duct (at arrow) of a beaver (*Castor*). (*b*) Median sagittal section of a cat kidney injected with India ink via the renal artery. There is a dense capillary plexus in the outer zone of the medulla (C. C. Gordon). (*c*) Median sagittal section of the outer zone of the renal medulla of an opossum (*Didelphis*) injected with India ink via the renal artery. There is a dense capillary plexus in the inner stripe and unbranched vasa recta passing through the zone. (*d*) Median sagittal section of a kangaroo rat (*Dipodomys*) kidney injected with India ink via the renal vein. The zonation of the medullary blood vessels is similar to that of the cat in (*b*) (M. O'Connor). (*e*) Median sagittal section of a kidney of a beaver (*Castor*) injected via the renal vein. There is no zonation of the medullary blood vessels.

medullary blood vessels to the individual nephrons.

Our observations indicate that the vasa recta arise in leashes from the efferent arterioles of the juxtamedullary glomeruli and descend, many as unbranched vessels, in parallel bundles to break up into capillary plexuses at different levels of the medulla. These plexuses drain via ascending, parallel vessels into branches of the arcuate and interlobular veins (Fig 1, *a* and *b*). We were unable to find any true loops (Fig. 2*a*), such as those figured in many physiology texts and reports, and described in detail by Trueta.

Moffat and Fourman show that, in the mammals they examined, the capillary plexuses that arise from the descending vasa recta differ in their arrangement so that there is a dense, peritubular capillary network in the inner stripe of the outer zone and a relatively sparse, spread-out plexus in the subcortical zone (outer stripe of the outer zone) and inner medullary zone. These authors suggest that such zonation occurs in all species, although they point out that in some of their animals the zones are more sharply demarcated than in others. In some of the species (kangaroo rat, gerbil, cat, and opossum) examined by us (Fig. 2, *b*, *c*, and *d*), there was a distinct zonation of the medullary blood vessels like that described by Moffat and Fourman. However, we were unable to observe zonation in the capillary plexus arrangement of the medulla of the pig or beaver (Figs. 1*b* and 2*e*). Previous studies have shown that the primitive rodent, *Aplodontia*, also lacks such zonation (*7*). In these animals the same type of plexus arises from each vas rectum whether in the inner, middle, or outer area of the medulla, and there are more branches and anastomoses of vasa recta than in the species showing zonation.

The zonation of medullary blood vessels in some animals is apparently correlated with the zonation that results from nephron architecture. In many mammals, the proximal tubules end at approximately the same level in the outer zone of the medulla to form the outer stripe (*8*). The thick segments of the ascending limbs of Henle's loops begin at about the same level somewhat lower to form the inner stripe and these two stripes form the outer zone. The inner zone, therefore, consists only of segments of Henle's loops and collecting ducts. The kidney of the opossum shows such zonation (*8*), and our dissections of the partially macerated specimens with blood vessels injected with India ink demonstrated a correlation between blood vessel and nephron zonation. The zone of dense capillaries occurs in the outer zone where the pars recta of the proximal tubule gives rise to the thin segment of Henle's loop (Fig. 1*a*). This part of the nephron and the distal portions of the nephron are surrounded by a dense, peritubular capillary plexus derived from short vasa recta. In the inner zone the vasa recta run as long unbranched vessels parallel to the thin segments of the loops and the collecting ducts. From Sperber's descriptions of the nephron architecture in the other species which show zonation of the medullary blood supply, it seems probable that correlations exist between nephron form and blood supply which are similar to those we observed in the opossum.

Aplodontia and the beaver show no zonation of nephron architecture because the transition from proximal tubule to loop of Henle occurs at different levels and the loop always occurs in the thick segment so that in these species there is no true inner zone of the medulla (*7, 8*). Apparently a similar arrangement occurs in the pig (*8*).

There appear to be functional differences between the kidneys of those mammals that show the zonation described above and those that do not. Thus in *Aplodontia* (*9*), beaver, and pig (*10*) a high protein diet does not enhance the renal concentrating ability, while in those mammals with zonation for which there are data [cat, rat, kangaroo rat (*10*), and rabbit (*9*)] such a diet increases the maximum urine concentration over that achieved on a low protein diet. It has been shown that urea accumulates in high concentration in the renal medulla of the dog and rat (*11*), and Berliner *et al.* (*12*) suggest that it is trapped there by the action of

the vasa recta countercurrent system. The vasa recta of *Aplodontia*, beaver, and pig may not be able to trap urea, or the difference in their form may produce differences in the way in which the nephron handles urea.

Schmidt-Nielsen and O'Dell (*13*) have shown that in the kidneys of sheep conserving urea, the urea concentration rises most dramatically in the inner stripe of the outer zone, and these authors state that this zone comprises a very important part of the concentrating mechanism. In the rat (*14*) the outer zone of the medulla, as well as the papilla, has a high NaCl concentration, and water is not reabsorbed although it is reabsorbed in the inner zone. It is, therefore, only in the inner zone of the medulla that the gradient of increasing osmolarity toward the papilla is seen. Histochemical studies of the rat renal medulla reveal that the concentration of plasma proteins in the plexuses of the inner stripe of the outer zone and of the apex of the papilla is higher than that of the vasa recta linking the two regions (*15*), and show that the vasa recta traversing the inner stripe of the outer zone are fully developed retia with two-directional flow (*16*).

The presence of a special capillary plexus around the segments of the nephron which comprise the outer zone supports the concept that it is very active physiologically in those species showing zonation. The lack of such zonation in species showing no enhancement of urine concentration after a high protein diet suggests that a segment of the nephron may function differently in these species as compared to those showing pronounced zonation.

It would, therefore, seem that there are species differences in the form of the vasa recta and associated vessels of the mammalian renal medulla. These differences may have as much significance in the urine concentrating process as do the anatomical differences in the nephron of different mammalian species.

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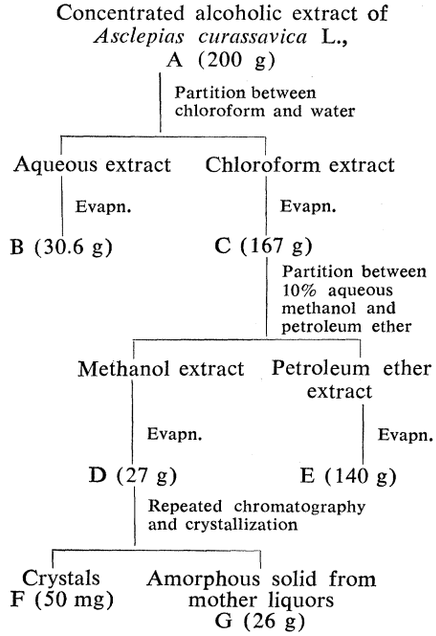
Calotropin, a Cytotoxic Principle Isolated from Asclepias curassavica L.

Abstract. *An alcoholic extract of* Asclepias curassavica *L., a plant widely used in folk medicine for treating cancer and warts, shows cytotoxic activity when tested in vitro against cells derived from human carcinoma of the nasopharynx. Systematic fractionation of the extract has led to isolation and characterization of calotropin as a cytotoxic principle. Calotropin is similar in structure to two cardiac glycosides recently shown to be responsible for the cytotoxicity of* Apocynum cannabinum *L.*

Asclepias curassavica L. (Asclepiadaceae), "cancerillo," and related species have been used for years to treat cancers, tumors, and warts in Costa Rica (*1*), Mexico (*2*), India (*3*), and elsewhere (*4*). During our search for tumor inhibitors from plant sources, alcoholic extracts of dried *A. curassavica* from Costa Rica (separated samples of roots, stems, and leaves) and from Mexico (a mixed sample of roots, stems, leaves, and flowers) (*5*) showed significant inhibitory activity when tested in vitro against cells derived from human carcinoma of the nasopharynx (*6, 7*). We report herein the fractionation of two active extracts and the isolation and characterization of a cytotoxic principle which is identified as calotropin.

Solvent partition of the alcoholic extract (*A* in Fig. 1) of the dried Mexican sample between water and chloroform resulted in a concentration of the activity in the chloroform phase (*C*). The dark gummy residue from the chloroform layer was de-fatted by partitioning between 10 percent aqueous methanol and petroleum ether (Skellysolve B), whereupon activity was concentrated in the aqueous methanol layer (*D*). Column chromatography of fraction *D* on silicic acid (using chloroform and chloroform-methanol as eluents) and analysis of the fractions by thin-layer chromatography and paper chromatography revealed at least seven components reactive to *m*-dinitrobenzene and sodium hydroxide (*8*). A compound of R_F 0.62 upon paper chromatography in the system chloroform-formamide was separated from the remainder of the material by further



Fraction	ED_{50} (*7*) (μg/ml)
A	1.3
B	9.8
C	1.9×10^{-1}
D	5.6×10^{-2}
E	5.1×10^{1}
F	3.7×10^{-3}
G	1.1

Fig. 1. Fractionation of a cytotoxic principle of *Asclepias curassavica* L., and cytotoxicity (*7*) of the fractions *A* through *G*.