JOURNAL OF MORPHOLOGY 224:57–63 (1995)

Structure of the Glomerular Capillaries of the Domestic Chicken and Desert Quail

GIOVANNI CASOTTI AND ELDON J. BRAUN
Department of Physiology, College of Medicine, University of Arizona, Tucson, Arizona 85724

ABSTRACT The glomerular capillary architecture of nephrons that include a loop of Henle (looped) and those that lack the loop (loopless) nephrons was examined qualitatively and quantitatively by electron microscopy in *Gallus gallus* and *Callipepla gambelii*. The glomerular capillaries of looped nephrons form a dichotomously branched network, while those of loopless nephrons are arranged loosely, and the majority are unbranched. There was no significant difference in the diameter of the glomerular capillaries between looped and loopless nephrons; however, in all cases the diameter of the afferent arteriole was significantly larger than that of the efferent arteriole. Based on size alone, the predicted blood flow rate in the efferent arteriole is 20% that of the afferent arteriole in *G. gallus* and 7% that of the afferent arteriole in *C. gambelii*. There was no significant difference in the volume density (Vv) of the glomerular capillaries between looped and loopless nephrons. However, the surface area density (Sv) of the glomerular capillaries in loopless nephrons of *C. gambelii* was significantly larger than for the looped nephrons, and for the loopless nephrons in *G. gallus*. This suggests that there may be a decrease in blood flow rate along the glomerular capillaries of the loopless nephrons in *C. gambelii*. Overall, the results indicate that the avian glomerular capillaries are less complex than those of mammals. Reasons may be that either avian blood is more viscous than that of mammals or that avian erythrocytes may be unable to fit physically through a tight intertwining network of capillaries due to the presence of a nucleus, which limits the tank-treading ability of avian erythrocytes.

The avian kidney contains two types of nephrons. These nephrons differ anatomically in that some nephrons contain a loop of Henle (looped) and others lack the loop (loopless) (Braun and Dantzler, '72). They also differ in having different-size renal corpuscles. Those of looped nephrons are more than twice the diameter of the loopless nephrons (Dantzler and Braun, '80). As the majority of the renal corpuscle consist of capillaries, this size variation may result in differences in blood flow and filtration between looped and loopless nephrons. Previous studies on the avian renal corpuscle have examined the simplicity of the capillary tufts qualitatively or the size of the pores of the capillary wall (i.e., glomerular filtration membrane) (Pak Poy and Robertson, '57; Siller, '71). To date, no studies have examined the structure of the glomerular capillaries quantitatively, or differences in glomerular structure between looped and loopless nephrons.

In birds, the complexity of the glomerular capillaries is closely related to the size of the renal corpuscle. For example, Dantzler and Braun ('80) found that the renal corpuscles of the peripherally located loopless nephrons have a single, unbranched capillary coiled around the periphery of the capsule. No other group of vertebrates shows such a simple capillary loop design (Dantzler, '85). In contrast, the renal corpuscles of the juxtamedullary looped nephrons possess an anastomotic network of capillaries, although not as complex as found in mammals (Braun, '82).

Previous studies on the Gambel's quail (*Callipepla gambelii*) and the European star-

Address reprint requests to Dr. Eldon J. Braun, Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724.

ling (*Sturnus vulgaris*) have shown that the single nephron glomerular filtration rate (SNGFR) is more than twice as high in the looped nephrons compared to loopless nephrons (Braun and Dantzler, '72; Braun, '78). However, as the glomerular capillaries of looped nephrons are more complex, they are also longer than those of the loopless nephrons. This additional length should reduce the rate of blood flow along these capillaries and hence the rate of filtration. As no quantitative data are available on the size and complexity of avian glomerular capillaries, this contradiction between SNGFR and glomerular capillary structure cannot be explained.

In mammals, the diameter of the glomerular capillaries is less than the diameter of the erythrocytes (Gaehtgens et al., '92). Hence, to travel along these capillaries, the erythrocytes undergo deformation by radial folding in a manner known as tank-treading (Gaehtgens et al., '81a). Larsson and Maunsbach ('80) and Tisher and Madsen ('86), studying rats, illustrated this phenomenon clearly. Avian erythrocytes are elongated compared to those in mammals and each contains a nucleus. Owing to the presence of a nucleus, avian erythrocytes have a limited tank-treading ability and hence cannot fit physically through the same-size capillaries as mammalian erythrocytes (Gaehtgens et al., '81a). In addition, previous studies have found that avian whole blood is more viscous than that of mammals (Usami et al., '70; Chien et al., '71). These properties may account for the simple structure of the glomerular capillaries found in birds.

The present study examined the structure of the glomerular capillaries in the domestic fowl *Gallus gallus* and the Gambel's quail *Callipepla gambelii,* both qualitatively and quantitatively. Our aims were to describe the complexity of the glomerular capillaries in both looped and loopless nephrons, and to quantify the size of the capillaries relative to the size of the erythrocytes. From these data we suggest why there is a difference in glomerular capillary complexity between birds and mammals.

MATERIALS AND METHODS

Glomerular casts

The architecture of the glomerular capillaries was examined in two specimens each of *Gallus gallus* and *Callipepla gambelii.* Birds were killed with an overdose intravenous injection of sodium pentobarbital. One specimen of each species was used to make a renal vascular cast. The dorsal aorta just cranial to the kidneys was cannulated and the kidneys were infused by light thumb pressure with phosphate buffer to clear the blood and then with methyl methacrylate monomer (Batson's No. 17). The resin was allowed to cure overnight, after which the kidneys were dissected from the synsacrum. The kidneys were placed in 40% potassium hydroxide for 72 hr to digest the tissue. The vascular casts were rinsed in water, air dried and groups of glomerular capillaries were dissected carefully from both the peripheral and juxtamedullary regions of the kidneys. They were placed onto scanning electron microscopy stubs, sputter-coated with gold, and viewed in a scanning electron microscope.

To identify possible differences in the sizes of the capillaries between adjacent glomeruli, groups of glomeruli were isolated in segments branching off the intralobular artery (Fig. 1). The diameters of the afferent and efferent arterioles and the glomerular capillaries were measured from both looped and loopless nephrons. In the case of the glomerular capillaries, several measurements were taken and a mean value obtained for each glomerulus.

Stereology

Birds were killed with an intravenous injection of sodium pentobarbital and the kidneys perfused with half-strength Karnovsky's fixative. The kidneys were dissected from the synsacrum and immersed in the perfusion fixative for 24 hr to ensure optimum fixation. Tissue blocks (1 mm^3) were sampled from both the peripheral and juxtamedullary regions of the kidneys.

Tissue blocks were sampled randomly (Østerby and Gundersen, '80), then processed routinely for transmission electron microscopy. Thick sections (500 nm) were cut and stained with toluidine blue. Approximately 50 renal corpuscles of looped nephrons and 50 renal corpuscles of loopless nephrons were photographed. A polygon was drawn around the periphery of the podocytes, which served as the glomerular reference space. The volume density (Vv) of the glomerular capillaries (excluding the glomerular filtration membrane) was measured from point counts and estimated as

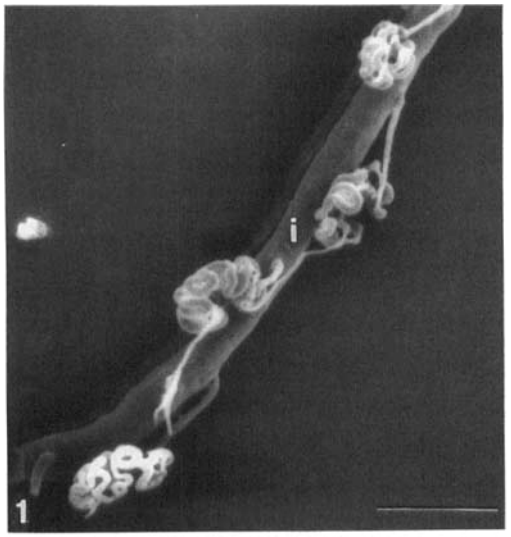
$$Vv(\text{capillary/glomerulus}) = \frac{\Sigma P(\text{capillaries})}{\Sigma P(\text{glomerulus})}\ mm^3/mm^3$$

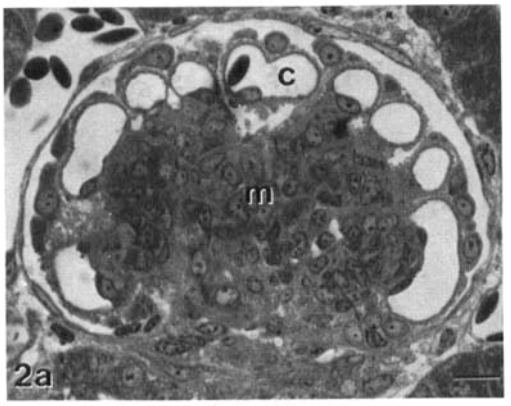
where ΣP(capillary) is the total number of points hitting the capillaries and ΣP(glomerulus) is the number of points hitting the reference space. The surface area density (Sv) of

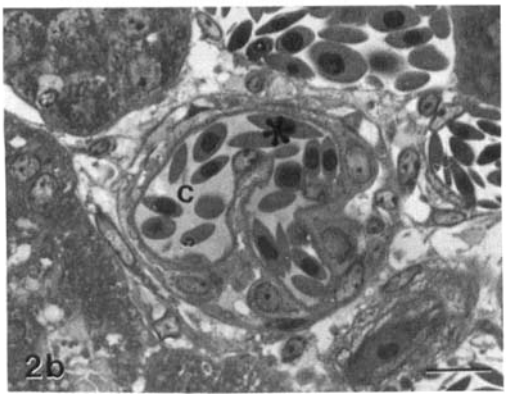
the capillaries was estimated as

$$Sv = \frac{2 \times \Sigma I(\text{capillary})}{k \times \Sigma P(\text{glomerulus})} \text{ mm}^2/\text{mm}^3$$

where ΣI(capillary) is the total number of intersections between the capillaries and the horizontal test lines (Fig. 2a), and k is the real distance in millimeters between two adjacent test points. Estimating both the volume and surface area density enabled us to determine whether the glomerular capillaries were short and wide or long and narrow. Differences in the sizes of the capillaries may affect the rate of blood flow along the glomerular capillaries.







Statistics

Volume and surface area density data were analysed, using a three-way nested analysis of variance (ANOVA) with a mixed-effects model in SAS (Searle, '87). Data on the internal diameters of the afferent arteriole, efferent arteriole and glomerular capillaries were analysed using a one-way ANOVA.

RESULTS

Glomerular capillary complexity

In both *Callipepla gambelii* and *Gallus gallus,* the glomerular capillaries of looped nephrons were more complex than those of loopless nephrons. In looped nephrons, the glomerular capillaries were arranged concentrically around the mesangium, whereas in loopless nephrons, the capillaries were folded upon themselves with only a small amount of mesangial tissue present (Fig. 2a,b). Overall, the glomerular capillary network was approximately twice the size in the looped nephrons (Fig. 3a,b). In looped nephrons, the glomerular capillaries consisted of a dichotomously branched network (Fig. 3a). By contrast, in the majority of loopless nephrons, the glomerular vasculature consisted of a single capillary that was arranged loosely (Fig. 3b). On rare occasions, the glomerular vasculature of the loopless nephrons consisted of two separate capillary loops (Fig. 3c). Overall, the structure of the avian glomerular capillaries is not as complex as in mammals (Fig. 4).

Glomerular capillary size

In both *Gallus gallus* and *Callipepla gambelii,* there was no significant difference in

Fig. 1. *Gallus gallus.* Scanning electron micrograph of a methyl methacrylate cast, showing glomeruli from loopless nephrons branching off the intralobular artery. i, intralobular artery. Scale bar = 100 μm.

Fig. 2. *Gallus gallus.* **a:** Transmission electron micrograph of a glomerulus from a looped nephron. **b:** Transmission electron micrograph of a glomerulus from a loopless nephron. Note the lack of a mesangium. c, capillaries; m, mesangium; * lack of deformability of red blood cell. Scale bar = 10 μm.

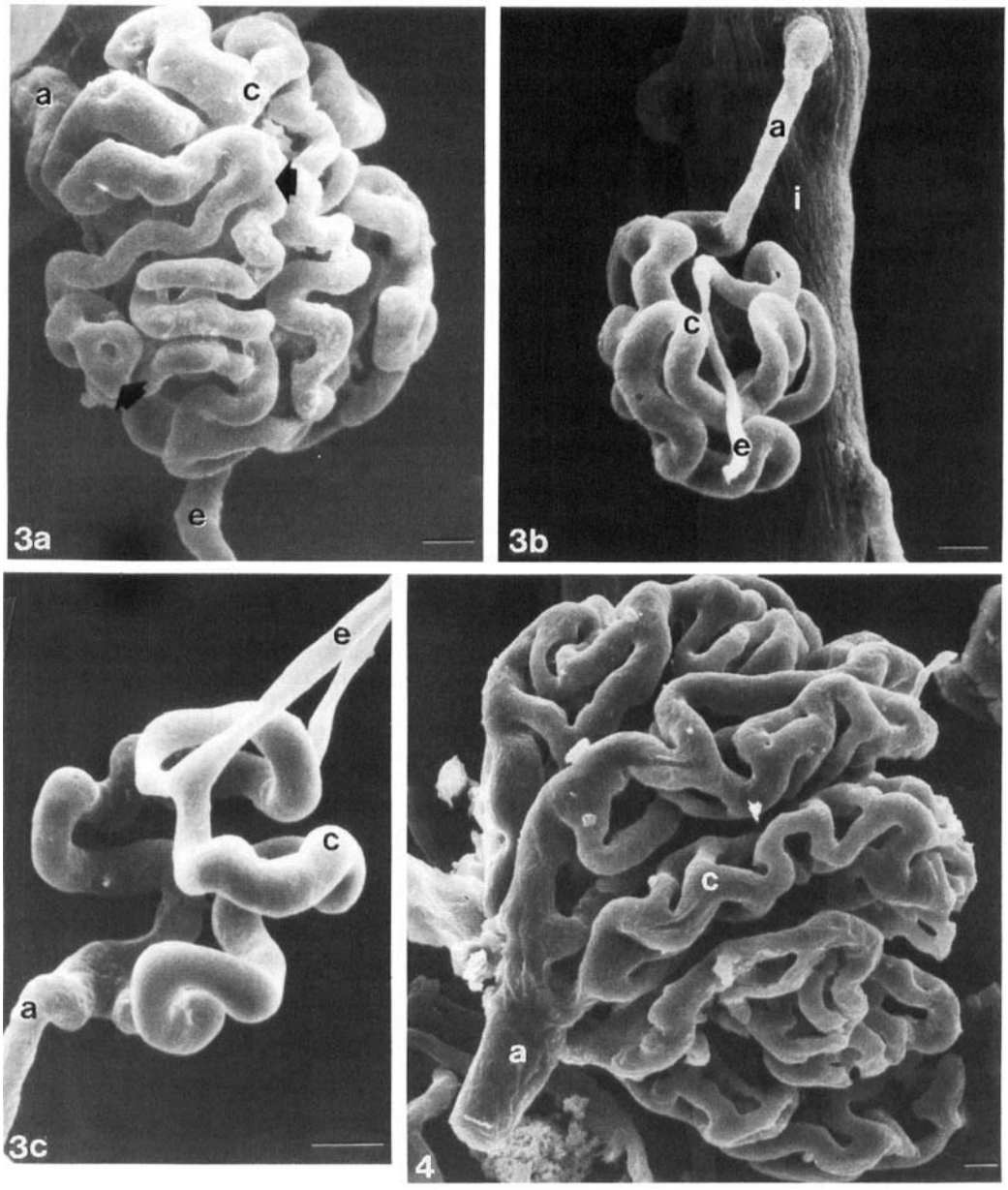


Fig. 3. *Gallus gallus.* Scanning electron micrographs of glomeruli from (**a**) looped, and (**b,c**) loopless nephrons. Note the difference in size as well as the complexity of the dichotomous branching in the looped nephrons. Arrows indicate dichotomous branching. a, afferent arteriole; c, capillaries; e, efferent arteriole; i, intralobular artery. Scale bars = 10 μm.

Fig. 4. *Gallus gallus.* Scanning electron micrograph of a methyl methacrylate cast of a rat glomerulus. a, afferent arteriole; c, capillaries. Scale bar = 10 μm.

the internal diameter of either the afferent or efferent arterioles between looped and loopless nephrons (Table 1). However, in all cases, the afferent arteriole had a significantly larger ($P < 0.05$) diameter than that of the efferent arteriole. In *C. gambelii,* the internal diameter of the glomerular capillaries was significantly larger ($P < 0.01$) in the looped nephrons (Table 1). In all cases, the internal diameter of the glomerular capillaries was

TABLE 1. Internal diameter (mean ±S.D.) of the glomerular capillaries from both looped and loopless nephrons in the avian kidney. Sample sizes are shown in parentheses

Species	Afferent arteriole[1]		Glomerular capillaries[2]		Efferent arteriole[1,2]	
	Looped	Loopless	Looped	Loopless	Looped	Loopless
G. gallus	6.2 ± 2.0 (35)	6.1 ±1.7 (62)	6.4 ± 1.4 (86)	6.6 ± 1.4 (92)	4.0 ± 1.4 (34)	3.9 ± 1.2 (39)
C. gambelii	4.8 ± 1.0 (66)	4.7 ± 1.3 (101)	5.0 ± 0.9[c] (89)	4.6 ± 1.0[c] (140)	2.4 ± 0.8 (54)	2.4 ± 0.7 (78)

[1] Significant difference ($P < 0.05$) between afferent and efferent arterioles.
[2] Significant difference ($P < 0.05$) between glomerular capillaries and efferent arterioles.
[3] Significant difference ($P < 0.01$) in the diameter of the glomerular capillaries between looped and loopless nephrons.

significantly larger ($P < 0.05$) than the efferent arteriole (Table 1).

For both *Gallus gallus* and *Callipepla gambelii*, there was no significant difference in the volume density of the glomerular capillaries between looped and loopless nephrons (Table 2). In *C. gambelii*, the glomerular capillaries of the loopless nephrons had a significantly higher ($P < 0.001$) surface density than that of the looped nephrons (Table 2). Among loopless nephrons, those of *C. gambelii* also had a significantly higher ($P < 0.001$) surface density than that in *G. gallus*.

DISCUSSION

This is the first detailed quantitative study of avian glomerular capillary architecture. In the looped nephrons of *Gallus gallus* and of *Callipepla gambelii*, the glomerular capillary network was larger and more complex than that of the loopless nephrons. The larger renal corpuscle of looped nephrons may on first glance suggest delivery of a greater amount of blood to the juxtamedullary area of the kidney. However, in the case of *G. gallus*, based on the glomerular volume there is no difference in either the volume or surface area density of capillaries between looped and loopless nephrons. That is, the increase in size of the capillaries and the size of the glomerulus are proportional. There is also no difference in the diameters of the glomerular capillaries, meaning that when compared to volume the lengths of the glomerular capillaries between looped and loopless nephrons are the same. The volume and length of capillaries are directly proportional only when there is no significant difference in tubular diameter. It should be noted that sodium pentobarbital was administered to kill the birds. Previous studies have shown that sodium pentobarbital depresses vascular smooth muscle (Rall, '93); hence, this may lead to vasodilation. However, in our study all birds were treated in the same manner and comparisons between glomerular types and species should remain unaffected.

TABLE 2. Volume (Vv) and surface area density (Sv) (mean ±S.D.) of the glomerular capillaries from looped and loopless nephrons in the avian kidney. Sample size are shown in parentheses

Species	Vv (mm^3/mm^3)		Sv (mm^2/mm^3)	
	Looped	Loopless	Looped	Loopless
G. gallus	0.3 ± 0.1 (57)	0.3 ±0.1 (49)	16.4 ± 5.5 (57)	16.4 ± 6.7[2] (48)
C. gambelii	0.3 ± 0.1 (54)	0.3 ± 0.1 (53)	17.8 ± 5.5[1] (54)	25.1 ± 7.3[1,2] (52)

[1] Significant difference ($P < 0.001$) between nephron types in *C. gambelii*.
[2] Significant difference ($P < 0.001$) in Sv between *C. gambelii* and *G. gallus*.

In *Callipepla gambelii*, the glomerular capillaries of the loopless nephrons had a significantly higher surface density (but not volume density) than that of the looped nephrons. This suggests that the glomerular capillaries of the loopless nephrons are narrower and longer than those of the looped nephrons. According to Poiseuille's law governing the flow of fluids through cylindrical tubes, a narrower and longer vessel should lead to a reduction in blood flow rate (Berne and Levy, '93). A slower blood flow rate in the glomerular capillaries of the loopless nephrons might be reflected in a lower SNGFR in these nephrons, as has previously been demonstrated in *C. gambelii* (Braun and Dantzler, '72). Furthermore it has been suggested that in some species SNGFR is flow rate dependent (Brenner et al., '72).

The glomerular capillaries of looped nephrons form a dichotomously branched network, while those of loopless nephrons are arranged loosely and have no dichotomous branches. Differences in the complexity of the glomerular capillaries may be related to filtration as capillary complexity increases the surface area available for ultrafiltration. This correlates with measurements of SNG-FR's

which are approximately twice as high in the looped nephrons (Braun and Dantzler, '72). Branching may result in an overall increase in the length of the glomerular capillaries, which according to Poiseuille's law would lead to a reduction in flow rate along the capillaries in the looped nephrons—a factor that would not enhance SNGFR (Berne and Levy, '93). However the reduction in flow rate along the glomerular capillaries may be overcome by smooth muscle contraction of the afferent arteriole. It has been demonstrated that renal blood flow and GFR are autoregulated in the domestic fowl (Vena et al., '90). In *Gallus gallus,* as the internal diameter of the glomerular capillaries was not significantly different between looped and loopless nephrons, the suggested reduction in blood flow in the looped nephrons could be significant if it were not for autoregulation. For *Callipepla gambelii,* the larger internal diameter of the glomerular capillaries of looped nephrons could lead to an increase in the rate of blood flow, offsetting any decrease in flow rate caused by the longer capillaries. However, no data are available in the literature on either blood pressure or rate of blood flow through the avian glomerulus. As the glomeruli are located below the surface of the kidney, obtaining blood flow measurements from birds is difficult.

In both *Gallus gallus* and *Callipepla gambelii,* the significant reduction in diameter of the efferent arteriole compared to the afferent arteriole may be related to the amount of plasma filtered. According to the Poiseuille's law describing the flow of fluids, based on the change in arteriole diameter from the glomerular capillaries to the efferent arteriole alone, and keeping all other factors constant, the predicted flow rate along the efferent arteriole is 20% that of the afferent arteriole in *G. gallus* and 7% that of the afferent arteriole in *C. gambelii.* However only about 20% of the blood volume entering the glomerular capillaries is filtered (Roberts et al., '93). The difference in arteriole diameter may be necessary to keep the pressure high to facilitate filtration.

As a result of the branched network of glomerular capillaries within the avian looped nephrons, erythrocytes would need to turn at angles of 90 degrees in some cases. Given that avian erythrocytes have at best a limited tank-treading ability (Gaehtgens et al., '81a), a highly branched glomerular capillary structure would make it difficult for blood to flow smoothly along the capillaries. Erythrocyte deformability is essential to enable blood cells to enter capillaries with a diameter of 8 μm or smaller (Secomb, '92). The glomerular capillaries in this study for both *Gallus gallus* and *Callipepla gambelii* had an average diameter of approximately 6.5 μm. Therefore, the simple structure would seem to be essential to enable the avian erythrocytes to pass along the glomerular capillaries.

The birds examined in this study did not have as complex a glomerular capillary network as found previously in mammals (Anderson and Anderson, '76; Tisher and Madsen, '86). Gaehtgens et al. ('81b), comparing the rheology of nucleated and non-nucleated erythrocytes, suggested that given the rheological properties of the different erythrocytes in birds and mammals one might expect the morphological design of the capillary network to differ. The results of the present study agree with this hypothesis.

The simple glomerular capillary structure in birds may also be related to the viscosity of the blood suspension. Because of the presence of the nucleated cells, avian whole blood may be up to twice as viscous as that of humans (Gaehtgens et al., '81b). This may cause a significantly larger hydrodynamic disturbance in blood flow. If the architecture of the glomerular capillaries were as complex in birds as that in mammals, according to the difference in viscosity, the rate of blood flow in the glomeruli of birds would be 50% that of mammals. Hence the simpler capillary network in birds may be necessary to maintain adequate blood flow along the glomerular capillaries. Tank-treading in mammals has little effect on the viscosity of blood flow, therefore mammals can accommodate a more complex glomerular capillary network (Secomb, '92).

Overall, this study found that the glomeruli of avian looped nephrons have a more branched capillary structure than those of loopless nephrons; however this branching is not as complex as occurs in mammals. This difference may be related to the rheological properties of avian erythrocytes. There are differences in the surface area density of glomerular capillaries between looped and loopless nephrons and these are consistent with measurements of SNGFR. Future studies are needed to examine both the blood pressure and blood flow rate along the glomerular capillaries because morphometric measurements alone cannot explain the differences in

SNGFR between glomeruli of looped and loopless nephrons.

ACKNOWLEDGMENTS

We thank Dr Denise Roe for doing the statistical analyses and Myra Pacelli for proofreading the manuscript. This research was supported by NSF IBN-9220241 and NIH 5R01DK16294-20.

LITERATURE CITED

Anderson, B.G., and W.D. Anderson (1976) Renal vasculature of the trout demonstrated by scanning electron microscopy, compared with canine glomerular vessels. Am. J. Anat. *145:*443–457.

Berne, R.M., and M.N. Levy (1993) Hemodynamics. In Physiology. Missouri: Mosby–Year Book, pp. 438–452.

Braun, E.J. (1978) Renal response of the starling (*Sturnus vulgaris*) to an intravenous salt load. Am. J. Physiol. *234:*F270–F278.

Braun, E.J. (1982) Glomerular filtration in birds—its control. Fed. Proc. *41:*2377–2381.

Braun, E.J., and W.H. Dantzler (1972) Function of mammalian-type and reptilian-type nephrons in kidneys of desert quail. Am. J. Physiol. *222:*617–629.

Brenner, B.M., J.L. Troy, T.M. Daugharty, W.M. Deen, and C.R. Robertson (1972) Dynamics of glomerular ultrafiltration in the rat. II. Plasma-flow dependence of GFR. Am. J. Physiol. *223:*1184–1190.

Chien, S., S. Usami, R.J. Dellenback, and C.A. Bryant (1971) Comparative hemorheology-hematological implications of species differences in blood viscosity. BRHLA *8:*35–37.

Dantzler, W.H. (1985) Comparative aspects of renal function. In D.W. Seldin and G. Giebisch (eds): The Kidney: Physiology and Pathophysiology. New York: Raven Press, pp. 333–364.

Dantzler, W.H., and E.J. Braun (1980) Comparative nephron function in reptiles, birds and mammals. Am. J. Physiol. *239:*R197–R213.

Gaehtgens, P., F. Schmidt, and G. Will (1981a) Comparative rheology of nucleated and non-nucleated red blood cells. I. Microrheology of avian erythrocytes during capillary flow. Pflugers Arch. *390:*278–282.

Gaehtgens, P., G. Will, and F. Schmidt (1981b) Comparative rheology of nucleated and non-nucleated red blood cells. II. Rheological properties of avian red cell suspensions in narrow capillaries. Pflugers Arch. *390:*283–287.

Larsson, L., and A.B. Maunsbach (1980) The ultrastructural development of the glomerular filtration membrane in the rat kidney: a morphological study. J. Ultrastruct. Res. *72:*392–406.

Østerby, R., and H.J.G. Gundersen (1980) Fast accumulation of basement membrane material and the rate of morphological changes in acute experimental diabetic glomerular hypertrophy. Diabetes *18:*493–500.

Pak Poy, R.K.F., and J.S. Robertson (1957) Electron microscopy of the avian renal glomerulus. J. Biophys. Biochem. Cytol. *3:*183–192.

Rall, T.W. (1993) Hypnotics and sedatives; ethanol. In A.G. Gilman, T.W. Rall, A.S. Nies, and P. Taylor (eds): The Pharmacological Basis of Therapeutics. New York: McGraw-Hill, pp. 345–382.

Roberts, J.R., B.C. Ford, and R.F. Wideman (1993) Blood flow to the avian kidney: Use of transit-time ultrasonic flow probes. *1270:*A220.

Secomb, T.W. (1992) Red blood cell mechanics and capillary blood rheology. Cell Biophys. *18:*231–250.

Searle, S.R. (1987) Linear Models for Unbalanced Data. New York: John Wiley & Sons, pp. 504–506.

Siller, W.G. (1971) Structure of the kidney. In D.J. Bell and B.M. Freeman (eds): Physiology and Biochemistry of the Domestic Fowl. London: Academic Press, pp. 197–231.

Tisher, C.C., and K.M. Madsen (1986) Anatomy of the kidney. In B.M. Brenner and F.C. Rector (eds): The Kidney. Philadelphia: W.B. Saunders, pp. 3–60.

Usami, S., V. Magazinovic, S. Chien, and M.I. Gregersen (1970) Viscosity of turkey blood: Rheology of nucleated erythrocytes. Microvasc. Res. *2:*489–499.

Vena, V.E., T.F. Lac, and R.F. Wideman (1990) Dietary sodium, glomerular filtration rate autoregulation, and glomerular size distribution profiles in domestic fowl (*Gallus gallus*). J. Comp. Physiol. *B160:*7–16.