ELECTRON MICROSCOPY OF THE AVIAN RENAL GLOMERULUS

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PLATES 36 TO 41

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The renal glomerulus has recently been extensively investigated with the electron microscope, and there is now fairly general agreement on the basic features of normal glomerular structure. Work has, however, as yet been almost confined to mammals. We considered that similar studies on other vertebrates would be useful, and the present paper reports work on the avian renal glomerulus. In this Class the metanephric glomerulus is small, with fewer and much simpler capillaries than in the mammalian glomerulus; and it has in its centre a large cellular mass not present in the mammalian glomerulus. Study of this mass of cells might be expected to throw some light on the relation of glomerular capillaries to the interstitial tissue of the glomerulus.

Materials and Methods

Young chickens (5 days to 3 months) were anaesthetised with intravenous nembutal supplemented by inhaled ether. The capsule was gently peeled off the exposed kidney, and the cortex bathed for 20 minutes in 1 per cent osmium tetroxide buffered by isotonic sodium phosphate (15) at pH 7.4. The cortex was then removed, sliced into 1 mm. cubes in the above fixative, and left for 2 hours at 4°C. Some animals were killed, the kidney rapidly removed, sliced in a drop of fixative, and thereafter fixed for 4 hours at 4°C. Fixation in these kidneys was not as good in those fixed by the previous in vivo method.

After fixation the tissues were washed in distilled water and dehydrated by successive transfer through 20, 30, 50, 70, 90 and 100 per cent alcohol, remaining 10 minutes in each solution. They were then placed for 10 minutes in equal parts of butyl methacrylate and absolute alcohol, then 10 minutes in pure methacrylate, and embedded in 20 per cent methyl methacrylate in butyl methacrylate. Polymerisation was carried out either in an oven at 47°C for 24 to 48 hours, or by ultraviolet irradiation for 4 to 8 hours at room temperature. Sections, cut with glass knives in a thermal-expanding microtome, were mounted on parlodian-covered grids and examined at 60 kv. in a Philips EM 100 electron microscope.

Thicker sections (0.5 μ) were mounted on glass slides, dipped in toluene to remove the methacrylate, stained by Jones's (16) silver method, and viewed by phase contrast. This procedure clearly delineated the basement membrane of the capillary loops. It was found that the thickness of the section was critical for optimum results. A similar technique using Wilder's (36) silver stain has been briefly mentioned by Mueller et al. (22).

RESULTS

Central Cell Mass.—At the hilum and expanding into the centre of the glomerulus there is a conspicuous mass of cells which we shall hereafter term
the "central cell mass" (Fig. 1). Marshall and Smith (19) thought this mass was not connective tissue but was a syncytium, and that the kidney of the bird represented a degenerative step in glomerular phylogeny. Smith (34) later elaborated this point and regarded the avian glomerulus as tending to evolutionary disappearance. He stressed the reduction in complexity of the capillary system and its partial replacement by indifferent connective tissue.

The cells of the central cell mass are comparable in size to endothelial cells, and have large nuclei with double nuclear membranes and one or two nucleoli. The cytoplasm is relatively scanty and forms projections which interdigitate with those of other cells to form a most complex lattice, especially in the region just beneath the capillary lumen (Figs. 3 and 7). Mitochondria are present but are not numerous. Cytoplasmic granules tend to form clumps and occasionally the elongated form of endoplasmic reticulum, with granules attached as described by Palade (24) may be seen. These cells are in direct contact with the adventitial cells of the glomerular arteriole, and show little morphological difference from them.

The intercellular spaces of the central cell mass contain material which is less dense than the cellular cytoplasm, but parts of it are denser and sometimes form a definite layer which may be double (one component to each cell surface) but more often is amorphous and sponge-like. This denser layer is continuous with the denser central layer of the basement membrane of the glomerular capillary loops. The intercellular material shows a variable affinity for silver impregnation (see Fig. 12). Collagen fibres have not been identified in it.

The central cell mass is by no means uniformly globular. Its surface shows projections, each made up of many cells (Fig. 1). While some of these projections form a support of capillaries, others project into Bowman's space between capillary loops. Here the surface of the mass exposed to Bowman's space is covered by the major component of the basement membrane of the glomerular capillary loop, for the membrane divides as it reaches the surface of the mass (Figs. 2 and 7). Pedicels of the epithelial cells rest on this component of the basement membrane as it lies on the surface of the mass; however in places epithelial cells sit flat on this surface.

The main morphological distinction between cells which are obviously endothelial cells of glomerular capillaries and contiguous cells which are equally obviously cells of the central cell mass is the less dense and more vacuolated cytoplasm of the endothelial cells. The endothelial nuclei are usually but not invariably located against the supporting cells of the central mass. The central mass is separated from direct contact with blood by an obvious endothelial cell or by the attenuated endothelial cytoplasm (Figs. 2 and 7).

Glomerular Loop.—The glomeruli are much smaller than in mammals, and the loops in an individual glomerulus are fewer, simpler, less tortuous, and are spread over the surface of the central cell mass. Between the loops there are
projections of the central mass. The endothelial nucleus is large and usually distinctly lobular (Fig. 7). Sometimes one nucleolus can be seen. Characteristically the peripheral cytoplasm away from the nucleus spreads as a thin attenuated sheet over the inner surface of the basement membrane. The endothelial sheet is of variable thinness (approximately 200 to 2,000 A) and scattered small ridges project into the capillary lumen. Dense cytoplasmic granules and occasionally endoplasmic reticulum are present in the thinner portions, while larger cell organelles (mitochondria) are usually found in the thicker portions near the nucleus. In places the endothelial cell membranes bounding the attenuating cytoplasmic sheet come together to form what appears in the photographs as a single line some 200 to 400 A in length (Fig. 6). In other places even this is missing, leaving a complete space or “pore” 300 to 800 A in width (Fig. 4). Similar “defects” in the endothelial lining have been reported by Hall in rats and humans (10), by Rhodin (30) (animal not stated), by Pease (26, 27) in rats, by Yamada (37, 38) in mice, by Mueller et al. (22) in dogs and humans. Rinehart and Farquhar (32) in rats regarded the endothelium as vesicular, and later Rinehart (33) in rats considered that the endothelium was given a pseudoporous appearance by vesicles. Dalton (6) in the mouse had earlier regarded the pores as artefacts. Whether or not they are to be regarded as artefacts (and careful study of thin sections shows that at least some of them are directly in the line of, and presumably due to small defects in the knife edge), pores are not numerous in the endothelium of the glomerular capillaries of the bird.

Vacuoles are more numerous in the main mass of endothelial cytoplasm than in other cells (Figs. 5 and 8). They are seen even though the tissue in close proximity shows good fixation. They may spread into the adjacent attenuated endothelial sheet. Some of them are probably sections cutting through the cytoplasmic ridges above the level of the endothelial sheet. The main body of endothelial cytoplasm contains mitochondria, small dense granules, and endoplasmic reticulum in elongated form with granules attached. The cytoplasm is less dense (in the sense of being less opaque to the electron beam) than that of the cells of the central cell mass. This phenomenon is not due to varying section thickness, but is consistently seen. The line of separation between the endothelial cell and the underlying central mass cell is frequently blurred owing to tangential sectioning, but normal sections through this region show that the endothelial cell membrane is clearly separated from that of the central mass cell by the intercellular material previously described.

Basement Membrane of the Glomerular Loop.—Most light microscopists have described this as a single refractile membrane, but Jones (17) regarded it as double, with an inner component derived from the capillary while the outer component was Bowman’s capsular membrane reflected over the glomerular surface. Churg and Grishman (5) using phase contrast methods also described two basement membranes, one endothelial and the other epithelial. Among
electron microscopic studies Oberling et al. (23), Rinehart et al. (31), Rinehart and Farquhar (32), Rinehart (33), Hall et al. (12), Hall (10, 11, 14), and Policard et al. (28) considered this membrane to be single in rats. Hall and Roth (13) found it to be single in rabbits, Reid (29) in mice, and Hall (10) in humans. More recently better techniques have shown it to comprise three layers in rats (27) and in mice (37). Rhodin (30) described these three layers but did not state the animal.

In the bird the membrane also has three layers as in mammals. (Fig. 9). There is a central, very finely granular, dense layer of material which divides at the surface of the central cell mass, the major component continuing over the surface of the mass to the next capillary (Fig. 12), while the minor component becomes continuous with the dense material in the intercellular spaces of the central cell mass. On the side nearest the lumen of the glomerular capillary there is a less dense layer, corresponding to the lamina rara interna of Yamada (37) and to the inner cement layer of Pease (27), which in some places may be obliterated by the widening of the dense middle layer which then comes into contact with the uniformly thin endothelial cell membrane (Fig. 6). There is a similar outer less dense layer, corresponding to the lamina rara externa of Yamada and to the outer cement layer of Pease, against which the pedicels of the epithelial cells are applied. At the line of contact of the pedicel there is a marked increase in density, which Rhodin (30) has shown to result from the very close apposition of two membranes. In the bird both the inner and outer less dense layers of the capillary basement membrane are continuous respectively with the two layers of less dense material on either side of the dense layer in the intercellular spaces of the central cell mass. Thus all three layers of the basement membrane fuse with the intercellular zone of the central mass, the dense layer becoming, through its minor component, continuous with the dense layer of the intercellular material.

Yamada (37) described delicate filaments extending from the cell membrane of the pedicel across the lamina rara externa to the dense central layer of the basement membrane, and Rinehart et al. (31) described epithelial cytoplasmic mucoid secretion as actually penetrating the basement membrane. These appearances were not noted in the avian glomerulus.

**Epithelial Cells.**—These are separate large cells, with large ovoid regular nuclei, forming an incomplete covering to the glomerulus as in mammals (Fig. 1). The cell bodies vary in shape, and send out large projections from which the smaller pedicels arise. Pedicels may also spring from the main cell body and may sometimes be very long and filamentous. They interdigitate with one another to form a complex maze and terminate either on the outer surface of the basement membrane of the capillary loops or on the extension of the membrane covering the surface of the central cell mass. Between adjacent pedicels as they rest on the outer surface of the capillary basement membrane a
TEXT-FIG. 1. A semischematic diagram taken from a slightly macerated specimen in which the intercellular substance is swollen. Fig. 11 represents part of this area. The continuity of the intercellular material with the capillary basement membrane and Bowman’s capsular membrane is shown.

TEXT-FIG. 2. An endothelial stalk of three endothelial cells (E) separated by intercellular material (I) which is continuous with the capillary basement membrane (B). The capillary lumina (C) of three capillary loops are shown. The potential intercapillary space in which mesangial cells would necessarily lie is at I. The thin lines indicate the cell membranes.

TEXT-FIG. 3. This diagram shows the separation of the three endothelial cells (E) by the cells of the central cell mass (S) in the avian glomerulus. The major component of the capillary basement membrane covers the surface of the mass and N, the minor component separating endothelial cell from the mass, and I, the intercellular material. This diagram shows the direct continuity of B, M, N, and I. Cell membranes are indicated by thin lines.
short thin membrane can occasionally be seen lying slightly further out in Bowman's space than the line of the pedicel basement membrane junctions (Fig. 10). Where it is not visible the basement membrane is apparently exposed to Bowman's space. This membrane has been called the filtration slit membrane by Yamada (37) in the mouse.

The epithelial cell nuclei contain one or two nucleoli and have double nuclear membranes with cytoplasmic granules attached to the outer component. Dense granules, endoplasmic reticulum, and mitochondria are present in the cytoplasm. Mitochondria can occasionally be seen in pedicels.

**Bowman's Capsule.**—Bowman (4) described the capsular basement membrane in 1842 as "a simple homogeneous and perfectly transparent membrane in which no structure could be discovered." McGregor (20) considered it to have two layers, an inner homogeneous layer continuous with the basement membrane of the glomerulus and an outer composed of argyrophilic fibres which mingled with those of the tubular stroma. Gersh and Catchpole (8) regarded the reticular fibres as embedded in the homogeneous component. Mueller et al. (22), using an electron microscope, described the capsular basement membrane as 3,500 to 5,000 Å in thickness, fairly dense and having a laminated structure.

As seen in Fig. 11, in the bird the parietal capsular membrane is composed of three layers, resembling therefore the basement membrane of the glomerular capillaries, though all three layers are somewhat less wide. All three are continuous with the intercellular substance between the adventitial cells of the arterioles, which in turn is continuous with the intercellular material of the central cell mass. In the hilar region the capsular basement membrane becomes continuous with the basement membrane of the glomerular loop. This is also well shown by the thin section-silver impregnation-phase contrast method.

The parietal epithelial cells which line the capsular space are flattened cells closely applied to the basement membrane. Their nuclei are also flattened and possess the double nuclear membrane. Endoplasmic reticulum, cytoplasmic granules, and mitochondria are relatively scarce.

**Hilum.**—In this region Bowman's capsular membrane joins the intercellular material between the adventitial cells of the arterioles, and then passes into the more intricate arrangement of the intercellular substance of the central cell mass. The basement membrane of the glomerular loop can also be traced into this maze (see Text-fig. 1). The cells of the central cell mass are in contact with the adventitial cells of the afferent and efferent arterioles.

**DISCUSSION**

There is still some debate on whether mammalian glomeruli possess an inter-capillary space and whether this space (if it exists) may contain non-endothelial connective tissue cells. Even the electron microscope has not yet finally resolved this question to the complete satisfaction of all workers on the subject. Among
workers who affirm the presence in mammals of an intercapillary tissue are
Zimmerman (39), Kimmelstiel and Wilson (18), Bensley and Bensley (3),
Goormaghtigh (9), McManus (21), Ehrich (7), Yamada (38), and Policard et al.
(28), while Allen (1), Bell (2), Hall (10), Pease and Baker (25), Rinehart et al.
(31), and Mueller et al. (22) are in disagreement. Vimtrup (35) regarded only the
larger glomerular branches near the hilum as being surrounded by connective
tissue. It should not be forgotten that there may be species differences even in
mammals which further work will bring to light. However in the bird a definite
and large mass of cells which are obviously not endothelial cells occupies the
central part of the glomerulus, the cells being contiguous to the adventitial cells
of the arterioles. The central cell mass may thus be regarded, in an important
sense, as a large intercapillary space filled with connective tissue cells, and the
study of the relations of this mass of cells to the glomerular capillary endothe-
ilium and to the capillary basement membrane may be expected to throw some
light on the "mesangial" question, for the highly complex arrangement of the
mammalian glomerular capillaries makes it very difficult to unravel their fine
structural relationships.

It is indeed particularly instructive to compare the avian glomerulus with the
mammalian. Recent work in mammals (Mueller et al. (22)) suggests that the
glomerular basement membrane invests the glomerulus as a whole, and that
groups of glomerular capillaries lie closely apposed to each other within the
investment; and further that endothelial cells of adjacent capillaries are often
grouped together to form an endothelial "stalk" (see Text-fig. 2). The inter-
capillary space is thus a potential space. Mesangial cells as reported by Yamada
(37) in mice would necessarily lie in this space.

The appearance in the bird is consistent with this interpretation, for a mass
of indifferent cells has been "inserted" into the centre of the endothelial stalk,
and separates the capillaries from each other, but still within the basement
membrane investing the whole glomerulus (see Text-fig. 3). The endothelial
nuclei also usually lie towards the centre of the glomerulus and thus against
the central mass.

McManus (21) considered that his third type of cell, (the axial cell) may have
knobs which project into the capillary lumen. Policard et al. (28) described in
rats projections of mesangial cells into the lumen of the capillary. These pro-
jections, which pushed the endothelial lining of the capillary before them, were
composed of a clear zone proximal to the endothelium and a dense zone near
the mesangium. Yamada (37) found in the mouse that the mesangial cells may
project into the endothelial cells, and may even penetrate them, thus being
exposed to blood. Zimmerman (39) also observed these projections and he
called them "Intrakapillarhöckerchen." In the avian glomerulus the central
cell mass may project into the lumen of the capillary but it always carries a
lining of endothelium before it and is never exposed directly to blood. This
arrangement of intracapillary as well as extracapillary projections of the central
cell mass makes the interpretation of electron micrographs of sections through these regions most difficult; it is often particularly difficult to distinguish between a cross-section of the terminal portion of a projection and endothelium. This is shown in Fig. 5 which we have interpreted as an intracapillary projection.

Gersh and Catchpole (8), discussing ground substance and basement membrane in general, considered basement membrane to be a concentrated form of the ground substance of intercapillary space, separating connective tissue from ectoderm, entoderm, and mesoderm. Morphologically the basement membrane of the glomerular loop and Bowman's capsule in the bird have the same appearance as the intercellular substance of the central cell mass and of the adventitia of the arterioles and they can be traced into direct continuity with them.

SUMMARY

Electron microscopy of sections of chicken glomeruli shows them to possess a large central cell mass, occupying the hilum and the centre of the glomerulus, and continuous with the adventitia of the afferent and efferent arterioles. The glomerular capillaries form a much simpler system than in mammals and are spread over the surface of the central cell mass. Between the capillaries the mass is limited externally by the major component of the glomerular capillary basement membrane, which continues over the surface of the mass from one capillary to the next. Projections of the central cell mass characteristically form the support for glomerular capillaries, and smaller knobs of the central mass may project actually into the lumen of the capillaries, but always carry a layer of endothelial cytoplasm before them. They are never in direct contact with blood. The basement membrane of the glomerular capillary loop has a central dense layer and two lateral less dense layers as in mammals. The central dense layer is continuous with similar appearing dense material in the intercellular spaces of the adventitia of the arterioles, and also with that of the central cell mass. The two less dense layers can also be traced into direct continuity with the less dense regions of this intercellular substance. The endothelial cytoplasm is spread as a thin sheet over the inner surface of the capillary basement membrane, and shows scattered "pores" resembling those described in mammals. Epithelial cells with interlacing pedicels are at least as prominent as those in mammals. Bowman's capsular membrane also possesses three layers similar to but less wide than those of the capillary basement membrane, and all three layers can be traced into continuity with the dark and light regions of the intercellular material of the adventitial cells of the arterioles, and beyond them with that of the central cell mass. At the hilum Bowman's capsular membrane also fuses with the capillary basement membrane.

We are grateful to Dr. S. G. Tomlin for access to the electron microscope and to Mr. J Orsula for assistance in its operation.
REFERENCES

34. Smith, H. W., Lectures on the Kidney, University of Kansas, Lawrence, Kansas, 1943.
EXPLANATION OF PLATES

PLATE 36

Fig. 1. Low power view of avian renal glomerulus showing the hilum on the left hand side and the prominent cell mass in the centre with projections to the top, bottom, and right. Epithelial cells with pedicels are prominent in the upper half and they can be seen resting on the central cell mass between capillary loops in the lower half. An endothelial cell applied to the central cell mass is indicated by the arrow in the lower right hand corner. Red blood cells are present in the capillary lumen.
FIG. 2. The micrograph shows the central cell mass occupying the left two-thirds and its relationship with the capillary endothelium. The cells with large nuclei (k) in the lower half are central mass cells, and interdigitating processes (i) form a complex lattice in the upper half. The intercellular space is represented at (s), endoplasmic reticulum with granules at (r), mitochondria in the processes at (m). The glomerular capillary lumen (c) is separated from the central mass by the endothelial cell (e), containing a tip of the nucleus (n), mitochondria, and endoplasmic reticulum. The thin attenuated endothelial cytoplasm lining the lumen shows at es. Pedicels (p) are present in Bowman’s space. The major component (mc) of the capillary basement membrane covers the surface of the central cell mass. × 24,000.
(Pak Poy and Robertson: Avian renal glomerulus)
PLATE 38

Fig. 3. The micrograph shows an intercapillary projection (w) of the central cell mass with a central mass cell nucleus (k) and interdigitating processes (i). A red blood cell is shown (rbc) lying in the capillary lumen (c) above an endothelial cell (e) and an epithelial cell (h). Pedicels (p) in Bowman’s space attach themselves to the surface of the central cell mass. X 13,000.

Fig. 4. The micrograph shows “pores” indicated by arrows in the capillary endothelial lining (es). Pedicels are indicated by p. X 40,000.
(Pak Poy and Robertson: Avian renal glomerulus)
Fig. 5. The micrograph shows a transverse section through the tip of a projection of the central cell mass. It is uncertain whether the mass of cytoplasm (w) is endothelial or a portion of the central cell mass. We have interpreted it as a projection of the central cell mass with capillary basement membrane (b) on two sides and endothelial cytoplasm (e) on the other two sides. Whether this is an inter- or intracapillary projection is another difficulty. We consider it to be an intracapillary projection. Vacuolation of the endothelial cytoplasm is shown at v. The capillary lumen is at (c). X 30,000.

Fig. 6. The attenuated endothelial sheet membranes joining together to form a single line (arrows). Pedicels (p) are clearly shown. At (l) the inner less dense layer of the basement membrane has disappeared and the central dense layer touches the endothelial lining (es). X 43,000.
PLATE 39
VOL. 3

(Pak Poy and Robertson: Avian renal glomerulus)
Fig. 7. An endothelial cell (e) rests on a projection (w) of the central cell mass. The lobulated nucleus is shown at n and mitochondria (m) and endoplasmic reticulum (r) are present in the cytoplasm. The capillary basement membrane divides at x into the major component (mc), which covers the surface of the projection and the minor component (y). Continuity of the capillary basement membrane (b) with the intercellular material (s) can be seen on the right side. The projection is composed of interdigitating processes (i) of central mass cells. An epithelial cell (h) rests on the surface of the projection. Epithelial pedicels are at p. × 30,000.

Fig. 8. The micrograph shows the vacuolation (v) of the endothelial cell (e) spreading into the attenuated endothelial lining, while the pedicels (p) and the epithelial cell nucleus (z) show relatively good fixation. The double nuclear membrane is shown around the nucleus (z) and the endothelial nucleus lies at n. × 33,000.

Fig. 9. The outer less dense, inner less dense, and central dense layer of the capillary basement membrane are indicated by arrows. The endothelial lining (es) shows variable thickness and microprojections and a mitochondrion (m) in a thickened portion. A red blood cell lies at rbc and epithelial pedicel's at p. × 35,000.
PLATE 41

Fig. 10. The arrows show thin membranes stretching across between adjacent pedicels (p). Notice that they are situated slightly away from the line of the pedicel-basement membrane junction. Endothelial cytoplasm lies at o. × 30,000.

Fig. 11. The micrograph shows the junction of Bowman’s capsular membrane (d), which has three layers—a thin dense layer, a narrow inner less dense, and relatively wide outer less dense layer—and the wall of an arteriole occupying the upper left hand corner. A leucocyte (q) and an endothelial cell (a) are present in the lumen of the arteriole, and (g) represents interdigitating processes of adventitial cell with their intercellular spaces (o). The continuity of intercellular material with capsular basement membrane can be seen. A flattened cell on Bowman’s capsular membrane is shown at f and epithelial cells at h. × 25,000.

Fig. 12. The micrograph demonstrates the capillary basement membrane by the thin section–silver impregnation–phase contrast technique. The continuity of the basement membrane from loop to loop around the central cell mass is clearly shown. The variable affinity for silver of the intercellular substance of the central cell mass is also shown.
(Pak Poy and Robertson: Avian renal glomerulus)