

**Don't know your amyloid from your epithelium?**

**You need our.....**

## **Basic Renal Electron Microscopy Training Day**

**Presented by Bart Wagner  
Chief Biomedical Scientist  
at Sheffield Northern General Hospital**

**Biomedical Imaging Unit**

<http://www.som.soton.ac.uk/research/sites/biu>

**Where: Southampton General Hospital, UK**

**When: Friday 30th September 2011**

**How much: £40.00 (£30.00 IBMS)**

**Contact: [a.page@soton.ac.uk](mailto:a.page@soton.ac.uk)**

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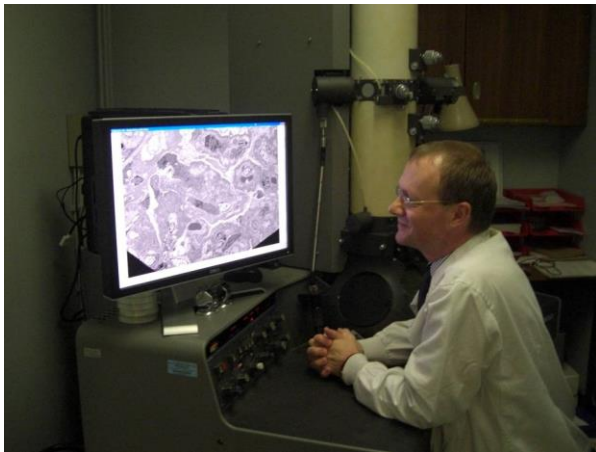


Basic Renal EM workshop

Southampton

September 30<sup>th</sup> 2011

# Renal Ultrastructure Normal



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# Sheffield

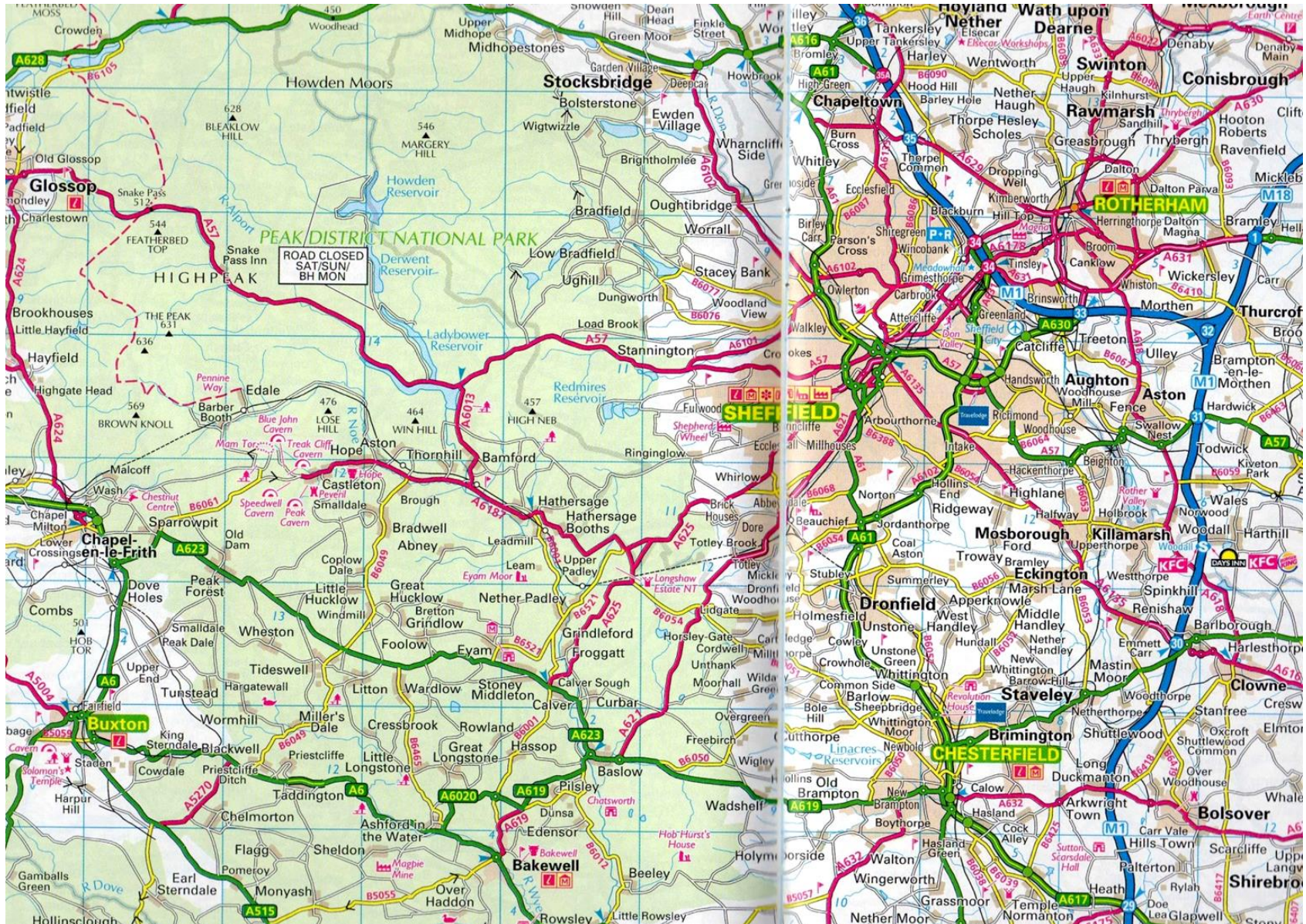


- In the centre of England, in South Yorkshire.
- A post-industrial city.
- Population of half a million.
- Two large universities & hospitals.
- Built on 7 hills.
- Manchester, Leeds, Nottingham - all nearby.
- But immediately adjacent to the Peak District





# Sheffield is very close to the Peak District National Park





## Near where I live... The Peak District National Park





If you are visiting Sheffield



Chatsworth House. Near Sheffield - built circa 1560





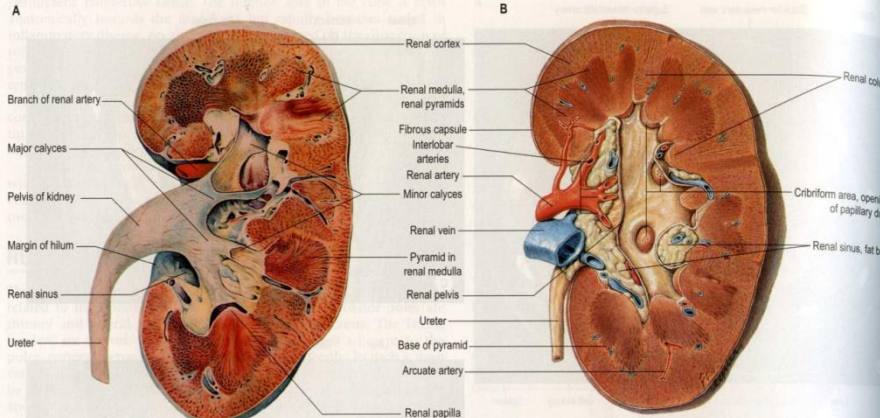
## CHAPTER 74

salaries excrete the end products of metabolism and excess water. Minerals are essential for the control of concentrations of various ions in the body, maintaining electrolyte and water balance. Minerals are constantly in the tissue fluids. The kidneys also have endocrine functions: they secrete renin, which has a profound effect on blood cell formation; renin, which influences blood pressure; and 1,25-dihydroxycholecalciferol (the metabolically active form of vitamin D<sub>3</sub>) which is involved in the control of calcium absorption and mineral balance; and various other soluble factors with metabolic actions. In the foal state, the kidneys are reddish-brown. They are situated on either side of the vertebral column, between the 12th and 13th thoracic vertebrae. Superiorly they are level with the 12th thoracic vertebra. Inferiorly with the 13th thoracic vertebra. The right is usually slightly inferior to the left, reflecting the position of the liver.

ing its relationship to the liver. The left is a little longer and narrower than the right and lies nearer the median plane (Fig. 74.1). The long axis of each kidney is directed inferolaterally and the transverse axis posteromedially, which means that the anterior and posterior aspects are not in the same plane as in fact anterolateral and posteromedial. An appreciation of this orientation is important in percutaneous and endoscopic renal surgery.

Each kidney is typically 11 cm in length, 6 cm in breadth and 3 cm anteroposterior dimension. The left kidney may be 1.5 cm longer than the right but the right kidney is by some 1 cm longer than the left. The average weight is 150 g in men and 135 g in women. In thin individuals with a lax abdominal wall the lower pole of the lower right kidney may just be felt in full inspiration by bimanual palpation; this is unusual.

In the fetus and newborn the kidney normally has 12 lobules. These are fused in adults to present a smooth surface although traces of lobulation may remain.



**Fig. 74.8** Left kidney, oblique vertical hemisection: normal macroscopic appearance of the renal cortex and renal medulla and the major structures at the hilum of the kidney. In **A**, the fat body of the renal sinus and most of the major vessels at the hilum have been removed, and the renal pelvis has not been opened. In **B**, the renal pelvis has been opened to reveal the interlobar arteries. (**B** from Sobotta 2006.)

FORTIETH EDITION

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Ultrastructure  
is an extension  
of histology  
and anatomy

cytogen, through which filtrate must pass to enter the urinary space. The differentiation of the adult podocyte phenotype is associated with the presence of several specific proteins, including nephrin, podocin, podocalyxin, and glomerular endothelial glycocalyxin (GEEG). Mutations in these proteins can cause congenital proteinuria and proteinuria in acquired forms of glomerular disease. Nephrotic syndrome is caused by a mutation of NPHS2 coding for nephrin. The luminal membrane and the slit diaphragm are covered by a dense surface coat rich in sialoglycoproteins, which gives this surface a negatively charged character. This is the key to the barrier of the perm-selectivity barrier. Differentiated podocytes cannot migrate.

The glomerular endothelium is finely fenestrated. The principal route for the passage of fluid from capillary lumen to urinary space is the fenestral basal lamina, the fused endothelial and podocyte basal lamina. This is usually 0.33  $\mu\text{m}$  thick in man, and acts as a selective barrier allowing the passage from blood to urinary space of small solutes, water and electrolytes and the circulation of *Haemoglobin* made in the liver, but larger molecules and those of similar size with a negative charge, are largely retained. Most protein that does enter the urine is actively reabsorbed and degraded by cells of the proximal tubule.

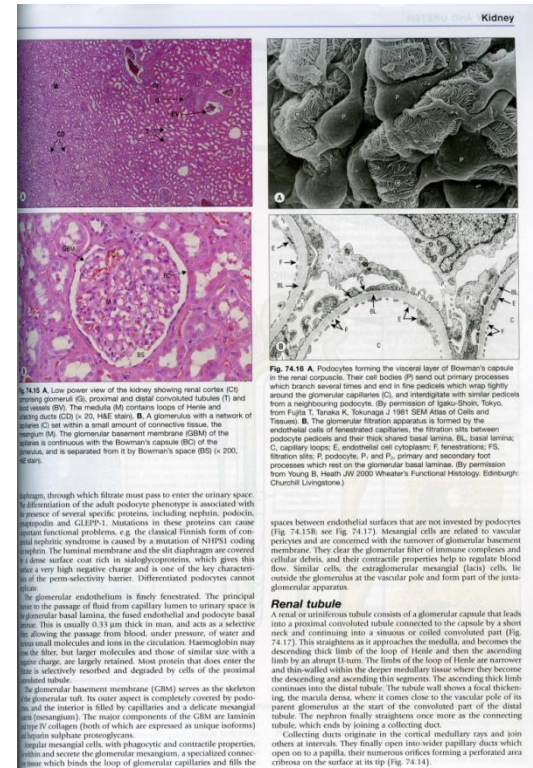
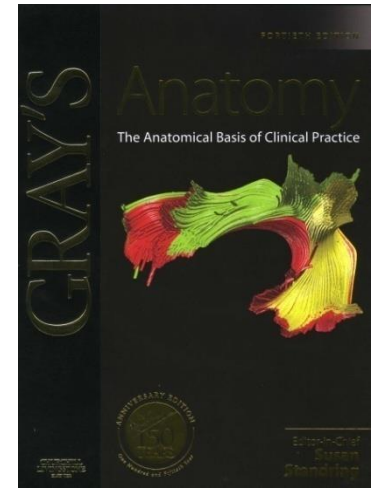
The glomerular basement membrane (GBM) serves as the skeleton for the glomerular tuft. Its outer aspect is completely covered by podocytes, and the interior is filled by capillaries and a delicate mesangial matrix (mesangium). The major components of the GBM are laminar type IV collagen (both of which are expressed as unique isoforms of heparin sulphate proteoglycans).

Regular mesangial cells, with phagocytic and contractile properties, surround and secrete the glomerular mesangium, a specialized connective tissue which binds the loop of glomerular capillaries and fills the

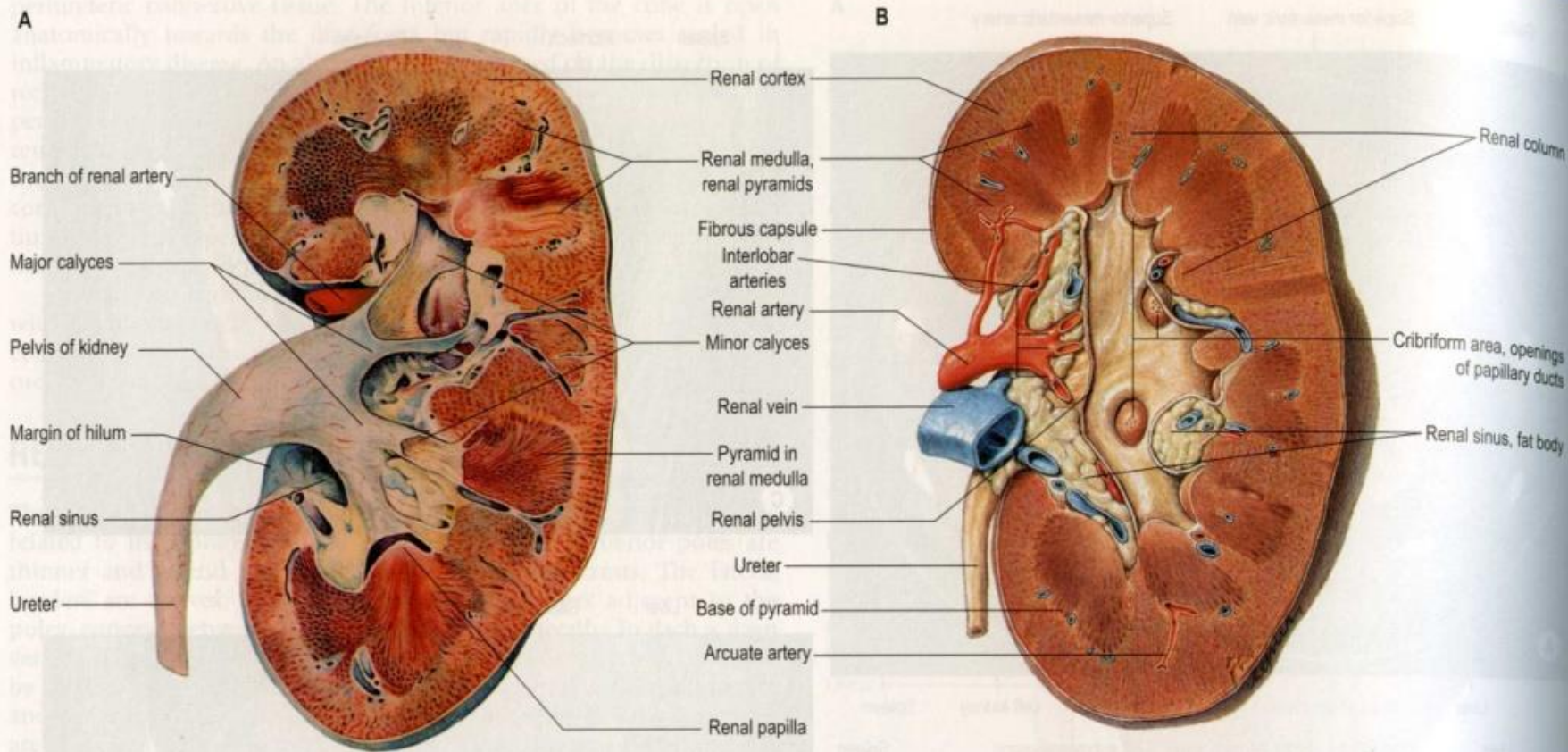
spaces between endothelial surfaces that are not invested by podocytes (Fig. 74.15B; see Fig. 74.17). Mesangial cells are related to vascular pericytes and are concerned with the turnover of glomerular basement membrane. They clear the glomerular filter of immune complexes and cellular debris, and their contractile properties help to regulate blood flow. Similar cells, the extraglomerular mesangial (laci) cells, lie outside the glomerulus at the vascular pole and form part of the juxtaglomerular apparatus.

**Renal tubule.** A renal or uriniferous tubule consists of a glomerular capsule that leads into a proximal convoluted tubule connected to the capsule by a short neck and continuing into a sinusoid or coiled convoluted tubule (Fig. 74.17). The descending limb of the tubule becomes the descending thick limb of the loop of Henle and then the ascending limb by an abrupt U-turn. The limbs of the loop of Henle are narrower and thin-walled within the deeper medullary tissue where they become the descending and ascending thin limbs. The ascending thick limb continues into the distal tubule. The tubule wall shows a focal thickening, the macula densa, where it comes close to the vascular pole of the parent glomerulus at the start of the ascending limb. The distal tubule descends straighter, one more as the connecting tubule, which ends by joining a collecting duct.

Collecting ducts originate in the cortical medullary rays and join others at intervals. They finally open into the papillary ducts that open on the papilla. Later in life, the papillary ducts form a perforated area, the cribrosa on the surface at its tip (Fig. 74.14).

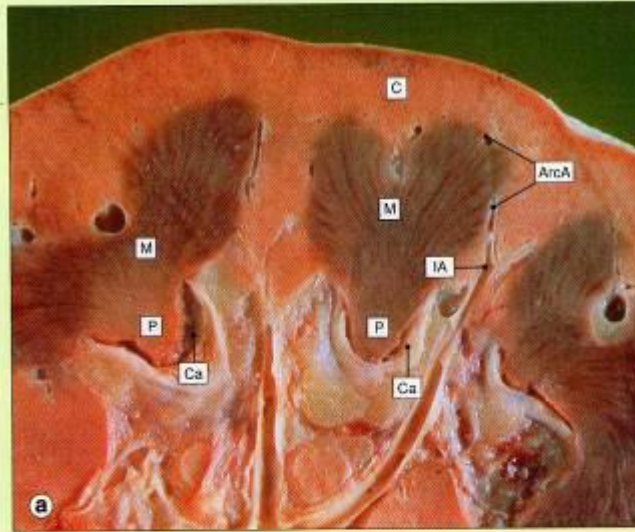
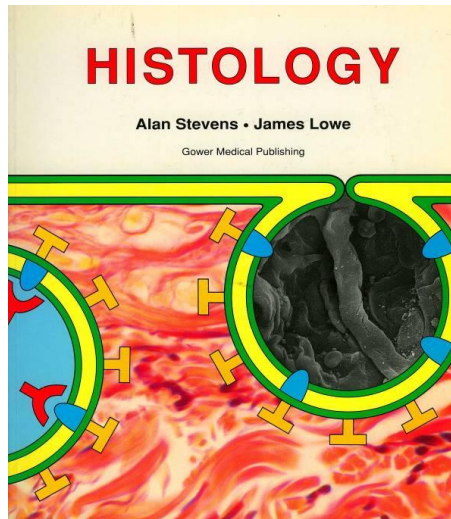






**Fig. 74.8** Left kidney, oblique vertical hemisection: normal macroscopic appearance of the renal cortex and renal medulla and the major structures at the hilum of the kidney. In **A**, the fat body of the renal sinus and most of the major vessels at the hilum have been removed, and the renal pelvis has not been opened. In **B**, the renal pelvis has been opened to reveal the interlobar arteries. (**B** from Sobotta 2006.)





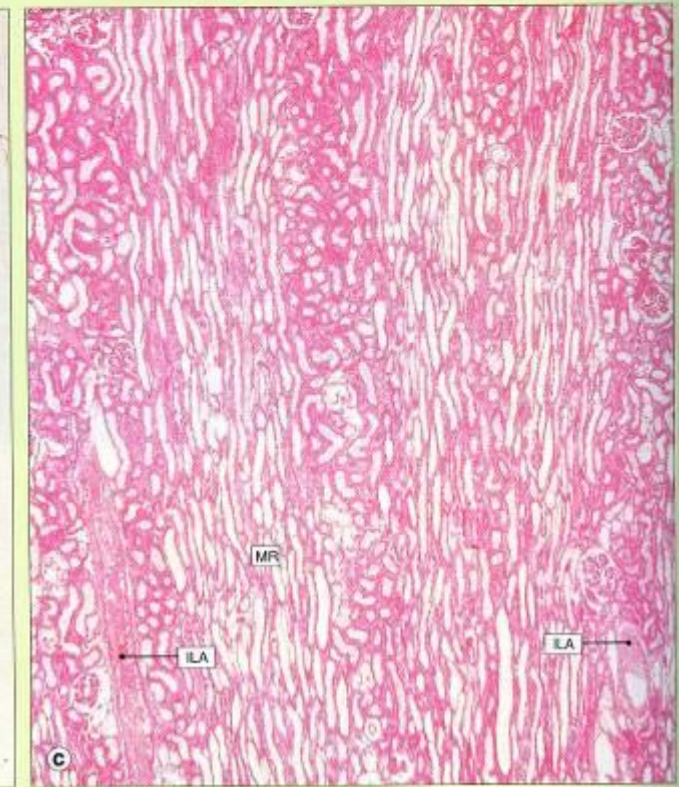
**Fig. 16.30 Anatomy of adult kidney.**

**a** Photograph of sectioned adult kidney, which has been fixed in formalin and the near natural colour restored in alcohol. Note the cortex (C), the medullary pyramid (M) culminating in the papillary tip (P), which protrudes into the lumen of a calyx (Ca). Interlobular arteries (IA) and arcuate arteries (ArcA) can also be seen. Little detail of cortical structure is visible with the naked eye, but the vertical linearity of the components of the medulla is highlighted by clusters of prominent blood vessels (vasa recta).

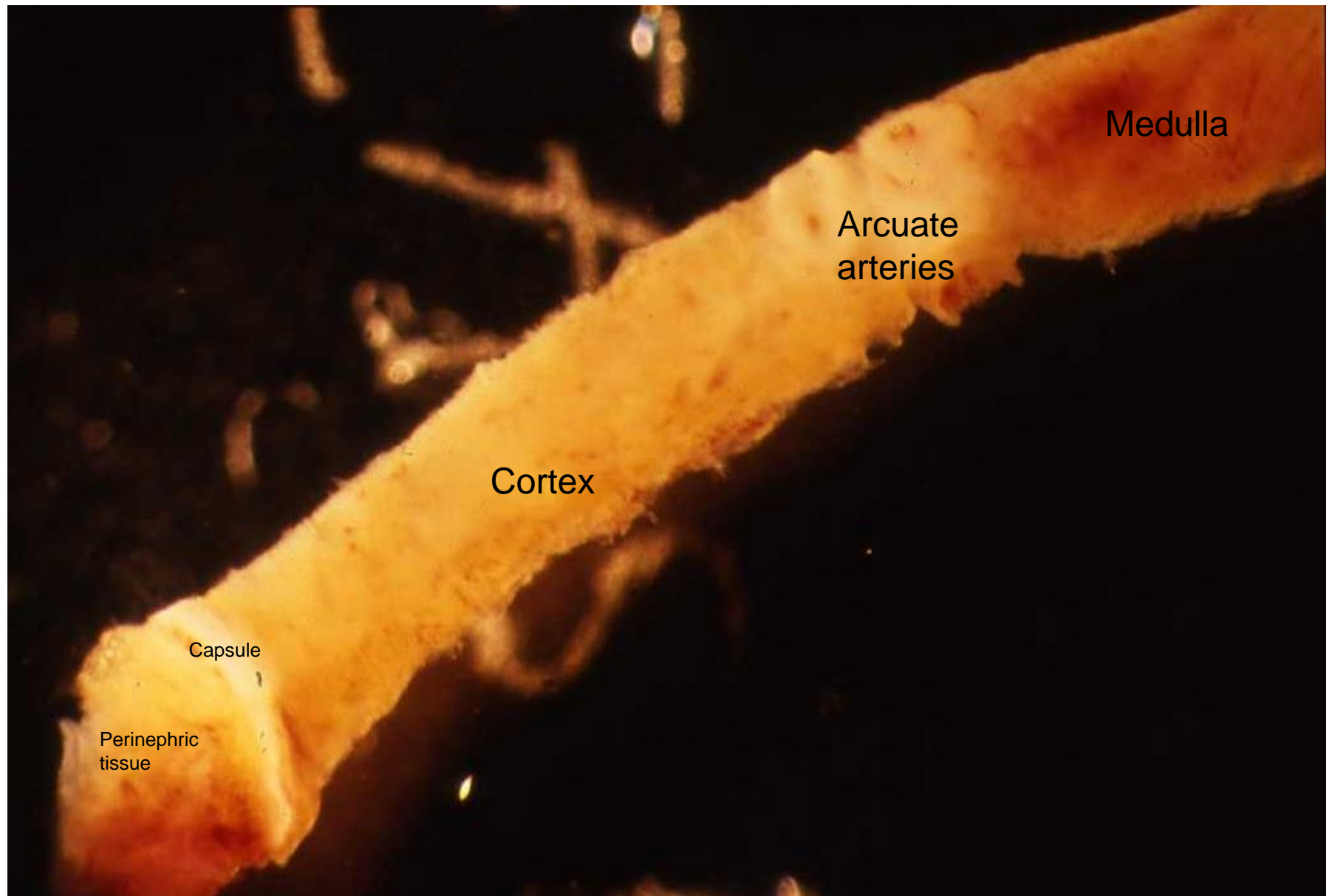
**b** In this H&E stained paraffin section prepared from the tissue block shown in **a**; the distinction between cortex (C) and medulla (M) can be easily seen. This section also shows the vertical linearity of the components of the medulla, both tubules and vessels.

At this low magnification, glomeruli can be seen as small dots in the cortex. Note that some areas of the cortex are free of glomeruli, but contain vertically running duct systems; these areas are known as medullary rays and represent the sites where cortical tubules drain into the collecting ducts.

**c** In this micrograph of cortex at a higher magnification than in **b** it can be seen that the medullary ray (MR) area is devoid of glomeruli and that the interlobular arteries (ILA) run in the glomeruli-rich area.





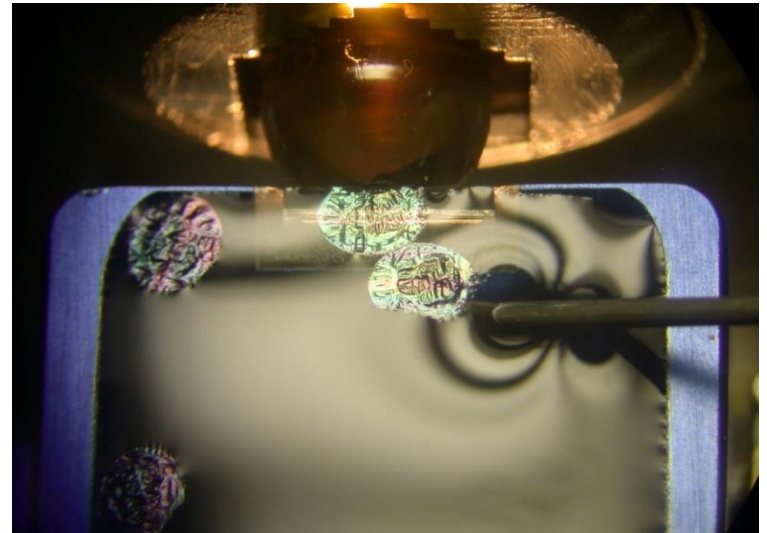


View of renal biopsy core as seen using stereo microscope

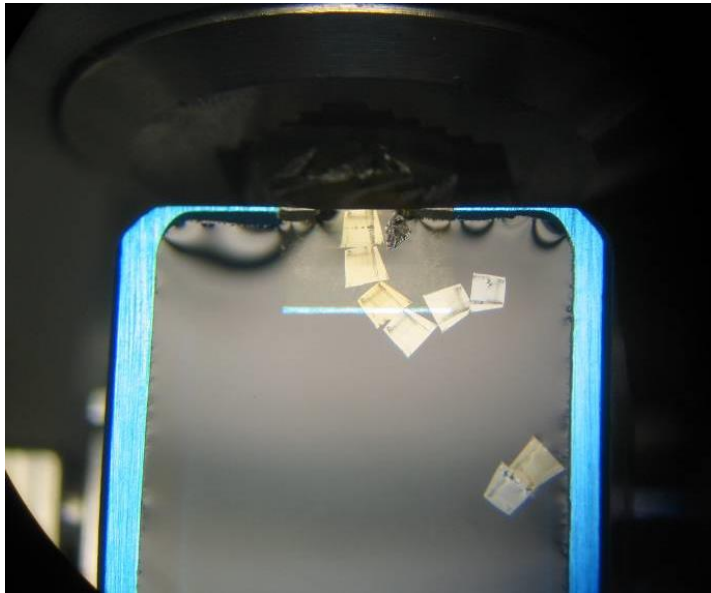




Resin blocks and their moulds



Picking up semi-thin sections



Cutting thin sections

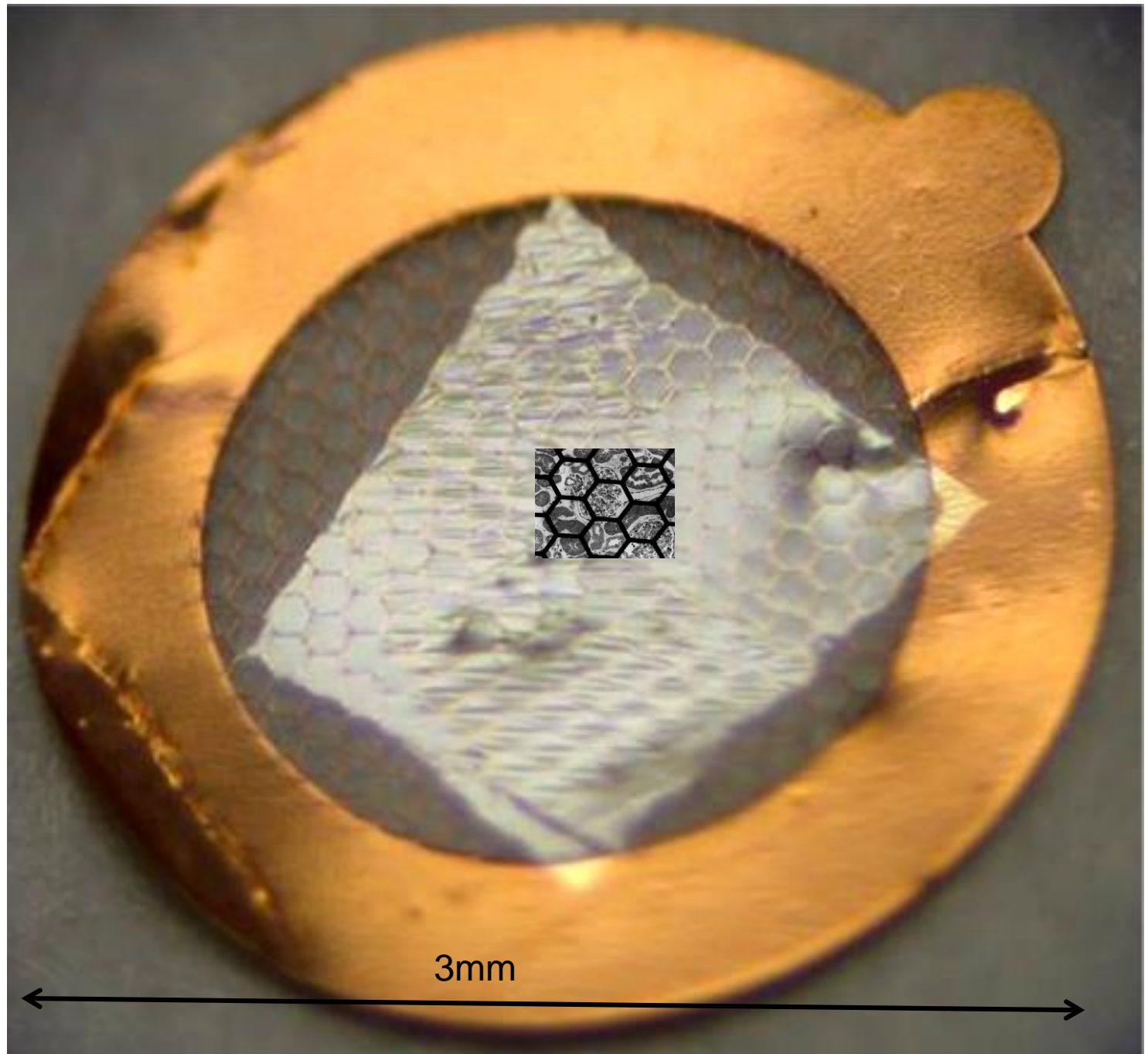


Picking up thin sections onto copper grids



Thin section  
on 200 mesh  
hexagonal  
high  
transmission  
copper grid

Section  
should  
have as  
many  
glomeruli  
as possible

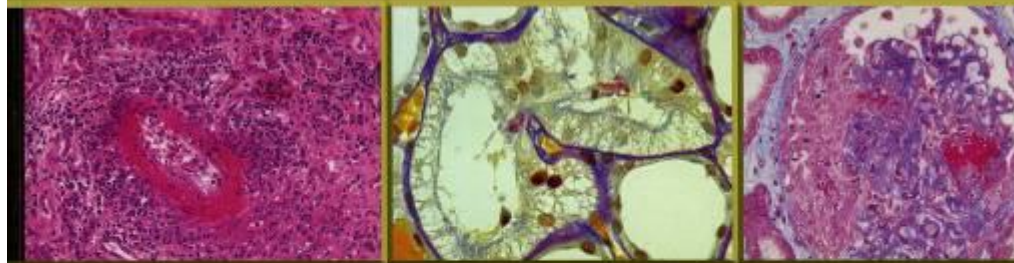




HEPTINSTALL'S

# PATHOLOGY OF THE KIDNEY

SIXTH EDITION



VOLUME I

J. Charles Jennette  
Jean L. Olson  
Melvin M. Schwartz  
Fred G. Silva

 Wolters Kluwer | Lippincott  
Health Williams & Wilkins

First chapter  
on renal  
anatomy and  
histology

2007



## Normal glomerulus – H&E

Parietal  
epithelial cell/  
cell lining  
Bowman's  
capsule

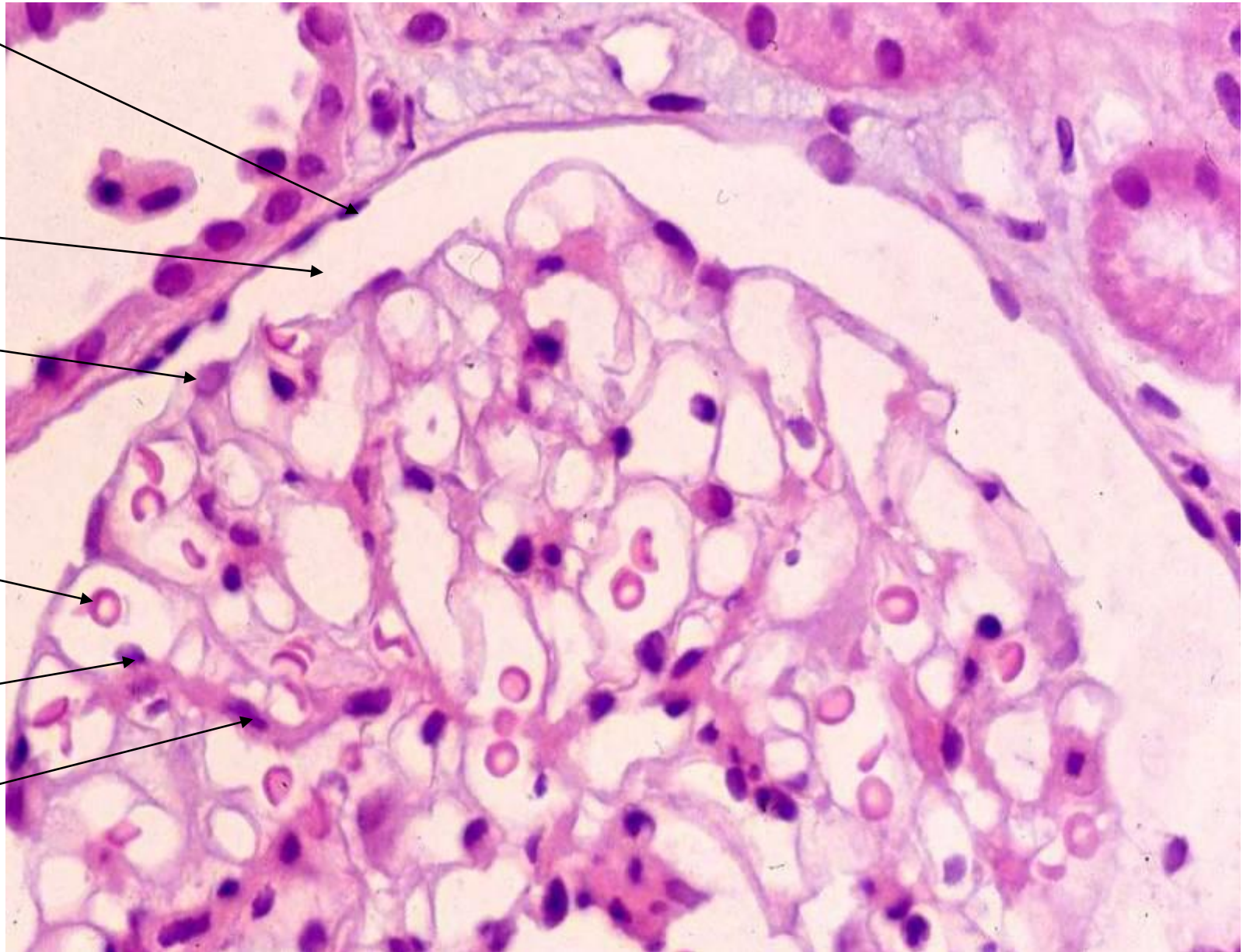
Bowman's  
space

Visceral  
epithelial  
cell/podocyte

Red Blood  
Cell/erythrocyte  
in capillary loop

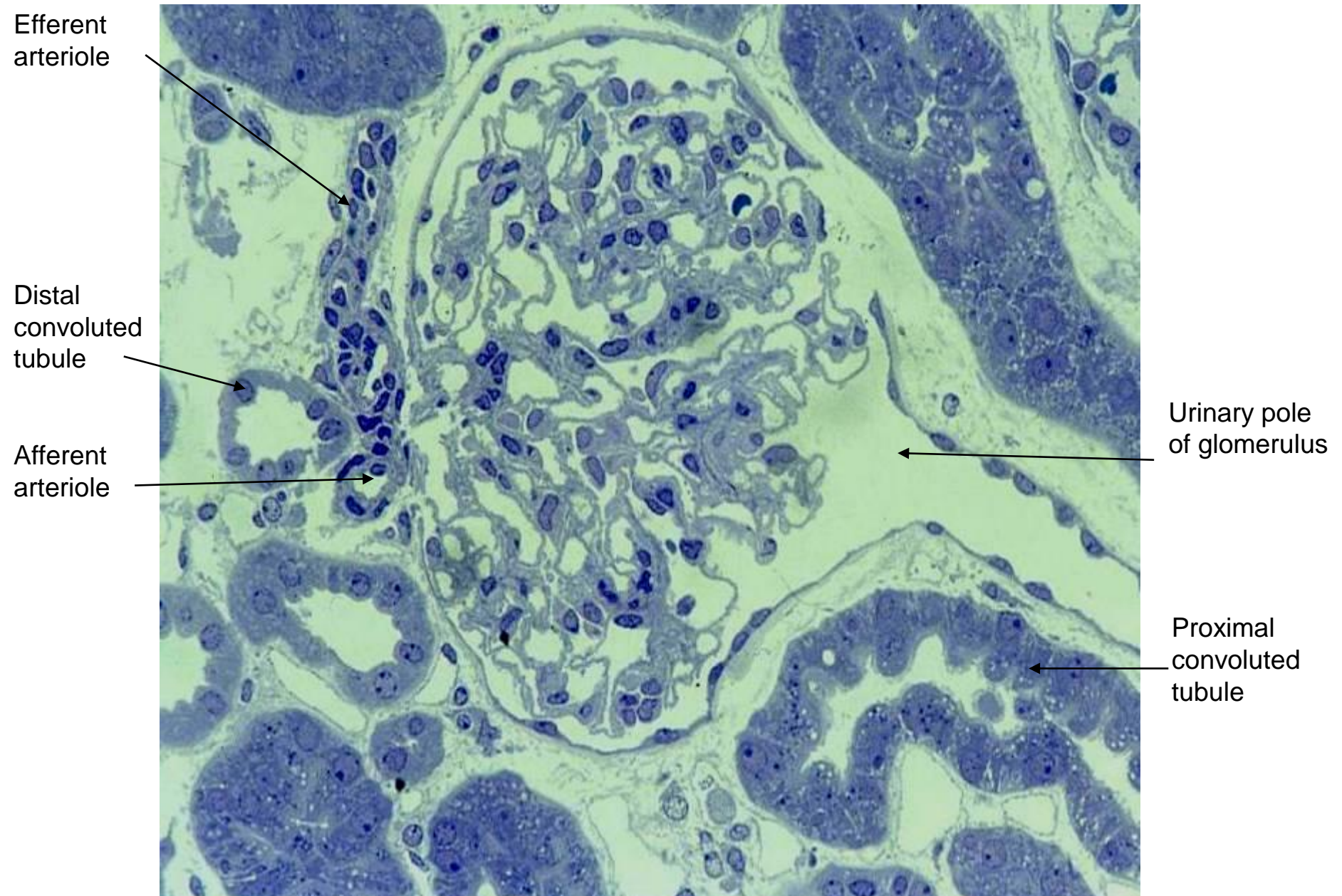
Endothelial  
cell

Mesangial cell



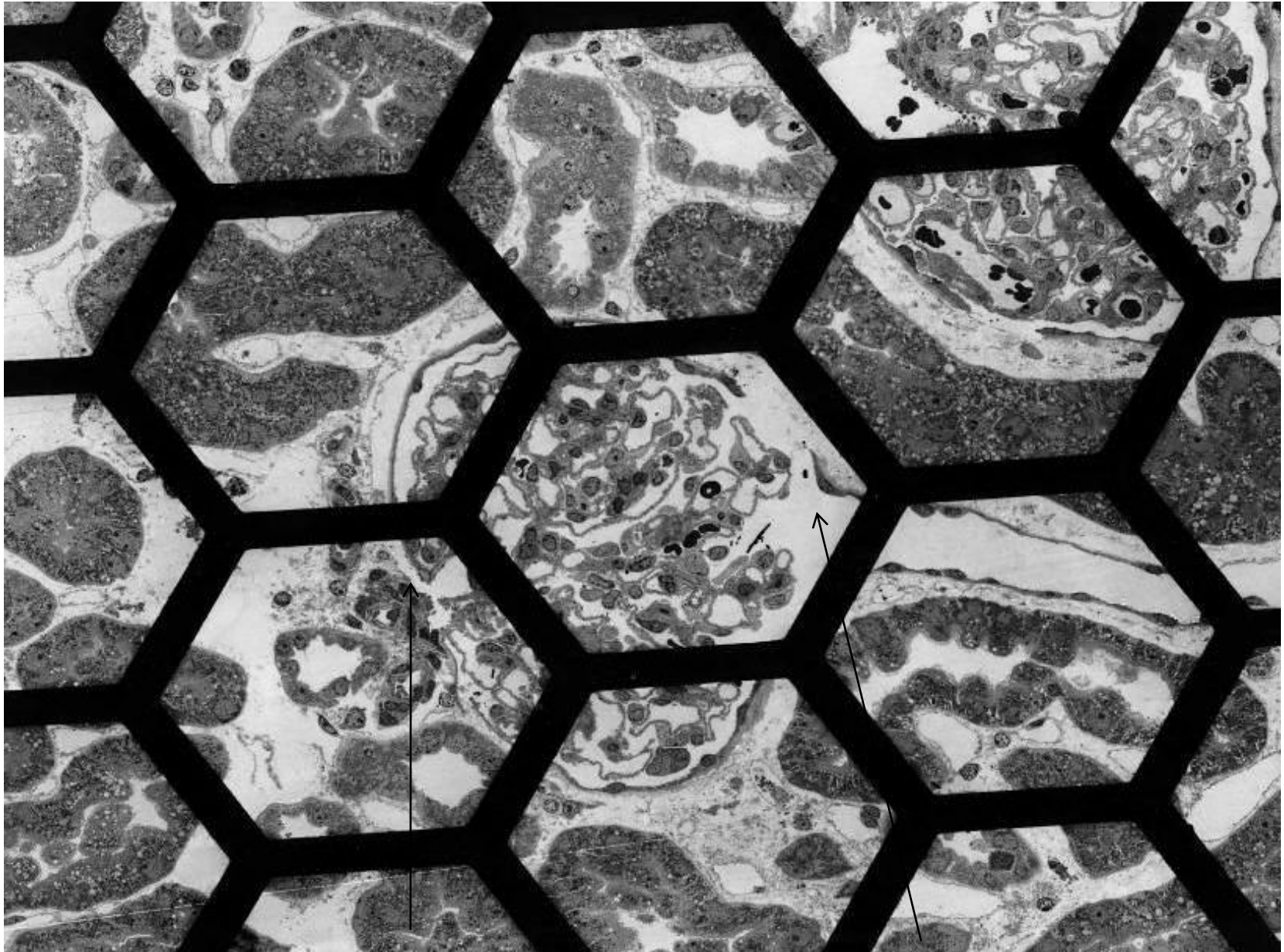


## Normal glomerulus – Toluidine Blue stained plastic section





Thin-section electron micrograph of same block as previous slide

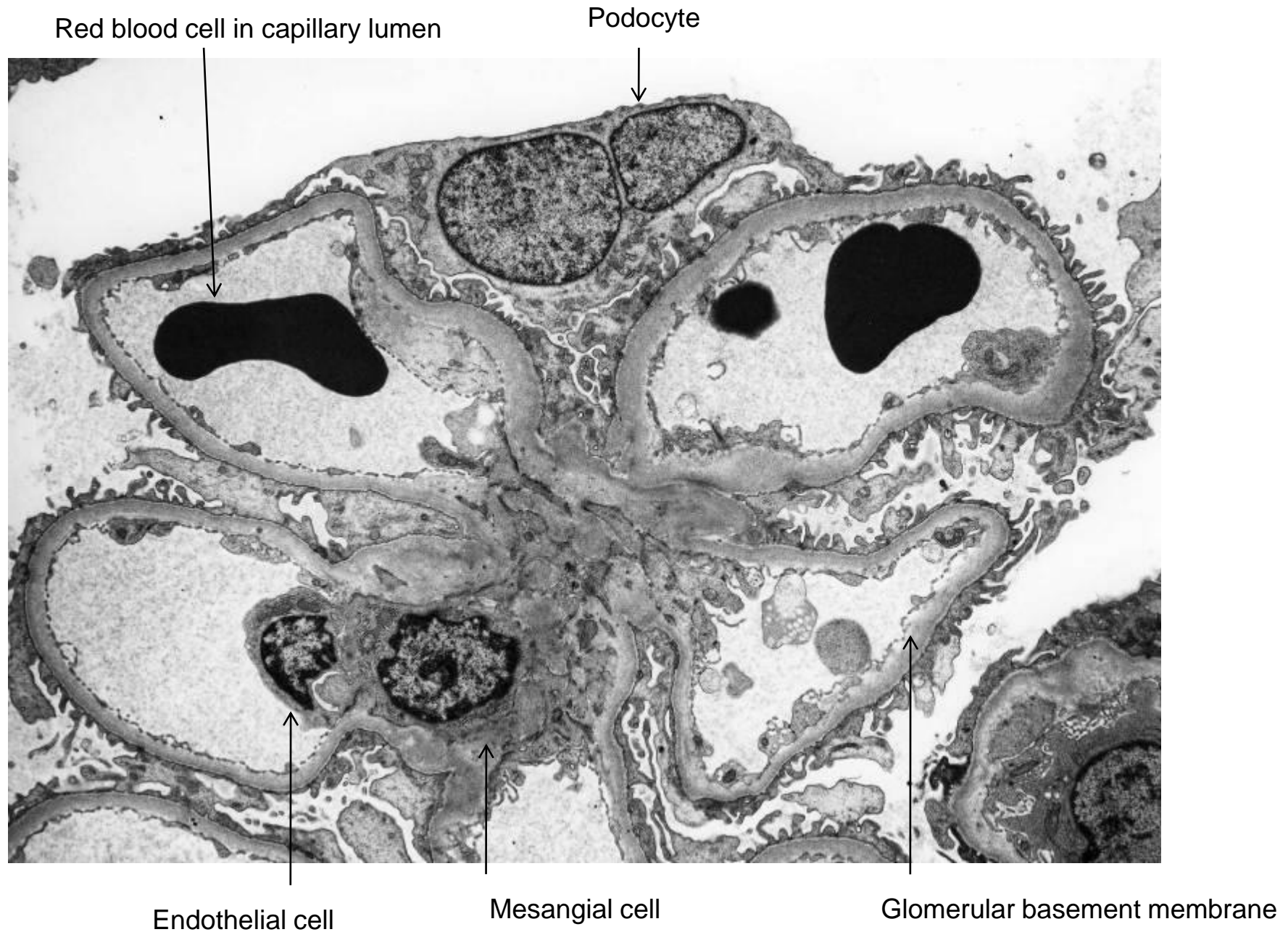


Vascular pole of glomerulus

Urinary pole of glomerulus

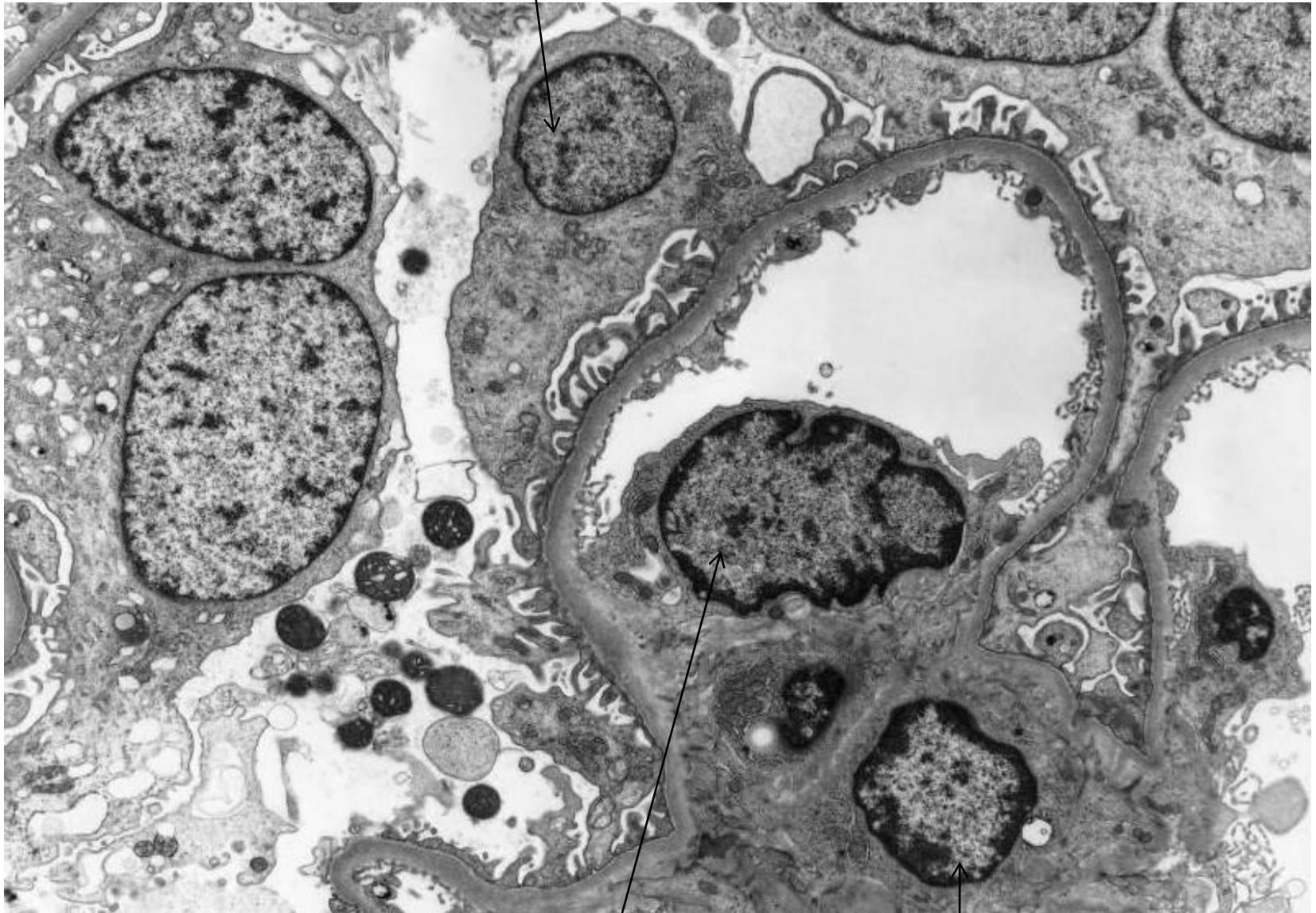


## Mesangial cell surrounded by 5 capillary loops



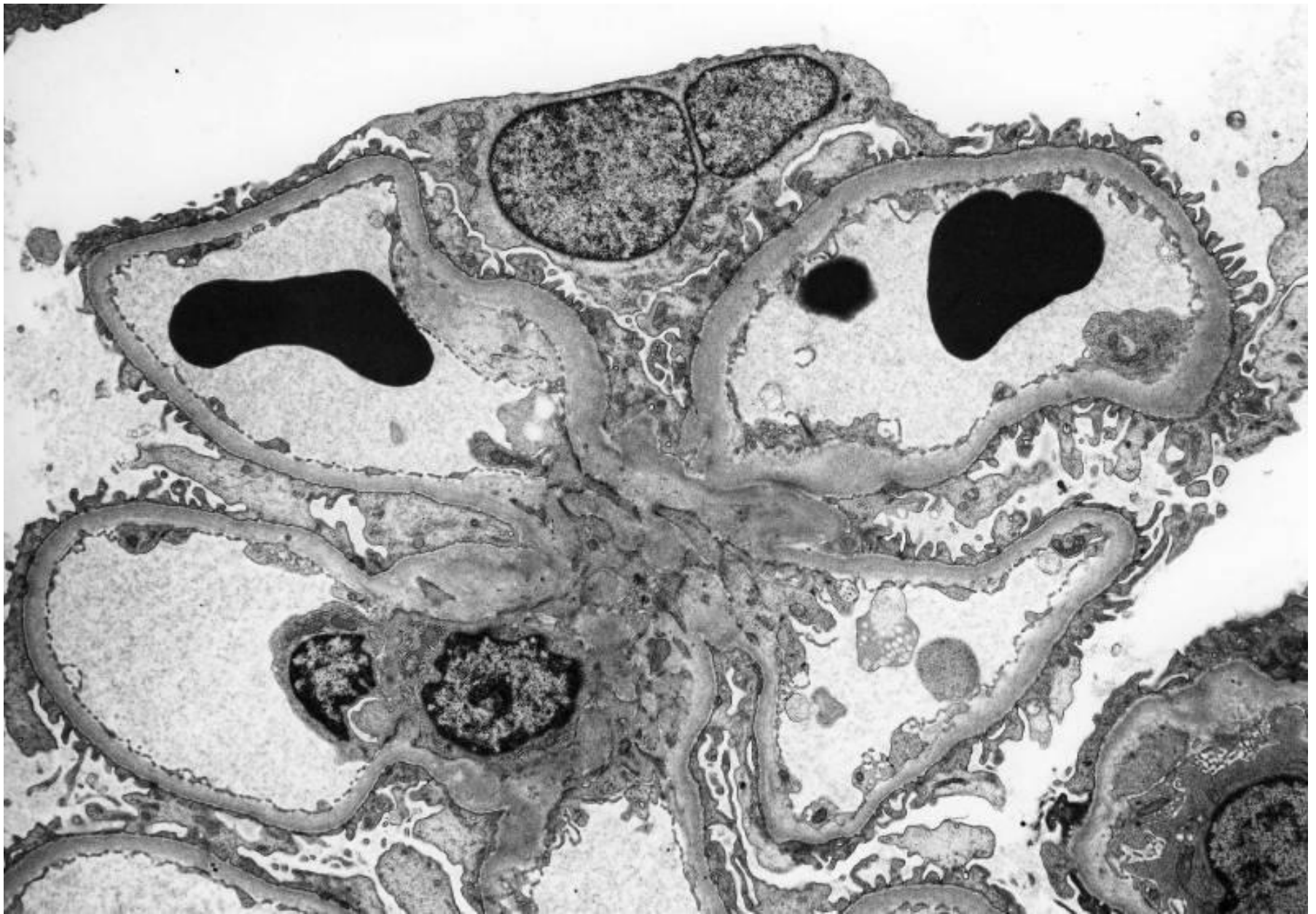


Podocyte



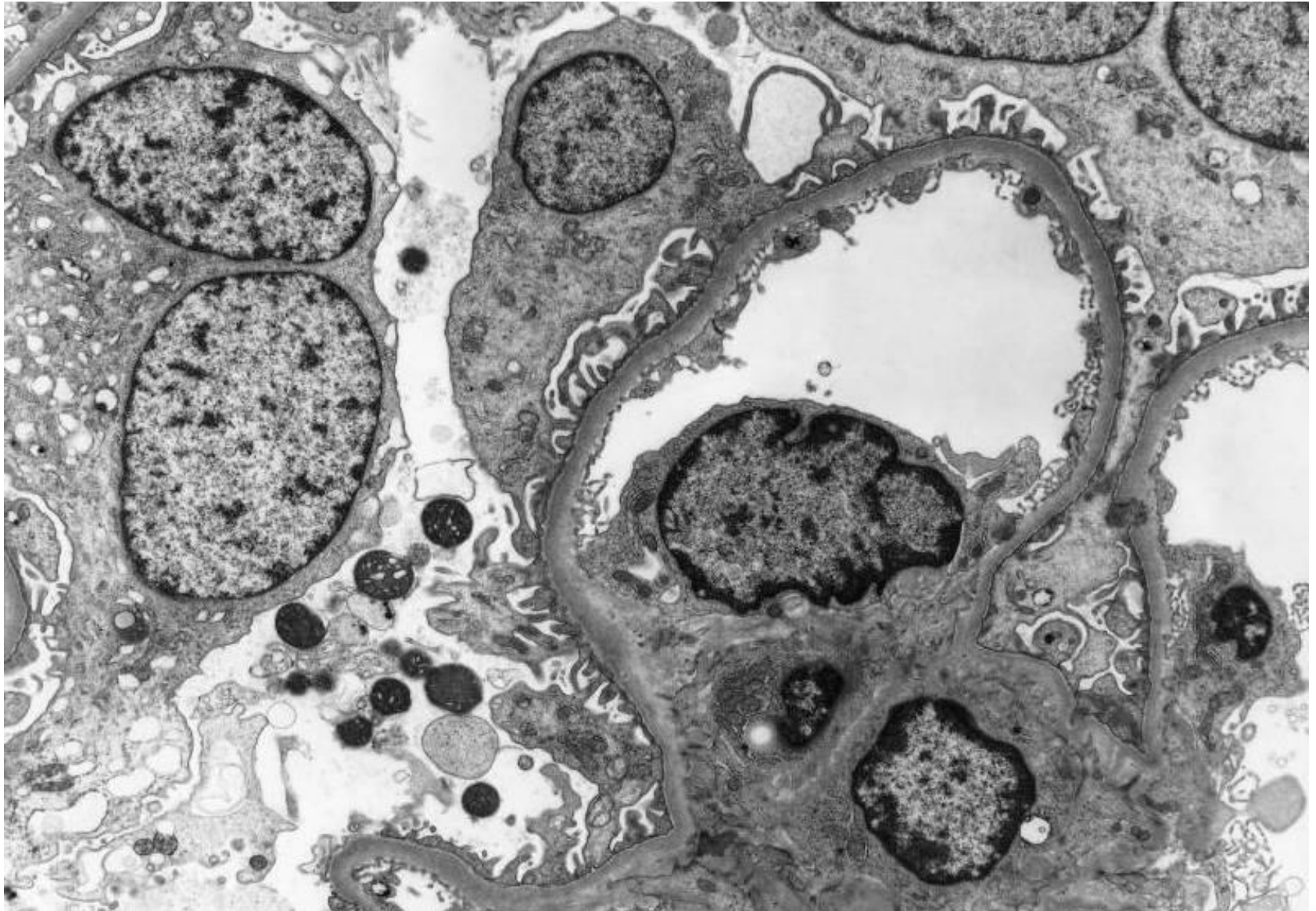
Endothelial cell

Mesangial cell



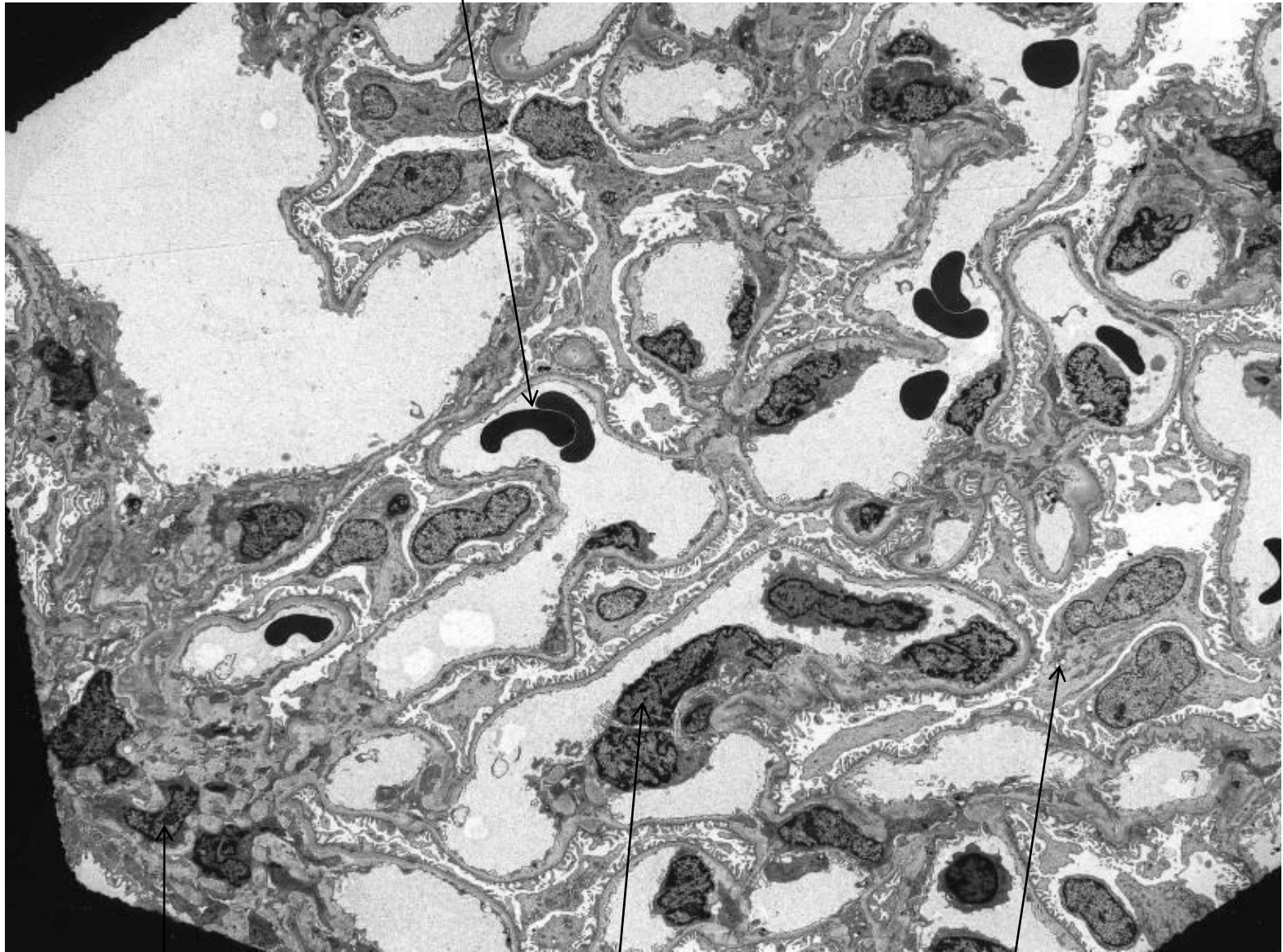
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Can you label this slide?

Erythrocyte within capillary lumen



Mesangial cell

Endothelial cell

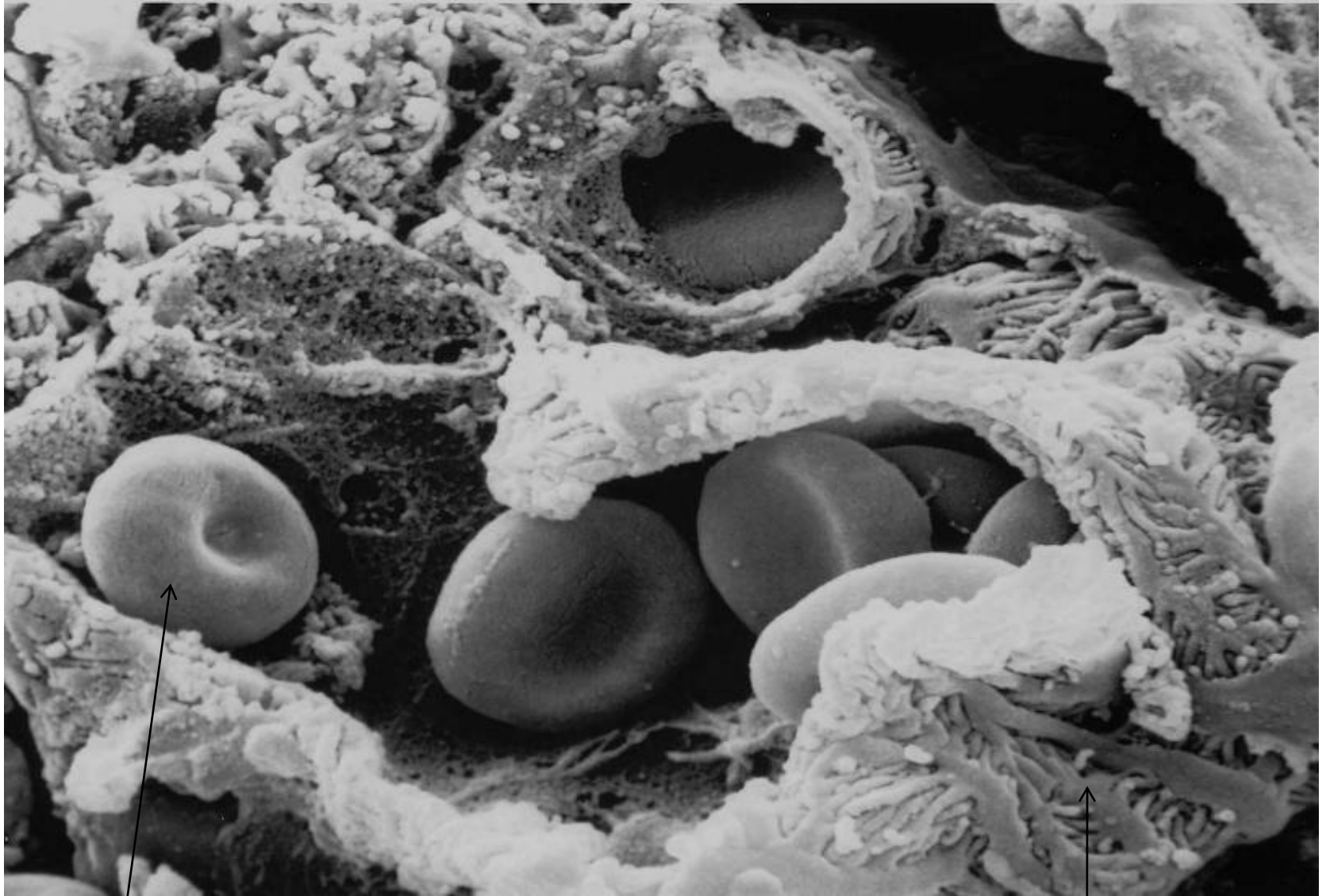
Podocyte



Scanning electron microscopy of exterior of whole rat glomerulus



Scanning electron microscopy of rat glomerulus cut in half

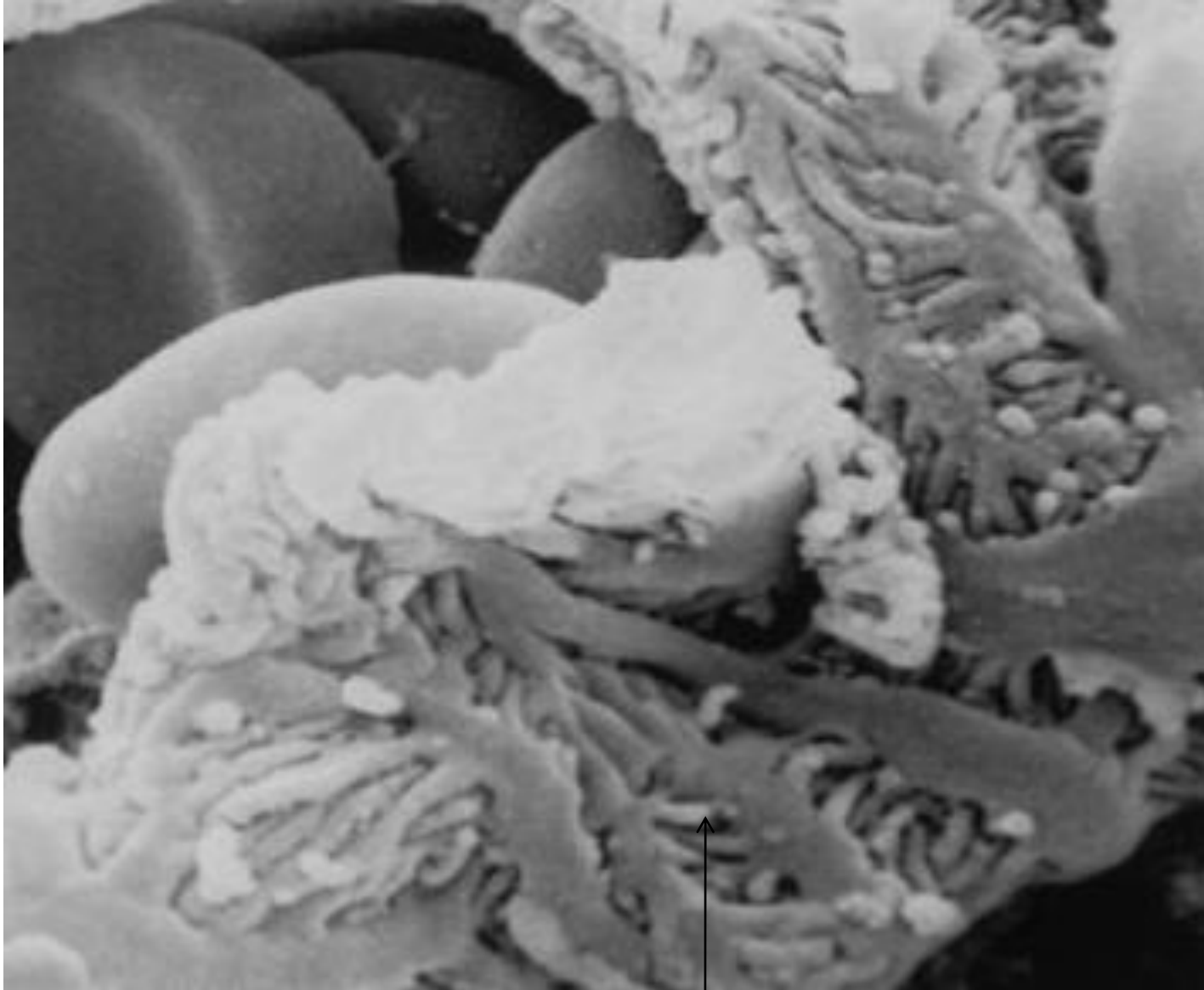


Erythrocyte

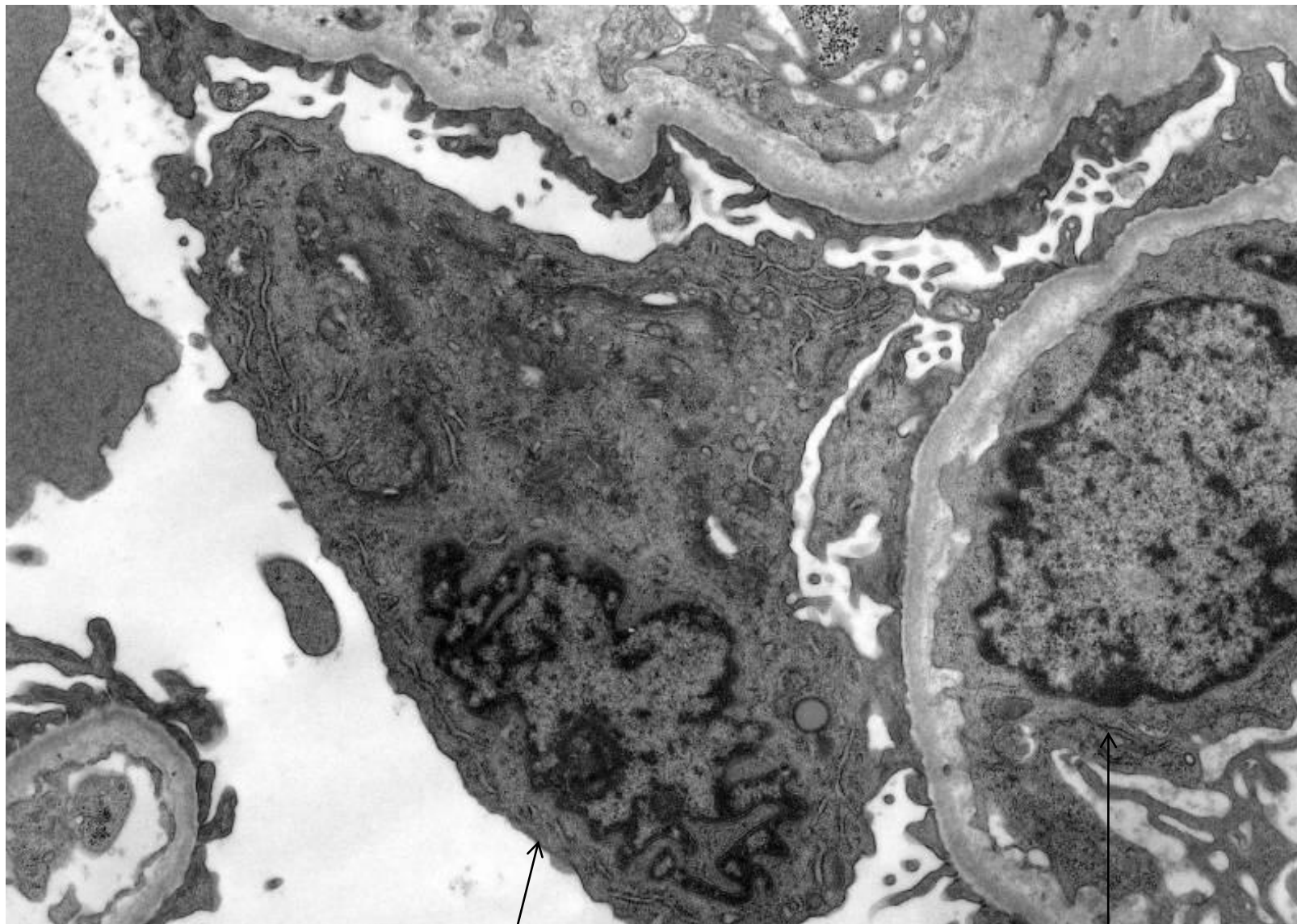
Podocyte



Higher magnification of previous slide



Interdigitating foot processes

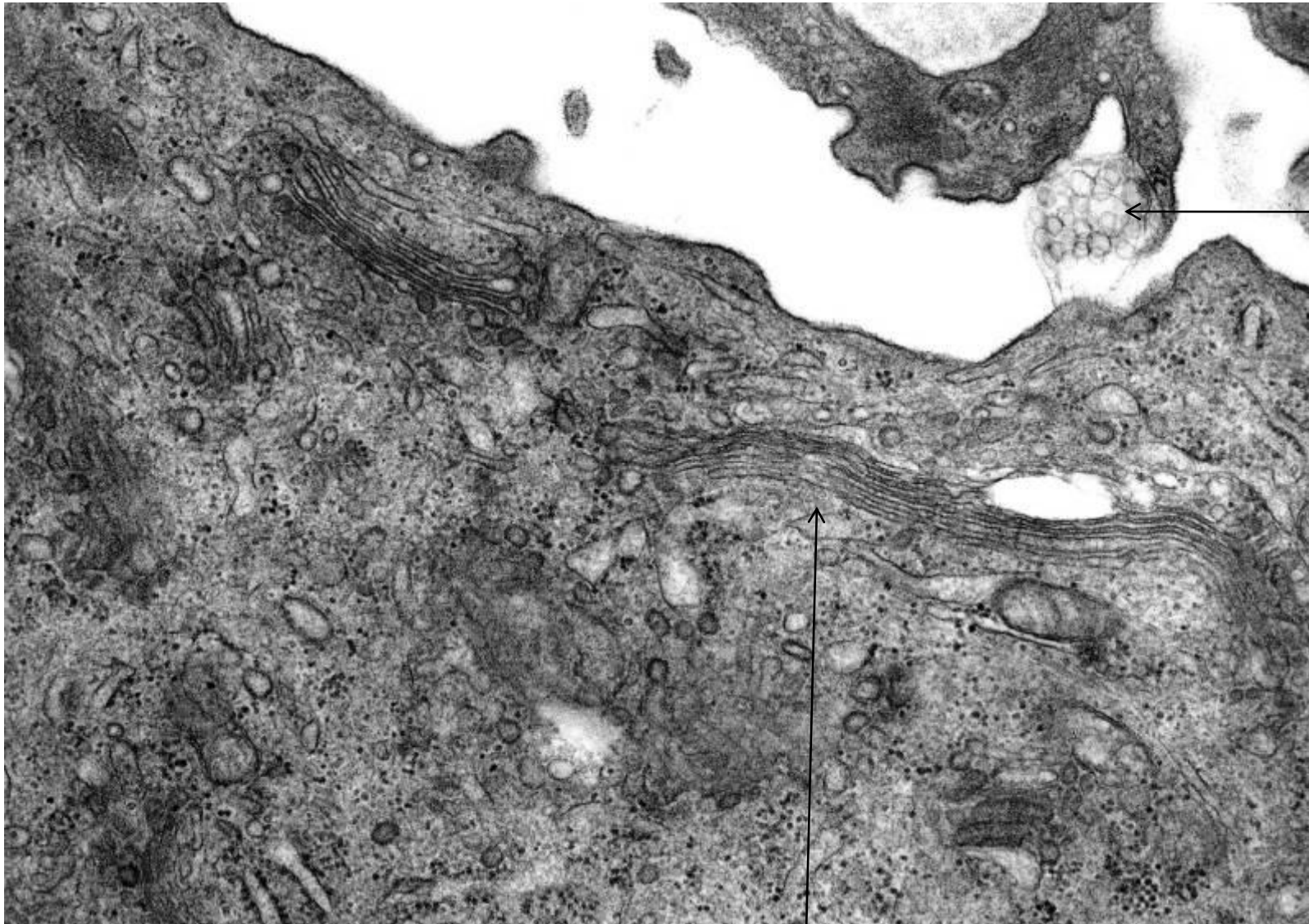


Podocyte

Endothelial cell



Higher magnification of previous image

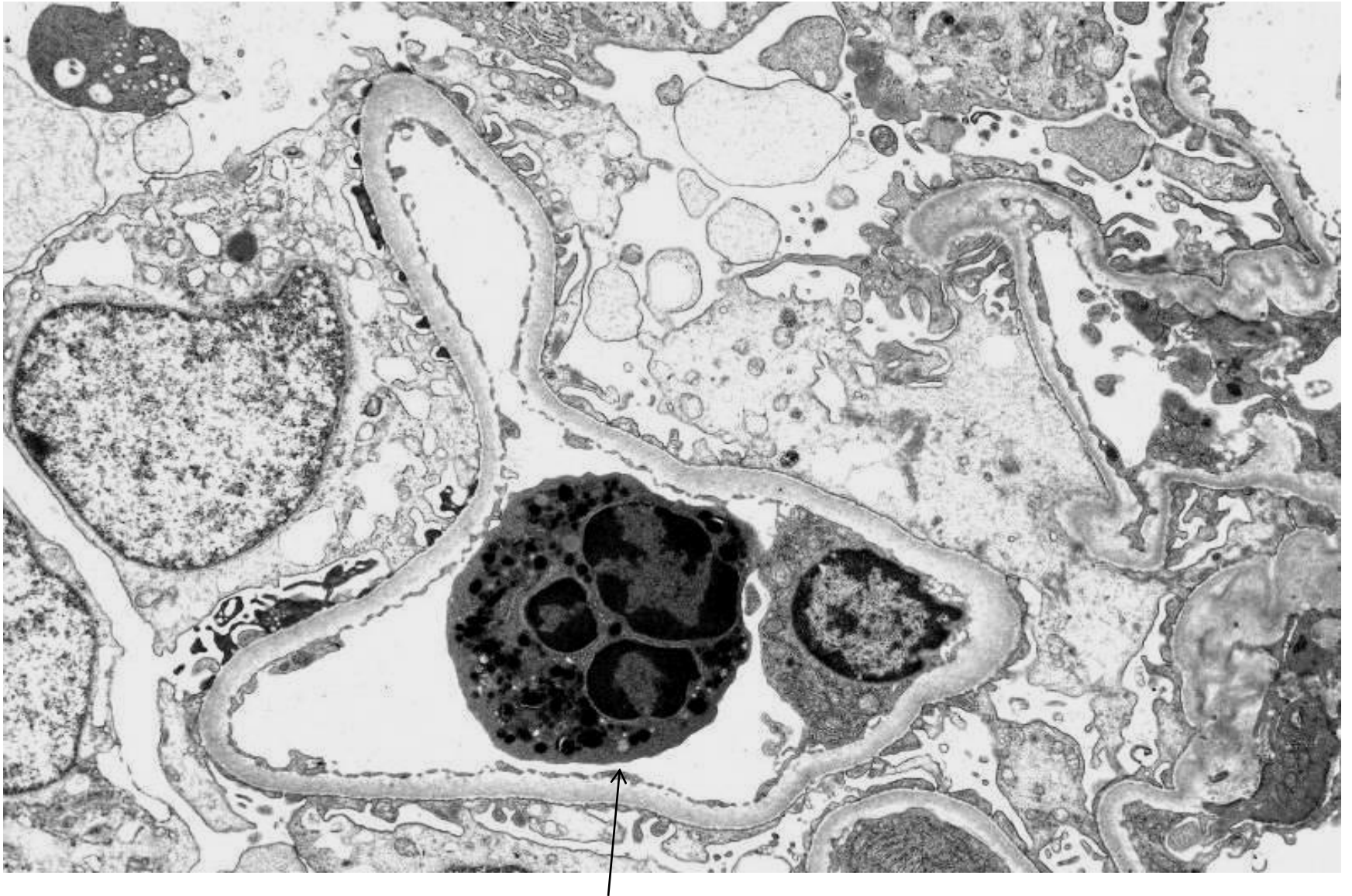


Fixation  
artefact

Podocyte cytoplasm

Golgi apparatus

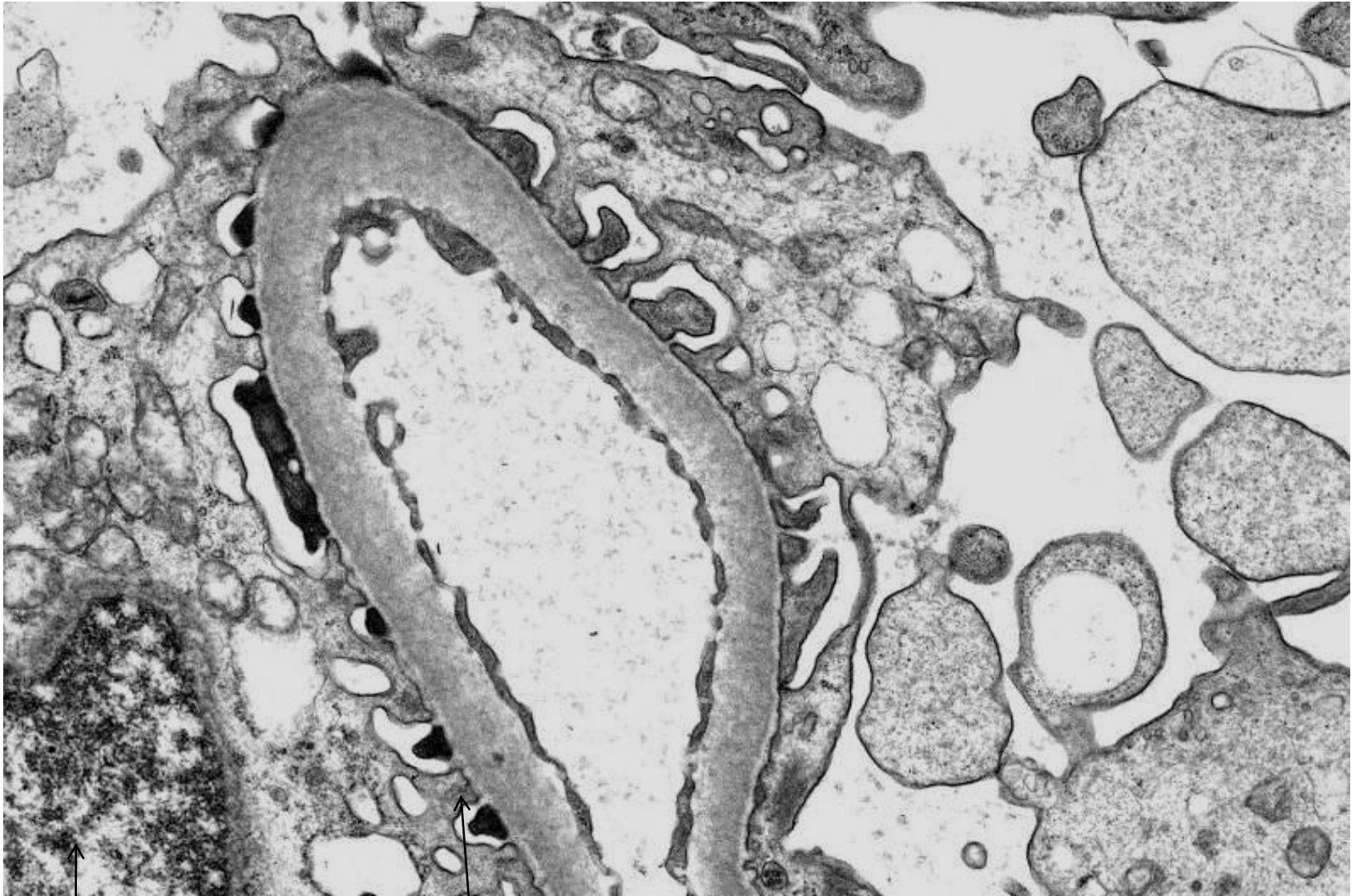
Podocyte – light cell dark cell artefact caused by hypoxia



Neutrophil polymorphonuclear leukocyte

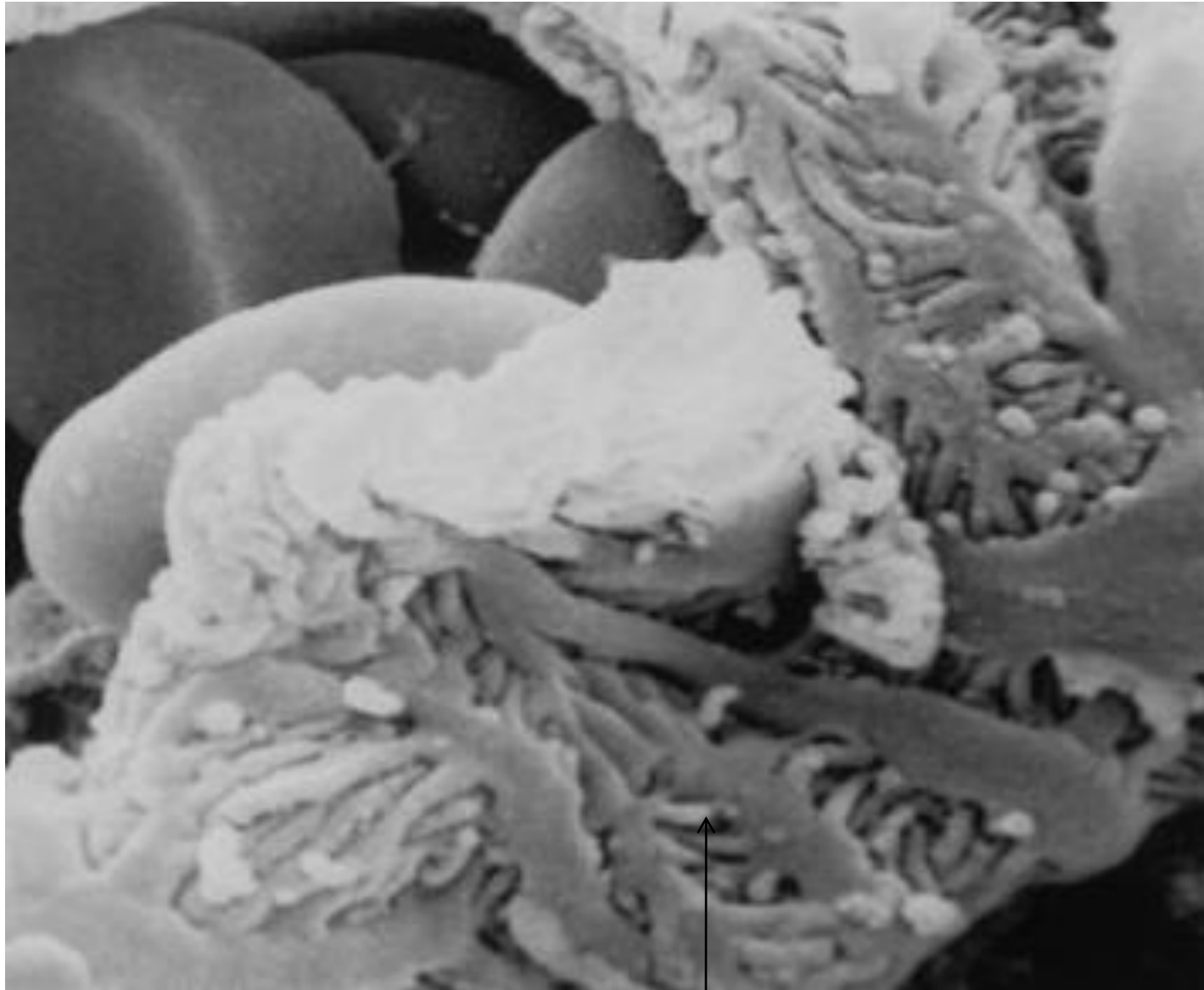


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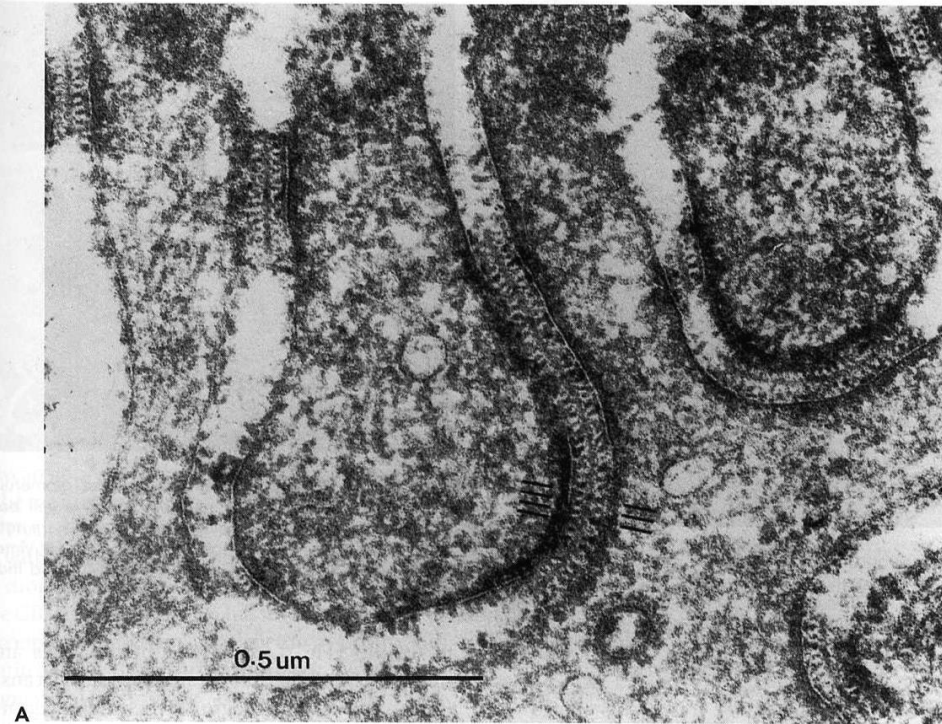
Podocyte

Interdigitation of foot processes



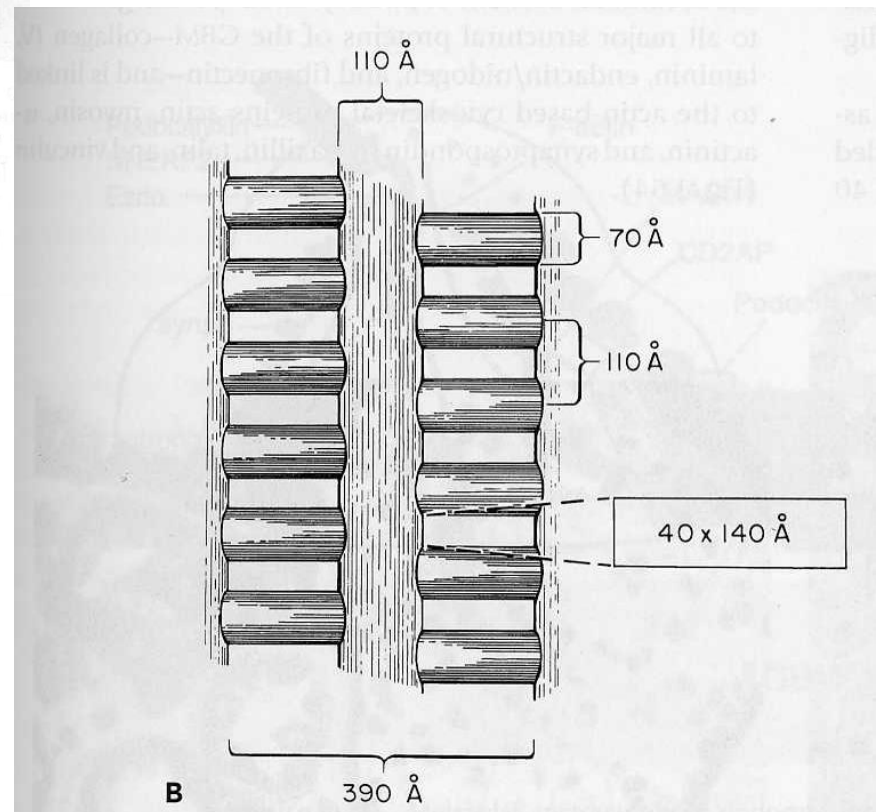
Interdigitating foot processes





Slit diaphragm viewed *en face*  
Rat glomerulus

Ladder shaped  
diaphragm with  
central band



Slit  
diaphragm  
human  
kidney

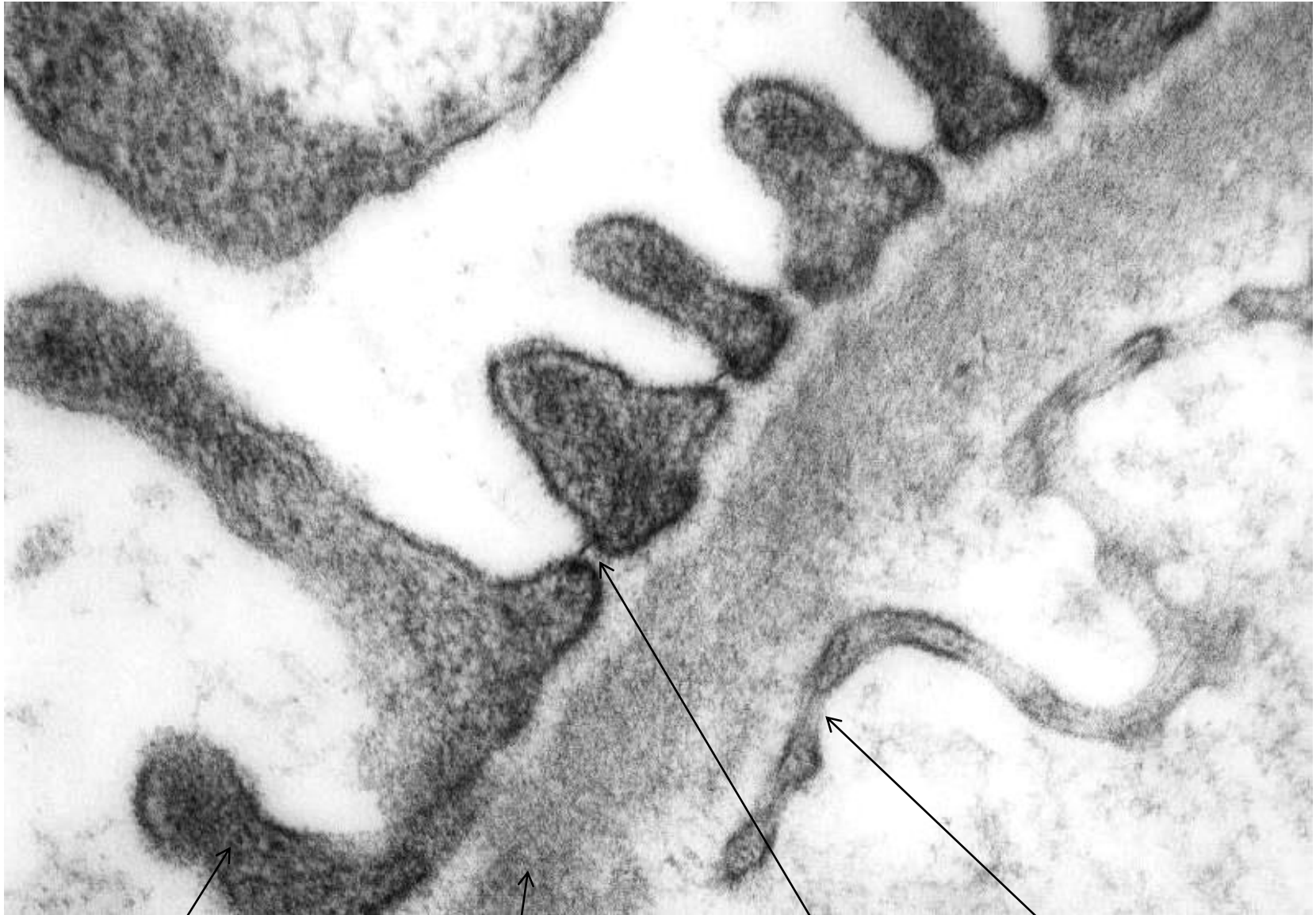


Glomerular  
basement  
membrane

Note: no central band



## Glomerular capillary wall

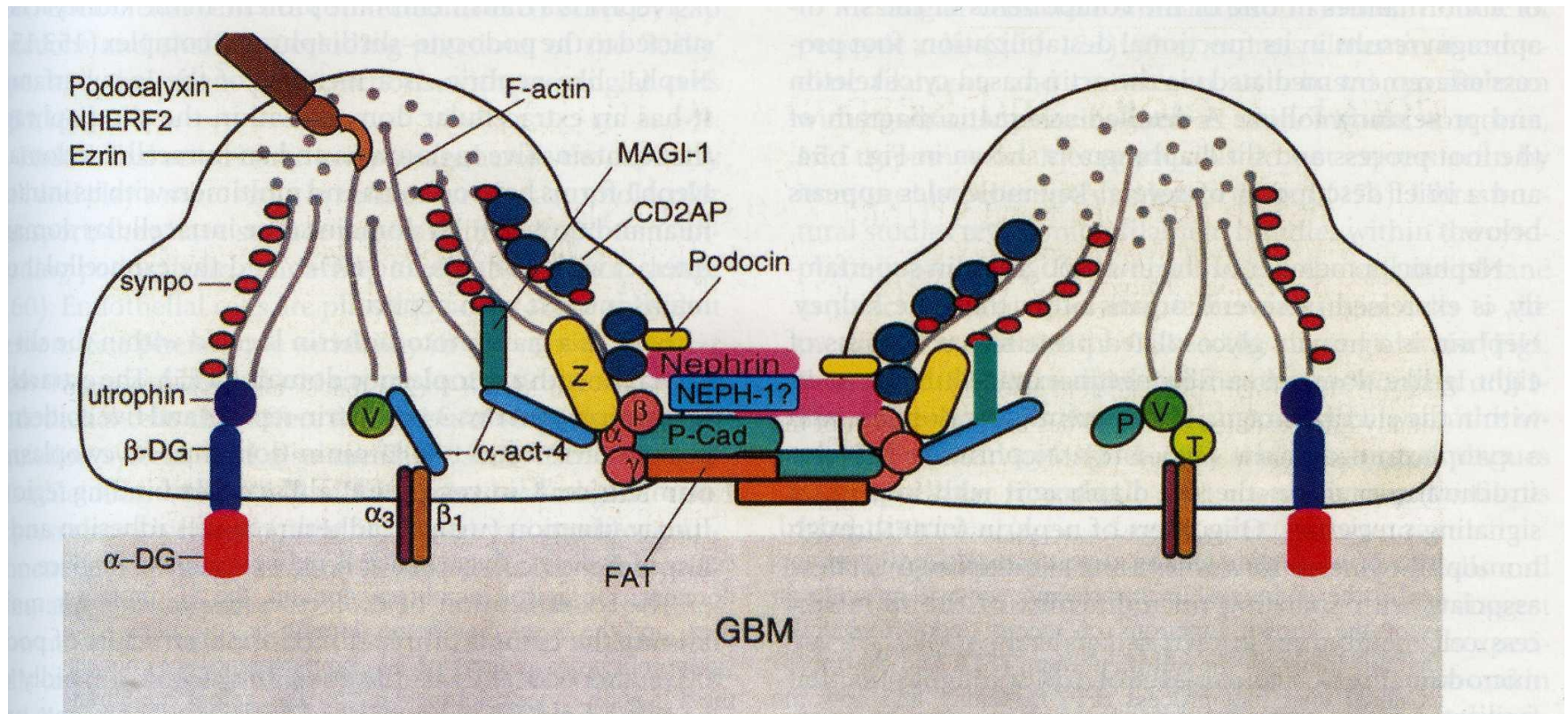


Foot processes

Glomerular basement  
membrane

Slit diaphragm

Fenestrated endothelium



**Figure 1.54** Molecular anatomy of the podocyte foot process (FP) and actin cytoskeleton. This schematic shows two adjacent podocyte FPs with the interposed slit diaphragm (SD) complex. The localization of NEPH-1 at the SD and its heterophilic interaction with nephrin remain to be established. The actin cytoskeleton is the common downstream pathway and receives input from three podocyte domains: the apical domain, the lateral SD-containing domain, and the basal domain of the FP sole plate, which links the podocyte to the GBM. Interference with any of the three domains will ultimately cause FP effacement and proteinuria/nephrotic syndrome.  $\alpha$ -act-4,  $\alpha$ -actinin-4;  $\alpha$ 3 $\beta$ 1,  $\alpha$ 3 $\beta$ 1 integrin;  $\alpha$ -DG,  $\alpha$ -dystroglycan;  $\beta$ -DG,  $\beta$ -dystroglycan; NHERF2, Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor 2; P, paxillin; P-cad, P-cadherin; synpo, synaptopodin; T, talin; V, vinculin. (From Mundel P, Shankland SJ. Podocyte biology and response to injury. *J Am Soc Nephrol* 2002;13:3005.)



Nephrin & p Cadherin

Podocalyxin

Alpha & Beta  
Dystroglycan

Alpha3 Beta1  
integrin

Actin

Collagen IV & Laminin

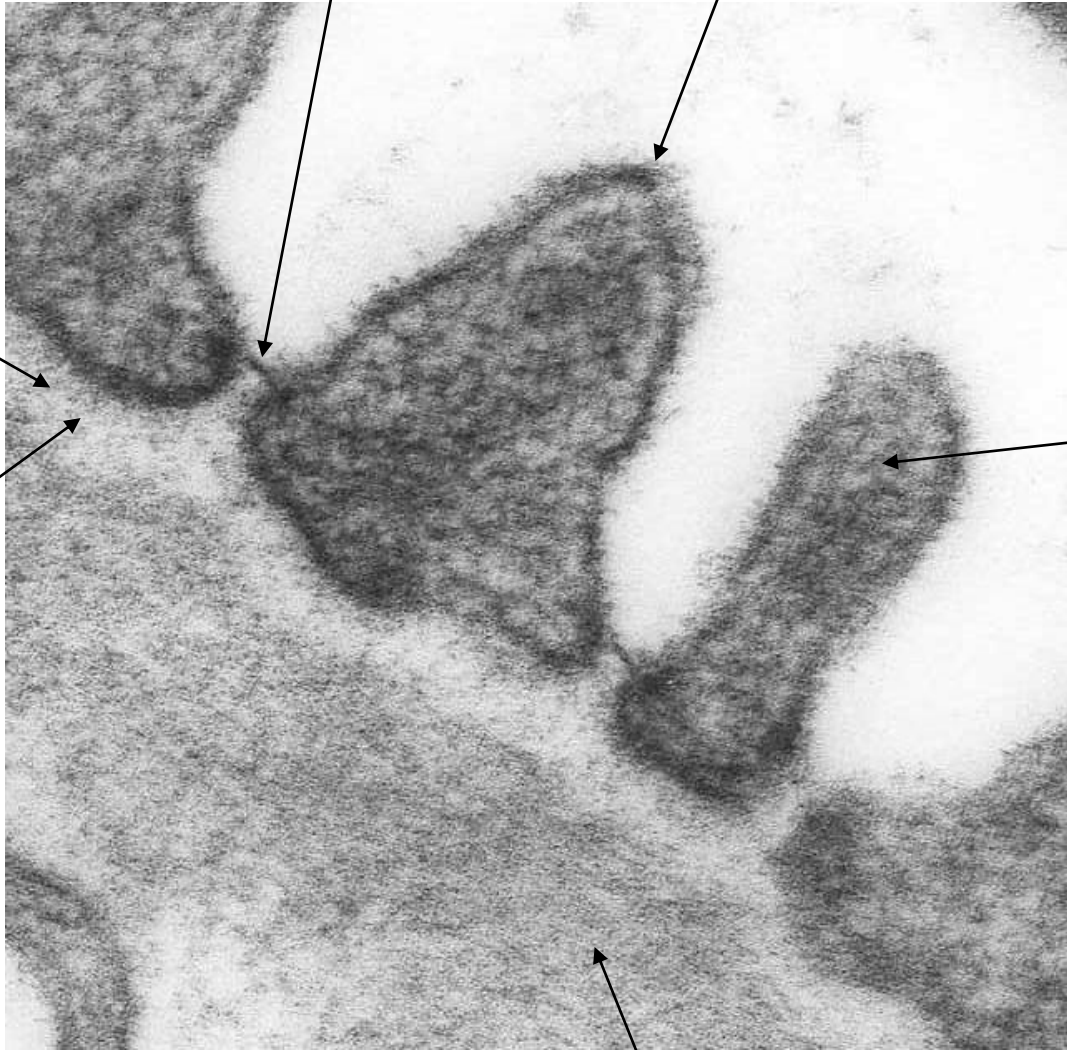
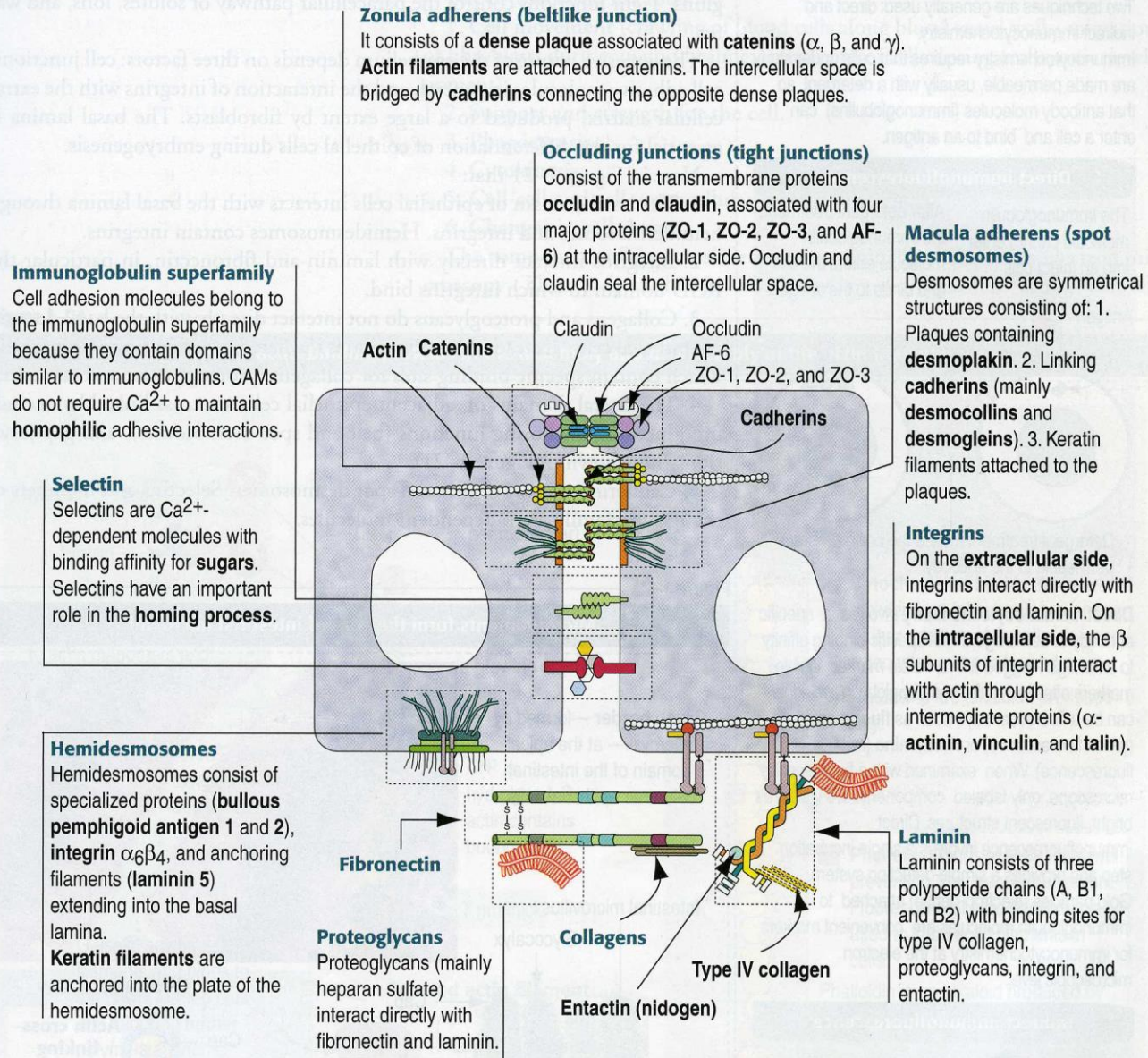
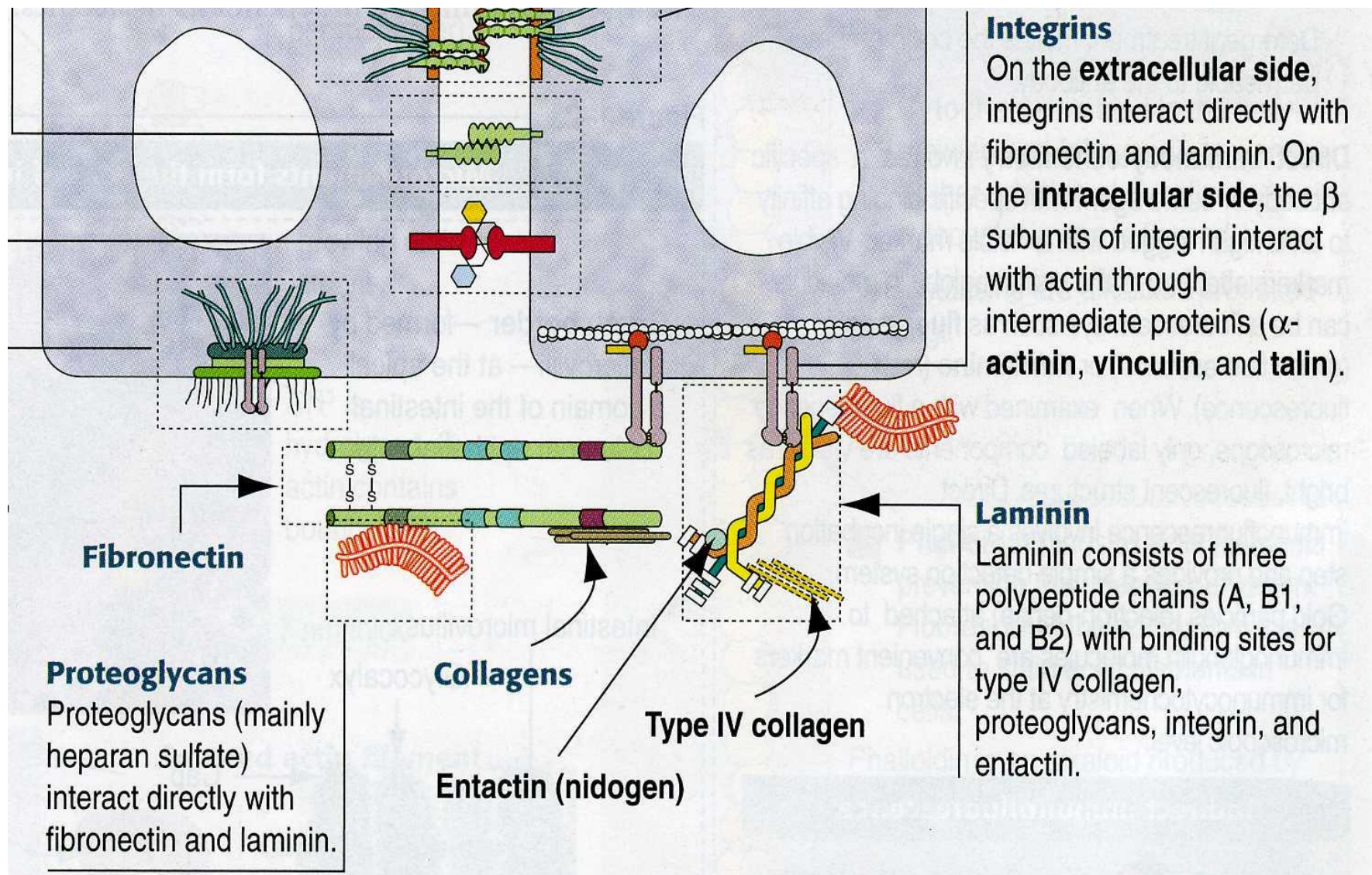


Figure 1-21

## Summary of cell junctions and cell adhesion molecules



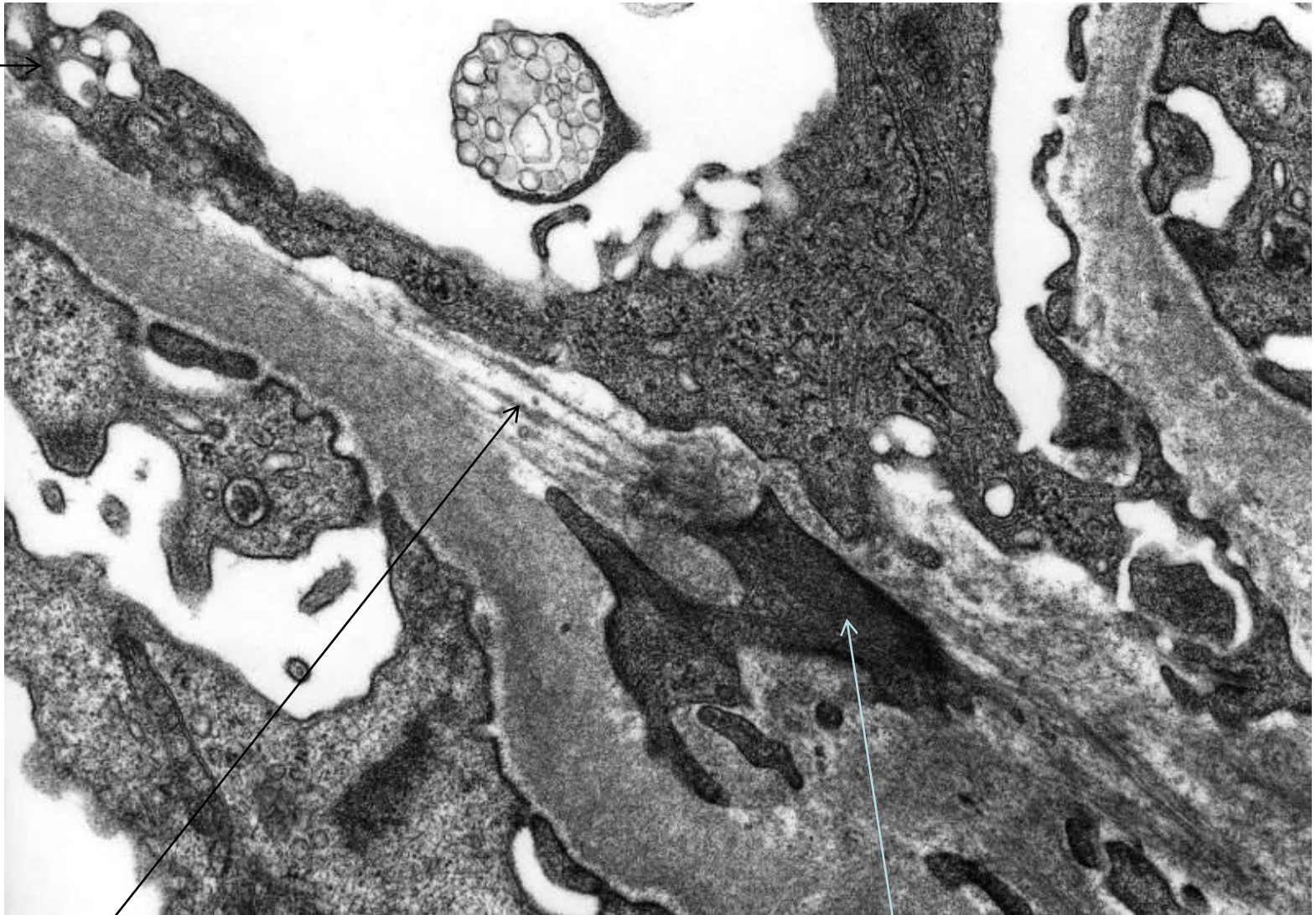






## Paramesangial region

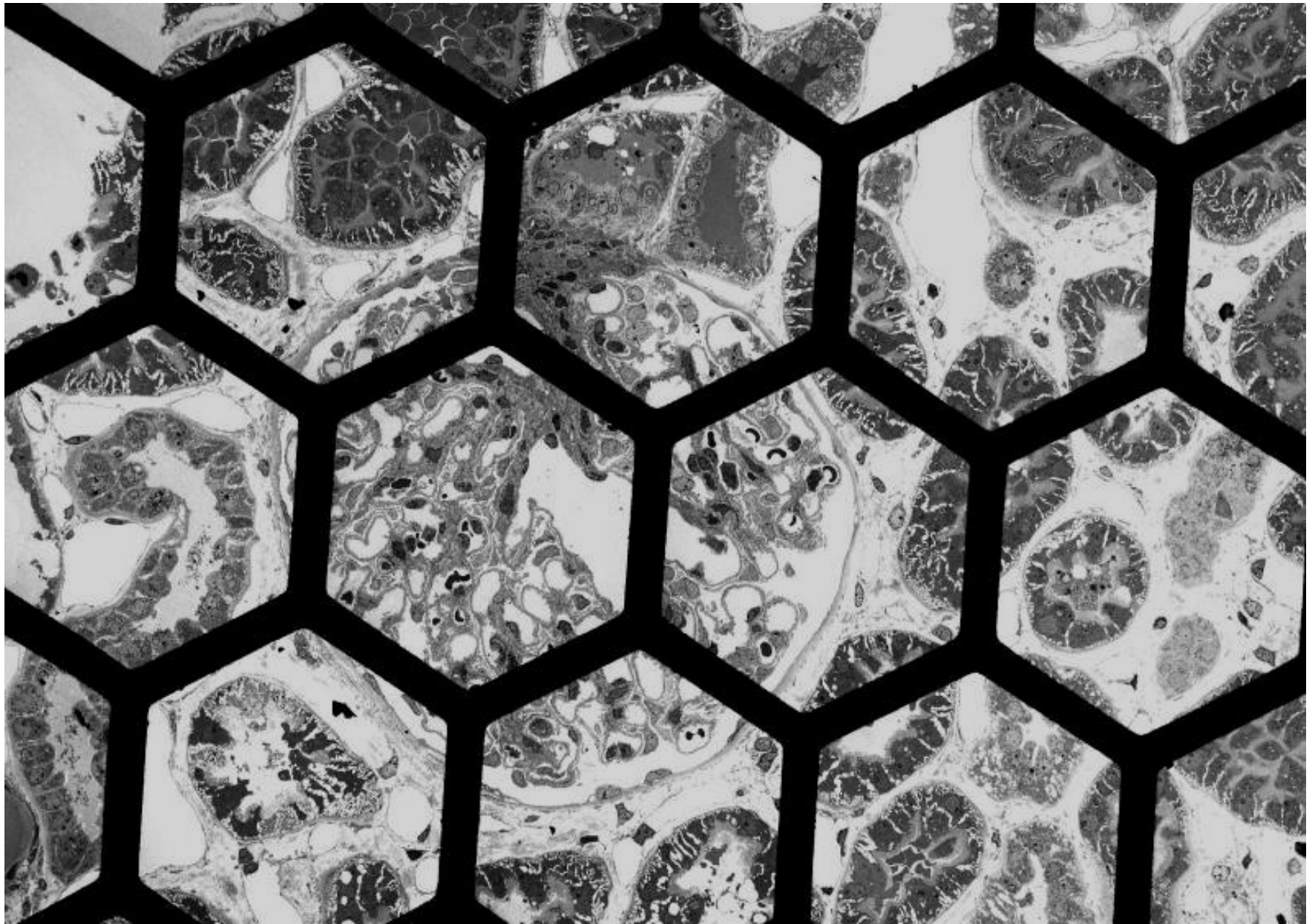
Endothelial  
cell



Fibronectin fibrils

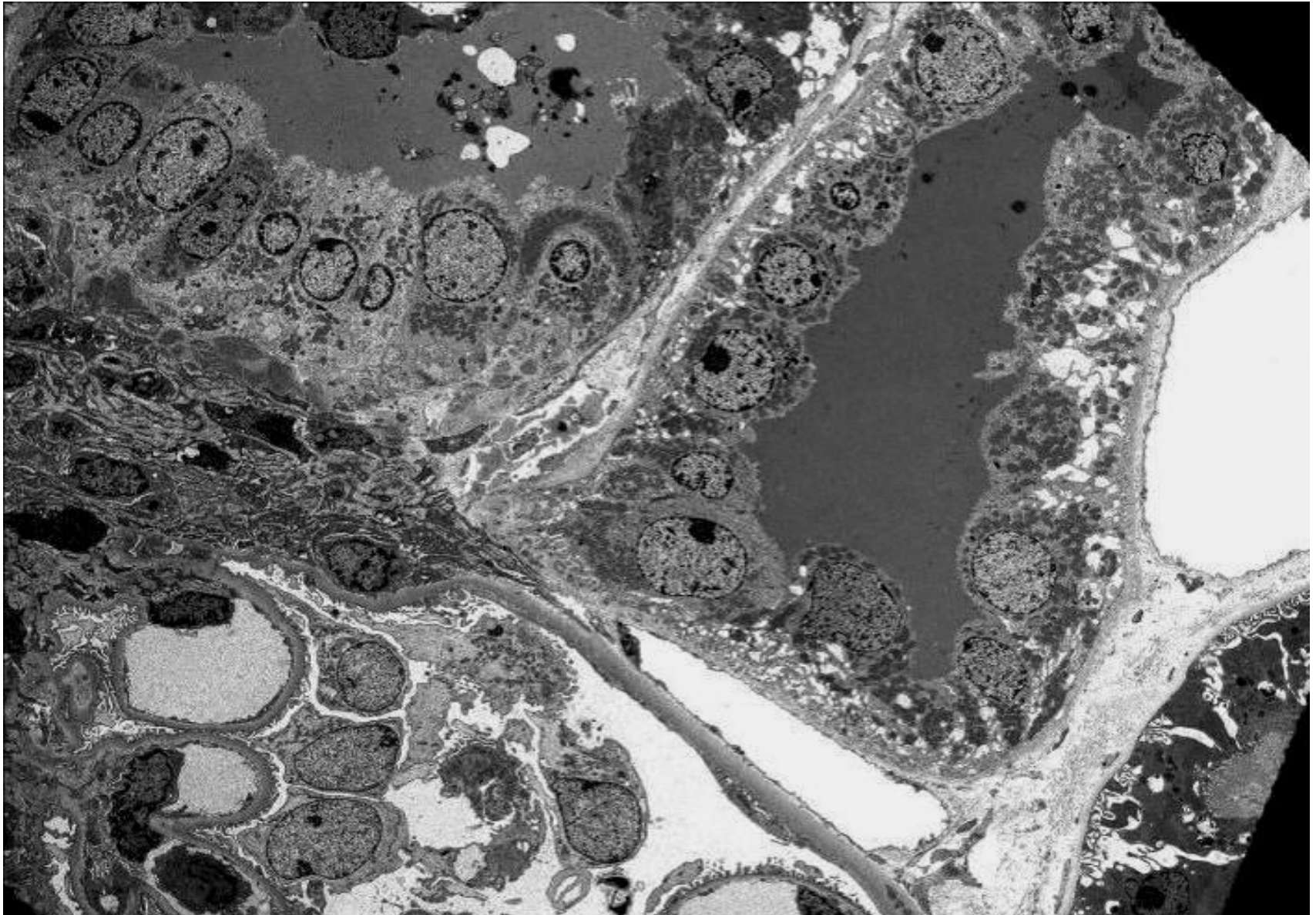
Portion of mesangial cell cytoplasm





Juxtaglomerular apparatus

Macula densa at end of thick ascending part of loop of Henle and beginning of distal convoluted tubule

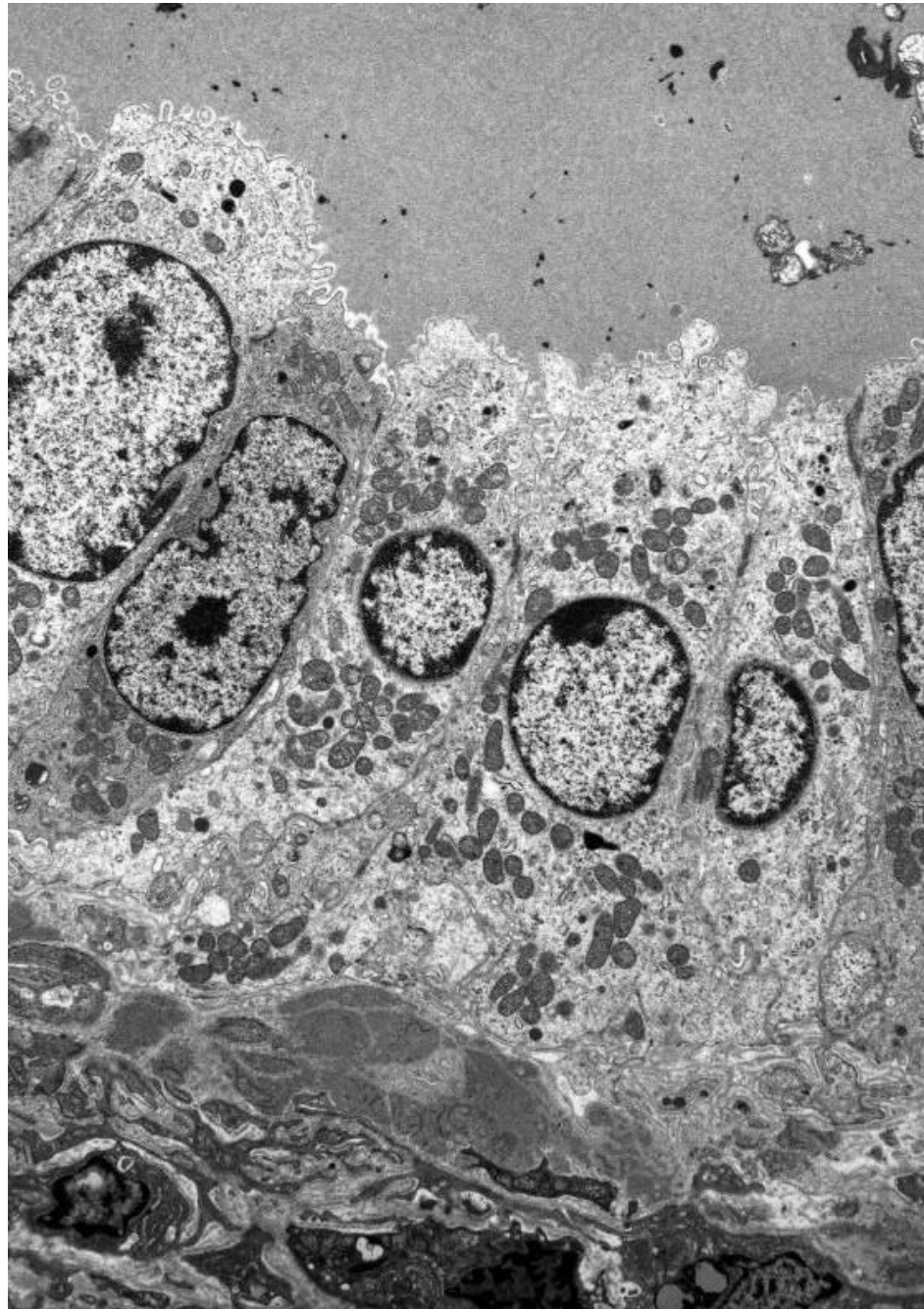


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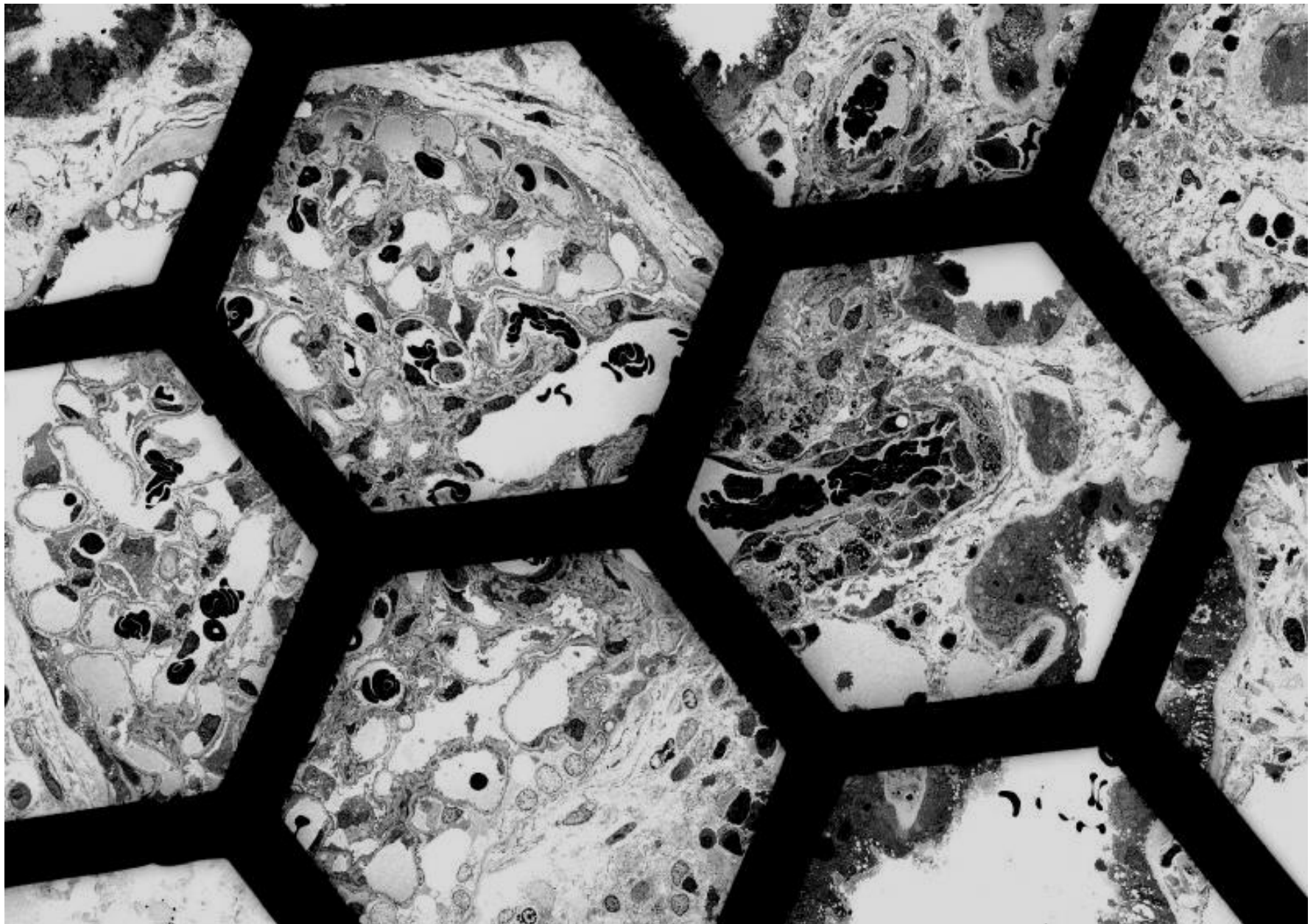
Macula densa part of distal convoluted tubule  
adjacent to extraglomerular mesangial/lacis cells



Macula densa cells

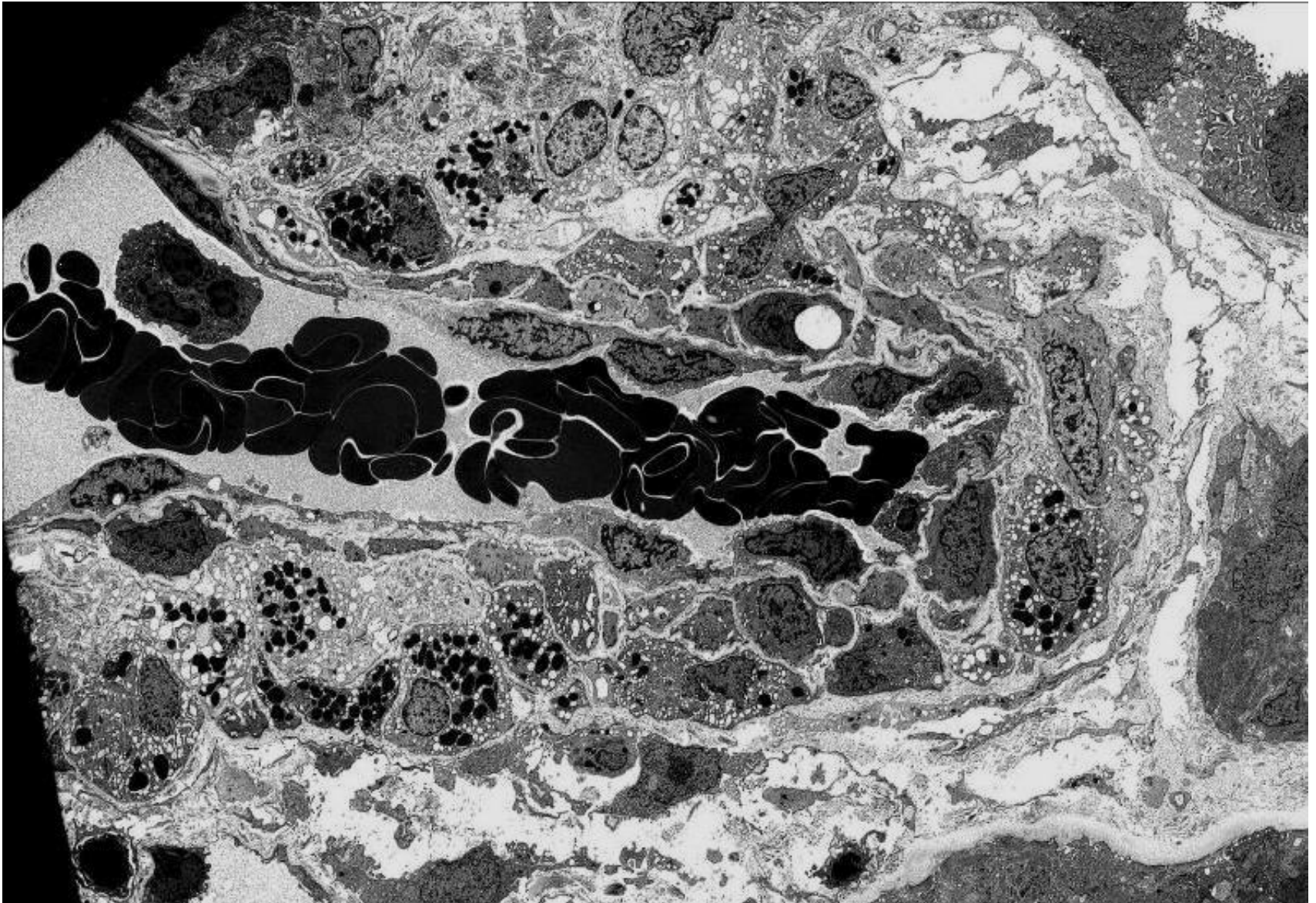


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Afferent arteriolar cells at point of juxtaglomerular apparatus contain renin and angiotensin II granules

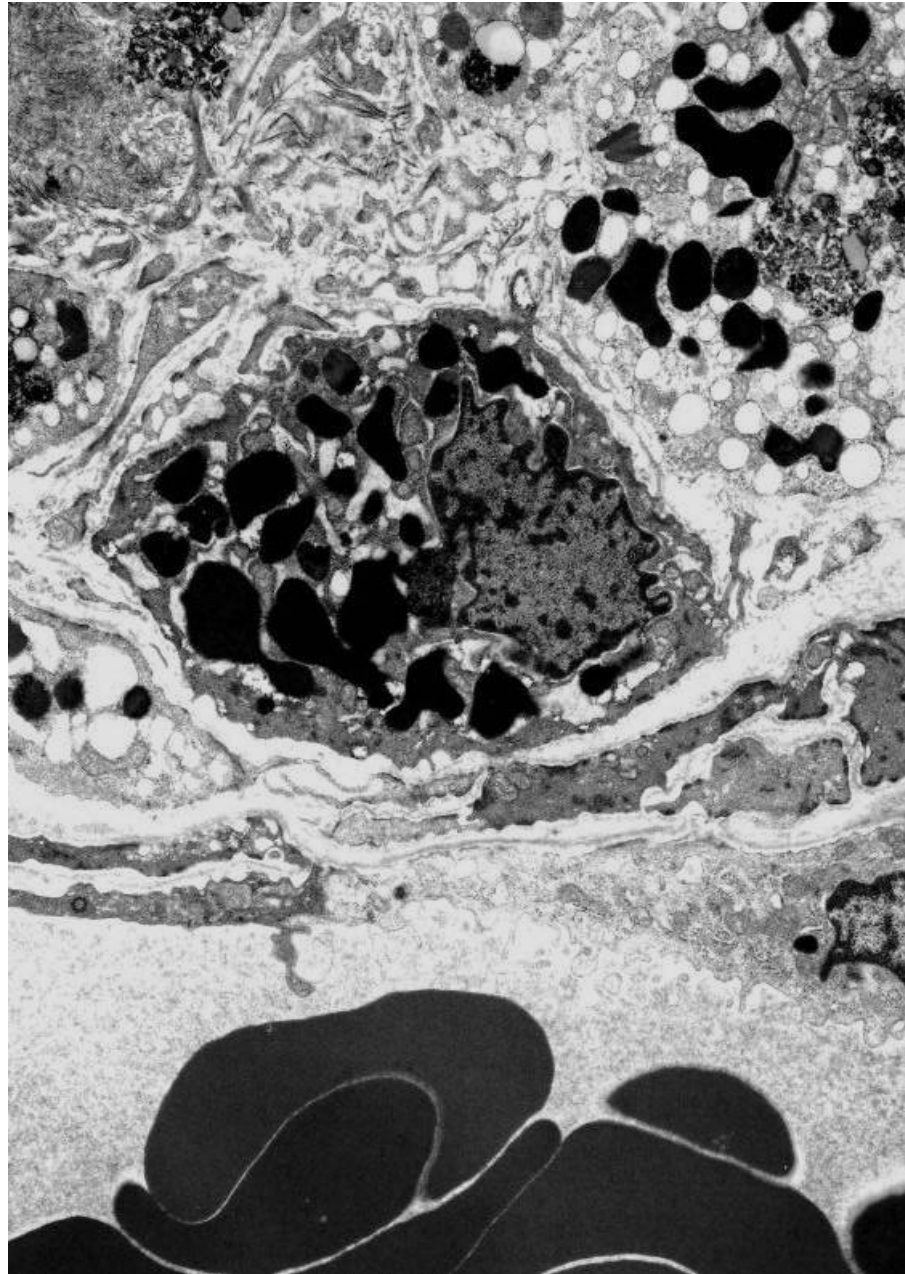




Afferent arteriole

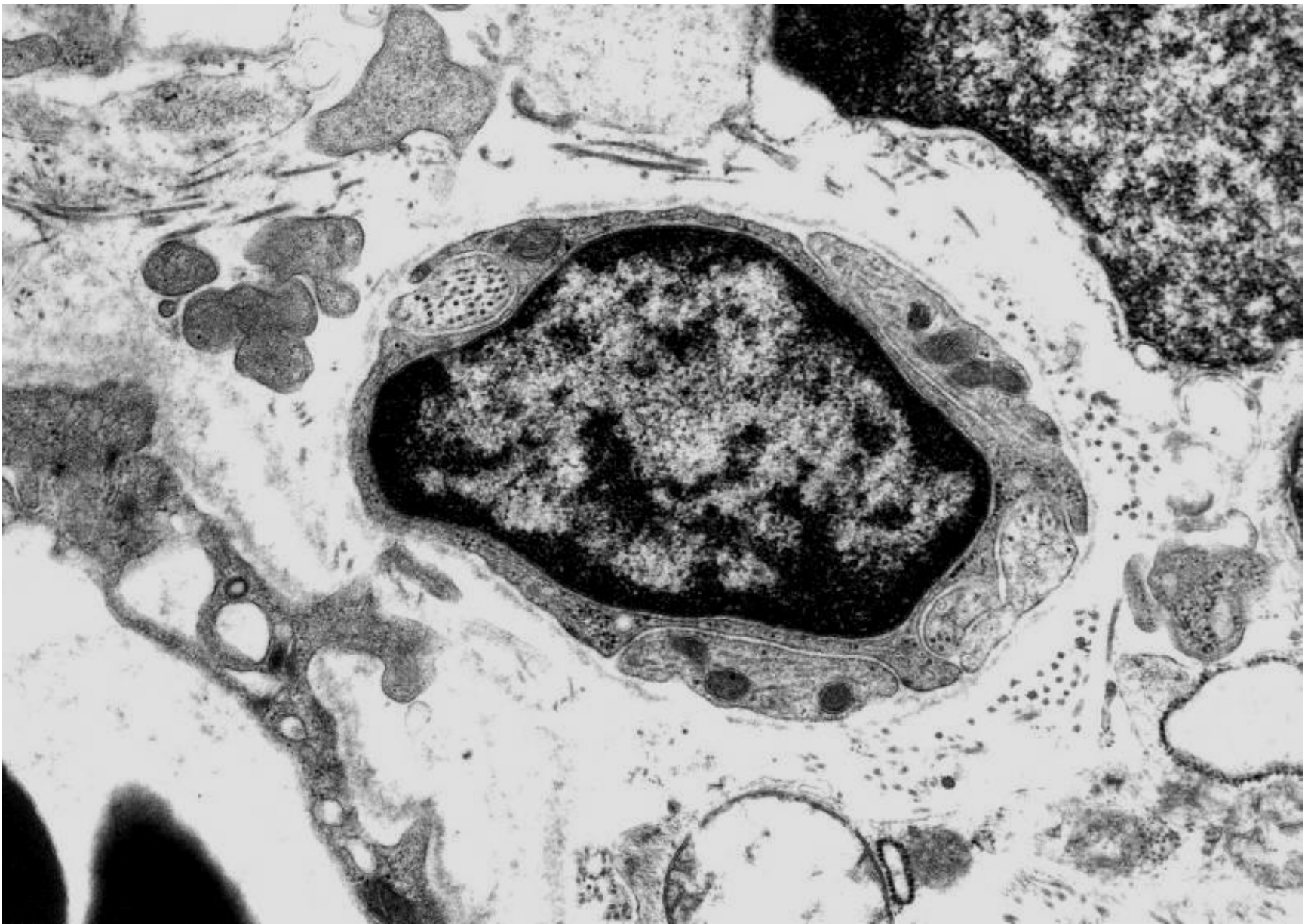
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Granular  
myoepithelial  
cells



Granules contain renin

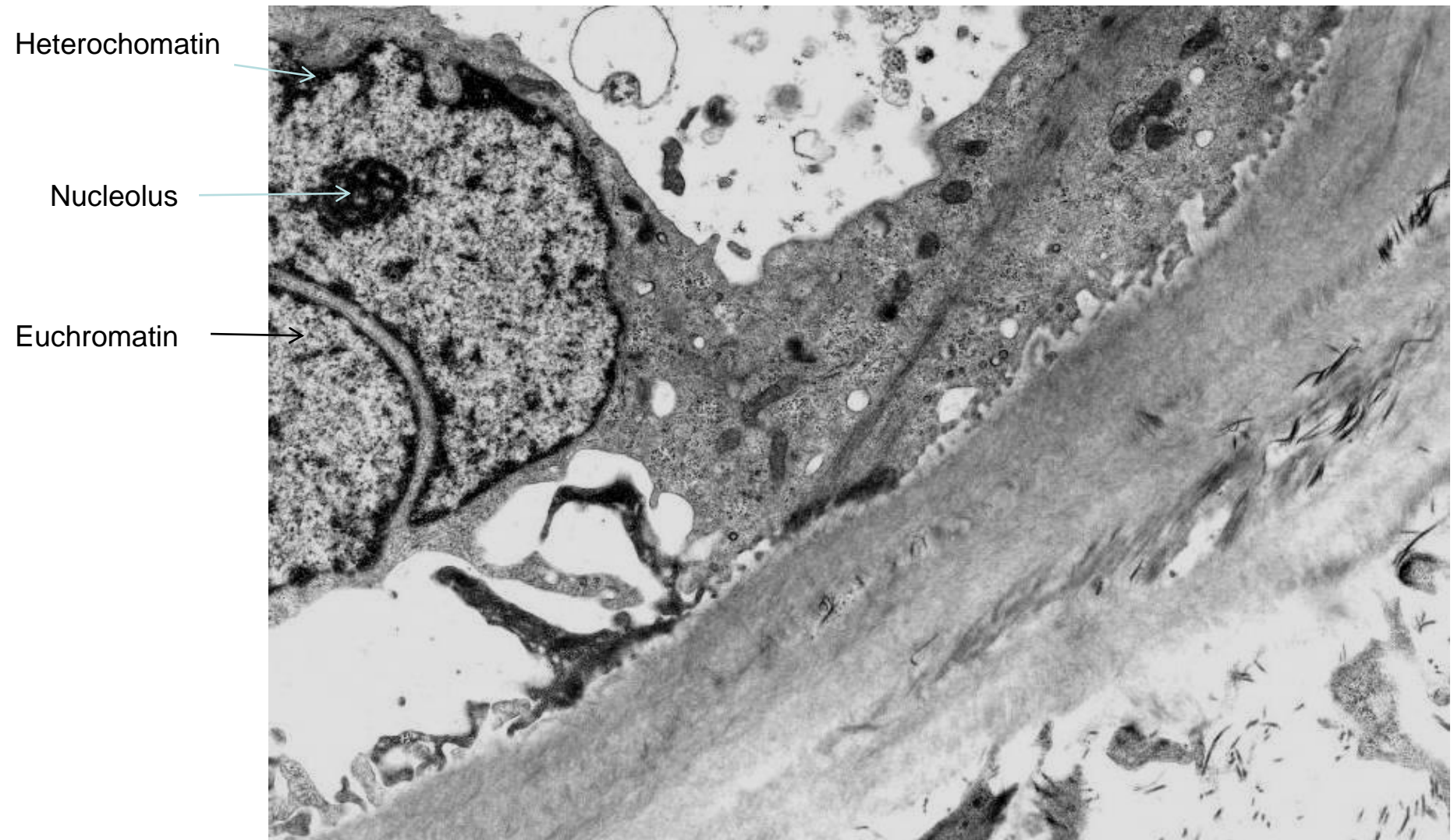




Schwann cell partly wrapping around neuronal cell processes  
Non-myelinated nerve in renal cortical interstitium

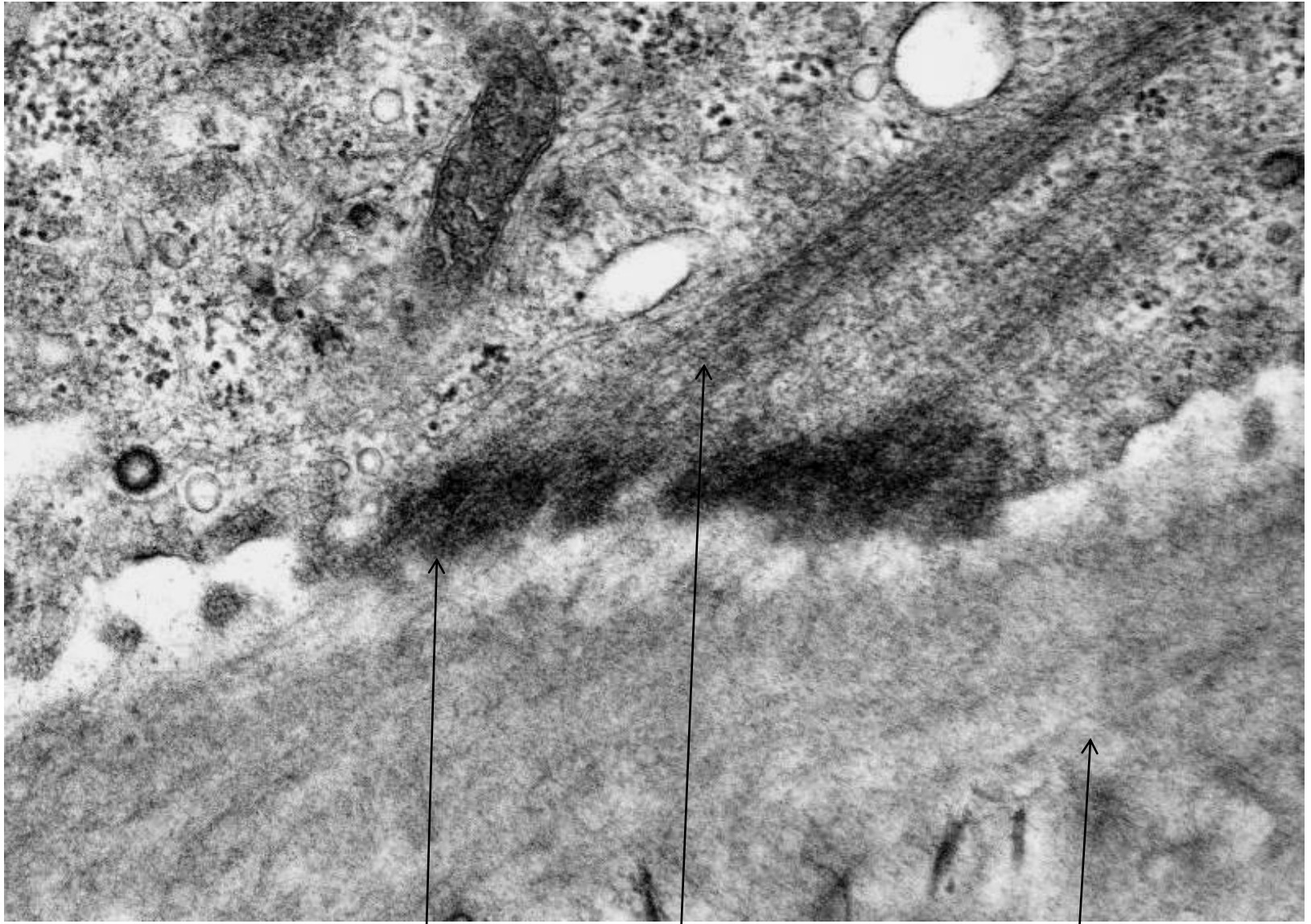
Innervation of smooth muscle  
cells of afferent and efferent  
arterioles

Cells lining Bowman's space/parietal epithelial cell sitting on Bowman's capsule





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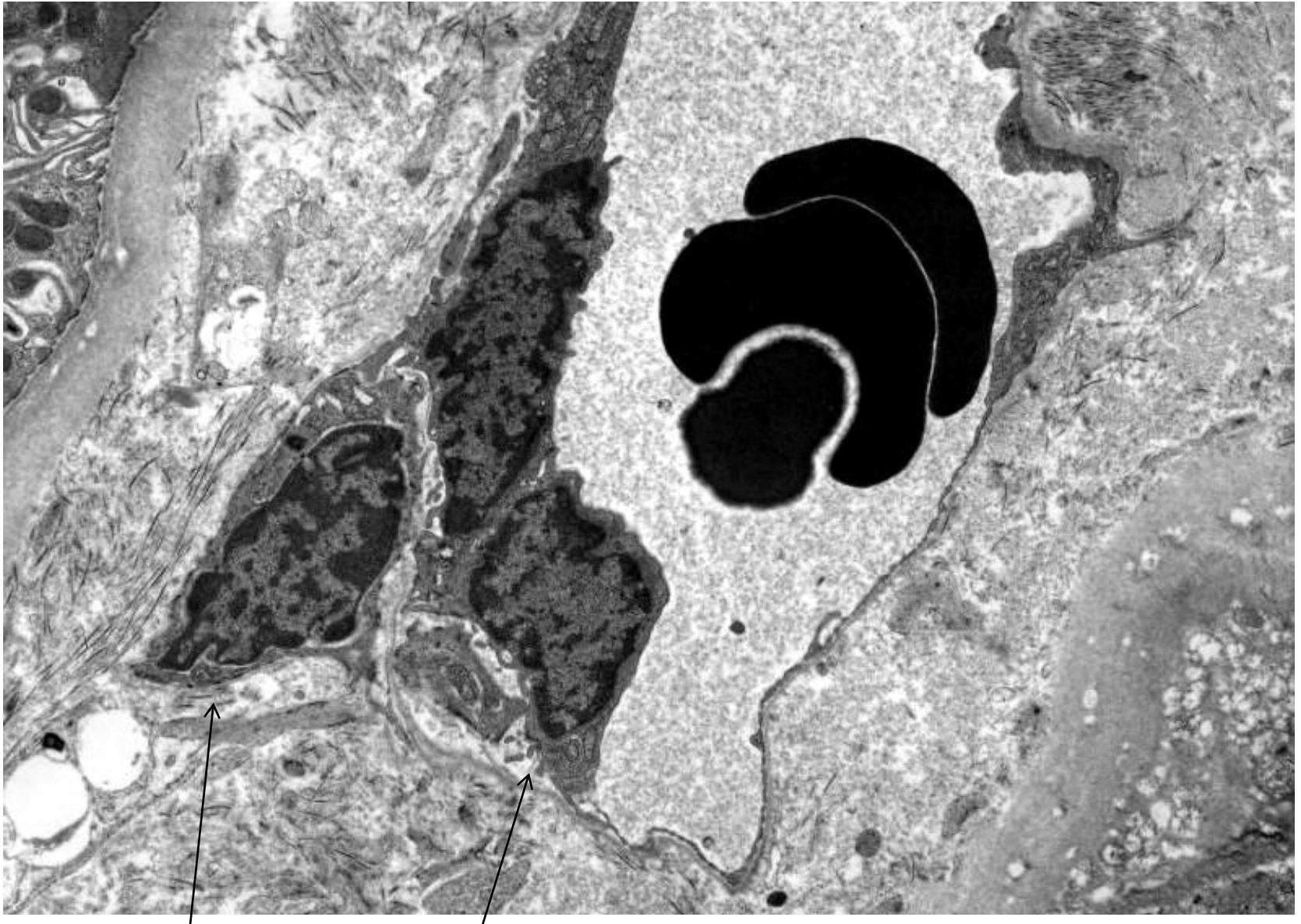


Matrix attachment point/plaque

Cell cytoskeleton

Bowman's capsule

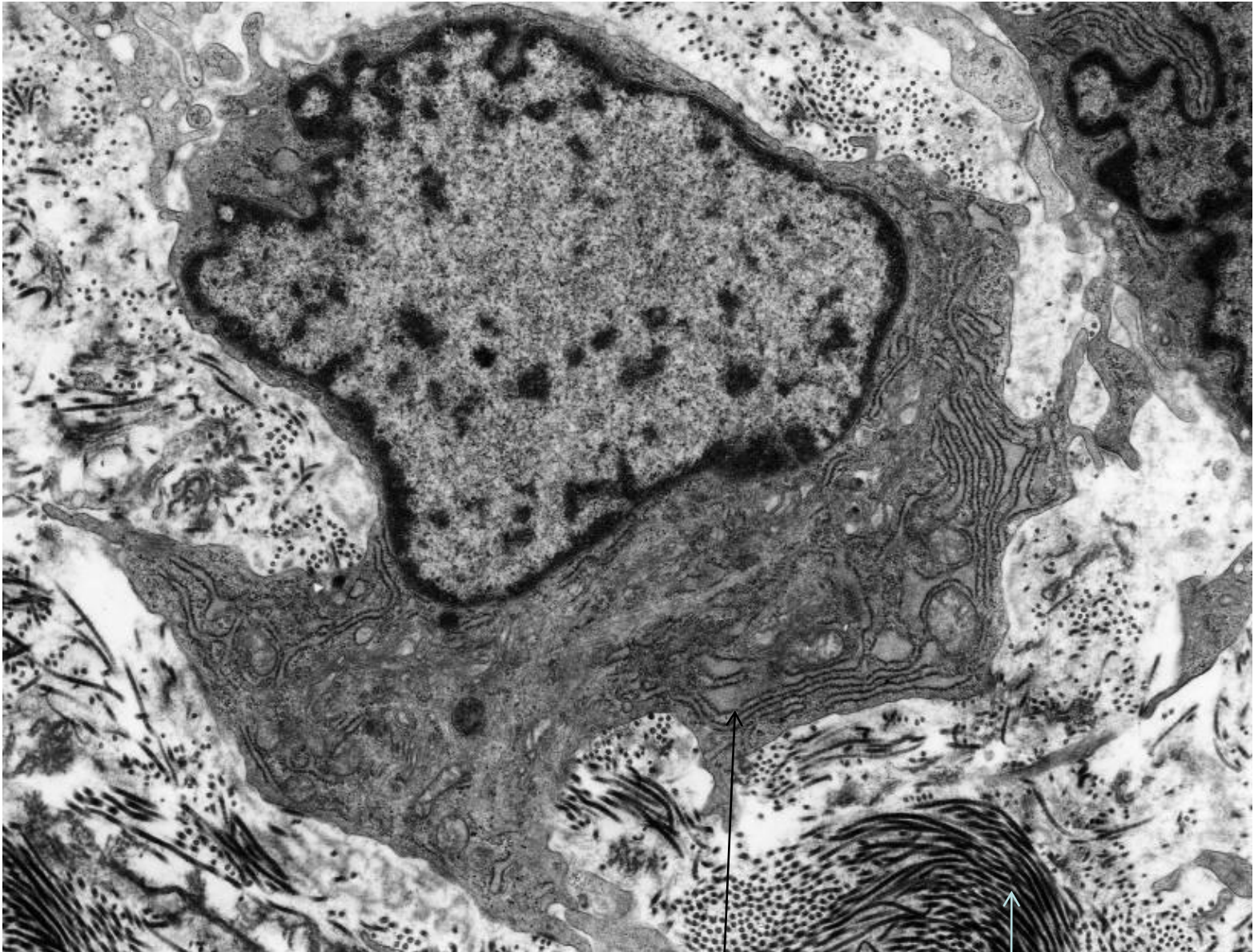
Peritubular capillary



Pericyte and endothelial cells



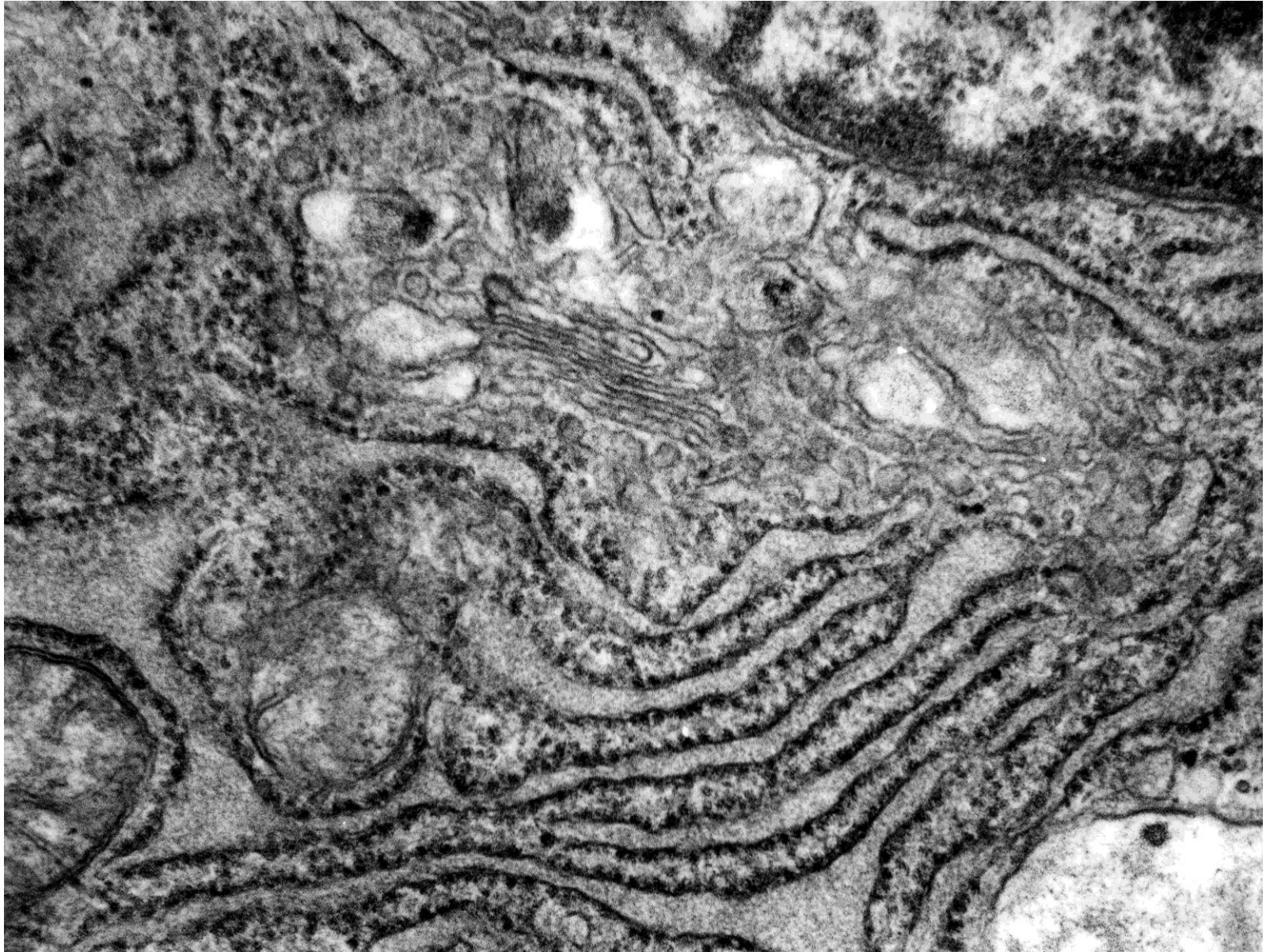
## Renal cortical interstitial fibroblast



Abundant rough endoplasmic reticulum

Fibrous collagen

Higher magnification of previous slide



Renal interstitial fibroblast



## Toluidine blue stained plastic section of renal cortical tubules

Distal  
convoluted  
tubule

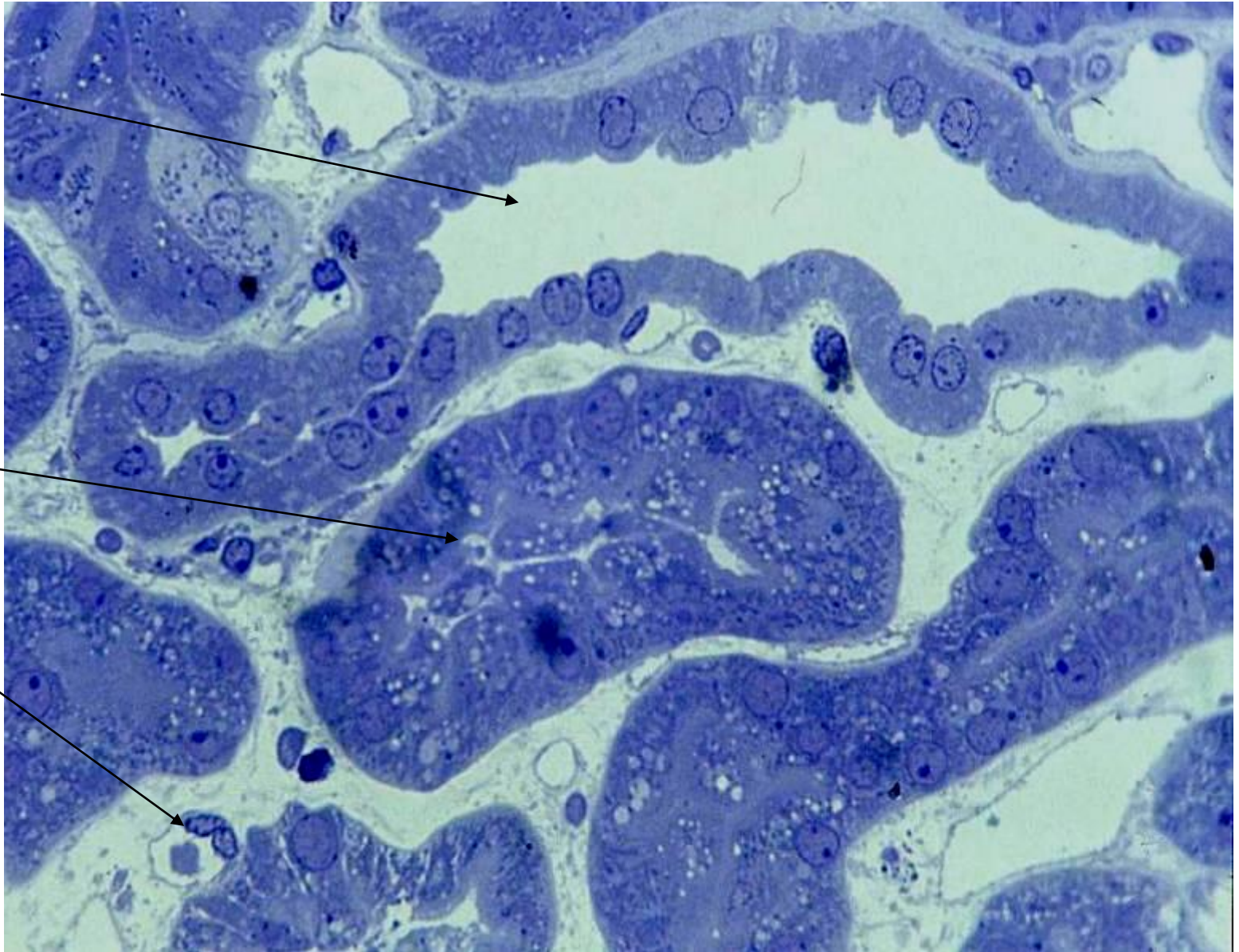
DCT

Proximal  
convoluted  
tubule

PCT

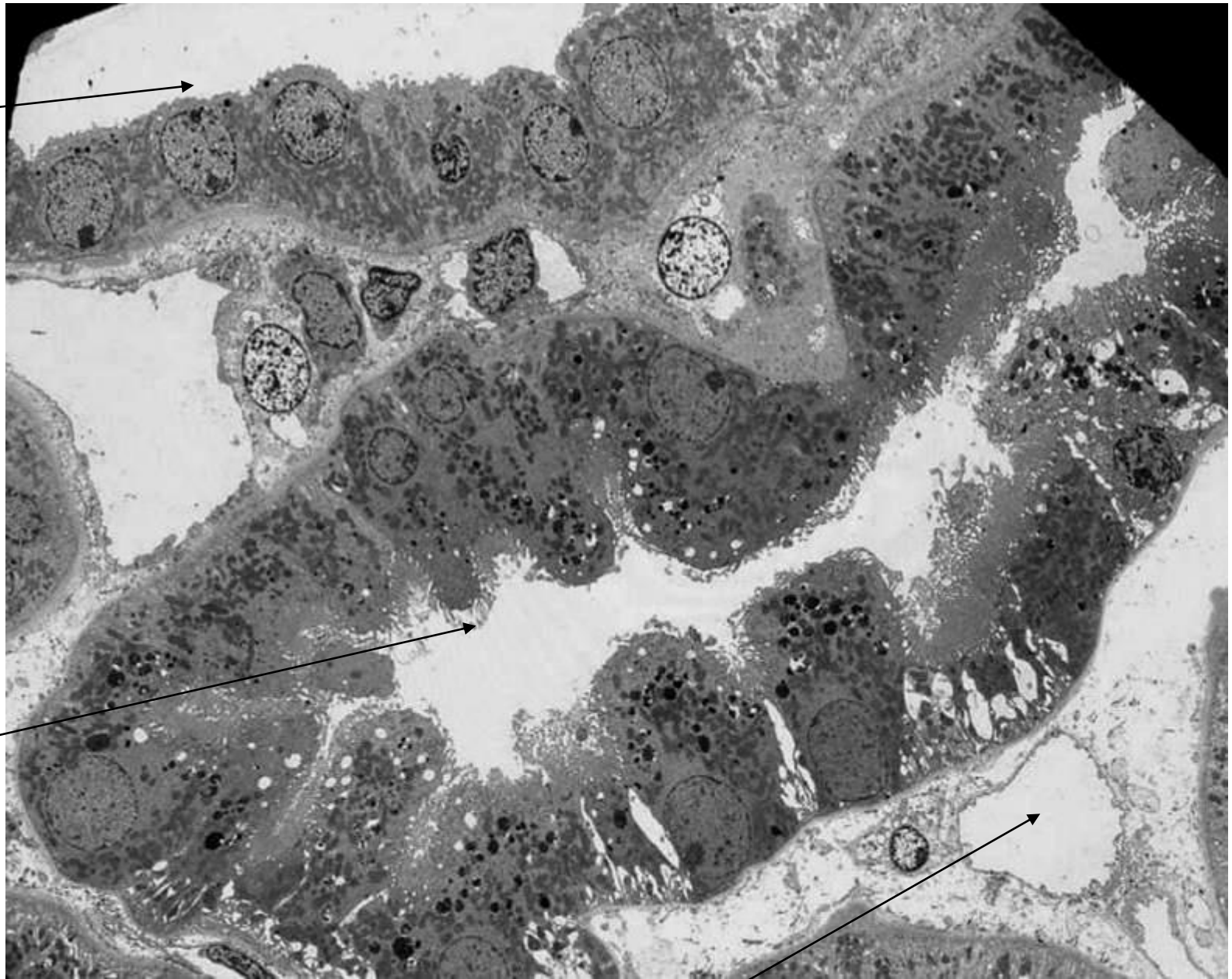
Peritubular  
capillaries

PTC



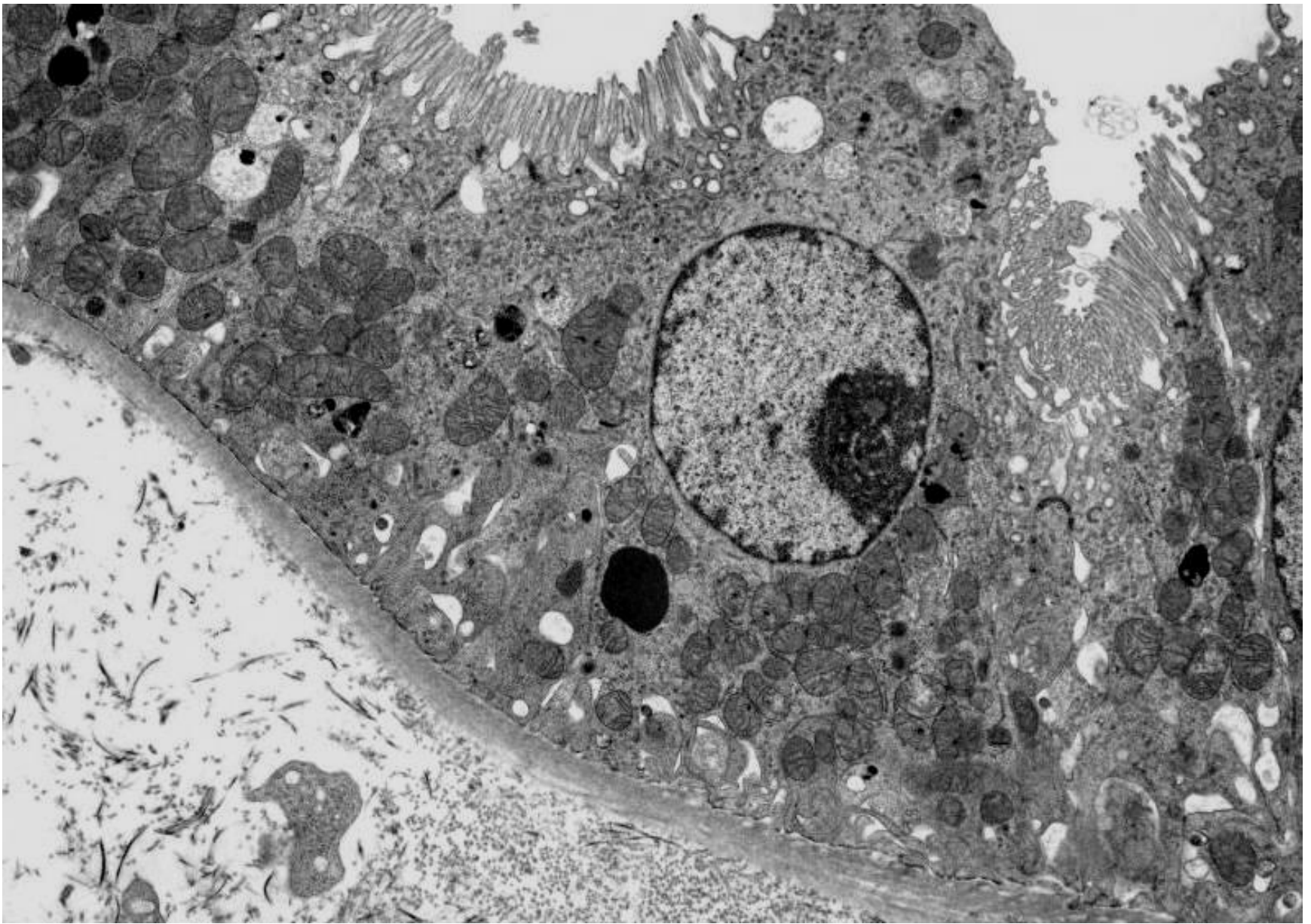
Distal  
convoluted  
tubule

Proximal  
convoluted  
tubule  
Note:  
apical  
microvilli



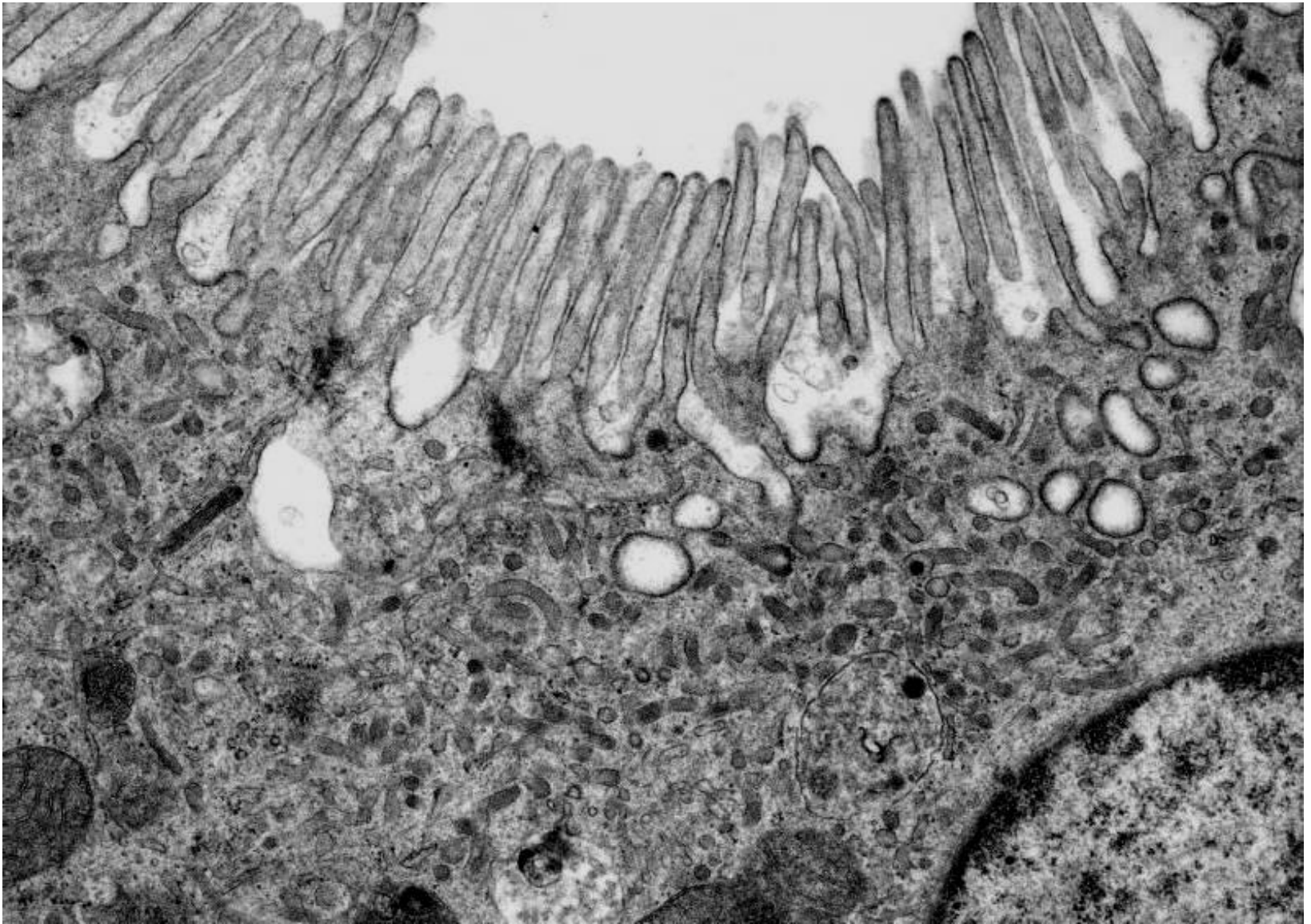
Peri-tubular capillary





Proximal convoluted epithelial cell

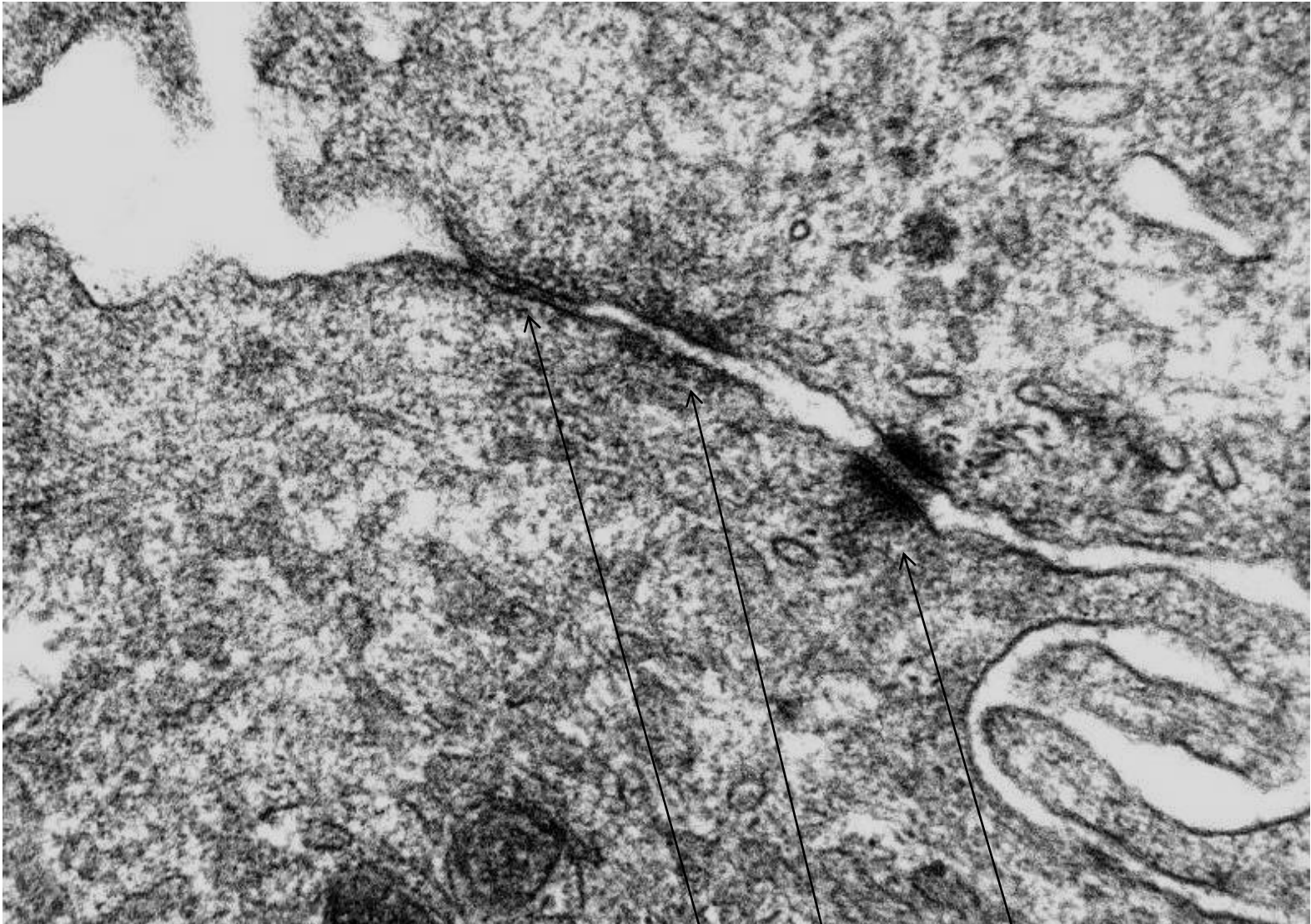
## Apex of proximal convoluted epithelial cell



Microvilli to resorb water via aquaporins, apical vesicles to resorb and transport peptides, smooth endoplasmic reticulum to replace cell membrane.



# Apex of tubular epithelial cell



Junctional complex

Tight, intermediate, desmosome junctions

Zonula occludens, zonula adherens, macula adherens

Tight junction

Intermediate junction

Gap junction

Desmosome



Junctional complex - endocervix



Zonula occludens

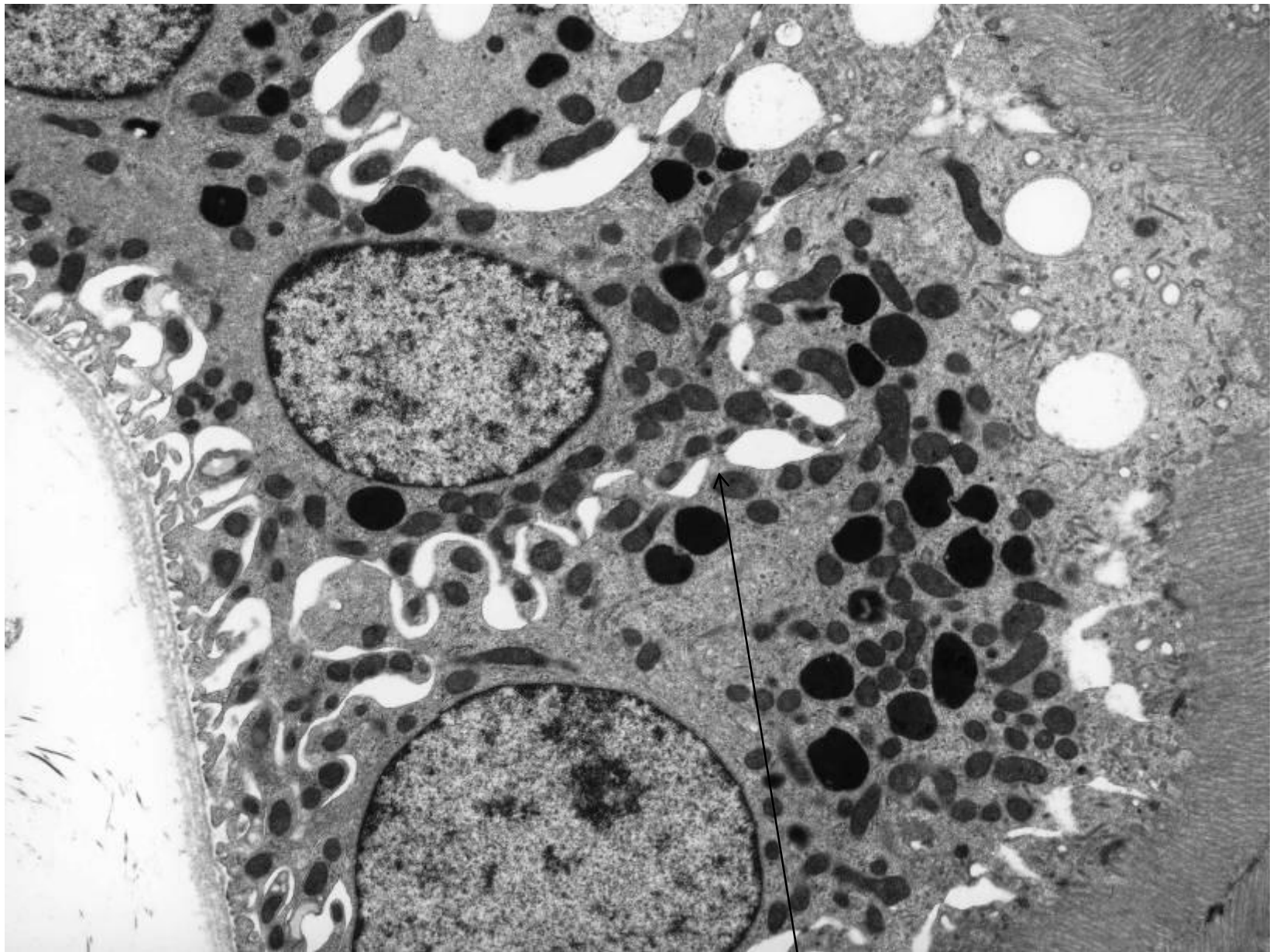
Zonula adherens

Gap junction

Macula adherens



Junctional complex - endocervix

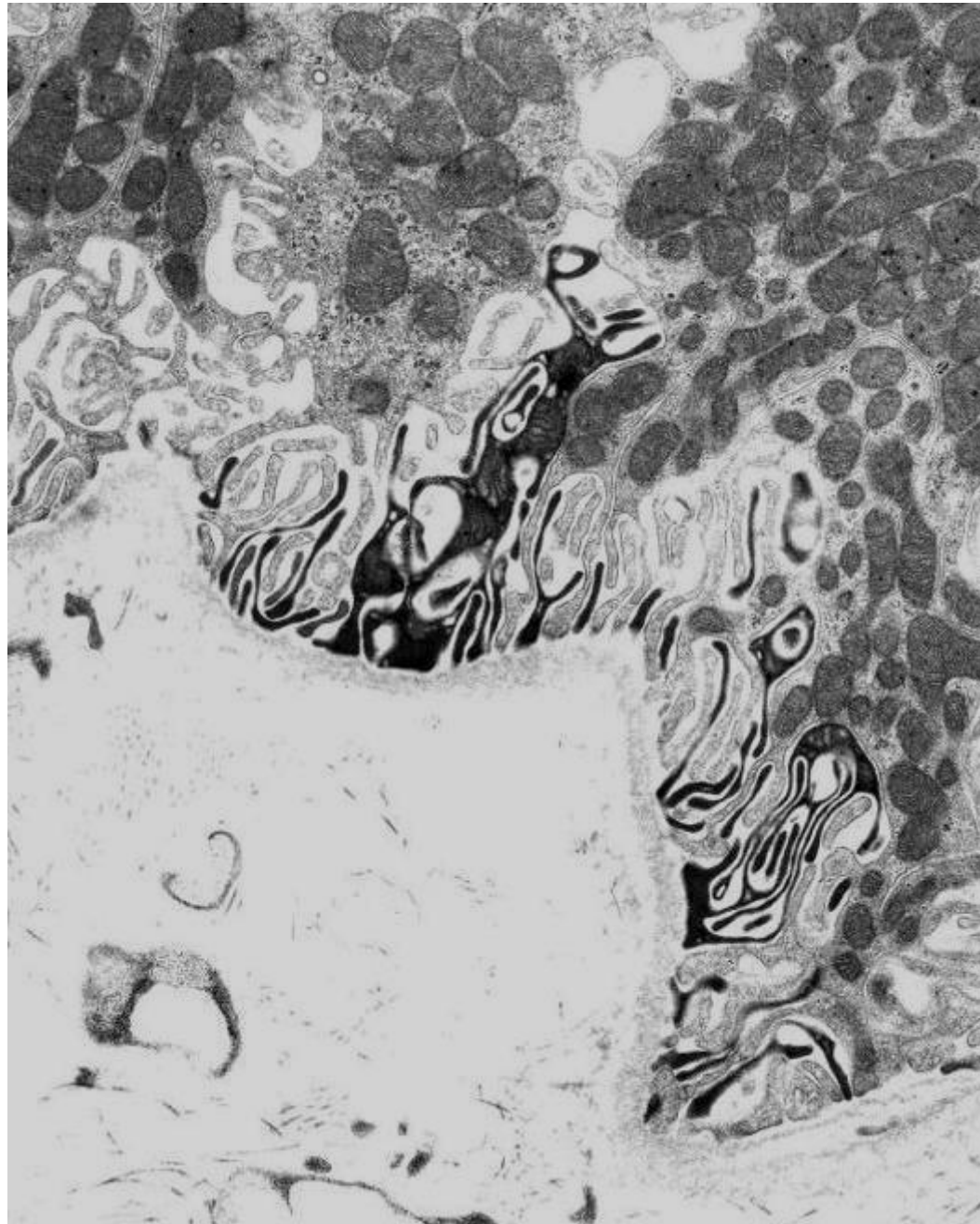


Proximal convoluted tubular epithelial cell

Cell shrinkage demonstrating lateral border attachment points

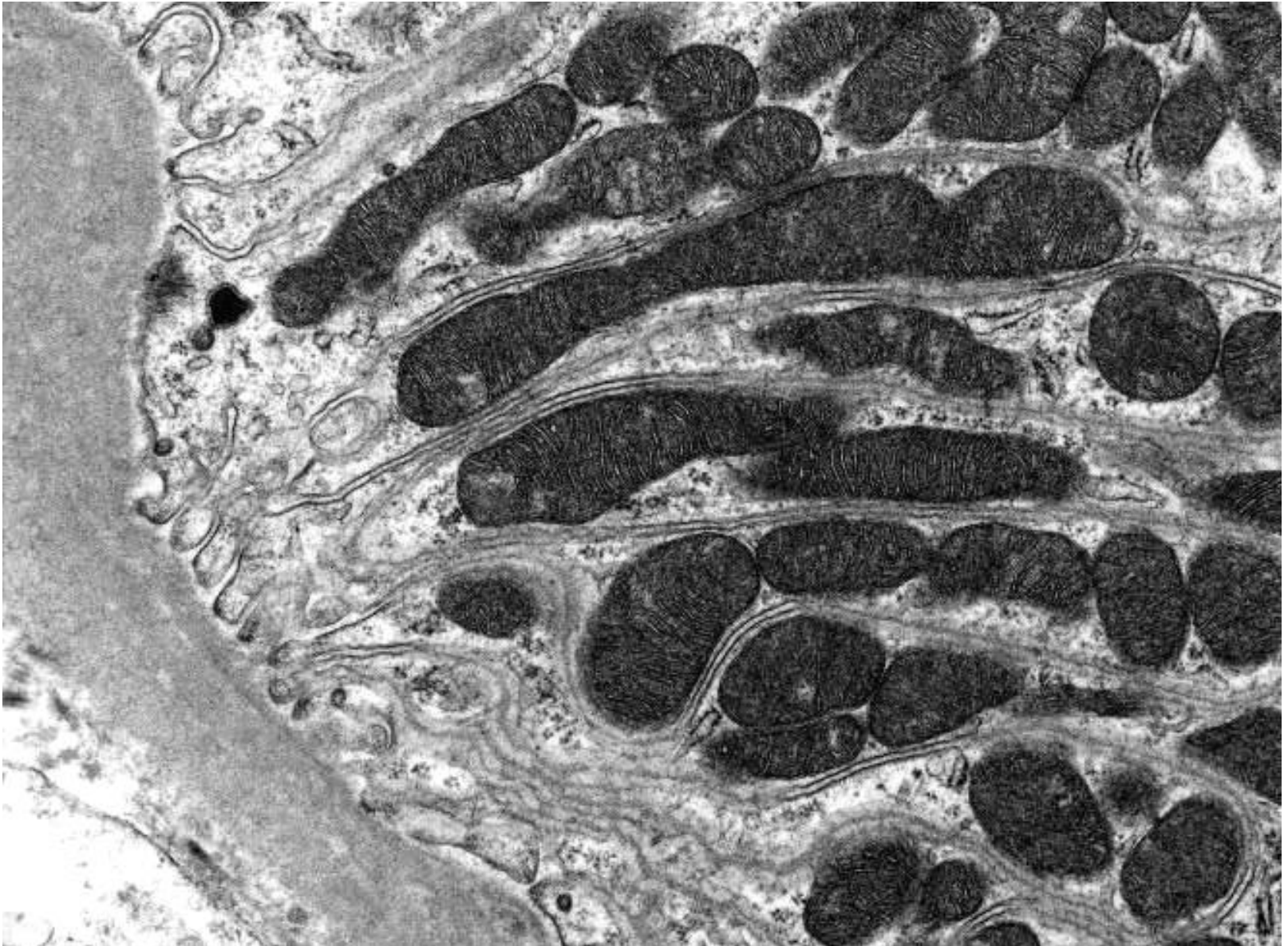


Base of tubular  
epithelial cell,  
folding and  
interdigitating with  
neighbour



Light cell dark cell  
phenomenon

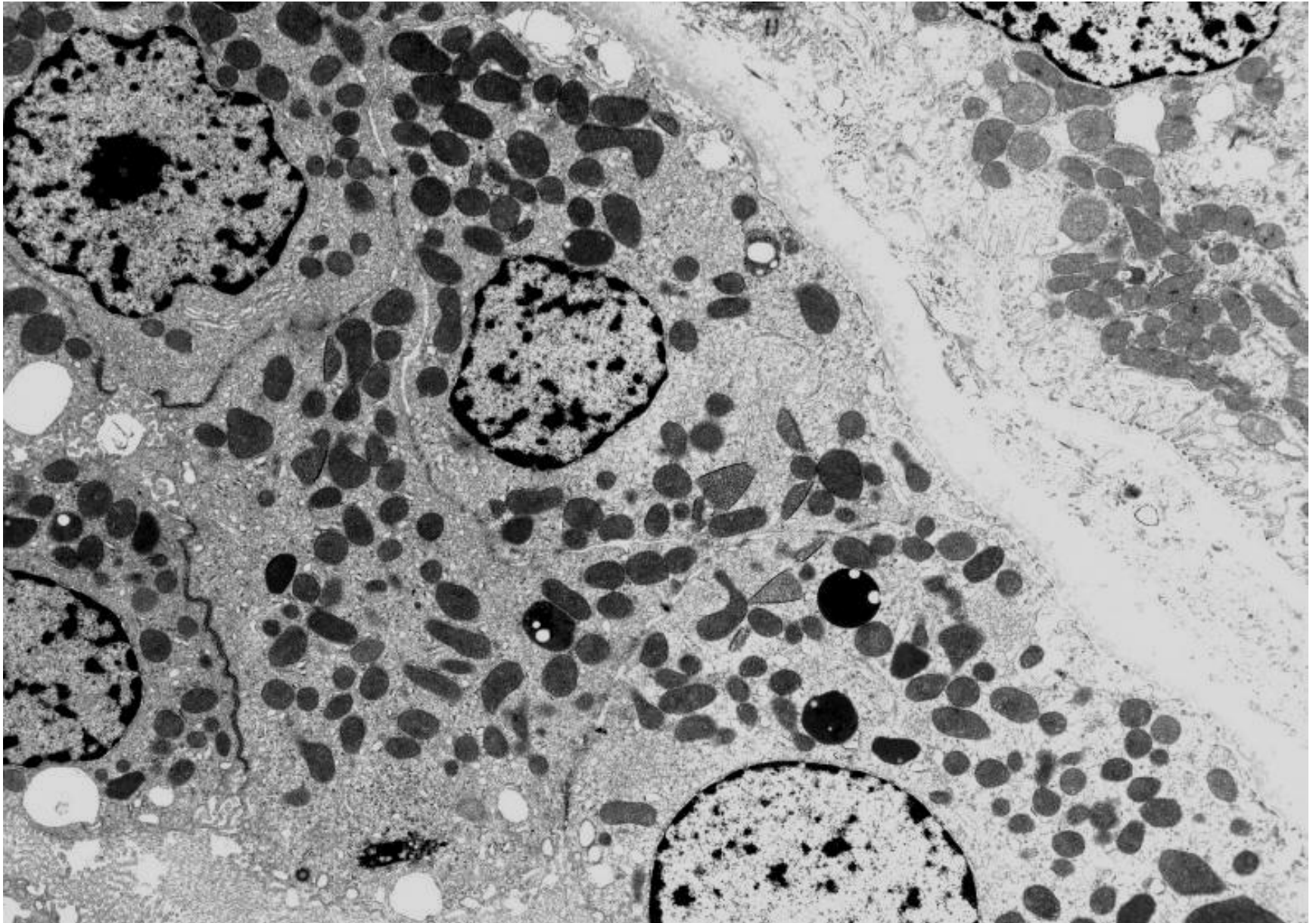
Base of tubular epithelial cell



Numerous mitochondria

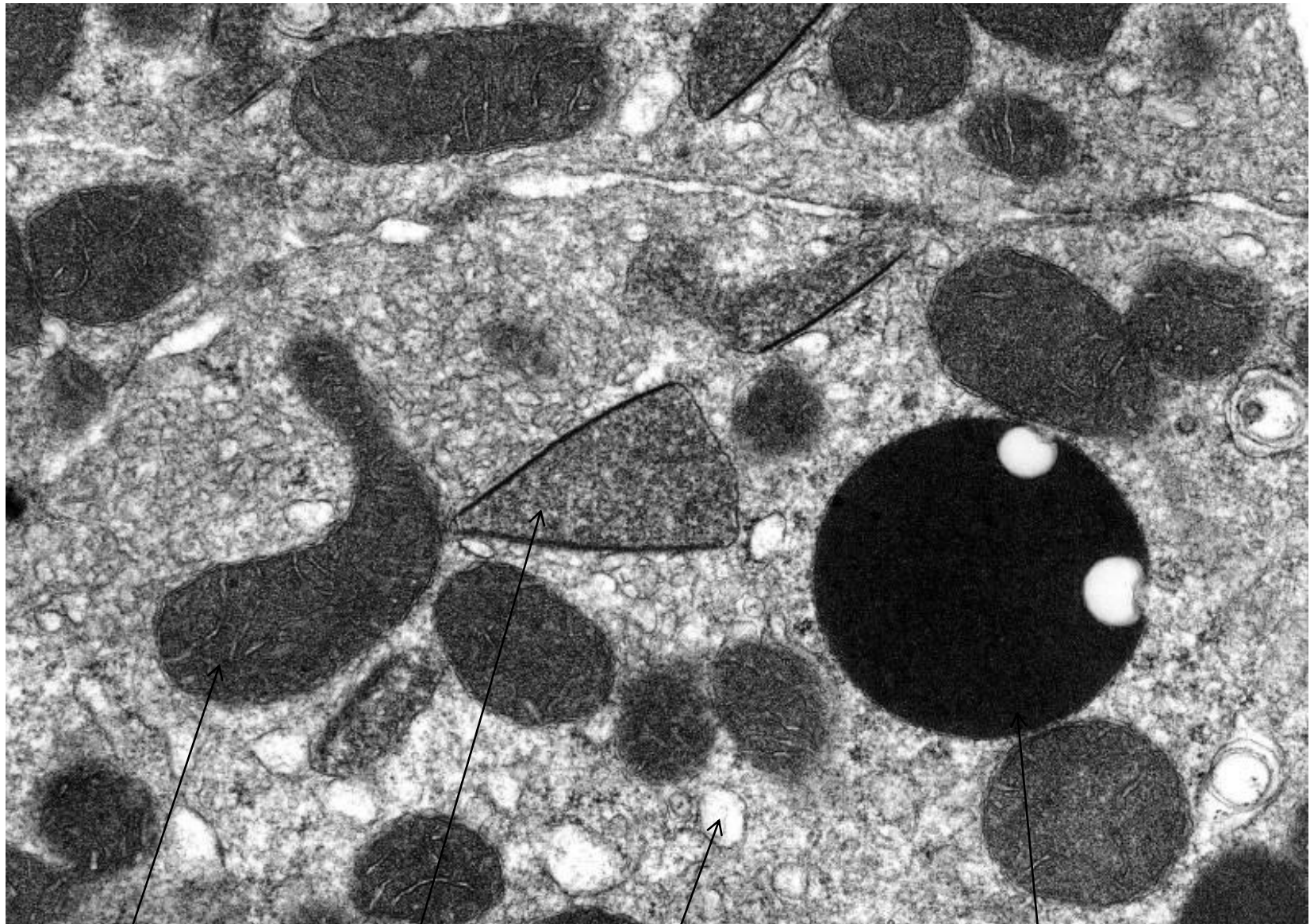


Proximal convoluted tubular epithelial cell



Proximal tubular epithelial cell

Higher magnification of previous slide



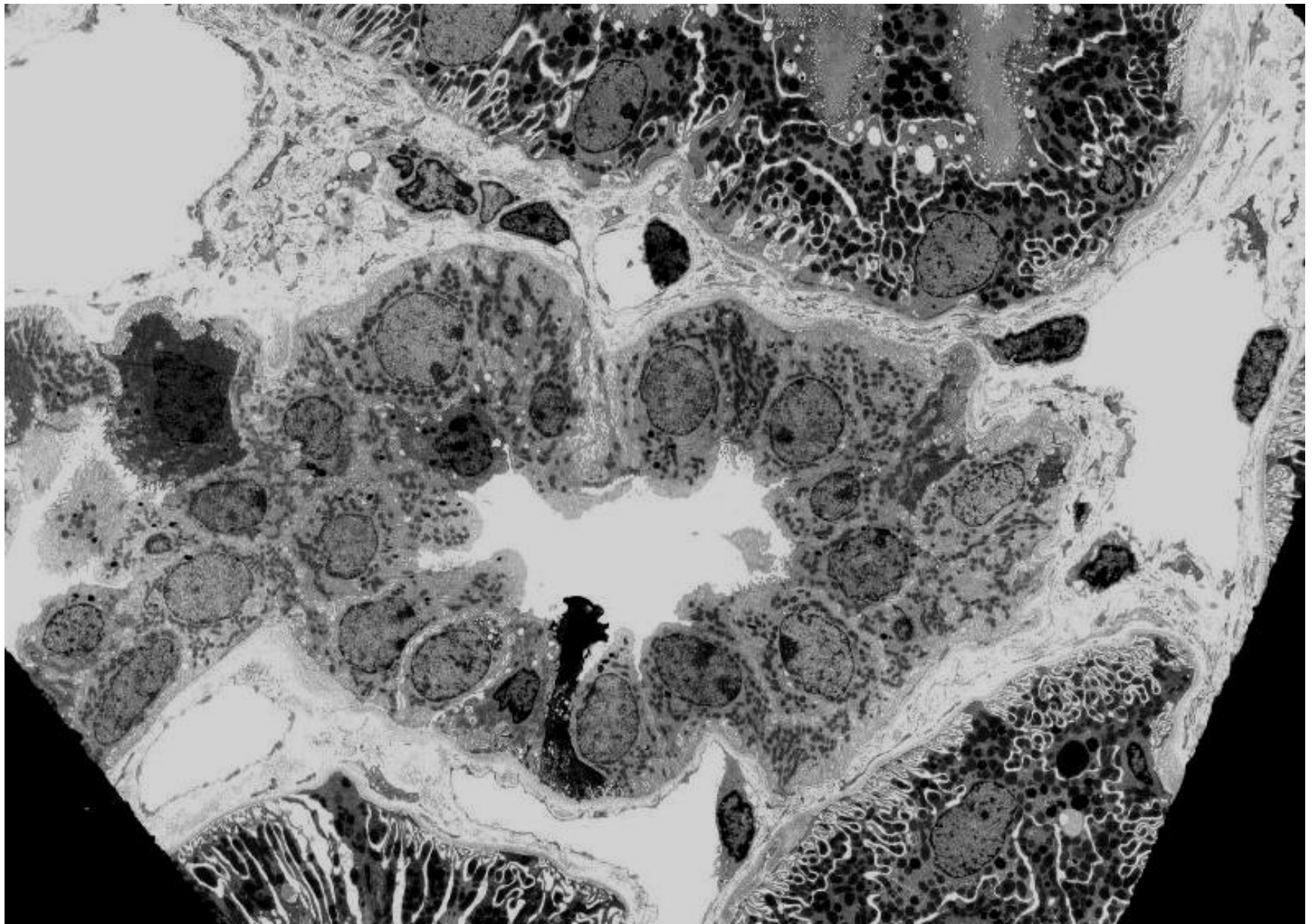
Mitochondrion

Peroxisome

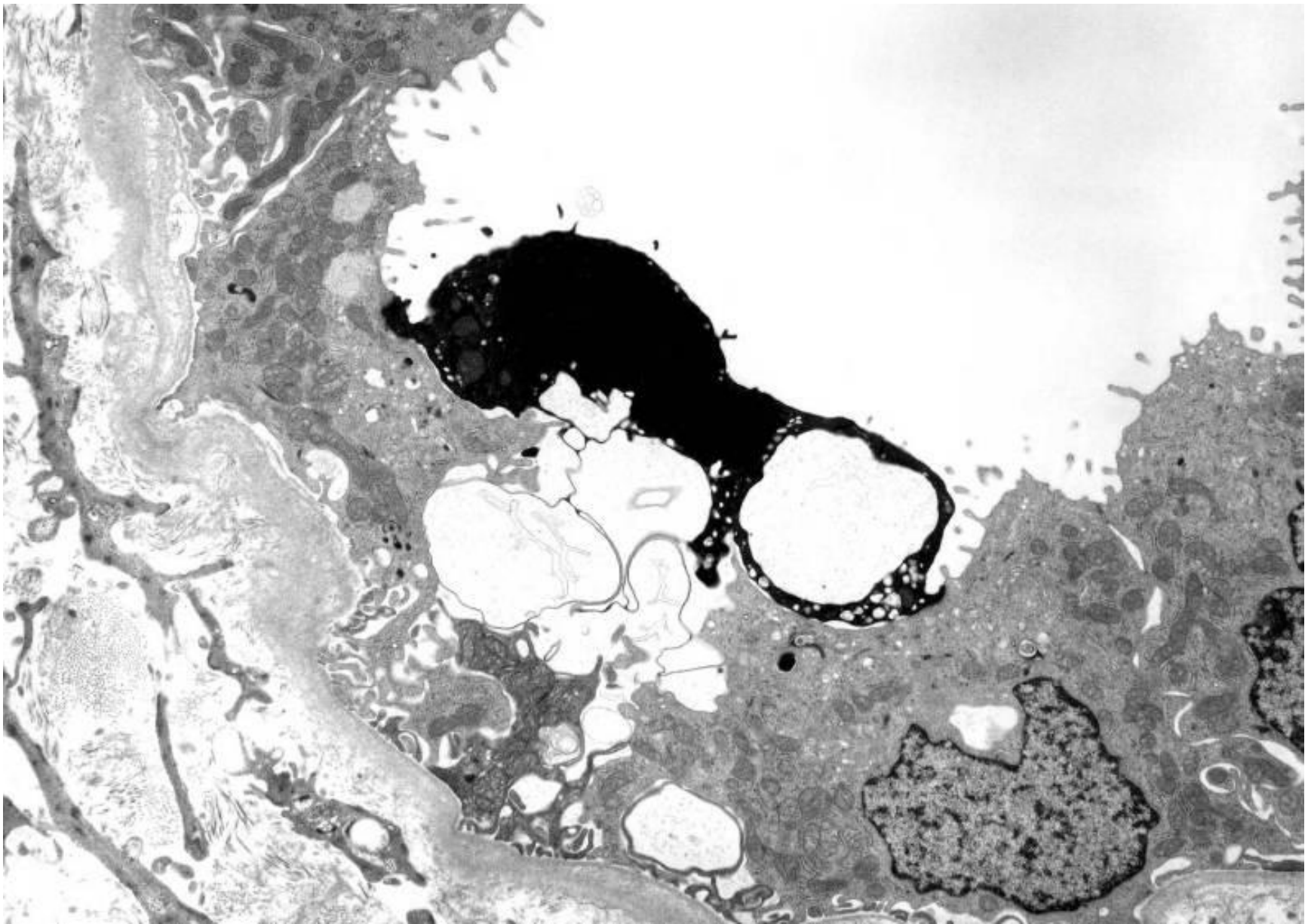
Smooth endoplasmic  
reticulum

Lysosome





Distal convoluted tubule



Distal convoluted tubule cell possibly  
having undergone apoptosis

Time for a quick break?

‘The mind cannot absorb what the backside cannot endure’



Prince Philip ,The Duke of Edinburgh.