

1962 Lophius study except that urine here was obtained directly in chronic preparations by ureteral catheterization in order to shorten the collection periods and more accurately measure urinary volumes. Observations of excretion rates were made with falling plasma concentrations of iodopyracet and PAH subsequent to intramuscular injections of the compounds 3-4 hours before taking the first plasma sample and starting urine collections. In a typical experiment to determine iodopyracet plasma values needed to obtain saturation of the carrier mechanism 40 mg/Kg injection into a 4.2 Kg fish gave a plasma iodopyracet concentration of .04 μ mole/ml at the midpoint of a 2.25 hr urine collection period during which the urine flow was 1.5 ml/Kg x hr. The iodopyracet excretion rate in this instance was 5 μ mole/Kg x hr. The average Tm_I for three separate determinations on this animal was 6.25 μ mole/Kg x hr, which is comparable to average values of 6.05, 5.62, and 5.51 μ mole/Kg x hr for 3 fish in the 1962 series. Saturation of the iodopyracet transfer system occurred when plasma concentrations were approximately .02 μ mole/ml, and when values fell below this there was a linear decline in excretion rates extrapolating to zero.

Far below saturation of the common carrier iodopyracet was found to be excreted more rapidly than PAH, either when each was presented alone or simultaneously in plasma concentrations lower than those needed to provide maximal transport rates. Thus, both at high saturation of carrier as noted in the 1962 series, and currently with low saturation the behavior of these transport competitors appears to obey Michaelis-Menton kinetics as discussed by Rosenberg and Wilbrandt (Exptl. Cell Research 9:49-67, 1955). Iodopyracet seems to have the higher affinity for the common carrier involved in the rate limiting step of this transcellular secretory process. At low saturation it would be predicted that transfer rates of various competitors would depend on the reciprocal of K_m and hence be proportional to the respective affinities; whereas with high saturation the rates of penetration would be proportional to K_m , i.e., inversely proportional to carrier affinity. The transport characteristics of PAH and iodopyracet at both high and low plasma levels comply with this interpretation.

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THE ANATOMY, MICRO-ANATOMY, AND ULTRASTRUCTURE OF THE KIDNEY OF THE DOGFISH, Squalus acanthias

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An increasing interest in kidney function in the spiny dogfish, Squalus acanthias, and the initiation of micropuncture studies in this experimental animal have prompted us to examine the microanatomy of its nephron. This was accomplished by a combined approach using light and electromicroscopy and microdissection.

Microdissection of the nephron of Squalus acanthias is more difficult than in mammalian kidneys because of its greater length, its more intricate coiling and looping, the intermingling of loops of one portion amongst loops of a separate but adjoining portion of the same nephron,

and the extreme fragility of its second or thinnest segment. Observations were made on approximately 125 nephrons incompletely dissected, and on 26 complete nephron dissections.

Gross Morphology

The dogfish kidney is a paired structure situated paravertebrally in close approximation to the dorsal body wall. The renal mass is about 2.8 gm/kg body weight. Each kidney in the male begins as a ribbon-like structure about the middle of the abdominal visceral compartment and gradually widens as it extends caudally to form a roughly triangular mass which partially fuses with the other kidney dorsally to overlap the cloaca. The intervening paravertebral tissue mass filling the interval cephalad of the kidney is comprised of the coiled up epididymis. In the female, the kidney substance begins as a slender ribbon about 1.5 cm caudal to the coracoid cartilage. The Wolffian duct in the female is a vestigial simple straight tube which runs caudally along the ventral surface of the kidney. The adrenal chromaffin tissue in the dogfish is a solid rod of endocrine tissue, quite separate from the inter-renal gland, lying imbedded in the caudal portion of the renal mass.

Arterial Supply (Fig. 1)

The renal arteries arise as ventral branches of segmental arteries of the dorsal aorta. Their distribution to the long thin cephalic portion of the kidney is markedly different from that to the thicker caudal region.

On the average the slender cephalic portion of the kidney (2/3 of its total length) is supplied by 4 renal arteries of medium size. They arise irregularly, divide, and anastomose within the

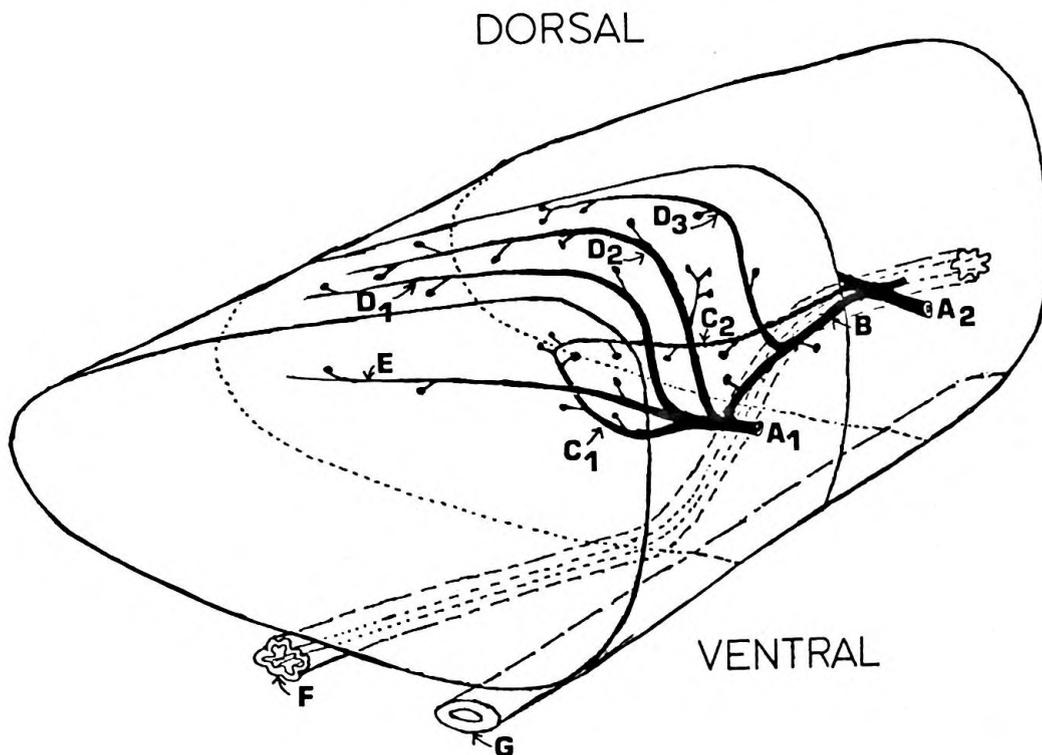


Figure 1. Schema of arterial supply to the kidney. A₁, A₂, renal arteries; B, intrarenal arterial chain; C₁, C₂, ventral arcuates; D₁, D₂, D₃, dorsal arcuates; E, horizontal artery; F, archinephric Wolffian duct; G, ureter.

kidney parenchyma near its medial border. The branches give rise to afferent arterioles supplying glomeruli distributed randomly in the renal substance. This entire portion has approximately 100 glomeruli.

The caudal 7-8 cm of the kidney receives 14-16 large renal branches (Fig. 1, A₁ and A₂) from the segmentals. These enter the kidney near its dorso-medial border. Each gives off a cephalic and a caudal branch which anastomose, forming a longitudinal arterial chain (Fig. 1, B) within the parenchyma. Branches from the renals and the arterial chain supply this portion of the kidney.

Further branching of renal arteries gives rise to 3 levels of arcuates, one ventral, two dorsal and one horizontal branch (Fig. 1, C, D, E). The ventral arcuates of 2 renal arteries join to form a loop (Fig. 1, C₁ and C₂) close to the ventral surface of the kidney. The dorsal aspect of the kidney is supplied by the dorsal arcuate branches. The horizontal branch generally arises from the renal artery and courses deep into the renal mass (Fig. 1, E). In the more caudal region this general pattern is preserved but each of the above branches may subdivide further. Afferent arterioles arise from all subdivisions of renal arteries and the intrarenal arterial chain. In the aggregate these combined afferents of the caudal portion give rise to approximately 4300 glomeruli. The total glomerular count per kidney is, therefore, 4400.

Venous Supply

The kidney has a renal portal circulation. The veins of the tail join to form a large trunk, the caudal vein, which divides dorsally near the cloaca to form the renal portal veins. These course cephalad along the dorsal lateral border of the kidney giving off numerous branches which enter the renal parenchyma. The two renal portal veins along their course receive segmental veins from the body wall in the region of the kidney. Within the renal parenchyma blood from the renal portal veins mixes freely with that derived from the vasa efferentia of the glomeruli in the large intra-renal sinuses.

Description of the Nephron

The dogfish nephron consists of a glomerulus and a very lengthy convoluted tubule ending at the collecting duct (Fig. 2). In 26 completely dissected specimens the nephron averaged 3.3 cm in length. In sections, the glomerulus, its afferent and efferent vessels and interposed portion of a distant segment of the tubule form a composite structure in two dimensions that is very similar to the mammalian glomerulus and juxtaglomerular apparatus. Bowman's capsular space empties into the tubule which emerges from the anti-hilar pole of the Malpighian corpuscle. The course of the tubule is relatively long and elaborately convoluted and is composed of six morphologically distinct segments. The last portion returns to approach the Malpighian corpuscle, being applied directly against Bowman's capsule, before emptying into a branch of the collecting duct. The extreme convolution of the tubule does not allow a clearly discernible division of the renal parenchyma into cortex and medulla.

Glomerulus

The glomerulus resembles the mammalian glomerulus closely, in dimensions and in arrangement of capillary and mesangial tissue, and in form of the capsular space. The average of thirty measurements made on macerated specimens are as follows:

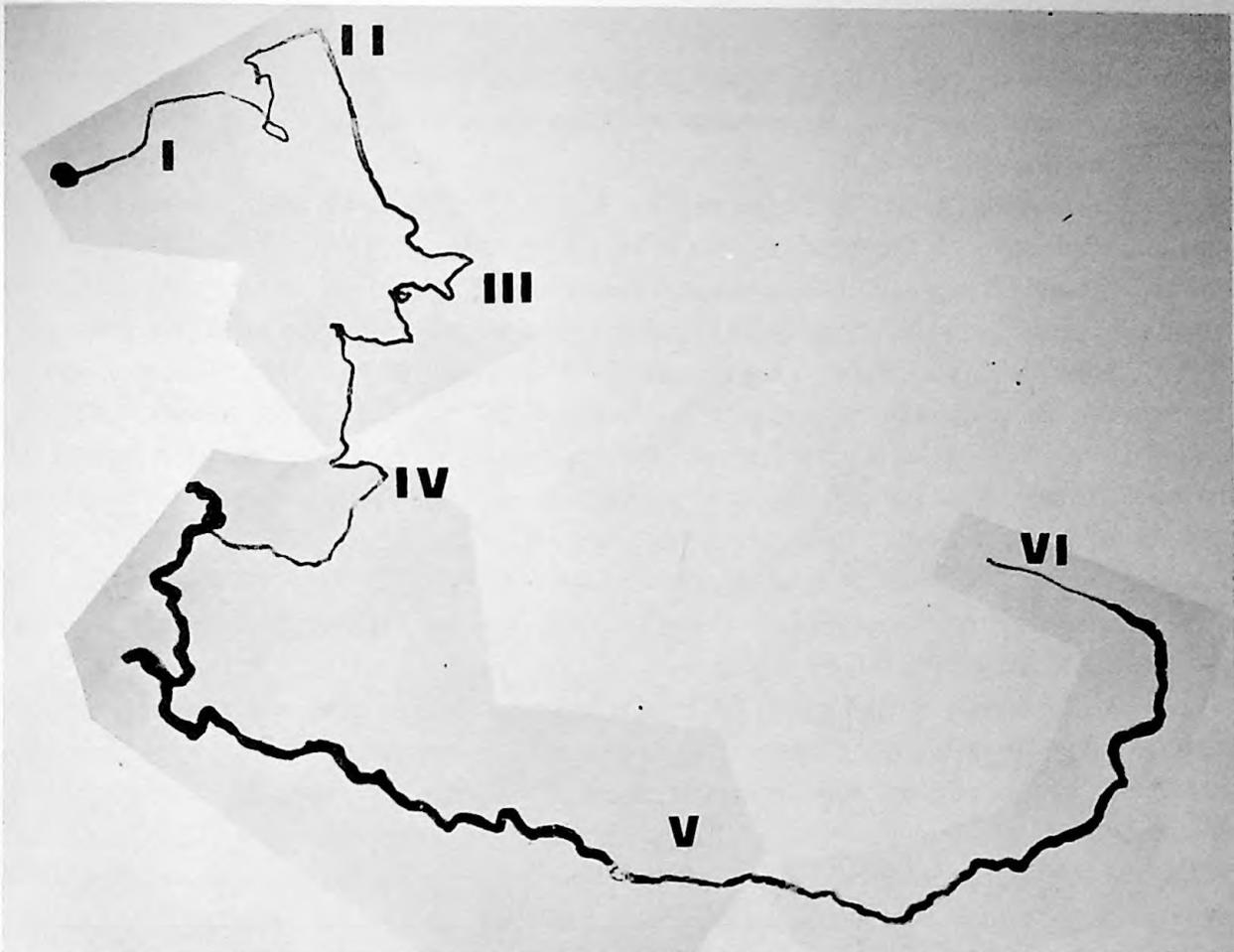


Figure 2. Complete nephron microdissected intact and photographed with reflected light, in water (x 15).

Polar axis of glomerular tuft - 139 microns,
 Transverse axis of glomerular tuft - 169 microns,
 Polar axis of Bowman's capsular space - 230 microns,
 Transverse axis of Bowman's capsular space - 180 microns.

Glomerular cellularity, including epithelial, endothelial and mesangial cells ranged from 130 to 170 cells per tuft (blood cells were not counted). The visceral epithelium covering the capillary loops is applied to a well-formed basement membrane, and on Bowman's capsule the parietal epithelium is applied to a fibrillar connective tissue basement membrane, which is in turn applied against the homogeneous basement membrane of a tubule. The capillary loops of the tuft are usually widely patent and mostly free of blood cells. The basement membrane is in general more dense than its mammalian counterpart, and more fibrillar than homogeneous in composition. The mesangial or stalk tissue has the usual arborising form and is sparsely cellular. This arborization creates a lobular form of the tuft. The tissue about mesangial cells is usually fine and spongy but occasionally is thick and hyalin. Bowman's capsular epithelium is flat and non-ciliated except at the margins of the exiting tubule where the cells are cuboidal and ciliated.

The Tubule

We have divided the tubule of the dogfish kidney into six segments, based on differences evident from the combined observations made by micro-dissection, light microscopy and electron microscopy (Fig. 2).

Segment I. (Average length of Segments I and II is 1.52 ± 0.3 mm). The tubule emerges from the antihilar aspect of the capsular space, runs perpendicularly away from the glomerular tuft, for an average 0.3 mm and ends by gradual transition of form into segment II. The lumen is at first very small, widens gradually and then narrows somewhat again as it merges into segment II. The lumen of this segment is narrowest of the nephron. The outside diameter averages 31 microns. The lining cells at the origin in Bowman's capsule ("neck") are closely packed, cuboidal acidophilic cells having a cytoplasm of medium density, a central vesicular nucleus, a definite microvillous brush border, and many large cilia. Gradually the cells become low cuboidal, less microvillous, non-ciliated and the lumen widens. The cilia of this portion are very large, approximately 2 microns in diameter, and of a length equal to 3-6 cell transverse diameters. On microdissection this portion is dense and quite opaque following staining, and terminates by transition to the translucent segment II.

On electromicroscopy of Segment I, a laminated basement membrane of moderate density is seen supporting the epithelium. Externally the basement membrane is frequently in direct contact with the basement membrane of interstitial thin-walled blood vessels. The epithelium of this part of the tubule is regularly cuboidal having straight lateral margins. The basal cell membrane shows slight to moderate infoldings. Luminal surface microvilli are numerous. Numbers of large well-formed cilia are present in the lumen. These arise singly from occasional separate cells. The cells of origin of the cilia appear to be smaller and with a smaller nucleus than the non-ciliated epithelium, and appear to occur at intervals separated by 4-6 non-ciliated cells. Mitochondria occur in moderate numbers, are evenly distributed and mostly elongated in form. The endoplasmic reticulum is rather sparse and the Golgi apparatus not exceptionally developed. Vacuoles are small, moderate in number, and irregularly distributed in the cytoplasm. Occasional lysosomes occur in the cytoplasm on the luminal side of the nucleus. The nucleus is of light density with irregular areas of increased density at its periphery.

Segment II. Segment II commences as a gradual transition from the ciliated dense segment I, continuing perpendicularly away from the glomerulus to become a translucent, generally thinner, and non-ciliated tubule. At roughly about the first quarter of its length segment II abruptly turns back on itself and proceeds toward the glomerulus. Close to the glomerulus it merges into the thicker, more opaque and somewhat wider segment III. On the average segment II measures 1.1 mm long and has a diameter of about 0.03 mm.

The most typical area of this segment occurs at the loop it makes on itself. Here the tubular cells become very flat, irregular in size, have no brush border and possess a very simple cytoplasm with a relatively large nucleus (Fig. 3).

On electronmicroscopy the basement membrane is constantly applied to voluminous thin-walled blood vessels. The cells are less cuboidal and become flattened progressively along its length. The cytoplasm shows some marginal overlapping, is generally pale with fine vacuolation, and has a very sparse endoplasmic reticulum with few small mitochondria arranged peripherally. The nuclear material is lightly stained with areas of increased density at its periphery. There are no microvilli.

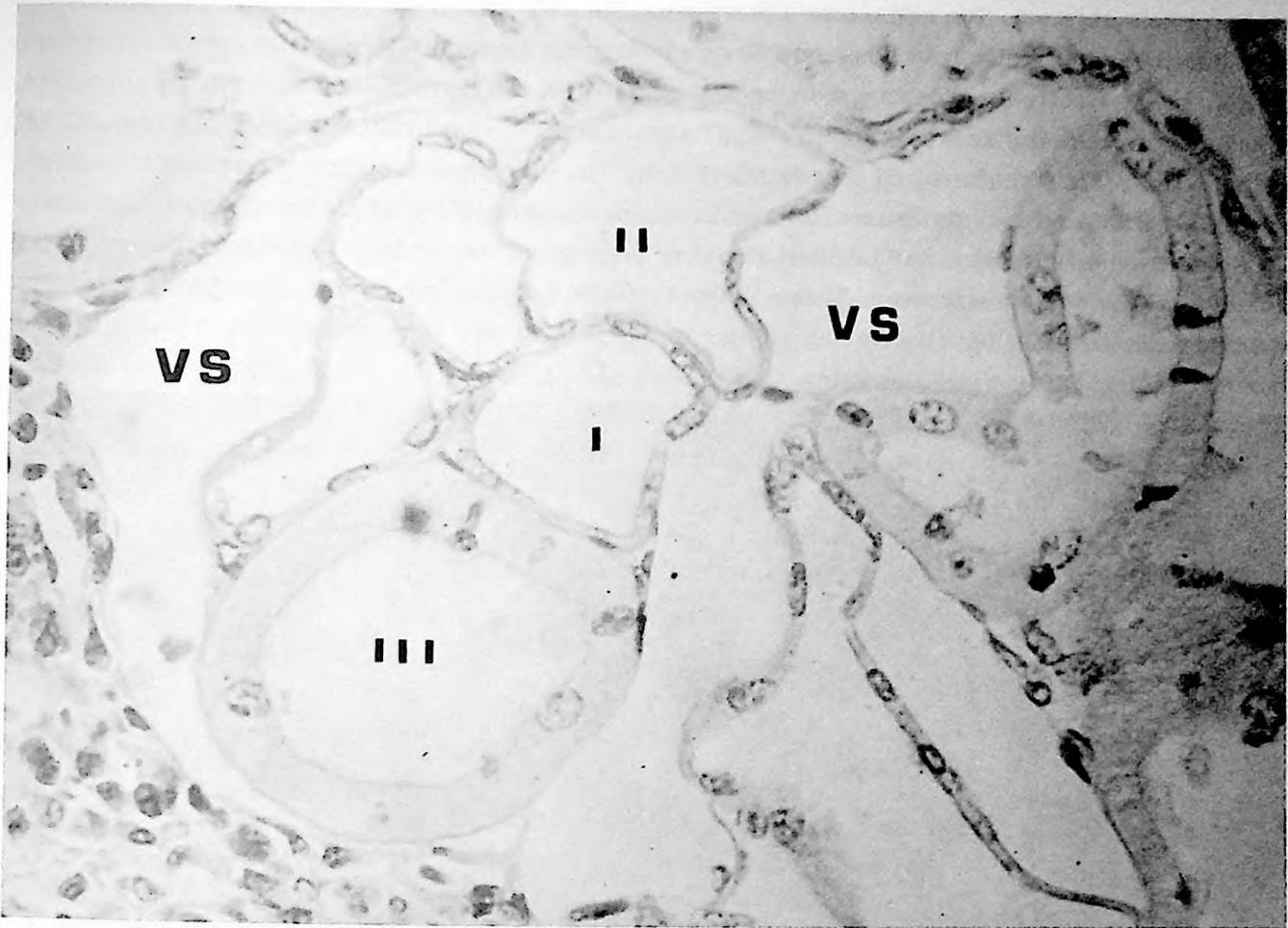


Figure 3. This intimate grouping of Segments I, II, and III is characteristic, as is the close apposition of venous sinuses (VS).

Segment III. (Average length of segments III and IV is 10.72 mm, average diameter is 0.04 mm.) Segments III and IV have the same external diameter and are convoluted. The greater part of these two segments lie within a cup-shaped hollow formed by the convolutions of segment V, and are intimately entwined with it.

In segment III the flat cells of segment II give way to very regular cuboidal dense cells with a definite brush border. These apparently are non-ciliated cells and closely resemble mammalian proximal tubular cells in light microscopy sections (Fig. 4). In cross-sections of segment III the lumen is wider than in segment II and is lined by 6-8 cells. The nuclei of the cells are round or oval, are located toward the luminal side of the cytoplasm, appear vesicular and have a prominent nucleolus.

On electronmicroscopy, the cells are cuboidal with no marginal overlap. The luminal surface is flat in some and bulging in others; it has well-developed microvilli. The cytoplasm has greater density due to prominent electron dense bodies of the lysosomal type. There is sparse endoplasmic reticulum, with a moderate number of mitochondria (greater than in segment II). Few supranuclear vacuoles are present.

Segment IV. The segment IV is more translucent at microdissection than segment III. The tall cuboidal cells of the preceding segment become flattened, resulting in a relatively wider

lumen for this segment. The cells have a faint brush border, and have long cilia. The nuclei bulge into the lumen. They have less cytoplasm and appear less dense than the cells of segment III (Fig. 4). On electronmicroscopy the characteristic features of the cells are the large nucleus relative to the cell size; variation in shape, size, infoldings and vacuolation from one cell to another. The cells have short and sparse microvilli. The cytoplasm is filled with mitochondria especially in the region of the numerous basal cell membrane infoldings. These infoldings are distinct and extend almost to the luminal surface. The cytoplasm shows large membrane-bound vacuoles in the supranuclear zone. There are occasional lysosomes. The Golgi apparatus is in a paranuclear position.

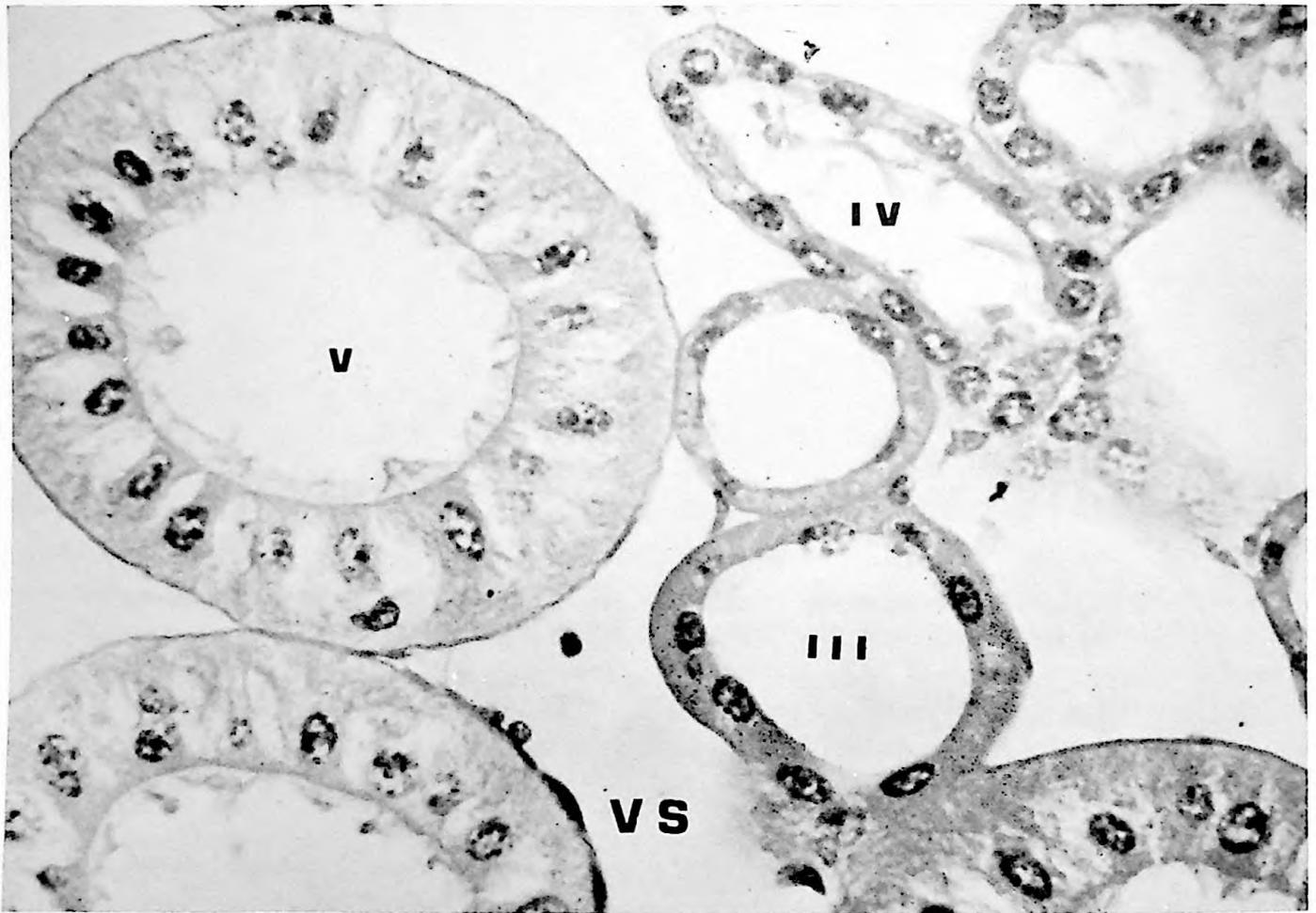


Figure 4. Section showing Segments III, IV, and V. Intertubular spaces are endothelial-lined venous sinuses (VS).

Segment V. (Average length of segment V is 18.56 mm and average diameter 0.08 mm.) Segment V begins as the abrupt enlargement of the distal end of segment IV. It is a tightly coiled, thick, almost opaque elastic tube. This part of the nephron is convoluted in a tight bundle about segments III and IV, forms the periphery of the individual nephron mass, and serves roughly to delineate one nephron from another. This tubule is obvious as the largest portion of the nephron. It is lined by tall, bulky, columnar cells and has a relatively small lumen. Large fusiform spaces seem to separate adjoining cells. The luminal surfaces of the cells have prominent mi-

crovilli and an occasional long cilia. Nuclei stain evenly but lightly (Fig. 4).

The ultrastructure of these cells is characterized by very dense and richly-developed basal cell infoldings paralleling the well-developed, elongated mitochondria which occur in all the cell zones, basal, paranuclear and supranuclear. The endoplasmic reticulum is smooth and often whorling and dense supranuclearly. The cytoplasm exhibits large parallel vesicular spaces. An occasional Golgi apparatus lies near the nucleus and there is an infrequent lysosome.

Segment VI. (Segment VI is very variable in length, 0.05 to 0.15 mm and has an average diameter of 0.03 mm.) The terminal part of segment V courses across the whole width of the nephron toward its glomerulus, where it narrows rapidly and ends in the thin-walled, relatively wide and short segment VI. The transitional zone of segment V into segment VI approaches the glomerulus intimately on its lateral and hilar aspects. Located at this site in this segment are a group of cells resembling the cells of the mammalian macula densa. The segment elsewhere is lined by cuboidal translucent cells without a brush border. It has a variable course; it may empty into a collecting tubule at almost a right angle, or may run parallel to it for a short distance before joining.

The Collecting Duct System

The collecting tubule is lined by simple flat non-ciliated epithelium, which becomes cuboidal but remains non-ciliated as it is traced into the main collecting duct. Further along the main collecting duct the cells become columnar and ciliated, and the duct also acquires a sparse investment of smooth muscle. At this point the main collecting duct comes to lie on the dorsal surface of the kidney in the midline. It then turns caudally, coursing medial to the primary branches of the ureter for a short but variable distance before emptying into one of them. The primary branches of the ureter pass caudally, joining with each other along their course to form the definitive ureter. These primary ureteric structures as well as the ureter have a prominent smooth muscle coat and a wide lumen, and they are lined by low columnar non-ciliated epithelium.

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UREA METABOLISM AND OSMOREGULATION IN THE SKATE, Raja erinacea

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Skates were transferred gradually during one week from 100% to approximately 50% seawater. Steady-state conditions with respect to solute and water balance were established by the end of the dilution program. Hematocrits of the fish in 50% seawater were somewhat lower than those in 100% seawater (12.5 ± 0.1 vs 15.5 ± 0.7) indicating that the skates did not osmoregulate completely in the dilute environment. Plasma concentrations of urea, trimethylamine oxide (TMAO) and chloride were 45, 23, and 30% lower respectively in the skates in 50% seawater than in the same fish in 100% seawater (Table 1). The rate of urea excretion (which reflects urea production in the steady-state) in skates in 100% seawater was 239 ± 42 μ moles/kg x hr (mean \pm S.E. of six fish), 126 ± 16 in 50% seawater ($P < .05$) and 214 ± 29 when returned to 100% seawater. Urine flow was increased nearly sixfold and glomerular filtration rate doubled (although not significant statis-