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Morphology of the kidney in a nectarivorous bird, the Anna's hummingbird *Calypte anna*

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Abstract

The kidneys of Anna's hummingbird (*Calypte anna*) differ in several significant ways from those of other birds that have been examined. The kidneys of this nectarivore contain very little medullary tissue; 90% of the total volume of the kidneys is cortical tissue, with medulla accounting for only an additional 2%. More than 99% of the nephrons are the so-called 'reptilian type', which lack the loop of Henle. The few looped ('mammalian type') nephrons are incorporated into only a few medullary cones per kidney. The loopless nephrons are similar to those of other birds. However, the looped nephrons differ in that they lack the thin descending limb of the loop of Henle, which is found in other birds and is thought to play an important role in the countercurrent multiplier system in the avian kidney. Instead, the cells of the nephron segment following the pars recta of the proximal tubule resemble those of the thick ascending limb, with the large populations of mitochondria that are typical of transporting epithelia and no reduction in cell height. The absence of a descending thin limb in Anna's hummingbird is not necessarily a correlate of the hummingbird's liquid diet, because thin limbs have been documented in the kidneys of two other hummingbird species, the rufous hummingbird (*Selasphorus rufus*) and the broad-tailed hummingbird (*Selasphorus platycercus*). The functional correlates of the unique renal morphology in Anna's hummingbird warrant further study.

Key words: *Calypte anna*, hummingbird, kidney, nephron, renal morphology

INTRODUCTION

Hummingbirds are among the smallest birds, ranging in body mass from 2–20 g (Brown & Bowers, 1985). They are largely nectarivorous and, because of their small size and high mass-specific metabolic rate, they must consume each day amounts of nectar greater than their body mass to meet their energy requirements (Lasiewski, 1963; Hainsworth & Wolf, 1972; Beuchat, Chaplin & Morton, 1979; Powers & Nagy, 1988; Weathers & Stiles, 1989).

High rates of nectar intake by hummingbirds can necessitate excretion of substantial amounts of excess water (Beuchat, Calder & Braun, 1990), so the kidneys must be capable of rapidly processing large volumes of very dilute urine. Calder & Hiebert (1983) have measured osmolalities of urine samples collected from birds in the field and noted that they were typically very dilute (<100 mOsm), although they did measure some urine samples that were probably isosmotic or even slightly hyperosmotic (reported in Beuchat *et al.,* 1990). The concentrations of electrolytes in the urine of the birds (4–19 mM Na^+, 10–27 mM K^+) mirror the low electrolyte levels typical of the nectar produced by hummingbird-pollinated plant species (Baker & Baker, 1975; Hiebert & Calder, 1983).

Despite the high rates of fluid intake by hummingbirds, there are environmental situations in which the birds must deal with potential dehydration. Rates of evaporative water loss in these small birds are very high, even at modest ambient temperatures (Lasiewski, 1964; Powers, 1992), and the amount of nectar required for energy balance can be inadequate to maintain positive water balance (Calder, 1979). In these circumstances, hummingbirds must be able to minimize urinary water loss through renal or post-renal mechanisms.

Hummingbirds and other nectarivorous birds face an unusual and challenging suite of osmoregulatory problems, and we might expect their kidneys to differ structurally from those of non-nectarivorous birds.

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Considerable attention has been directed at documenting renal structure of other species of birds in the context of increasing our understanding of the urinary concentrating mechanism (see reviews by Dantzler, 1989; Braun, 1993; Nishimura, 1993). Only one study, on the other hand, has addressed the anatomy of the hummingbird kidney. Johnson & Mugaas (1970*b*) examined the kidneys of two species of hummingbirds, the broad-tail hummingbird (*Selasphorus platycercus*) and the rufous hummingbird (*Selasphorus rufus*). They noted that hummingbird kidneys "contain a poorly developed renal medulla consisting almost entirely of collecting ducts with only a few associated looped nephrons". The only quantitative data they report, for a single rufus hummingbird, are sizes of cortical and medullary regions, diameters of thick and thin limbs of the loops of Henle, and number of collecting ducts.

The goal of this study was to document aspects of renal structure that might reflect the unique physiological demands that must be met by the kidney in a nectarivorous bird, with a special emphasis on those features related to the concentrating and diluting capacity of the kidney.

MATERIALS AND METHODS

Animals

Male Anna's hummingbirds were collected in San Diego and Riverside Counties, California, U.S.A. They were housed in the laboratory in flight cages and provided a commercial hummingbird diet *ad libitum.* (Nektar-Plus, Nekton). Kidneys were removed from the birds and processed within 72 hours of capture. Mean body mass was 5.2 ± 0.4 g ($n = 17$).

Renal composition

Birds ($n = 5$) were killed with carbon dioxide, the abdominal cavity was opened, and the dorsal aorta was cannulated. The kidneys were flushed with 0.2 M phosphate buffer, followed by half-strength Karnovsky's fixative (350 mOsm). The kidneys were then dissected from the synsacrum.

The length and width of the right kidneys were measured using vernier callipers to $\pm$ 0.01 mm and their volumes estimated by water displacement (Scherle, 1970). The tissue was then processed routinely for light histology, after which length and width were measured again. Linear tissue shrinkage (length and width) due to histological processing ranged from 22–27%. This is within the range reported for honeyeater kidneys (Casotti, Richardson & Bradley, 1993). The kidney tissue was embedded in paraffin wax and a series of transverse serial sections were cut at 10 equally-spaced intervals along its length using stereological procedures outlined in Mayhew (1991). The resulting sections were stained with haematoxylin and eosin.

The relative volumes of the cortex, medulla and vasculature were estimated using point-counting techniques (Gundersen & Jensen, 1987; Gundersen *et al.*, 1988). The volumes of components of the nephron (the renal corpuscle, proximal tubule, limbs of Henle, distal tubule, and cortical and medullary collecting ducts) were estimated in the same way.

Nephron morphology

To examine the gross morphology of the nephron, 2 birds were killed with carbon dioxide and the kidneys dissected from the synsacrum. The tissue was placed in a solution of alcoholic ferric chloride (95 ml ethyl alcohol, 5 ml concentrated HCl, 30 g ferric chloride) overnight at 4 °C. This was followed by digestion for 2 h in 20% HCl at 37 °C, after which the tissue was placed in cold (4 °C) acid ferric chloride (200 mg ferric chloride, 0.2 ml acetic acid, 100 ml distilled water). After soaking the kidney in water for 4–12 h to soften, individual nephrons were dissected free using finely drawn glass needles and were transferred to a drop of 50% glycerol on a microscope slide. Nephrons were viewed under a compound microscope, and computer-digitized images were captured and stored for later analysis.

The left kidneys of the birds were used to examine the cellular ultrastructure of the loop of Henle and collecting duct. The kidneys were fixed as described above. Individual medullary cones were identified by the connective tissue sheath that surrounds the loops of Henle of looped nephrons, as well as the collecting ducts originating from both looped and loopless nephrons. Each medullary cone, minus its associated cortical tissue, was removed from the kidney and its length measured ($\pm$ 0.01 mm) using an ocular micrometer on a dissecting microscope. This measurement approximates the length of the longest loops of Henle within each cone (Boykin & Braun, 1993). The tissue was then processed routinely for transmission electron microscopy; it was postfixed in 1% Dalton's osmium tetroxide, washed in a series of graded alcohols, then infiltrated with propylene oxide and embedded in epon resin. Sections were cut to a thickness of 90 nm, stained with uranyl acetate and lead citrate, and viewed on a transmission electron microscope.

Enumeration of nephrons

To determine the total number of nephrons in each kidney, 5 birds were killed and the kidneys flushed with phosphate buffer as described above. To make the glomeruli easier to count, they were stained by perfusing the kidneys with 5% alcian blue 8GS until the dye exited the caudal vena cava and the kidneys turned blue. Both kidneys were dissected from the synsacrum and fixed in 50% ethanol for 24 h, then transferred to a mixture of 50% ethanol and 1% ammonium hydroxide for 1.5 h. Following digestion in 20% HCl for 2 h at 37 °C, each

kidney was transferred into a volumetric flask containing 10 ml of distilled water. The kidney was agitated using a magnetic stir plate and bar until the tissue was broken up and individual glomeruli could be identified under the microscope. Once fragmented, the suspension was brought to a final volume of 100 ml with 10% neutral buffered formalin. For both kidneys, the number of glomeruli (both stained and unstained) in three 1 ml aliquots were counted in a Sedgwick-Rafter counting chamber. The mean count of the replicates was multiplied by the volume of the suspension to compute the total number of glomeruli in each kidney, which is equal to the total number of nephrons.

To estimate the number of looped nephrons, medullary cones were dissected from the kidneys of 9 birds and processed routinely for transmission electron microscopy as described above. A 1 μm transverse section was cut at the corticomedullary junction of each cone (the 'base' of the cone) and stained with toluidine blue. The number of looped nephrons was determined by counting the total number of limbs of Henle apparent and dividing by 2 (because each loop of Henle has a descending and ascending limb). The number of loopless nephrons in each kidney was estimated by subtracting the number of looped nephrons from the mean value for total number of nephrons estimated by the glomerular counts.

Statistics

All values are reported as mean ± standard deviation.

RESULTS

On average, 89.7% (± 3.0) of the volume of the kidney in Anna's hummingbird was occupied by cortex, with major vasculature accounting for another 7.8% (± 2.4), and medullary cones for only 2.4% (± 0.7) (Table 1, Fig. 1a). More than half (60%) of the cortical volume was occupied by proximal tubule, 19.3% was capillaries, 15% was distal tubule, 3.6% was collecting duct, and 2.1% was renal corpuscle (Fig. 1b). There were only 3–7 medullary cones per kidney (Table 2). The collecting ducts accounted for the largest proportion of the total volume (38.4%) of the cones, followed by descending and ascending thick limbs of Henle (32.3%), capillaries (19.8%), and proximal tubules (9.5%). The thin descending limb of the loop of Henle was absent (Fig. 1c).

The total number of nephrons per kidney (both loopless and looped) averaged 10,624 (± 1,192; Table 1), and 99.6% of these were the short, loopless nephrons found only in the cortex. Cones averaged 0.7 mm in length (range: 0.5–1.3 mm) and contained an average of 13.5 looped nephrons (Table 1). Because the number of cones per kidney ranged from 3–7, the number of looped nephrons per kidney varied from 40–131.

The structure of the loopless nephrons of Anna's hummingbird was like that of other birds, with a renal corpuscle, and proximal and distal convoluted tubules, the latter of which attached to the collecting ducts at a right angle (Fig. 2b).

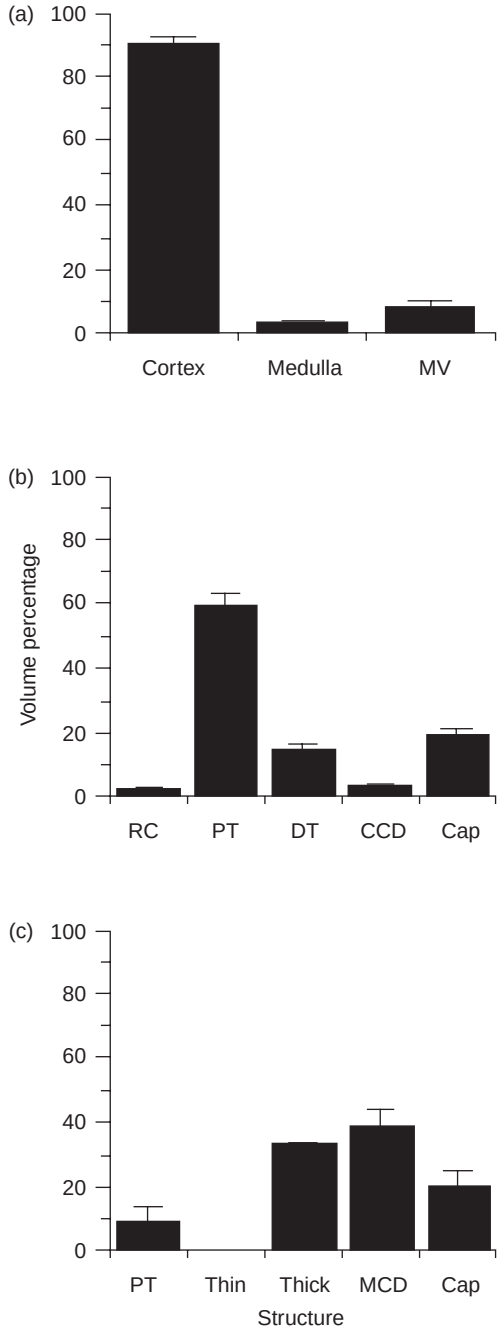

Fig. 1. Mean (± S.D.) volume percentage of: (a) kidney components; (b) cortical nephron tubules and capillaries; and (c) medullary nephron tubules and capillaries in Anna's hummingbird. MV, major vasculature; RC, renal corpuscle; PT, proximal convoluted tubule; DT, distal convoluted tubule; CCD, cortical collecting duct; MCD, medullary collecting duct.

In the looped nephrons, the proximal tubule could be quite long, extending in some nephrons as far as two-thirds the length of the descending limb of Henle. The descending and ascending limbs of the loops of Henle

Table 1. Total kidney volume and relative volumes of kidney components in birds. Sources: 1) This study; 2) Warui, 1989; 3) Casotti, Richardson, & Bradley, 1993; 4) Casotti & Richardson, 1992

Species	Body mass (g)	Kidney volume (mm^3)	Cortex (% of total)	Medulla (% of total)	Blood vessels (% of total)	Glomeruli (% of cortex)	Proximal tubule (% of cortex)	Distal tubule (% of cortex)	Loop of Henle (% of medulla)	Cortical collecting duct (% of cortex)	Medullary collecting duct (% of medulla)	Source
Anna's hummingbird (*Calypte anna*)	5.2	43.8	89.7	2.4	7.9	2.10	60.0	15.0	32.3	3.8	38.4	1
Budgerigar (*Melopsittacus undulatus*)	47.0	376.0	71.3	13.7	14.6							2
Collared turtle dove (*Streptopelia decaocto*)	160.7	1385.7	78.4	10.1	10.6							2
Common quail (*Coturnix coturnix*)	136.5	1385.0	78.5	8.8	11.9							2
Common starling (*Sturnus vulgaris*)	69.3	1145.0	72.5	14.9	11.9							2
Cut throat (*Amadina fasciata*)	20.8	188.3	75.9	11.7	11.8							2
Domestic fowl (*Gallus gallus*)	1644.8	11890.0	80.2	6.9	11.5							2
Great-crested grebe (*Podiceps cristatus*)	640.0	6480.0	78.7	6.7	12.1							2
Grey-fronted honeyeater (*Meliphaga plumula*)	12.0	140.0	76.1	12.9	11.0	2.58	50.4	14.8	39.0	3.9	25.3	3
Helmut guinea-fowl (*Numida meleagris*)	1685.0	12466.7	76.4	7.5	15.1							2
House sparrow (*Passer domesticus*)	27.5	230.0	73.8	12.8	12.7							2
Little wattlebird (*Anthochaera chrysoptera*)	62.0	424.6	81.0	4.7	14.3	3.0	50.9	10.5	38.6	6.4	35.6	4
Mallard duck (*Anas platyrhynchos*)	1185.0	7246.7	79.7	5.4	12.2							2
New Holland honeyeater (*Phylidonyris novaehollandiae*)	21.9	196.6	83.2	4.5	12.3	2.53	64.6	11.0	27.1	3.8	54.0	4
Red wattlebird (*Anthochaera carunculata*)	88.0	779.8	77.1	6.7	16.2	2.55	47.7	9.0	28.5	7.9	37.3	3
Spiny-cheeked honeyeater (*Acanthogenys rufogularis*)	42.5	520.6	72.7	15.1	12.2	2.98	59.8	6.2	30.9	5.3	41.5	4
Spotted munia (*Lonchura punctulata*)	13.8	140.0	74.8	12.9	11.8							2
Common turkey (*Meleagris gallopavo*)	7583.4	32170.0	77.2	9.8	12.0							2
Western spinebill (*Acanthorhynchus superciliosus*)	9.2	110.2	78.6	5.8	15.6	2.95	48.8	13.5	40.8	5.3	37.6	3
White-cheeked honeyeater (*Phylidronyris nigra*)	18.3	177.0	79.0	6.1	14.9	2.87	50.4	15.6	37.2	4.0	33.3	3
White-fronted honeyeater (*Phylidonyris albifrons*)	16.9	177.0	75.8	8.0	16.2	2.89	49.7	12.8	35.8	8.7	34.0	4
White-plumed honeyeater (*Meliphaga pencillata*)	15.3	195.6	73.5	13.4	13.1	2.93	52.5	8.7	41.8	9.9	23.6	3
White-rumped munia (*Lonchura punctulata*)	14.7	176.7	80.5	5.8	13.1							2
Yellow-throated miner (*Manorina flavigula*)	45.0	667.4	75.3	10.8	13.9	2.43	46.7	7.7	30.5	8.6	37.1	3
Zebra finch (*Poephila guttata*)	14.3	150.1	73.4	13.7	12.2							2

Table 2. Components of the renal medulla in birds. Number of glomeruli and number of cones are for both kidneys. Sources: 1) This study; 2) Johnson & Mugaas, 1970*a*; 3) Goldstein & Braun, 1989; 4) Johnson & Skadhauge, 1975

Species	Body mass (g)	No. glomeruli	No. cones/ 2 kidneys	Cone length (mm)	No. looped nephrons/cone	% looped nephrons	% medulla of kidney volume	Source
Anna's hummingbird (*Calypte anna*)	5.2	21,248	14	0.7	13.5	0.4	2.4	1
Black-throated sparrow (*Amphispiza bilineata*)			125	2.1			15.4	2
Budgerigar (*Melopsittacus undulatus*)			128	2.4			13.3	2
Glaucous-winged gull (*Larus glaucescens*)	803.8	1,128,500	1391	2.5	240.2	29.6	10.5	3
House finch (*Passer domesticus*)			118	1.8			12.7	2
House sparrow (*Carpodacus mexicanus*)	24.3	71,374	122	2.3	103.9	17.8	8.5	3
Macaroni penguin (*Eudyptes chrysolophus*)	2500	2,445,466	2780	5.7	134.6	15.3	12.8	3
Ring-necked pheasant (*Phasianus colchicus*)	1278.8	789,000	434	2.6	123.6	6.8	7.3	3
Savannah sparrow (*Passerculus sandwichensis beldingi*)			375	1.7			22.2	2
Savannah sparrow (*Passerculus sandwichensis nevadensis*)			115	1.5			9.7	2
Singing honeyeater (*Meliphaga virescens*)	25		118	1.9			11.8	4
Song sparrow (*Melospiza melodia juddi*)			119	1.4			7.2	2
White-crowned sparrow (*Zonotrichia leucophrys*)	22.9	106,044	115	1.9	93.9	10.5	5.5	3
White-winged dove (*Zenaida asiatica*)	130.3	151,514	95	2.7	148.9	11.5	8.2	3
Zebra finch (*Taeniopygia castanotis*)	10.4	17,566	42	2.1	126	29.8	9.6	3

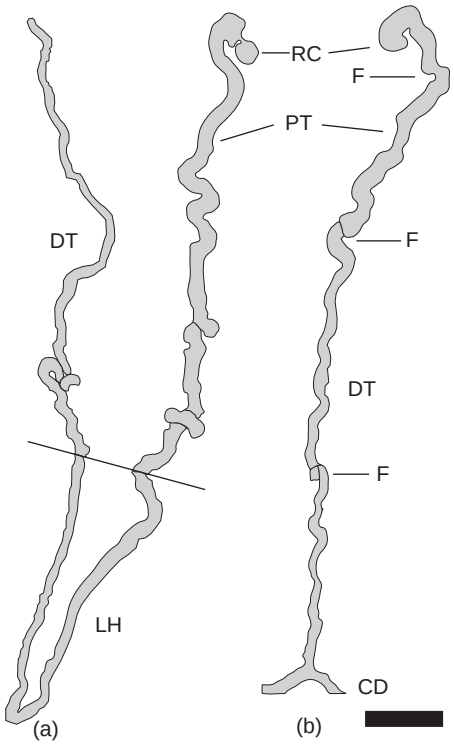

Fig. 2. Looped (a) and loopless (b) nephrons from Anna's hummingbird. (F) shows bends in the loopless nephron where it folded upon itself *in situ*. Solid line represents the boundary of the loop of Henle. (RC, renal corpuscle; PT, proximal convoluted tubule; DT, distal convoluted tubule; LH, loop of Henle; CD, collecting duct.) Scale bar = 100 μm.

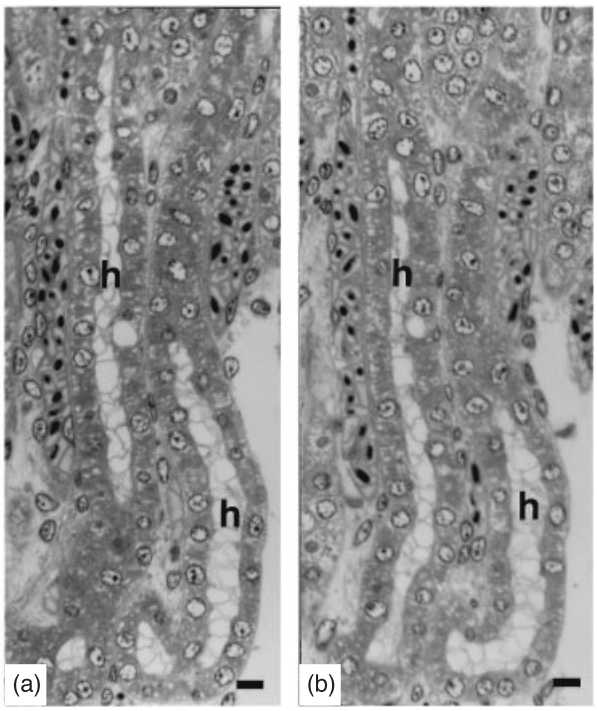

Fig. 3. Adjacent longitudinal sections of the (A) ascending and (B) descending limbs of the loop of Henle in a looped nephron of Anna's hummingbird, stained with toluidine blue. Note the similarity in ultrastructure and cell height between the two limbs. (h, loop of Henle.) Scale bar = 5 μm.

lay parallel to the collecting ducts in the medullary cones as observed in other species of birds (Braun & Dantzler, 1972).

We were unable to find the thin descending limb of the loop of Henle in Anna's hummingbird, despite diligent efforts that included examination of tissue sections and individual nephrons using both light and electron microscopy. The cells of the descending limb were indistinguishable from those of the thick ascending limb, with a large, round, centrally-located nucleus surrounded by cytoplasm containing numerous polyribosomes and vacuoles (Figs 2a, 3a, b, 4a). Extensive infoldings of the basal lamina formed a labyrinth in which elongated mitochondria were abundant (Fig. 4a). Tight junctions (zonula occludens) were present on the apical surface between adjoining cells. The lateral cell membranes lacked interdigitations between cells, and the apical surface was devoid of microvilli. The cells ranged from 5–7 μm in height, and there was no evidence of the intracellular structural heterogeneity observed in the descending limb in other species of birds (Nishimura *et al.*, 1989).

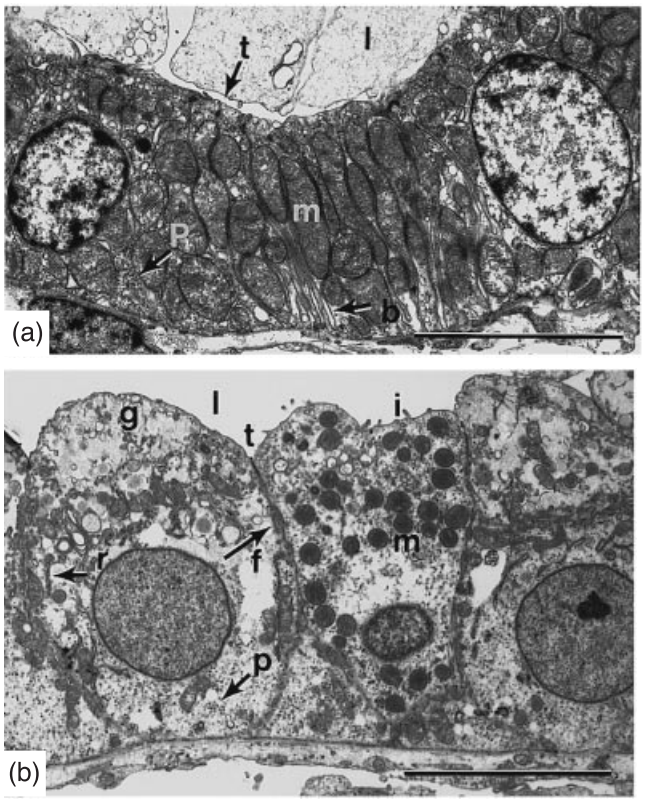

Fig. 4. Transmission electron micrographs of the kidney of Anna's hummingbird showing (A) the ultrastructure of the limbs of the loop of Henle and (B) principal and intercalated cells in the collecting duct. (m, mitochondria; p, polyribosomes; r, rough endoplasmic reticulum; b, basal labyrinth; t, tight junction; g, mucopolysaccharide granules; i, microvilli; f, interdigitated folding of the basolateral membrane; l, lumen.) Scale bar = 5 μm.

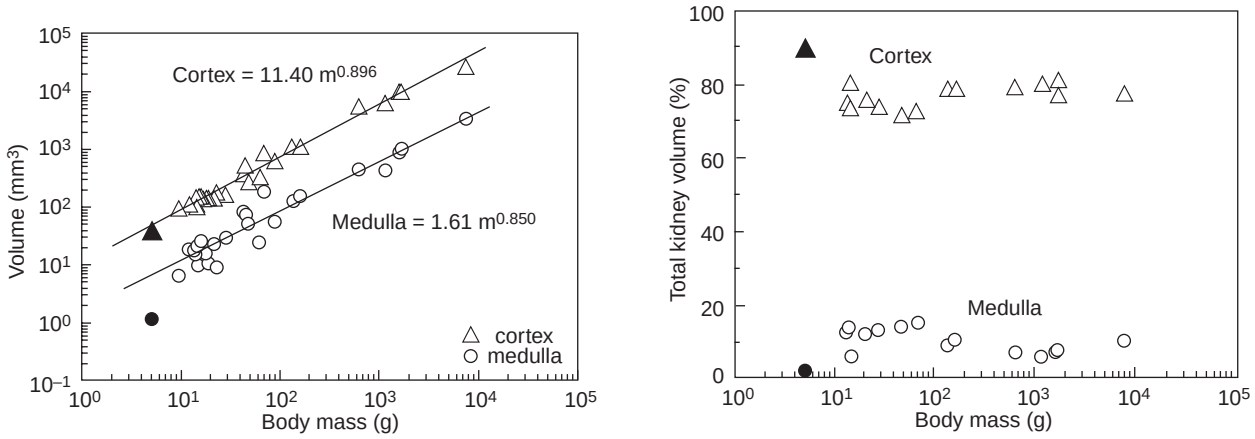

Fig. 5. The scalings of the amount of cortical and medullary tissue as (a) absolute volume (mm^3) and (b) percentage of total kidney volume in birds. Data are from Warui (1989), Casotti & Richardson (1992), Casotti *et al.* (1993), Goldstein & Braun (1989), and this study. The filled symbols depict our data for Anna's hummingbird.

The collecting ducts were composed of two cell types, principal (light) cells and intercalated (dark) cells (Fig. 4b). In both, the nucleus was large, round, and located centrally or basally in the cell, and polyribosomes and rough endoplasmic reticulum were apparent in the cytoplasm. Adjacent cells had interdigitated infoldings of the lateral membrane and were joined at their apices by tight junctions (zonula occludens; Fig. 4b). The principal cells, which ranged from 11–12 μm in height, contained few mitochondria, and these were mostly apically-located, as were numerous mucopolysaccharide granules (Fig. 4b). In intercalated cells, the mitochondria were numerous and distributed throughout the cell, and there were apically-located vacuoles. The apical surface of the principal cells was flattened and had a few microvilli, while that of intercalated cells was rounder and lacked microvilli (Fig. 4b). The intercalated cells were 8–9 μm in height.

DISCUSSION

The structure of the kidney in Anna's hummingbird differs in some significant ways from that of other birds. In the typical avian kidney, 70–85% of the total volume is cortical tissue, another 5–15% is medullary tissue, and blood vessels make up the remainder (Johnson & Mugaas, 1970a; Warui, 1989; Casotti *et al.*, 1993; Table 1, Fig. 5). In Anna's hummingbird, however, 90% of the kidney was occupied by cortex and only 2% by medulla, the most extreme values ever recorded in a bird. Despite this, the absolute volume of cortical tissue in the kidney of Anna's hummingbird was very close to that predicted for a 4.5 g bird, based on the allometric relationship we calculated using data in the literature for other species (39 mm^3 actual vs. 42 mm^3 predicted; Table 2). The amount of medullary tissue, however, was only 25% of that predicted (1 mm^3 vs. 4 mm^3). Thus, the high fractional volume occupied by the cortex in the kidney of Anna's hummingbird is a consequence of the exceptionally small amount of medullary tissue. Indeed, the kidney of Anna's hummingbird had by far the fewest number of medullary cones and looped nephrons of any bird species examined thus far (Table 2).

Is the relatively small renal medulla in Anna's hummingbird peculiar to this species, or is it a characteristic of birds with high rates of water flux? In a study of 14 species of passerine and non-passerine birds, including representatives from six different orders, Warui (1989) found that birds from mesic and aquatic habitats had relatively more cortex (78% and 80%, respectively) and less medulla (9% and 6%) than birds from arid regions, in which only 72% of kidney volume was cortex and 14% was medulla. Within the Australian honeyeaters, which comprise a single family (Meliphagidae) that includes both nectarivorous and insectivorous species, the relative amount of cortex is similar in the nectarivorous (75–84%) and insectivorous species (73–80%), but medulla accounts for only 4–9% of renal volume in nectarivores while it ranges from 8–17% in insectivores (Casotti & Richardson, 1992; Casotti *et al.*, 1993).

Thus, it does seem likely that the relatively smaller fraction of medullary tissue in Anna's hummingbird is a general characteristic of nectarivorous birds, and perhaps also of other species (e.g. aquatic birds) that have high rates of water turnover. For these, we can speculate that the ability to produce a concentrated urine is generally less critical, and that adequate reductions in urinary water loss during periods of osmoregulatory stress can be accomplished with relatively less medullary machinery than is necessary in birds that must concentrate their urine to a greater degree to remain in water balance.

Even among nectarivorous and aquatic birds, the kidney of Anna's hummingbird appears to be unique in that the loop of Henle in the looped nephrons lacks the thin descending limb. In the looped nephrons of the avian species examined to date, the thin descending limb of the loop of Henle is interposed between the pars recta

of the proximal tubule and the descending segment of the thick limb at the apex of the loop (Nishimura, Imai & Ogawa, 1986; Braun & Reimer, 1988; Nishimura *et al.*, 1989; Braun, 1993). In these birds, the cells of the thin limb are ultrastructurally distinct from those of the proximal tubule and the thick limb. The cells of the pars recta are cuboidal and have a dense microvillus brush border, and there are many predominantly basal mitochondria (Nishimura *et al.*, 1986; Braun & Reimer, 1988; Casotti & Richardson, 1993). The cells of the thick limb also have large numbers of mitochondria, but they lack microvilli. In the thin limb, on the other hand, the cells are flattened, they have few mitochondria, and they lack microvilli. In Anna's hummingbirds, the cells distal to the pars recta in the descending limb were similar to those of the thick ascending limb, with the large numbers of mitochondria and apical tight junctions that are characteristic of transporting epithelia.

The concentration of urine by the avian kidney is thought to occur by a countercurrent multiplier system that is similar to that of mammals, except that urea plays no role in the development of the medullary osmotic gradient (Skadhauge & Schmidt-Nielsen, 1967). The thick ascending limb functions in birds as it does in mammals, reabsorbing sodium and chloride from the luminal filtrate without movement of water, thereby increasing the osmolality of the interstitial fluid (Rocha & Kokko, 1973; Stoner, 1977; Jamison & Kriz, 1982; Nishimura, Imai & Ogawa, 1983; Miwa & Nishimura, 1986). In mammals, the outer medullary region of the thin descending limb is permeable to water but not sodium. Because the interstitial fluid is hyperosmotic relative to the filtrate, water is passively removed from the fluid in the descending thin limb, thereby concentrating what remains in the tubule (Jamison & Kriz, 1982). In contrast, the thin descending limb in birds is highly permeable to sodium and chloride but not to water (Nishimura *et al.*, 1989), so concentration of its fluid is accomplished by diffusion of these electrolytes into the tubule rather than the reabsorption of water. In effect, this is a solute 'recycling' mechanism; sodium and chloride removed from the fluid in the ascending limb of the loop of Henle are returned to the filtrate in the descending limb. Although the movements of water and solutes in and out of the thin descending limb are opposite in birds and mammals, the net effect (concentration of the tubular fluid) is the same.

Given the absence of the thin descending limb of the loop of Henle in Anna's hummingbird, the presence of a solute recycling mechanism similar to that of other birds (or mammals) seems doubtful. Indeed, it would appear that both the descending and ascending limbs of the loop in Anna's hummingbird are involved in active, energy-dependent transport of solutes, because the ultrastructure of the cells in both limbs is similar to that of transporting epithelia (Nishimura *et al.*, 1989). If this is the case, the tubular fluid would be diluted along the entire course of the loop of Henle, and addition of reabsorbed solutes to the medullary interstitial fluid would increase its osmolality. However, this system would not allow the development of a cortico-medullary osmotic gradient because the tubular fluid at the apex of the loop would not become concentrated. Furthermore, the solute gradient against which the sodium chloride transporter must pump would increase along the distance of the descending and ascending limbs as the luminal fluid becomes more dilute. The degree to which the luminal fluid can be diluted will depend on the rate of back flux of sodium and chloride, which depends on the permeability of the cell membrane to these solutes and the magnitude of the gradient. This arrangement is much less efficient than a counter-current multiplier, in which the magnitude of the gradient between luminal fluid and the interstitium is maintained at a modest level along the length of the ascending limb by the addition of solute to the fluid in the descending limb.

From these considerations, we predict that the urinary concentrating ability of Anna's hummingbird should be modest at best. Excreted urine collected from Anna's hummingbirds in the field was uniformly dilute; most samples were less than 100 mOsm, and all but one (out of 32) was less than 200 mOsm (Beuchat *et al.*, 1990). The most concentrated sample was 322 mOsm, which was probably only isosmotic at best. In contrast, urine samples collected from rufous hummingbirds (*Selasphorus rufus*) in the field were as high as 380 mOsm, and it is interesting to note that the looped nephrons in this species do contain the thin descending segment documented in other birds (Johnson & Mugaas, 1970*b*).

Hummingbirds and other nectarivorous birds have exceptionally high rates of water turnover. Does processing this water load require a greater filtration capacity of the kidney? Beuchat *et al.* (1990) have argued that the glomerular filtration rate predicted for Anna's hummingbird from the allometry for birds should be adequate to filter ingested water, even at the highest rates of consumption. From this, we would expect that the number of glomeruli in the kidneys of Anna's hummingbird should be consistent with that predicted from the allometry for number of glomeruli in birds. Anna's hummingbird has an average of 10,624 glomeruli per kidney, or about 21,248 total. This is by far the lowest number of glomeruli found in any bird studied thus far (Table 2). Yokota, Benyajati & Dantzler (1985) have computed the allometric equation relating total number of glomeruli in birds with body size as: # glomeruli = $3090\ m^{0.766}$, where m is body mass in g. From this, the predicted total number of glomeruli for a 5 g bird is 10,600, which is only 50% of the actual value. The glomerular count for Anna's hummingbird falls outside the 95% confidence interval for the regression at 5 g (i.e. 7,289–15,522 glomeruli). However, because the smallest bird in their data set weighed about 10 g, predicting the number of glomeruli for a 5 g hummingbird requires extrapolation beyond the limits of their data, which must be done with caution. A more rigorous analysis awaits the enumeration of nephron number in larger nectarivores or smaller non-nectarivores.

Because of its small body size, Anna's hummingbird

has the smallest kidney with the fewest looped nephrons and medullary cones of any bird examined to date. None the less, its rate of water intake is among the highest of any vertebrate, terrestrial or aquatic. Hummingbirds afford comparative biologists a fascinating model organism in which to study the structure and function of the kidney in a vertebrate near the lowest limits of body size.

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