The Journal of Experimental Biology 204, 1201–1206 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3130

EFFECTS OF SEASON ON KIDNEY MORPHOLOGY IN HOUSE SPARROWS

GIOVANNI CASOTTI*

Department of Biology, West Chester University, West Chester, PA 19383, USA

*e-mail: giovanni@bio.wcupa.edu

Accepted 14 December 2000; published on WWW 26 February 2001

Summary

Seasonal variability in kidney morphology of the house sparrow *Passer domesticus* was examined using light microscopy. Sparrows were captured from the wild in winter, spring, summer and autumn. The kidneys were perfused with half-strength Karnovsky's fixative and processed for light microscopy by embedding in either paraffin wax or JB4 acrylic resin. Absolute volumes of the kidneys, their components (cortex, medulla and blood vessels), components of the nephron (renal corpuscles, proximal tubules, loops of Henle, distal tubules and collecting ducts) and the capillaries surrounding the nephron were quantified using stereology. Tissue processed in paraffin wax had a mean shrinkage of 17.7 % compared with 10.1 % in JB4 resin. Absolute volumes of the kidneys and nephrons were compared statistically between tissue processing methods and among seasons. Absolute volumes of the structures within the kidneys were not significantly different between treatments or among seasons. Within the nephron, the only measured variables to show significant differences were the absolute volumes of the distal tubules and cortical collecting ducts between tissue processing treatments. Thus, kidney morphology was relatively unaffected by changes in season. In addition, the results show that embedding tissue using acrylic resin causes less shrinkage, and it should therefore be the preferred embedding medium for quantitative morphologists.

Key words: kidney, stereology, histology, JB4, house sparrow, *Passer domesticus*.

Introduction

Kidney morphology varies among different classes of vertebrates. The kidneys of birds and mammals, unlike those of other vertebrates, contain both a cortex and a medulla. The renal medulla enables concentrated urine to be produced, so it is not surprising that birds and mammals are also the only classes of vertebrate that can produce such urine (Braun and Dantzler, 1997). The urine-concentrating ability of birds is limited compared with that of mammals. The maximum urine:plasma osmolality (U/P) ratio recorded in birds is approximately 5.0 in the savannah sparrow *Passerculus sandwichensis* (Poulson and Bartholomew, 1962), while in mammals the maximum recorded ratio is 26.8 in the desert hopping mouse *Notomys alexis* (MacMillen and Lee, 1967; MacMillen and Lee, 1969). The order of magnitude of this difference in maximal urine-concentrating ability between birds and mammals has prompted interest in investigating the morphology and physiology of the avian kidney.

Birds produce, at best, only a slightly hyperosmotic urine. Because the demand for conserving water is increased in an arid environment, one might expect birds inhabiting arid regions to produce a more concentrated urine, and those inhabiting a mesic environment to produce a less concentrated urine. However, a recent study has shown that this is not the case (Casotti et al., 2001). In fact, one study showed a slightly negative correlation between lack of water in the environment and the ability to produce a hyperosmotic urine (Goldstein and Braun, 1989). One might also expect that the morphology of the kidney would dictate the capability to regulate urine concentration. Thus far, the only anatomical index that has proved to be consistently correlated with water abundance in the environment in which a bird lives is the volume of the renal medulla (Warui, 1989; Casotti and Richardson, 1992; Casotti et al., 1993; Casotti et al., 1998). Birds inhabiting an arid environment and having a largely insectivorous diet have a greater volume of medulla than birds inhabiting mesic environments and eating a nectarivorous diet. For example, the Anna's hummingbird *Calypte anna* exists on a diet consisting almost entirely of nectar and has the most dilute urine ever recorded (U/P ratio as low as 0.1). In addition, this species also has the lowest volume of medulla recorded (2.4 %) for a kidney (Casotti et al., 1998). More dramatically, the black-chinned hummingbird *Archilochus alexandri* is reported to have no renal medulla (Beuchat et al., 1990).

One concern with the above studies, which examined the volume of renal medulla, is that the animals were collected at different times of the year (i.e. in different seasons). Therefore, one cannot rule out the possibility that season (or diet, which may change with season) may have an effect on the morphology of the kidney. Previous studies on gut tissue in birds (Richardson and Wooller, 1986; Richardson and

Wooller, 1988; Richardson and Wooller, 1990), mammals (for reviews, see Borkowska, 1995; Gross et al., 1985; Karasov and Diamond, 1983) and fish (Ciccotti et al., 1993) have shown that gut morphology changes considerably with changes in diet. These changes can occur as soon as 3 days following a change in diet (Karasov and Diamond, 1983). However, no one has yet examined whether the morphology of avian kidney tissue varies with changes in either diet or season.

All previous studies examining quantitative renal histology in birds have used paraffin wax to embed the tissue (Johnson and Mugaas, 1970; Warui, 1989; Casotti and Richardson, 1992; Casotti et al., 1993). However, for many histological studies, investigators have chosen to embed biological tissue in acrylic resins (Higuchi et al., 1979; Cole, 1982; Helander, 1983; Ladekarl, 1994; Miller and Meyer, 1990). These resins offer a number of advantages over paraffin wax, including less tissue shrinkage, less tissue distortion (as a result of reduced compression during cutting) and a shorter staining time because deparaffinating agents such as toluene or xylene are not required (Higuchi et al., 1979; Cole, 1982; Ladekarl, 1994). For quantitative morphological studies, a reduction in tissue shrinkage and distortion are by far the most important advantages.

By examining the kidney tissue in a single species (the house sparrow *Passer domesticus*), the aim of the present study was to examine whether changes in season have an effect on the morphology of the avian kidney. In addition, renal tissue was embedded in paraffin wax and in the acrylic resin JB4 to determine whether embedding in different media had any effect on the quantitative morphology of the tissue. The results show that the volume of some components within the nephron varies with season. In addition, data are presented which indicate that embedding tissue in acrylic resin produces results superior to the previously more commonly used paraffin wax because there is less tissue shrinkage in the resin.

Materials and methods

Animals

House sparrows (*Passer domesticus*) were collected from the wild, in Pennsylvania, under license using mist nets. Six males were collected in each of the four seasons, giving a total of 24 birds for the study. Birds were immediately transported back to the laboratory for kidney tissue processing. The mean body mass for the birds in winter was 27.1±1.9 g, in spring 27.3±1.6 g, in summer 24.9±1.7 g and in autumn 26.1±1.4 g (means ± S.E.M.).

Tissue preparation

Captured birds were killed with an intraperitoneally injected overdose of sodium pentobarbital, the abdominal cavity was opened and the dorsal aorta cannulated. The kidneys were flushed with 0.2 mol l^{-1} phosphate buffer (pH 7.4), followed by half-strength Karnovsky's fixative (350 mosmol l^{-1}). The kidneys were dissected from the synsacrum and immediately placed into the fixative for 24 h to ensure optimum fixation.

Table 1. *Sampling strategy for light microscopic analyses*

Season	Number of birds	Number of kidneys	Processing method	
			Wax	Acrylic resin
Winter	6	12	3 left, 3 right	3 left, 3 right
Spring	6	12	3 left, 3 right	3 left, 3 right
Summer	6	12	3 left, 3 right	3 left, 3 right
Autumn	6	12	3 left, 3 right	3 left, 3 right

Following fixation, the volume of the kidneys was measured by water displacement (Scherle, 1970).

Light microscopy

Kidney tissue was processed using two different techniques and embedded in two different media. The purpose of this portion of the study was to examine whether different techniques of processing and embedding of the tissue had any effect on the morphology of the tissue. In addition, to ensure that any possible differences in the morphology between the left and right kidney within an individual bird did not bias the results of the present study, tissue was sampled as shown in Table 1. Briefly, equal numbers of kidneys (left and right) were processed in paraffin wax and acrylic resin for each season.

Paraffin wax

Prior to processing in wax, the lengths and widths of each kidney were measured using vernier calipers to an accuracy of ±0.1 mm. All measurements were taken at the same location for all kidneys. Length measurements were taken from the anterior tip of the cranial division to the most posterior tip of the caudal division. Width measurements were taken from the lateral to the medial side of the cranial division (because this division showed the maximum width of the kidneys). For embedding in paraffin wax, kidneys were processed routinely through a series of alcohol solutions of increasing strength, transferred to toluene and finally into paraffin wax (Paraplast Plus, Oxford Labware, St Louis, MO, USA). Prior to embedding in wax, the length and width of each kidney were re-measured and the degree of shrinkage due to processing was calculated (see Table 2). Statistical comparisons of the amount of tissue shrinkage caused by processing were performed using a one-way analysis of variance (ANOVA). The tissue was cut in an unbiased manner at 10 equally spaced intervals along its entire length, sectioned at a thickness of 5 μm using steel knives and mounted onto glass slides (Mayhew, 1991). The resulting sections were stained with hematoxylin and eosin.

JB4 acrylic resin

Prior to processing in JB4 acrylic resin, the length and width of each kidney were measured using vernier calipers to an accuracy of ±0.1 mm. Measurements were taken as described for paraffin wax. For processing, kidneys were placed into increasing concentrations of alcohol solutions, then infiltrated in JB4 resin overnight at 4 °C on a tissue rotator. Prior to embedding, the length and width of each kidney were re-

measured, and the degree of shrinkage due to processing was calculated (see Table 2). Statistical comparisons of the amount of tissue shrinkage caused by processing were performed using a one-way ANOVA. The tissue was re-immersed in fresh JB4, and the resin was allowed to cure for 48 h at room temperature (25 °C). Sections were cut at a thickness of 5 μm using steel knives and mounted on glass slides. As with paraffin sections, the tissue was cut in an unbiased manner at 10 equally spaced intervals along its entire length (Mayhew, 1991). Sections were adhered onto the slides using Tissue-Tack adhesive (Electron Microscopy Sciences, Ft Washington, PA, USA) and stained with hematoxylin and eosin.

Stereology

The volume densities of (i) kidney components (cortex, medulla and major blood vessels), (ii) nephron components (renal corpuscle, proximal tubule, loops of Henle, distal tubule and collecting ducts) and (iii) cortical and medullary capillaries were estimated by point counting using the Cavalieri principle (Gundersen et al., 1988). Volume densities were converted to absolute volumes by taking into account the volume of the kidney estimated by water displacement (Scherle, 1970).

Statistical analyses

Absolute volume data were $\log_{10}$-transformed and analyzed using analysis of covariance (ANCOVA) to correct for variations in body mass using the statistical software program Statistica. Different independent variables were used depending on the dependent variable being measured. For example, when analyzing differences in kidney volume, bird body mass was used as the independent variable. When comparing the volumes of the cortex, medulla and major blood vessels, kidney volume was used as the independent variable. When comparing the volumes of the renal corpuscle, proximal tubules, distal tubules, cortical collecting duct and cortical capillaries, the volume of the cortex was used as the independent variable. When comparing the volumes of the limbs of Henle, collecting ducts and medullary capillaries, the volume of the medulla was used as the independent variable. To compare differences between slopes and elevations of regression lines, a Student–Newman–Keuls test was performed. Values are presented as means ± S.E.M.

Results

Tissue shrinkage

Tissue shrinkage due to processing for paraffin wax ranged from 12 to 23 %, and these values did not differ significantly among seasons. Tissue shrinkage for processing in JB4 acrylic resin ranged from 8 to 14 %. These values also did not differ significantly among seasons (Table 2). However, when data for the amount of shrinkage due to processing for paraffin wax (17.7±4.7 %) and those for JB4 (10.1±3.6 %) were grouped and compared with one another, there was a significantly greater (P<0.05) degree of shrinkage due to processing for paraffin wax.

Table 2. *Linear percentage tissue shrinkage of house sparrow kidneys*

	Length (%)		Width (%)	
Season	Paraffin wax	JB4	Paraffin wax	JB4
Winter	21.9±1.0	10.8±0.4	14.8±0.9	8.5±1.6
Spring	20.0±1.2	10.6±1.1	14.4±3.1	8.2±1.5
Summer	18.1±1.8	9.6±0.7	22.7±1.6	11.2±2.1
Autumn	17.4±1.5	8.5±1.2	12.4±1.2	13.5±2.2

Values are means ± S.E.M. (N=6).

Absolute volume data

The kidneys of house sparrows consist mostly of cortex with smaller amounts of medulla and blood vessels (Table 3). There were no significant differences in the absolute volume of the kidneys among season or between treatments (Table 3A,B). The majority of the cortex consist of the proximal tubules, followed by the capillaries, distal tubules, collecting ducts and renal corpuscles (Table 3). In most cases, there were no significant differences in the absolute volumes of components within the cortex. Two exceptions were the distal tubules and cortical collecting ducts. In the case of the distal tubules, values for kidneys harvested in the spring and summer and processed with paraffin wax were significantly higher (P<0.05) than those for autumn birds processed in either paraffin wax or JB4 (Table 3A,B). For the collecting ducts, data for birds collected in the winter and spring and processed in wax were significantly higher (P<0.05) than those of summer and autumn birds processed in JB4 (Table 3A,B). The majority of the medulla of the sparrow kidney consisted of thick limbs of Henle, followed by collecting ducts, capillaries and thin limbs of Henle (Table 3). There were no proximal tubules within the medulla of the house sparrow kidney. There were no significant differences in the absolute volume of components within the medulla.

Discussion

Studies examining the effects of season on the morphology of biological tissue have not been widespread. However, data on gut tissue show that morphology may change with differences in diet corresponding to differences in season (Gross et al., 1985; Richardson and Wooller, 1986; Richardson and Wooller, 1988; Richardson and Wooller, 1990; Borkowska, 1995). Given the importance of the kidney in osmoregulation and the fact that different seasons may pose different osmoregulatory demands on animals, it is reasonable to speculate that kidney morphology might show seasonal changes, but to date no studies have examined this relationship. Instead, previous studies on kidney morphology in both birds and mammals have examined differences related to habitat. In birds, there is a tendency for species inhabiting arid environments to have a greater medullary thickness (Braun, 1985); amongst mammals, those inhabiting an arid environment tend to have the longest medullary papillae (for

Table 3. *Mean absolute volumes of components within the kidney, cortex and medulla in house sparrows processed in paraffin wax (A) and JB4 acrylic resin (B)*

Structure	Volume (mm^3) Winter[1]	Spring[2]	Summer[3]	Autumn[4]	Significance
A					
Kidney	130.8±8.1 (2.11)	155.8±8.1 (2.18)	143.8±7.1 (2.16)	122.8±4.4 (2.10)	NS
Cortex	108.8±6.7 (2.05)	125.3±6.0 (2.04)	114.5±5.3 (2.04)	99.3±3.7 (2.04)	NS
Medulla	9.2±1.3 (0.97)	12.6±1.3 (1.01)	16.4±1.9 (1.17)	12.0±1.1 (1.13)	NS
Blood vessels	12.9±1.3 (1.11)	17.8±1.5 (1.21)	12.9±0.7 (1.09)	11.5±1.3 (1.07)	NS
Cortex					
Renal corpuscle	3.0±0.1 (0.48)	3.4±0.4 (0.49)	3.4±0.3 (0.52)	2.6±0.2 (0.44)	NS
Proximal tubule	61.3±4.2 (0.80)	68.9±4.1 (0.78)	59.2±3.1 (0.76)	53.0±2.8 (0.78)	NS
Distal tubule	15.9±1.9 (1.20)	22.7±1.6 (1.31)	22.7±1.9 (1.33)	14.7±1.4 (1.21)	*
Collecting duct	6.0±0.8 (0.77)	6.1±0.6 (0.73)	5.5±1.0 (0.68)	4.7±0.4 (0.72)	**
Capillaries	22.6±1.2 (0.36)	24.2±1.6 (0.34)	23.6±2.2 (0.36)	24.2±1.5 (0.42)	NS
Medulla					
Proximal tubule	0	0	0	0	
Thin limb of Henle	1.0±0.2 (0.93)	1.0±0.1 (0.95)	1.8±0.3 (0.99)	1.2±0.1 (1.06)	NS
Thick limb of Henle	4.2±0.6 (0.71)	5.8±0.6 (0.71)	7.3±0.8 (0.70)	5.2±0.6 (0.68)	NS
Collecting duct	2.2±0.2 (0.42)	3.2±0.4 (0.46)	4.1±0.5 (0.48)	3.2±0.3 (0.49)	NS
Capillaries	1.9±0.4 (1.38)	2.6±0.3 (1.36)	3.2±0.5 (1.31)	2.4±0.3 (1.31)	NS
	Winter[5]	Spring[6]	Summer[7]	Autumn[8]	
B					
Kidney	136.8±6.0 (2.13)	146.8±6.2 (2.16)	138.2±6.5 (2.14)	126.5±2.5 (2.10)	NS
Cortex	112.1±4.9 (2.05)	119.5±5.7 (2.04)	113.7±6.9 (2.05)	105.7±2.5 (2.06)	NS
Medulla	9.6±1.4 (0.96)	13.0±1.1 (1.06)	11.3±0.7 (1.04)	10.0±0.8 (1.03)	NS
Blood vessels	14.7±1.2 (1.16)	13.9±0.7 (1.11)	13.1±1.1 (1.11)	11.2±1.2 (1.05)	NS
Cortex					
Renal corpuscle	2.5±0.4 (0.37)	2.5±0.2 (0.39)	2.2±0.2 (0.32)	2.1±0.3 (0.32)	NS
Proximal tubule	61.8±3.1 (0.79)	71.7±3.5 (0.82)	69.2±6.5 (0.82)	61.7±1.8 (0.82)	NS
Distal tubule	18.9±1.5 (1.27)	17.7±1.5 (1.21)	18.2±1.4 (1.25)	14.5±1.2 (1.17)	*
Collecting duct	4.5±0.5 (0.64)	5.0±0.9 (0.63)	2.9±0.3 (0.44)	3.2±0.3 (0.53)	**
Capillaries	24.4±0.8 (0.39)	22.7±1.4 (0.33)	21.3±2.3 (0.30)	24.2±2.2 (0.39)	NS
Medulla					
Proximal tubule	0	0	0	0	
Thin limb of Henle	1.1±0.2 (1.11)	1.2±0.2 (1.00)	1.0±0.2 (0.99)	1.1±0.2 (1.06)	NS
Thick limb of Henle	4.7±0.8 (0.73)	6.3±0.7 (0.73)	5.2±0.4 (0.71)	5.7±0.8 (0.74)	NS
Collecting duct	2.1±0.2 (0.37)	2.8±0.2 (0.40)	2.8±0.2 (0.44)	2.5±0.3 (0.43)	NS
Capillaries	1.7±0.3 (1.30)	2.6±0.2 (1.35)	2.3±0.3 (1.34)	2.0±0.2 (1.34)	NS

Values are means ± S.E.M.
NS, not significant
*2,3>4,8; **1,2>7,8.

reviews, see Braun, 1985; Beuchat, 1996). For both birds and mammals, those species that live in arid habitats tend to be better at concentrating their urine than those inhabiting freshwater habitats, although there are many exceptions (Braun, 1985; Beuchat, 1996).

In addition to season, the current study also examined the effects of tissue shrinkage caused by processing using paraffin wax and acrylic resin. Paraffin wax is the most commonly used embedding medium among histologists, but results in a significant amount of tissue shrinkage (Warui, 1989). In the present study, tissue shrinkage due to processing in paraffin wax ranged from 12 to 23%. These values are in agreement with data from previous published studies also using paraffin wax (Casotti and Richardson, 1992; Casotti et al., 1993). In contrast, some investigators have changed to using acrylic resin as an embedding medium. One advantage of acrylic resin is that it results in less shrinkage of the tissue; this is preferable when obtaining quantitative data on variables such as volumes, surface areas or lengths (Miller and Meyer, 1990; Ladekarl, 1994). Results from the present study on the amount of shrinkage due to processing in acrylic resin show shrinkage that ranged from 8 to 14%, and these values are significantly

lower than shrinkage due to paraffin wax. These data are comparable with those of previous published studies (Miller and Meyer, 1990; Ladekarl, 1994).

The morphology of the kidney of house sparrows was relatively unaffected by changes in season. None of the variables examined showed any significant changes with season. Although, at first glance, absolute volumes of some variables appear to be significantly different among seasons, when regressed against their appropriate covariate they are not statistically significant. These results are somewhat surprising for two reasons. First, previous studies have shown morphological differences in the amount of medulla with changes in habitat (Warui, 1989; Casotti and Richardson, 1992; Casotti et al., 1993; Casotti et al., 1998), and furthermore, I hypothesize that a change of season may cause the same effect. Thus, data from the present study show that kidney morphology may not be affected by changes in season, and the morphological differences in kidney structure found by previous authors may, in fact, be the result of differences in diet or perhaps inherent phylogenetic differences among species. Second, tissue processing using paraffin wax caused significantly more tissue shrinkage than processing in JB4 resin. Despite this, the absolute volume data in this study show no significant differences in the medulla processed by either method.

Two components of the nephrons (the distal tubules and collecting ducts) showed significant differences with respect to the method of processing the tissue. On occasion, the volumes of distal tubules and cortical collecting ducts were significantly higher in tissue processed with paraffin wax. That volumes of nephron components were significantly higher when processed with wax than when processed with resin is unusual, given that data on tissue shrinkage show that the tissue shrank more when processed in wax than in resin. Hence, differential tissue shrinkage must have occurred either in the wax-embedded or in the resin-embedded tissue. Thus, in some instances, processing method had an effect on the amount of structure measured within the kidney. The suggestion that methods used to process biological tissue can affect an anatomical variable has been documented previously (Miller and Meyer, 1990; Ladekarl, 1994).

The volumes of distal tubules and cortical collecting ducts were not consistently higher in any one season; hence, there was no correlation between anatomical structure and season. As in the mammalian nephron, the distal tubule in the avian nephron passively reabsorbs water and actively reabsorbs sodium chloride from the nephron tubule (Braun and Dantzler, 1997). This additional reabsorption within the cortex aids in conserving fluid that might otherwise be excreted. Hence, the act of reabsorption by the distal tubule saves both water and solutes. In the avian nephron, absorption of sodium chloride from the distal tubule may, in some instances, proceed without water reabsorption, further increasing the concentration gradient along the length of the nephron tubule and thus allowing water to be reabsorbed distally along the nephron at the medullary collecting ducts. In mammals, water is reabsorbed along the collecting ducts in the presence of antidiuretic hormone (ADH). In birds, the ADH is adenine vasotocin (AVT). Although presumed to act along portions of the distal nephron (i.e. the collecting duct system and cortical collecting duct), the site of action of AVT has not yet been identified. This action has not been directly demonstrated.

As with the distal tubules, the cortical collecting tubules also play a role in producing a concentrated urine by reabsorbing water from the tubular lumen. In addition, they are also known to secrete mucin, which may aid in eliminating uric acid from the kidney (Nicholson, 1982). They play a role in producing a concentrated urine, so their volume might have been expected to vary with season.

In summary, there appear to be no significant changes in the morphology of the sparrow kidney with season. This study did find some significant differences in kidney structure related to processing method, so the method by which histological tissue is processed may affect the quantitative outcome. JB4 acrylic resin produces less tissue shrinkage than paraffin wax so it should be the preferred embedding medium for quantitative morphological studies.

This study was funded by a Faculty Development Grant from West Chester University, FD 2112-001.

References

Beuchat, C. (1996). Structure and concentrating ability of the mammalian kidney: correlations with habitat. *Am. J. Physiol.* **270**, R157–R179.

Beuchat, C. A., Calder, W. A. I. and Braun, E. J. (1990). The integration of osmoregulation and energy balance in hummingbirds. *Physiol. Zool.* **63**, 1059–1081.

Borkowska, A. (1995). Seasonal changes in gut morphology of the striped field mouse (*Apodemus agrarius*). *Can. J. Zool.* **73**, 1095–1099.

Braun, E. J. (1985). Comparative aspects of the urinary concentrating process. *Renal Physiol.* **8**, 249–260.

Braun, E. J. and Dantzler, W. H. (1997). Vertebrate renal system. In *Handbook of Physiology: Comparative Physiology* (ed. W. H. Dantzler), pp. 481–576. New York: Oxford University Press.

Casotti, G., Beuchat, C. A. and Braun, E. J. (1998). Morphology of the kidney in a nectarivorous bird, the Anna's hummingbird, *Calypte anna*. *J. Zool., Lond.* **244**, 175–184.

Casotti, G., Lindberg, K. and Braun, E. J. (2000). Functional morphology of the avian medullary cone. *Am. J. Physiol.* **279**, R1722–R1730.

Casotti, G. and Richardson, K. C. (1992). A stereological analysis of kidney structure of honeyeater birds (Meliphagidae) inhabiting either arid or wet environments. *J. Anat.* **180**, 281–288.

Casotti, G., Richardson, K. C. and Bradley, J. S. (1993). Ecomorphological constraints imposed by the kidney component measurements in honeyeater birds inhabiting different environments. *J. Zool., Lond.* **231**, 611–625.

Ciccotti, E., Macchi, E., Rossi, A., Cataldi, E. and Cataudella, S. (1993). Glass eel (*Anguilla anguilla*) acclimation to freshwater and seawater: morphological changes of the digestive tract. *J. Appl. Ichthyol.* **9**, 74–81.

Cole, M. B. (1982). Glycol methacrylate embedding of bone and cartilage for light microscopic staining. *J. Microsc.* **127**, 139–148.

Goldstein, D. L. and Braun, E. J. (1989). Structure and concentrating ability in the avian kidney. *Am. J. Physiol.* **256**, R501–R509.

Gross, J. E., Wang, Z. and Wunder, B. A. (1985). Effects of food quality and energy needs: changes in gut morphology and capacity of *Microtus ochrogaster*. *J. Mammal.* **66**, 661–667.

Gundersen, H. J. G., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sorensen, F. B., Vesterby, A. and West, M. J. (1988). The new stereological tools: Dissector, fractionator, nucleator and point sampling intercepts and their use in pathological research and diagnosis. *Acta Pathol. Microsc. Immunol. Scand.* **96**, 857–881.

Helander, K. G. (1983). Thickness variations within individual paraffin and glycol methacrylate sections. *J. Microsc.* **132**, 223–227.

Higuchi, S., Suga, M., Dannenberg, A. M. and Schofield, B. H. (1979). Histochemical demonstration of enzyme activities in plastic and paraffin embedding tissue sections. *Stain Tech.* **54**, 5–12.

Johnson, O. W. and Mugaas, J. N. (1970). Some histological features of avian kidneys. *J. Anat.* **127**, 423–435.

Karasov, W. H. and Diamond, J. M. (1983). Adaptive regulation of sugar and amino acid transport by vertebrate intestine. *Am. J. Physiol.* **245**, G443–G462.

Ladekarl, M. (1994). The influence of tissue processing on quantitative histopathology in breast cancer. *J. Microsc.* **174**, 93–100.

MacMillen, R. E. and Lee, A. K. (1967). Australian desert mice: Independence of exogenous water. *Science* **158**, 383–385.

MacMillen, R. E. and Lee, A. K. (1969). Water metabolism of Australian hopping mice. *Comp. Biochem. Physiol.* **28**, 493–514.

Mayhew, T. M. (1991). The new stereological methods for interpreting functional morphology from slices of cells and organs. *Exp. Physiol.* **76**, 639–665.

Miller, P. L. and Meyer, T. W. (1990). Methods in laboratory investigation. Effects of tissue preparation on glomerular volume and capillary structure in the rat. *Lab. Invest.* **63**, 862–866.

Nicholson, J. K. (1982). The microanatomy of the distal tubules, collecting tubules and collecting ducts of the starling kidney. *J. Anat.* **134**, 11–23.

Poulson, T. L. and Bartholomew, G. A. (1962). Salt balance in the savannah sparrow. *Physiol. Zool.* **35**, 109–119.

Richardson, K. C. and Wooller, R. D. (1986). The structures of the gastrointestinal tracts of honeyeaters and other small birds in relation to their diets. *Aust. J. Zool.* **34**, 119–124.

Richardson, K. C. and Wooller, R. D. (1988). The alimentary tract of a specialist frugivore, the mistletoebird, *Dicaeum hirundinaceum*, in relation to its diet. *Aust. J. Zool.* **36**, 373–382.

Richardson, K. C. and Wooller, R. D. (1990). Adaptations of the alimentary tracts of some Australian lorikeets to a diet of pollen and nectar. *Aust. J. Ecol.* **38**, 581–586.

Scherle, W. F. (1970). A simple method of volumetry of organs in quantitative stereology. *Mikroscopie* **26**, 57–60.

Warui, C. N. (1989). Light microscopic morphometry of the kidneys of fourteen avian species. *J. Anat.* **162**, 19–31.