Luminal Morphology of the Avian Lower Intestine: Evidence Supporting the Importance of Retrograde Peristalsis for Water Conservation

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ABSTRACT

Tissue from the lower intestine of two species of sparrow, the house sparrow *Passer domesticus* and savannah sparrow *Passerculus sandwichensis* was sectioned in an unbiased manner and examined quantitatively using stereology. The tissue was processed for light microscopy, and scanning and transmission electron microscopy to examine the extent to which microvilli enhanced the epithelial surface area of the cecae, rectum, and coprodeum. Parameters measured included individual microvillus surface area, microvilli packing density, and absolute surface area. In both species, the average surface area, packing density, and absolute surface area of microvilli decreased distally along the rectum and coprodeum. All three measured variables were not statistically significant ($P > 0.05$) between species. Surface area amplification on the cecae due to microvilli was low, and approximated values equivalent to distal regions of the rectum and coprodeum. In the cecae, microvilli within the savannah sparrow had a significantly higher ($P < 0.05$) individual surface area, packing density, and absolute surface area than in the house sparrow. The functional implications of a change in microvilli population are discussed in relation to retrograde peristalsis within the lower intestine of birds. Anat Rec 263: 289–296, 2001. © 2001 Wiley-Liss, Inc.

Organs and organ systems that are involved in osmoregulation differ among vertebrates. For example, birds do not possess a urinary bladder but instead, urine formed by the kidneys travels along the ureters into the cloaca (terminal portion of the intestinal tract). From there, it may move in an orad direction by retrograde peristalsis into the cecae (Duke, 1989; Clauss et al., 1991; Brummermann and Braun, 1994). The urine is refluxed along the length of the rectum before being voided. Fluid from the upper gastrointestinal tract also enters the cloaca. Therefore, the cloaca receives an influx of water from the kidneys and the small intestine. The amount of time taken for urine reflux varies with the hydration state of the animal, with hydrated animals having a shorter transit time (Brummermann and Braun, 1994). Transit times in the lower intestine are short and normally only last for a few minutes (Nechay et al. 1968).

The influx of water into the cloaca can be reabsorbed through the epithelium of the lower intestinal tract to maintain hydration. In the lower intestine and cecae, water and sodium chloride are reclaimed by the process of sodium-linked water reabsorption (Thomas, 1982; Thomas and Skadhauge, 1982a, 1989; Anderson and Braun, 1985). Experiments with sparrows show that significant urine modification occurs in hydrated but not in dehydrated sparrows (Goldstein and Braun, 1988). In hens, salt-loading experiments show that on high salt diets the coprodeal epithelium absorbs virtually no salt, but on a lower salt diet, sodium absorption increases dramatically up to 100-fold (Chosniak et al. 1977; Thomas and Skadhauge, 1982b).

In birds, although ureteral urine is modified post renally, it is not known if reabsorption of water and solutes is evenly distributed along the length of the lower intes-

tine or localised in one region such as the cecae. Many birds have cecae that branch from the digestive tract at the ileorectal junction. These cecae vary in size from very small (< 1 mm) in some Passeriformes to very large (many centimeters) sacculated structures in Galliformes (McLelland, 1989). Thus, the amount of water reclaimed in cecae varies, and was found to be as high as 98% of all water entering the hindgut in the Rock Ptarmigan (*Lagopus mutus*) a member of the Galliformes (Gasaway et al., 1976). Sparrows (Order Passeriformes) possess only very small (approximately 1 mm) cecae and hence their functional capacity for sodium chloride and water reabsorption may be limited; however, this has not been investigated.

Diet has been shown to influence the microscopic structure of the avian lower intestinal tract. Generally, the more dilute the diet, the greater the surface area amplification due to villi and microvilli (Clauss et al., 1988; Dantzer et al., 1988; Brugger, 1991; Elbrønd et al., 1991; Ciccotti et al., 1993). Dietary changes causing an increase in microvilli abundance are associated with a 100-fold augmentation in the rate of NaCl flux across the epithelium (Dantzer et al., 1988). The principle mediator of these adaptations is the hormone aldosterone, which is known to affect Na^+ channels at the apical plasma membrane of coprodeal epithelial cells (Elbrønd et al., 1991).

Of the few studies examining changes in the lower intestine with diet, most have been laboratory based. One study has correlated changes in rectal epithelium microvilli with diet in wild birds (Goldstein et al., 1990). As that study was predominantly a physiological investigation, the authors presented only two electron micrographs and did not present any quantitation of their data. Another study by Amanova (1975) examined villi morphology within the lower intestine of wild desert birds. One other study has compared colonic surface area and habitat as extrapolated from data in the literature (Goldstein and Braun, 1986). The authors found that the galah (inhabiting an environment with less than 250 mm of annual rainfall and hence xeric dwelling) has a colonic surface area approximately one half that of the house sparrow and domestic chicken (both inhabiting environments having more than 500 mm of annual rainfall and hence mesic dwelling).

The present study employed stereology to quantitatively examine differences in the microvillus population along all regions of the lower intestinal tract (from the cecae to the cloaca) of two species of wild sparrows: the house sparrow, which is adapted to a mesic environment, and the savannah sparrow, which inhabits a salt marsh environment. The data show that microvilli surface area and packing density are greatest in proximal regions of the lower intestine. Both variables decline distally along the length of the intestine, with the decline being more pronounced in the intestine of the savannah sparrow. The implications of these anatomical findings with regard to retrograde peristalsis and environmental differences are discussed.

MATERIALS AND METHODS

Animals

Seven male house sparrows (*Passer domesticus*) were collected from the wild in Pennsylvania using mist nets. Birds were immediately transported to the laboratory for intestinal tissue processing. Mean body mass for the birds was 26.9 ± 2.1 g. The glutaraldehyde-fixed intestinal
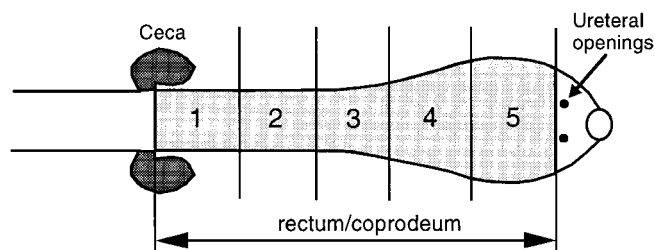

Fig. 1. Schematic representation of the lower intestine in the sparrows showing sampling strategy. The rectum/coprodeum (R-C) was defined as that area just distal to the attachment of the cecae on the intestine to the coprourodeal fold, just proximal to the ureteral openings. The R-C segment was divided into 5 subsegments and each was examined for their abundance of microvilli, as were the cecae.

tracts of three salt marsh savannah sparrows used in this study were made available by Prof. E. J. Braun from the University of Arizona. These birds were collected from Baja, California. Body mass data for these birds were not available. However, the intestinal tissue used in this study was taken from the same birds used in another study on osmoregulation and the average body mass was approximately 18 g (Goldstein, et al., 1990).

Tissue Preparation and Sampling

Captured birds were killed with an overdose intraperitoneal injection of sodium pentobarbital (65 mg/ml). The abdominal cavity was immediately opened and the lumen of the intestinal tracts from the ileum to the vent was flushed with half-strength Karnovsky's fixative (350 mOsm.). After flushing, the entire intestinal tract was immersed in fixative for 24 hr to ensure optimum fixation. The tissue was then cut horizontally along its long axis and rinsed in several changes of fresh phosphate buffer (pH 7.2) to remove any additional particulate matter. Morphological examination of the lower intestine revealed the absence of a rectocoprodeal fold in both species of sparrow. As it was not possible to accurately differentiate these regions, the area sampled for analysis was the rectum-coprodeum (R-C) segment (Fig. 1). The proximal end of this segment was located just distal to the point of attachment of the cecae. The distal end of the segment was easily identified at the coprourodeal fold just proximal to the ureteral openings (Fig. 2C). Thus, the R-C segment was terminated proximally at the cecae and distally at the coprourodeal fold (Fig. 1).

Prior to sectioning tissue, the arrangement of villi within the R-C region of the intestine was examined using a dissecting microscope. Examination revealed no difference in the surface architecture of villi between the two sparrow species, hence the R-C segment of one house sparrow was processed for scanning electron microscopy. The tissue was dehydrated in a series of graded alcohols, critically point dried, coated with gold, and viewed on a JOEL T220 scanning electron microscope.

After rinsing with phosphate buffer, the R-C segment of tissue from all remaining birds was divided into 5 smaller segments of approximately equal length. The length and width of each segment were recorded. Each segment was further subdivided into 5 equal subsegments and, of these, one segment was randomly selected and processed for
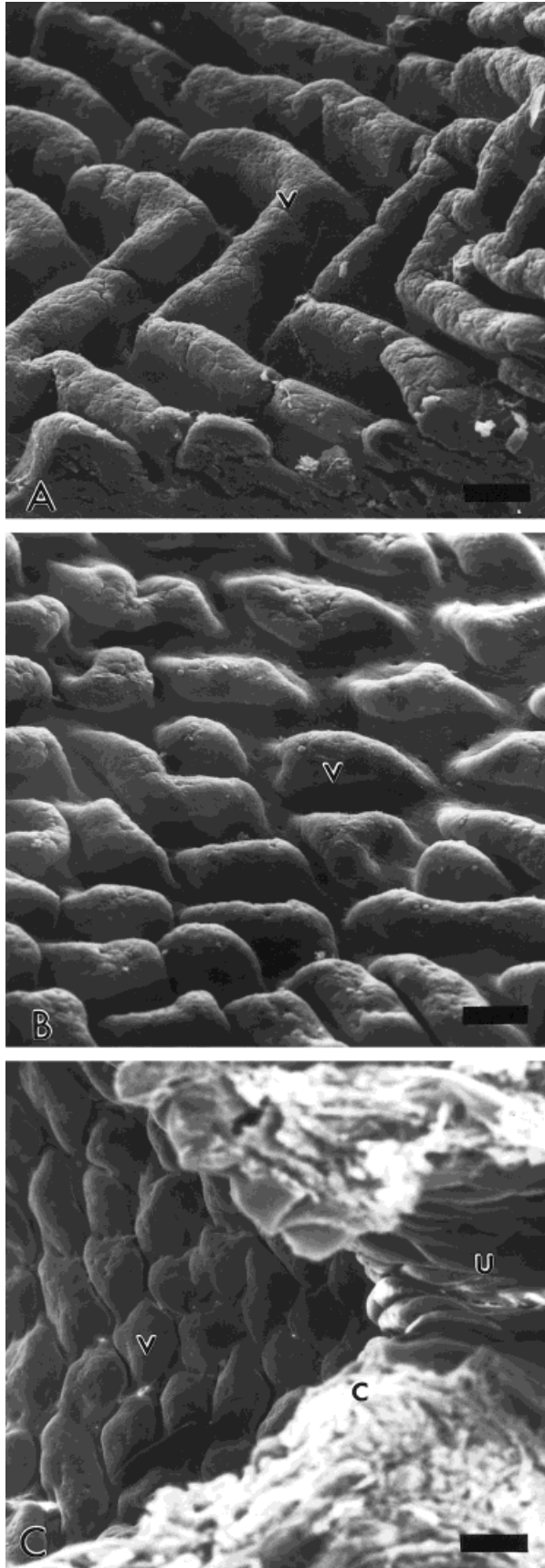
transmission electron microscopy (TEM). The tissue was dehydrated in a series of graded alcohols, then infiltrated with propylene oxide and embedded in epon resin. As intestinal tissue does not fulfill the requirements of being IUR (Isotopic, Uniform, Random) in space, the tissue was made IUR by sampling in the same manner as outlined in Makanya et al. (1995), so as to satisfy the requirements of vertical sectioning for estimation of surface area determination (Baddeley et al., 1986).

Vertical sections were cut at a thickness of 3 μm and stained with toluidine blue. The resulting stained sections were photographed using an Olympus MO 35-mm camera attached to an Olympus BX40 microscope. Micrographs were printed to a final linear print magnification of 120$\times$. Thin sections (90 nm) were then cut, mounted on TEM grids and stained with lead citrate and uranyl acetate. The resulting grids were viewed on a JEOL 100CX-2 electron microscope. A total of 5 micrographs per segment were prepared and printed to a final linear print magnification of 51,800$\times$.

Stereological Analyses

For stereological analysis of the R-C region, a three-level sampling scheme was used as described in detail by Makanya et al. (1995). Briefly, first the area occupied by the villi of the mucosa was estimated by calculating the circumference of each intestinal segment and multiplying by the length of the segment. These measurements were made at a gross macroscopic level using vernier calipers. Second, at the light microscopic level the surface area amplification due to villi S(v) was determined. Intersection counts were made using a cycloid test grid superimposed over light micrographs. Intersections between the test grid and the villi I(v) and mucosa I(m) were summed for each segment and the ratio I(v)/I(m) obtained. The total villi amplification S(v) was calculated using the ratio I(v)/I(m) and multiplied by the area occupied by the villi of the mucosa for each segment. Last, at the level of TEM, the extent to which the microvilli amplify the surface area of the mucosa S(mv) was determined using a cycloid test grid by counting the number of intersections between the test grid and traces of microvilli I(mv) and the apical epitheliocyte membrane I(em). From this, the absolute microvillus surface area was determined using the equation S(mv) = S(v) $\times$ I(mv)/I(em). Other parameters measured included the diameter d(mv) and height h(mv) of 5 microvilli from each segment. These measurements were obtained on whole microvilli whose boundaries (i.e., plasmalemma) were clearly outlined. These measurements were used to estimate the surface area of an average microvillus using the formula s(mv) = π $\times$ d(mv) $\times$ h(mv). Packing density of microvilli (i.e., the number of microvilli per unit surface area) was also estimated using the formula N(mv)/S(v) = [S(mv)/S(v)]/s(mv).

Fig. 2. Scanning electron micrographs showing the change in surface architecture of the villi of the house sparrow within the (**A**) rectum, (**B**) proximal portion of the coprodeum, and (**C**) distal portion of the coprodeum leading into the urodeum. Note the apparent decrease in the height of the villi distally along the intestine and the variation in villi morphology. V, villi; c, coprourodeal fold; u, urodeum. Scale bar = 100 μm.

Cecae

The left cecum of each bird was processed for light microscopy, embedded in JB4 acrylic resin, mounted on glass slides, and stained with hematoxylin and eosin. Ten parallel, equidistant sections (5-μm-thick) were taken throughout the length of the cecae to satisfy the unbiased tissue sampling techniques described by Gundersen et al. (1988). The luminal surface area S(l) of the cecae was estimated from 8 sections per cecum using the equation S(l) = 2 × 1/20 × mag^n × [I(l)/P(ep)], where 1/20 represents the ratio of test point number to cycloid arc length on the test grid, mag^n refers to the section magnification, and I(l) and P(ep) refer to the number of intersections hitting the cecal luminal surface and test points hitting the cecae, respectively.

The right cecae were processed routinely for TEM. Sections were cut at 90 nm, mounted on TEM grids, and stained with lead citrate and uranyl acetate. Sections were viewed in a JEOL 100CX-2 electron microscope and micrographs taken of the microvilli on the apical surface of the epithelial cells. For 50 microvilli from each sparrow species, data on diameter and height were calculated using the same criteria outlined for the intestine. Using the formulas outlined above for the intestine, these data were used to calculate the absolute surface area of microvilli and packing density of microvilli in the cecae.

Statistics

Data for average surface area of a microvillus, packing density of microvilli, and absolute surface area of microvilli were analyzed using a two-way ANOVA with a split-plot repeated measures design using the computer program Statistica. Differences in microvilli between species and along the R-C segments were tested using the Student-Newman-Keuls test on log transformed data. Significance levels were set at the 95% confidence interval for all variables.

Intestinal Fluid Sampling

Data on the concentration of intestinal fluid from the lower intestine of savannah sparrows was provided in Goldstein et al. (1990). The osmolality of intestinal fluid from wild house sparrows was sampled from the same house sparrows used for anatomical study. Once birds were captured in mist nets, intestinal fluid within the rectum and cloaca was immediately extracted from the animals by gently inserting a P200 Gilson pipette tip through the vent and into the rectum/cloaca. Fluid was drawn into the pipette by capillary action. Collected fluid was placed on ice and transported back to the laboratory where it was analysed for its osmolality using a Wescor 5520 Vapor Pressure Osmometer.

RESULTS

Villi Morphology

Luminal villi morphology varied greatly along the length of the rectum-coprodeum (R-C) segment in both species of sparrow. Villi within the first three sampling sections were oriented in a regular zigzag pattern and were separated by as much as 50 μm (Fig. 2A). Moving distally down the R-C segment (segments 4 and 5), as villi height decreased so did the space between them. In distal regions of the R-C segment, villi did not appear in the same ordered pattern as in the proximal regions of the rectum (Fig. 2B,C). There were no apparent differences in villi morphology between the two species of sparrow.

Microvilli Morphology: Rectum-Coprodeum

The height of microvilli decreased with length along the R-C segment from an average of 0.35 μm for all birds in segment 1 to 0 microvilli present distally along the segment. This trend was evident in both species of sparrow (Fig. 3). Microvilli appeared to be present in equal density in segment 1 in both species of sparrow; however, microvilli populations appeared to decrease more rapidly in the lower intestine of the savannah sparrow than the house sparrow (Fig. 3).

The surface area occupied by an individual microvillus decreased distally toward the coprodeum. For the house sparrow, segments 1 and 2 showed little variation in villi surface area, but this was followed by a sharp decline in subsequent segments (Figs 3, 4). In the savannah sparrow, the villi surface area of segment 1 was comparable to that of the house sparrow but showed a marked decrease in all subsequent segments (Fig. 4). Despite the variation in microvilli surface area between house and savannah sparrows in segment 2 (Fig. 4), total surface areas of the R-C segment did not differ significantly between the two species ($P = 0.24$). However, the decline in microvilli surface area along the lower intestine was significant from segment 1 to segment 5 in both species ($P < 0.001$) (Fig. 4).

Packing density gives an indication of the abundance of microvilli per unit surface area. For both species of sparrow, the packing density of microvilli was highest proximally in the R-C segment and decreased distally reaching its lowest density in the coprodeum (Fig. 5). Although the decrease in packing density was greater in the savannah sparrow than the house sparrow, the difference was not statistically significant ($P = 0.14$). This decline along the length of the intestine was significant for both species ($P < 0.001$).

The presence of microvilli greatly increased the absolute surface area of the R-C segment in proximal portion of the rectum in both species of sparrow (Fig. 6). In both species, microvilli surface area decreased significantly with distance along the R-C segment, reaching its lowest level in the coprodeum ($P < 0.001$). The decline in surface area was more rapid in the savannah sparrow than in the house sparrow. Despite the variation in absolute surface area between house and savannah sparrows in segment 2, when all areas of the R-C segment were grouped together, there was no significant difference in the absolute surface area of microvilli between the two species ($P = 0.12$).

Microvilli Morphology: Cecae

Microvilli were more abundant in the cecae of the savannah sparrow compared to the house sparrow. The surface area of the average microvillus was 33% greater in the savannah sparrow and this difference was significant ($P < 0.001$) (Table 1). In addition, the microvilli of the savannah sparrow cecae had significantly higher ($P < 0.001$) packing density. Finally, the absolute surface area occupied by the microvilli within the cecae was significantly greater in the savannah sparrow by 60% ($P < 0.001$) (Table 1).
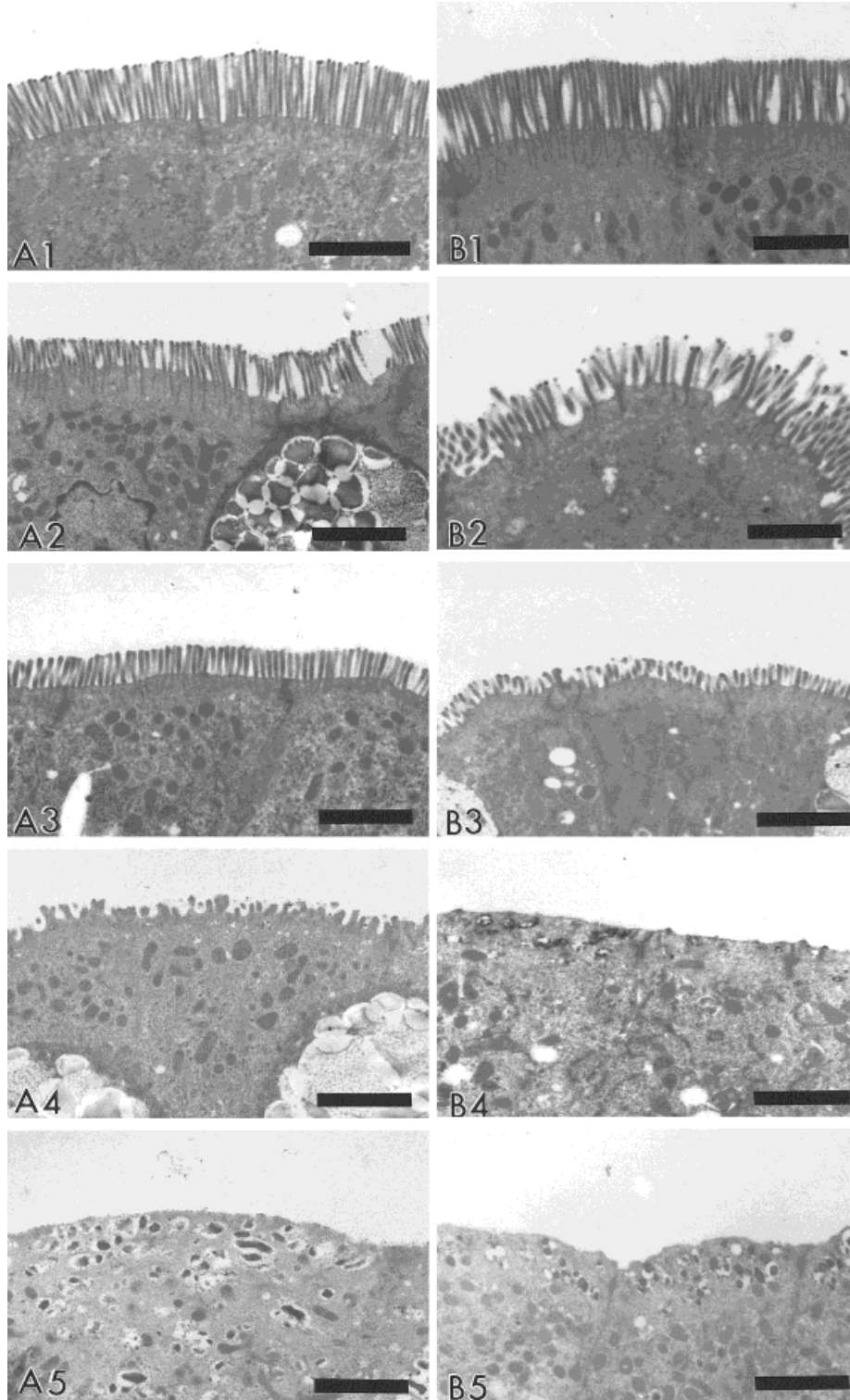
Fig. 3. Transmission electron micrographs showing the change in height of microvilli as a function of the level of sampling from segments 1 to 5 (i.e., proximal to distal) along the rectum/coprodeum (R-C) segment in both (**A**) house and (**B**) savannah sparrows. Scale bar = 0.5 μm.
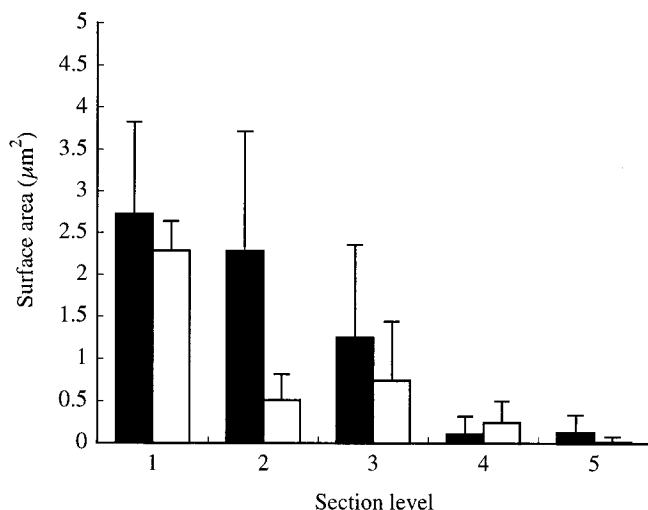
Fig. 4. Surface area of an average microvillus as a function of distance along the rectum/coprodeum (R-C) segment from sampling segments 1 to 5 (i.e., proximal to distal). Filled bars, house sparrows; open bars, savannah sparrows.

Fig. 6. Absolute surface area of microvilli as a function of distance along the rectum/coprodeum (R-C) segment from sampling segments 1 to 5 (i.e., proximal to distal). Filled bars, house sparrows; open bars, savannah sparrows.
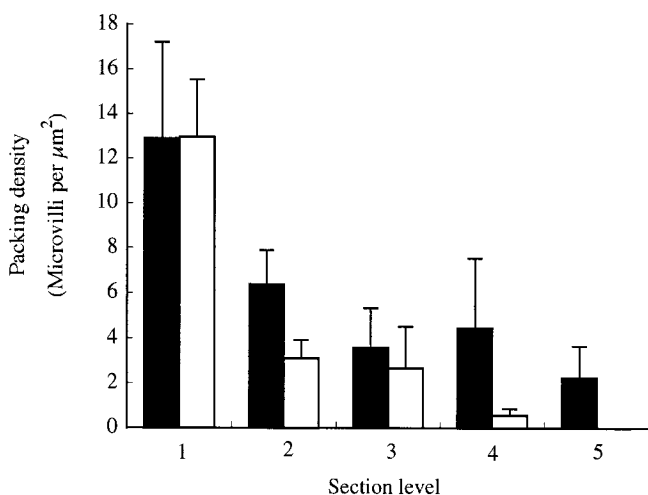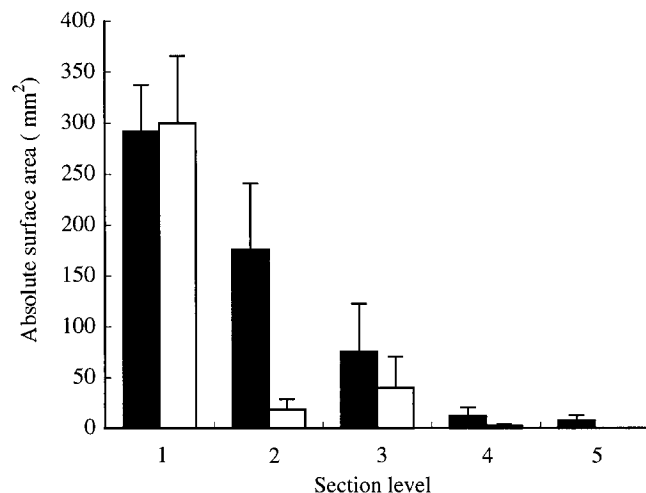

Fig. 5. Packing density of microvilli as a function of distance along the rectum/coprodeum (R-C) segment from sampling segments 1 to 5 (i.e., proximal to distal). Filled bars, house sparrows; open bars, savannah sparrows.

TABLE 1. Characteristics of microvilli (mv) located on the luminal surface of cecae in two species of sparrow*

Variable	House sparrow	Savannah sparrow
Surface area of mv (μm^2)	0.63 ± 0.15 (50)	0.81 ± 0.17 (50)
Packing density (mv/μm^2)	3.31 ± 0.1 (50)	5.54 ± 0.2 (50)
Absolute surface area (mm^2)	8.63 ± 2.81 (50)	13.86 ± 2.52 (50

*Data are means $\pm$ S.E. Sample sizes are shown in parentheses.

Intestinal Fluid

The concentration of lower intestinal fluid from the house sparrows used in this study was 420 ± 158 mOsm/kg H_2O. This compares with a concentration of 650 ± 228 mOsm/kg H_2O in the savannah sparrows reported by Goldstein et al. (1990). These values were statistically significant ($P < 0.05$).

DISCUSSION

The results of this study indicate that the number and height of villi decreased with distance distally along the lower intestinal tract of sparrows. In the proximal portion of the rectum, the villi were arranged in a zigzag pattern, similar to that seen for other species of sparrow (Amanova, 1975). This pattern presumably favours the retention of chyme for the processes of water and sodium chloride absorption within the rectum. In addition, the absolute surface area and packing density of microvilli in sparrows also decreased with distance distally along the lower intestine in both species, and this suggests that most reabsorption of ureteral urine may occur along proximal portions of the rectum and possibly the cecae. Many studies have shown that both NaCl and water are reabsorbed in the rectum and cecae (Thomas, 1982; Thomas and Skadhauge, 1982a, 1989; Anderson and Braun, 1985), but no studies of which this author is aware have reported regionally differential absorption.

The kidneys of birds are not as efficient as those of mammals in producing a concentrated urine. In fact, the maximum ureteral urine to plasma (U/P) ratio in birds is only 2.5, one-tenth of the maximum U/P ratio in mammals (MacMillen and Lee, 1967; Braun and Dantzler, 1972). Therefore, ureteral urine that empties into the urodeum of the cloaca has a relatively high water content. Since proximal regions of the rectum have the most abundant population of microvilli, the anatomical evidence suggests that this is the area where most reabsorption of ureteral urine may occur. These data emphasize the importance of retrograde peristalsis for water retention.

The results of the present study showed no significant differences in packing density or individual or absolute surface area of microvilli in the lower intestine of house and savannah sparrows. The results in the present study are contradictory to laboratory-based studies where differences in the number of microvilli were found on species feeding on different diets (Makanya et al., 1997). In addition, microvilli abundance has been shown to be greater in the lower intestine of hydrated animals compared to dehydrated animals (Mayhew et al., 1990, 1992; Elbrønd et al., 1991). This coincides with physiological experiments showing that a significant amount of postrenal urine modification occurs in hydrated animals but not in dehydrated animals (Goldstein and Braun, 1986, 1988). Savannah and house sparrows are granivores, but each species inhabits different environments. The savannah sparrows inhabit a salt marsh environment (and the animals may be prone to dehydration by their environment) compared to the house sparrows, which inhabit a mesic environment. Despite osmoregulatory differences brought about by their environment, both species of sparrow have about the same physiological capacity to cope with conditions of dehydration (due to a low availability of fresh water) (Goldstein and Braun, 1986, 1988; Goldstein, et al., 1990). Thus, it may not be surprising that microvilli populations in the lower intestine of these two species of sparrow are not statistically different.

Some of the savannah sparrows used in this study were the same as those used for the study by Goldstein et al. (1990). As part of that study, the authors measured the concentration of intestinal fluid in savannah sparrows and found it to be hyperosmotic to plasma (650 ± 228 mmol/kg). The concentration of intestinal fluid in house sparrows measured in the present study was (420 ± 158 mmol/kg). Thus, intestinal fluid in the house sparrows was more dilute than in the savannah sparrows. Although not statistically significant, the results of the present study showed a trend of a higher absolute surface area and packing density of microvilli in the house sparrow, especially in segments 2 to 5. These findings support the previously observed association of greater microvillar surface area and more dilute urine (Clauss et al., 1988; Dantzer et al., 1988; Brugger, 1991; Elbrønd et al., 1991; Ciccotti et al., 1993).

Although not statistically significant, data from the present study show that savannah sparrows have a lower microvilli packing density, hence absolute surface area than house sparrows. More dilute diets seem to be correlated with more microvilli per unit area in the lower intestine. Previous studies have examined the packing density of microvilli in the lower intestine of other birds. For example, Mayhew et al. (1992) reported a statistically higher packing density in the coprodeum of hens fed a low salt rather than a high salt diet. Makanya et al. (1995) showed that packing density of microvilli of insectivorous bats was less than that of frugivorous bats, although in this later study, the authors did not report any statistical analyses of their data.

In birds, ureteral urine travels orad in the lower intestine and can enter the cecae (Duke, 1989; Clauss, et al., 1991). As urine enters the cecae, sodium-linked water reabsorption occurs and this is presumably aided by an increase in microvilli surface area. In the present study, the surface area of the average microvilli in the cecae, packing density, and absolute surface area was greater in the savannah sparrow, and indicates the potential for a greater amount of water and solute reabsorption in the cecae of the savannah sparrows. However, the overall amount of water and solute reclamation is not known, and as the cecae in sparrows are very small organs, it may not amount to a significant contribution of the overall volume of fluid entering the lower intestinal tract.

The stereological data reported in this study provide quantitative estimates of actual anatomical measurements. As such, the results of this study are not free of bias. One such bias occurs in fixation and embedding of the tissue. However, this was kept to a minimum through the use of glutaraldehyde fixation and resin embedment, which introduce less distortion than other fixative-embedding combinations (Hayat, 1981; Burton and Palmer, 1988). Because of the small size of the microvilli, another bias that may lead to an overestimation of microvilli parameters is due to the Holmes effect, when the thickness of the specimen section approximates the dimension of the anatomical structure being measured (Gundersen, 1979).

Despite all of the studies conducted on the lower intestinal tract of birds, surprisingly little is known about the morphology of the epithelium. The present study shows that microvilli are present in greater numbers in proximal regions of the rectum and presumably this is the area where most reclamation of sodium chloride and water occur. In addition, species with a more dilute diet may possess more microvilli to amplify the epithelial surface area than species with a more concentrated diet. Data in the present study suggest this trend, but the results were not statistically significant at the 95% confidence interval, perhaps due to the small sample size available of the savannah sparrows. Like the rectum, the cecae have a well-defined layer of microvilli but in sparrows, as they are very small structures, they may play only a minor role in water and solute reabsorption from ureteral urine. In birds, if most reabsorption of ureteral urine occurs in proximal regions of the lower intestinal tract (as the anatomical data suggest), then the importance of retrograde peristalsis within the system cannot be understated. Further physiological studies on regional uptake differences within the lower intestine of birds are needed.

ACKNOWLEDGMENTS

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