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INFLUENCE OF DIETARY SODIUM AND OTHER FACTORS ON PLASMA ALDOSTERONE CONCENTRATIONS AND *IN VITRO* PROPERTIES OF THE LOWER INTESTINE IN HOUSE SPARROWS (*PASSER DOMESTICUS*)

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Summary

House sparrows (*Passer domesticus*) had plasma aldosterone concentrations of about 180pgml^{-1} while maintained on a low-sodium diet (LS, 0.1mequiv Na^+ ingested per day), 135pgml^{-1} on a sodium intake of 0.9mequivday^{-1} (high-salt diet, HS) and 45pgml^{-1} on a Na^+ intake of 3.8mequivday^{-1} (high-salt diet with saline drinking water, HSS). The plasma concentration of aldosterone changed to the LS or the HS level within 1 day of switching from the HS or the LS diet, respectively. Neither dehydration (22h, 14.5% loss in body mass) nor brief periods of stress (1–5min of handling) caused a change in circulating levels of aldosterone. The electrical properties of the lower intestine acclimated to the different sodium intakes with a time course similar to that of the changes in aldosterone levels. On the LS diet, the lower intestine generated an electrical potential difference (PD) of 5mV (lumen negative) and a short-circuit current (I_{sc}) of about 50 μA cm^{-2}; these were consistently inhibited by amiloride (resulting in a lumen-positive PD) and were stimulated by glucose or amino acids (leucine and lysine) in about half of the tissues. In HS birds, the PD and I_{sc} were abolished and the effects of glucose and amino acids were reduced, but amiloride still caused a significant change in transmural PD (to a mucosa-positive value). These properties resemble those of the chicken coprodeum more than they do those of chicken colon, although the tissues tested were from the mid-region of the large intestine and their histology resembled that of colon. Sparrows tested immediately upon capture from the wild had plasma aldosterone levels not significantly different from those of birds on the LS diet, which is consistent with the known diet of this species. However, I_{sc} was higher and tissue resistance was lower in wild birds compared with low-salt birds in the laboratory, perhaps indicating the influence of other hormones in addition to aldosterone.

Introduction

Osmoregulatory homeostasis is one of the important physiological requirements for a bird to survive in a changing environment. Avian hydromineral balance involves the interaction of numerous organ systems, including the kidneys, intestines, respiratory tract and skin, and these are regulated by a variety of physiological signals and neural and hormonal modulators. An evaluation of the osmoregulatory condition of birds in their

Key words: aldosterone, coprodeum, colon, intestinal transport, sparrow, *Passerdomesticus*.

natural habitat could potentially be based on examination of any of these organs and their regulators. Yet few studies have evaluated osmoregulatory condition in wild birds, and most of that work has been based on just a few physiological variables (such as total water turnover rate). In recent studies of small passerine birds, I have explored the use of additional indicators of hydromineral balance, including osmotic and/or electrolyte concentrations of plasma, urine and intestinal contents (Goldstein and Zahedi, 1990; Goldstein *et al.* 1990), haematocrit (Goldstein and Zahedi, 1990) and intestinal morphology (Goldstein *et al.* 1990). In the present study, this objective is extended to an evaluation of whether circulating levels of aldosterone and electrical properties of the lower intestine might also be useful indicators of water and electrolyte balance.

Aldosterone is one of the principal hormones involved in regulating the excretion of sodium in birds. However, the patterns of change in circulating levels of aldosterone have been quantified in just four avian species, including three gallinaceous birds [the chicken *Gallus domesticus* (e.g. Skadhauge *et al.* 1983; Arad *et al.* 1985; Rosenberg and Hurwitz, 1987), the turkey *Meleagris gallopavo* (Rosenberg and Hurwitz, 1987) and the Japanese quail *Coturnix coturnix* (Kobayashi and Takei, 1982)] and the domestic duck *Anas platyrhynchos* (Harvey *et al.* 1984; Klingbeil, 1985). In these studies, increases in plasma aldosterone concentration were consistently stimulated by low-sodium diets, and stress also stimulated release of the hormone in chickens, ducks and turkeys. However, interspecific variation is also evident. For example, the plasma concentration of aldosterone on a high-salt diet varied from $5\,\mathrm{pg\,ml^{-1}}$ in chickens (Arnason *et al.* 1986) to $35\,\mathrm{pg\,ml^{-1}}$ in domestic ducks (Klingbeil, 1985); furthermore, aldosterone concentration was elevated during dehydration in some species (Kobayashi and Takei, 1982; Klingbeil, 1985) but not in others (Arad *et al.* 1985).

One of the important physiological effects of aldosterone in birds is to promote sodium absorption by epithelia of the colon and coprodeum (Clauss *et al.* 1984). In chickens, injection of aldosterone into animals on high-salt diets induces many of the properties seen in animals eating diets low in sodium. Changes observed in the colon and/or coprodeum of chickens placed on a low-salt diet include an enlarged absorptive surface area (Clauss *et al.* 1988), stimulation of sodium absorption (Thomas and Skadhauge, 1979), an increase in electrical potential and short-circuit current (Thomas and Skadhauge, 1982) and a change from sodium absorption that is insensitive to amiloride and stimulated by glucose and amino acids to transport that is inhibited by amiloride but insensitive to the small organic compounds (Lind *et al.* 1980). As for aldosterone, studies of these changes are restricted to a few species, including the chicken (e.g. Thomas and Skadhauge, 1982), the duck (Skadhauge *et al.* 1984) and the galah *Cacatua roseicapilla* (Skadhauge and Dawson, 1980).

As in these other species, the lower intestine of the house sparrow is known to absorb sodium and water (Goldstein and Braun, 1986) and to have an important role in hydromineral balance (Goldstein and Braun, 1988). However, the *in vitro* properties of this tissue and their relationship to variation in plasma aldosterone levels have not been studied in any passerine or other small bird. The utility of these variables as indicators of osmoregulatory condition of wild-caught animals depends on their range and time course of variation. I therefore examined the relationship between dietary sodium intake, plasma

aldosterone levels and lower intestinal electrical properties in house sparrows, and also evaluated the effects of time of day, handling stress and dehydration on plasma aldosterone levels.

Materials and methods

Capture and maintenance of animals

House sparrows (*Passer domesticus* L.) were captured from wild populations in Greene County, Ohio. This capture area is characterized by agricultural land interspersed with native woodland and residences. Birds used for laboratory studies were kept one or two per cage with a photoperiod of 11h light (08:00–19:00h):13h dark and a temperature of approximately 24˚C. The birds were fed one of three diets. The low-sodium (LS) diet ($0.02 mequiv Na^{+}\,g^{-1}$ food) was a mixture of soy granules (20% by weight), wheat (40%) and barley (40%); these birds drank tap water. The high-sodium (HS) diet consisted of the low-salt food to which NaCl was added (final sodium content $0.2 mequiv\,g^{-1}$ food); these birds also drank tap water. The third group of birds (HSS birds) received the high-salt food and 1% NaCl drinking water. The food for all birds contained $0.12 mequiv\,g^{-1}$ K^{+}. Albon (sulfadimethoxine) was added to the drinking water as a prophylactic against coccidiosis, and vitamins were added to the water at least once per week. Birds were weighed after acclimation to their various diets (see below); the mean body mass was 23.9±3.3g (*N*=19), and was unaffected by diet.

Birds were maintained on either the LS or the HS diet for at least 1 week prior to switching to the opposite diet for measurements. Birds in the HSS group were first kept for at least 1 week on the HS diet. Birds were tested after being on the LS diet for 1, 3 or 7 days, on the HS diet for 4h, 24h or more than 3 days, and on the HSS diet for more than 3 days. These times were chosen based on the time course of changes in plasma aldosterone in chickens switched among diets (Thomas and Skadhauge, 1982). To test for diurnal variation in plasma aldosterone levels, birds on the LS diet for 7 days and on the HS diet for more than 3 days were sampled both in the first 0.5h of light (08:00–08:30h) and towards the end of the light phase of the photoperiod (17:30–18:30h). The 4h HS birds were measured between 13:00 and 13:30h. All other measurements were made in the morning (08:00–10:30h).

Dehydration was studied in birds maintained on the HS diet. I removed all drinking water, but not food, at 10:00h, and sampled blood at 08:00h the following morning. This regimen produced a significant increase in plasma osmolality (from 340±8 to $384±12 mosmol\,kg^{-1}$, *N*=5; see also Goldstein and Zahedi, 1990).

I assessed the effect of a brief stress (handling) on plasma aldosterone levels by collecting ureteral urine prior to blood sampling. Birds on the HS diet were removed from their cages and cloacal cannulas, constructed from polyethylene tubing (PE 300) and designed to collect ureteral urine (Goldstein and Braun, 1986), were inserted. Urine was collected for 1–4min. Occasionally a bird escaped from its cage and was captured by net in the laboratory. I also considered these birds stressed, and analysed data from them together with data from birds whose urine was sampled. The effect of these brief stresses was assessed by comparing plasma aldosterone in these birds with levels in HS birds.

Blood from wild birds was collected from animals captured in mist nets. I took blood samples immediately upon capture, and the time from capture to sampling was similar to that required for unstressed birds in the laboratory.

Measurement of aldosterone and plasma electrolytes

I collected blood for the aldosterone assay by puncture of a brachial vein. Birds were removed from their cages and a blood sample (approximately 150–200 μl) was immediately taken. This procedure took approximately 2min.

Blood was centrifuged and a sample of plasma was separated for measurement of Na^+ and K^+ concentrations by flame photometry (Intrumentation Laboratories 343). I stored the remaining plasma at −20°C until assay for aldosterone, which, for most samples, was carried out within 1 week of sample collection.

Aldosterone was assayed using a commercially available assay kit (Coat-a-Count aldosterone assay kit, Diagnostic Products Corp., Los Angeles, CA). The kit is designed for use with 200 μl samples of plasma. I was unable to collect samples this large from the sparrows, at least within a short enough time for the birds to be considered unstressed. Hence I brought the sparrow plasma samples up to a final volume of 200 μl by adding the appropriate volume of one of the assay kit's aldosterone standards (50pgml^{-1} aldosterone in reconstituted serum). I assayed these 'spiked' samples and then calculated the concentration of aldosterone in the original plasma sample. The characteristics of this solid-phase radioimmunoassay kit have been extensively tested (Diagnostics Products Corp. bulletin on the Coat-a-Count aldosterone assay kit). However, I conducted three checks on the use of this assay with our samples. First, I measured the intra-assay and inter-assay variation for 200 μl plasma samples taken from a pool of plasma collected from chickens maintained on the low-sodium diet for 4 days. Second, I compared the aldosterone concentrations calculated from 75 or 100 μl samples ('spiked' to a final volume of 200 μl) with concentrations measured in the same assay on 200 μl samples. Finally, I measured the intra-assay coefficient of variation for spiked 75 and 100 μl samples.

The results of these analyses were as follows. The inter-assay coefficient of variation for 200 μl plasma samples, based on five assays, was 11.9%, slightly higher than the value presented by the assay manufacturer for samples with a similar aldosterone concentration (89±10pgml^{-1} for the chicken plasma). The calculated aldosterone concentrations in 75 and 100 μl spiked samples did not differ significantly from each other, and averaged 90±10% (N=12) of the value measured in the same assay on 200 μl unspiked samples. Intra-assay coefficients of variation for aldosterone concentrations calculated for 75 and 100 μl spiked samples were 11.3 and 7.4%, respectively. These results suggest that the procedure for spiking samples yields consistent results, with variability no greater than that measured when using full 200 μl samples.

In vitro *characterization of the lower intestine*

I measured the electrical potential difference (PD) and short-circuit current (I_{sc}) of colonic tissue mounted *in vitro* in Ussing chambers. The chambers had a luminal

diameter of 4mm, exposing a tissue surface of 0.126cm^2. Electrical properties were measured using a Physiologic Instruments VCC600 voltage clamp. The chamber was connected to calomel reference electrodes *via* 3 % agar/Ringer's solution bridges. Before the start of each experiment the voltage clamp was adjusted, using a fluid-filled chamber, to compensate for the fluid resistance in the chamber. A sparrow was then rapidly decapitated and the colon was removed and mounted in the chamber without stripping the serosal smooth muscle layers. Avian Ringer's solution (composition in mmol l^{-1}: KCl 5.9, NaH_2PO_4 1.2, NaCl 115, $NaHCO_3$ 25, $CaCl_2$ 1.25, $MgCl_2$ 1.1) warmed to 41˚C and aerated with 95% O_2/5% CO_2 was circulated on both sides of the tissue. I recorded PD and I_{sc} with this initial solution and after sequentially adding (to the mucosal half of the chamber) glucose (5mmol l^{-1}), leucine and lysine (4mmol l^{-1} each, added together) and amiloride (10^{-4} mol l^{-1}).

Histology

I examined the histology of the lower intestine of two house sparrows (one freshly captured from the wild, the other a bird on the HS diet). I was particularly interested in examining the point of transition from colonic to coprodeal morphology; no obvious transition point is evident upon gross examination of the dissected lower intestine. I removed the lower intestine, fixed it in 4% cacodylate-buffered glutaraldehyde, rinsed it in sodium cacodylate buffer, pH7.2, and then cut the lower intestine into 2mm sections. The entire lower intestine measured 10–11mm in length. Pieces of intestinal tissue were then post-fixed in 1% cacodylate-buffered osmium tetroxide, dehydrated in a graded series of alcohols, and embedded in epoxy resin. Approximately 1 μm sections were cut and stained with Toluidine Blue.

Statistics

I tested for statistical differences between treatment groups using analysis of variance and Newman–Keuls *post-hoc* testing. Probabilities of less than 0.05 were considered to be significant.

Results

Dietary sodium intake and plasma electrolytes

The mass of food consumed did not differ between dietary treatments. However, birds on the HS and HSS diets drank significantly more than LS birds (Table 1). The patterns of food and water intake on the three diets resulted in substantially different sodium intakes but similar potassium intakes (Table 1). Birds on each of the diets were able to regulate their electrolyte balance, and I measured no significant differences in levels of plasma electrolytes between groups (Table 2).

Effects of dietary sodium intake on plasma aldosterone concentrations

The aldosterone concentration in HSS sparrows averaged 46pgml^{-1}, that for HS birds was 136pgml^{-1} and that for LS birds was 183pgml^{-1} (Table 2). The increase in plasma aldosterone from the HS to the LS condition was accomplished within the first 24h after

transfer between diets (the earliest time point examined). Similarly, the transition from the LS to the HS condition was also accomplished within the first 24h. Levels of plasma aldosterone in birds on the HS diet for just 4h were very variable, probably reflecting differences in the amount of food consumed, and hence of sodium ingested, during that short time interval. Nevertheless, the increased variability found in the LS condition suggests that aldosterone levels were already beginning to respond within 4h of switching diets.

Wild birds had plasma aldosterone levels averaging 290pgml^{-1} (Table 2), which differed significantly from the value for HSS and HS birds but not from that for LS birds.

Table 1. *Dietary electrolyte intake in house sparrows receiving a low-salt diet and tap water (LS), a high-salt diet and tap water (HS), and a high-salt diet with 1% NaCl water (HSS)*

	LS	HS	HSS
Food consumption (gday^{-1})	6.0±1.1 (8)	4.7±0.7 (6)	5.9±0.8 (5)
Drinking rate (mlday^{-1})	7.5±1.7 (4)	13.8±4.1 (6)	15.3±6.5 (9)
Na^+ intake (mequivday^{-1})	0.1	0.9	3.8
K^+ intake (mequivday^{-1})	0.7	0.6	0.7

Na^+ and K^+ intakes are calculated from mean food and water intake rates for each group.
Values are mean ± S.D. (*N*).

Table 2. *Plasma electrolyte and aldosterone concentrations in house sparrows under a variety of conditions*

	Na^+ (mequiv l^{-1})	K^+ (mequiv l^{-1})	Aldosterone (pgml^{-1})
LS diet, 1 day	173.5±5.0 (5)	1.8±0.6*,† (5)	207.3±14.4* (5)
LS diet, 3 days	150.0±6.1 (3)	3.3±0.5 (3)	296.2±89.9* (5)
LS diet, 7 days	152.6±4.3 (10)	4.5±0.4 (10)	182.7±28.3* (11)
HS diet, 4h	153.2±3.7 (6)	3.8±0.6 (6)	224.5±101.5 (6)
HS diet, 1 day	151.7±3.5 (6)	4.0±0.6 (6)	123.4±44.6† (6)
HS diet, ⩾4 days	149.1±6.0 (11)	4.8±0.4 (11)	136.3±33.6† (11)
HSS diet, ⩾4 days	156.3±7.1 (6)	2.6±0.7*,† (6)	45.9±13.2*,† (6)
Dehydration	161.0±11.3 (2)	4.6±0.1 (2)	86.1±28.8† (5)
Wild birds	151.3±1.8 (4)	4.4±0.4 (4)	289.8±65.3* (5)

Values are mean ± S.D. (*N*).
*Significantly different from HS, 4 days; †significantly different from LS, 7 days.

Effects of time of day, dehydration and handling stress on plasma aldosterone

Plasma aldosterone concentrations at the beginning and the end of the light phase of the photoperiod were the same for birds on the HS diet (135±41pgml^{-1} at 08:00h, 139±67pgml^{-1} at 18:00h) and for those on the LS diets (175±49pgml^{-1} at 08:00h, 189±40pgml^{-1} at 18:00h).

The sparrows lost 3.6±0.5g during their 22h dehydration (equivalent to 14.5% of body mass). Dehydration of HS birds did not result in any change in aldosterone concentration (Table 2, comparing dehydrated and HS birds). Similarly, the handling stress that I imposed on the birds (urine collection prior to blood collection) did not affect aldosterone concentrations (147.3±55.8pgml^{-1}, not significantly different from the value for HS birds). Confirming this, I found no significant correlation between plasma aldosterone levels and handling time (examined for handling times ranging from 1 to 5min).

Effects of dietary sodium intake on electrical properties of colon

The electrical PD and short-circuit current of the colon mounted *in vitro* tended to be highest early in an experiment and then to decline with time, as reported previously for lower intestinal tissues from the galah (Skadhauge and Dawson, 1980) and the chicken (Skadhauge, 1981, p. 116). The high early values are presented in Table 3. Addition of metabolic substrates (5mmol l^{-1} glucose or 1mmol l^{-1} hydroxybutyrate) to the perfusion solution either at the start of or during an experiment had no effect on the decline. Despite this, the effects, when present, of glucose, amino acids or amiloride were readily apparent, as they occurred rapidly compared with the time scale of the decline. The electrical variables were consistently more stable after amiloride had been added, even though the absolute magnitude of the PD was often similar before and after addition of this drug (though with sign reversed; see below).

The PD of colon from house sparrows acclimated to the LS diet for a full week averaged approximately 5mV, lumen negative, and I_{sc} was approximately 70 μA cm^{-2}.

Table 3. *Electrical variables of house sparrow lower intestine measured at the start of the experiment*

	PD (mV)	Percentage with PD<0	I_{sc} (μA cm^{-2})	Resistance (Ωcm^2)	*N*
LS diet, 1 day	−2.2±0.5*	100	−39.7±10.2*	60.1±4.9	5
LS diet, 3 days	−1.5±0.8	80	−42.2±21.8*	45.8±9.3	5
LS diet, 7 days	−5.1±2.0*	100	−69.0±23.1*	53.9±7.6	5
HS diet, 4h	−3.1±6.9	60	−12.5±21.7†	54.7±5.8	5
HS diet, 1 day	2.9±3.5†	40	69.0±56.8†	74.6±10.5	5
HS diet, ⩾4 days	−0.4±1.4†	33	15.2±21.1†	68.3±12.5	6
HSS diet, ⩾4 days	0.4±0.9	57	7.7±16.8†	58.1±2.9	7
Wild birds	−4.4±1.1	83	−137.3±52.5*,†	35.3±6.7*,†	6

PD, electrical potential difference; I_{sc}, short-circuit current; values of PD<0 indicate lumen negative; *N*, sample size.

*Significantly different from HS, 4 days; †significantly different from LS, 7 days.

In contrast, birds that had been on HS and HSS diets for 4 days had mean colonic PD and I_{sc} values not significantly different from 0, and approximately half of these birds had lumen-positive PDs (Table 3). The change from the LS to the HS condition was quite rapid; after 4h on the HS diet, 40% of birds already had a lumen-positive PD. The changes in PD and I_{sc} in the reverse direction (desalination) appeared to be complete by 1 day, by which time all birds measured had lumen-negative electrical potentials.

The colonic resistance, calculated from PD and I_{sc}, averaged $60\,\Omega cm^2$ and did not vary between dietary groups (Table 3).

The effects of glucose and amino acids on colon I_{sc} were variable (Table 4). Glucose had no effect on tissues from LS birds, except in one individual on that diet for 1 day. In contrast, glucose did affect approximately half of the HS birds within 4h on this diet. The time pattern of amino acid effects was similar to that for glucose. Amino acids had no effect on birds eating the LS diet but did affect approximately half of the birds on the HS diet. Approximately 60% of the birds that responded to glucose also responded to amino acids.

Amiloride had an immediate and rapid effect on almost all tissues studied, regardless of diet (Table 4). Application of amiloride consistently resulted in a lumen-positive PD, often of similar or greater magnitude than the lumen-negative PD that obtained before amiloride.

Birds measured freshly captured from the field had colonic electrical properties like those of LS birds: electrical potential was lumen-negative in all but one bird, amiloride reversed this PD, and glucose and amino acids were without effect (Tables 3, 4). Freshly captured birds had the lowest tissue resistances of all the groups.

Histology of the lower intestine

The intestinal villi in the first 5mm posterior to the juncture with the digestive caeca were parallel and tall, approximately 300 μm in height (Fig. 1A). The villar height decreased to about 125 μm in the caudal half of the intestine (Fig. 1B). The villi were shorter (85 μm in height) and irregular in the most caudal 2mm (Fig. 1C). This latter pattern resembles that observed in coprodeum of several other passerine species (Johnson and Skadhauge, 1975).

Discussion

Plasma aldosterone levels in house sparrows

Effect of dietary Na^+ intake and time course of change

The circulating levels of aldosterone vary among species. Values for birds on a high-sodium diet vary from 5 (Arnason *et al.* 1986) to $35 pgml^{-1}$ (Klingbeil, 1985); values for birds on a low-sodium diet range from 85 (Klingbeil, 1985) to $165 pgml^{-1}$ (Arnason *et al.* 1986). The sparrows were on the high side of this range (Table 2). In both chickens and ducks, the time course for changing plasma aldosterone concentration is more rapid upon switching from a low- to a high-sodium diet than after the reverse change (Thomas and Skadhauge, 1982; Harvey *et al.* 1984; Radke *et al.* 1984). I am unable to draw any

Table 4. *Effects of glucose (5 mmol l^{-1}), amino acids (4 mmol l^{-1} leucine plus 4 mmol l^{-1} lysine) and amiloride (10^{-4} mol l^{-1}) on short-circuit current (I_{sc}) of house sparrow lower intestine*

	Glucose			Amino acids			Amiloride		
	Percentage stimulation of PD	Percentage of birds responding	*N*	Percentage stimulation of PD	Percentage of birds responding	*N*	Percentage inhibition of PD	Percentage of birds responding	*N*
LS diet, 1 day	4.2±3.2	20	5	0*	0	5	75.2±48.5	60	5
LS diet, 3 days	0*	0	5	0*	0	5	423.3±201.5	100	5
LS diet, 7 days	0*	0	4	20.2±19.0	20	5	271.0±105.5	100	5
HS diet, 4 h	21.2±14.3†	40	5	15.6±15.0	40	5	157.2±40.3	100	5
HS diet, 1 day	91.9±57.3†	80	5	59.3±21.1	75	4	110.4±50.2	100	3
HS diet, ⩾4 days	16.1±11.1†	33	6	38.0±25.1	33	6	140.2±95.2	60	5
HSS diet, ⩾4 days	125.1±101.3	43	7	31.9±22.0	50	6	149.2±27.7	80	5
Wild birds	0*	0	5	0*	0	5	165.5±32.7	100	5

*Significantly different from HS, 4 days; †significantly different from LS, 7 days.

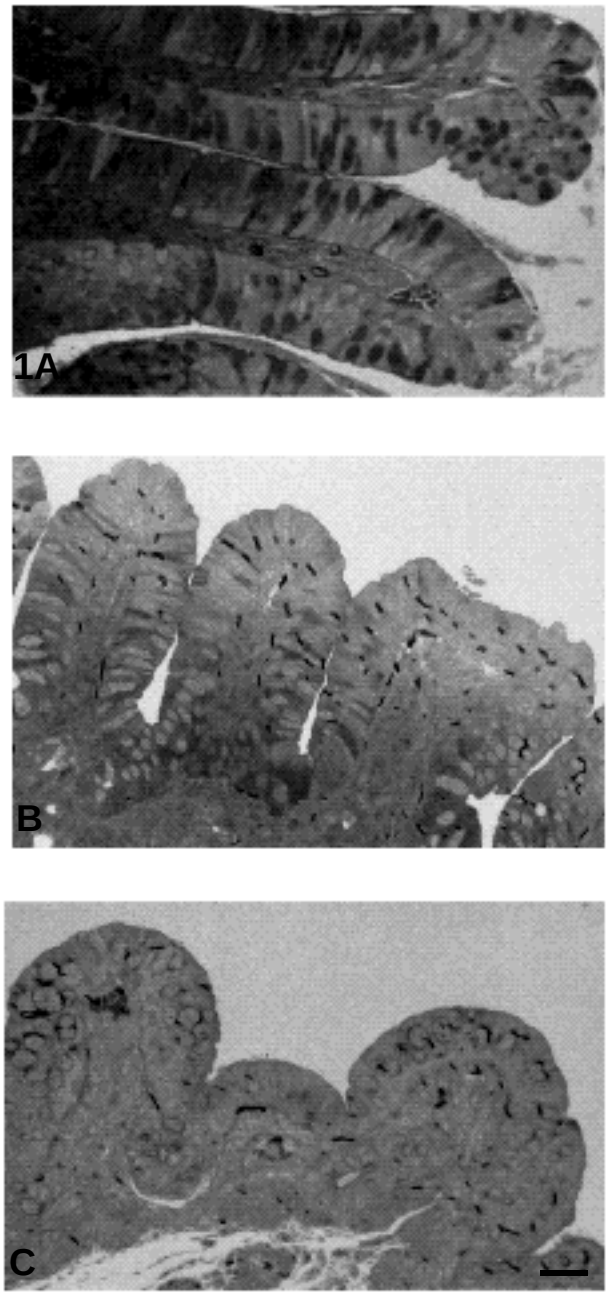

Fig. 1. Lower intestine of the house sparrow. Tissue sections were taken (A) 2mm, (B) 6mm and (C) 10mm distal to the ileocolic junction. All photographs are at the same magnification (the scale bar represents 25 μm). Note the decreasing height of the villi from the cranial to the caudal end of the organ.

firm conclusions on this point from my study of house sparrows, as the changes had begun or were complete in the shortest acclimation times examined. It does appear that house sparrows show more rapid changes in aldosterone concentration than either of the two larger species.

Effect of dehydration

Dehdydration had no significant effect on plasma aldosterone levels in house sparrows. Arad *et al.* (1985) obtained similar results for chickens, but this response contrasts with that of ducks (Klingbeil, 1985) and Japanese quail (Kobayashi and Takei, 1982). The reason for these different responses is not clear. Plasma osmolality was significantly increased during dehydration in all these species. In mammals, the increased plasma osmolality associated with dehydration inhibits aldosterone release, which would otherwise be stimulated by the enhanced angiotensin II levels that are also induced by dehydration (Schneider, 1990). In birds, too, the renin–angiotensin system is stimulated by dehydration (Kobayashi and Takei, 1982). However, the increased angiotensin levels may (Kobayashi and Takei, 1982) or may not (Gray *et al.* 1986) stimulate aldosterone release. The interplay between these various factors in birds thus remains unresolved.

Effect of handling

I could measure no effect of brief (1–5min) handling on plasma aldosterone levels in house sparrows. Longer periods of stress [15min with noise and blood sampling (Rosenberg and Hurwitz, 1987) or 45min of physical restraint and agitation (Klingbeil, 1985)] do increase aldosterone levels in other avian species. This may reflect the stimulation of aldosterone release by adrenocorticotropic hormone, ACTH (Radke *et al.* 1985), the release of which is induced by stress. In some small birds (Dawson and Howe, 1983), though not all (Wingfield and Farner, 1978), increased concentrations of corticosterone, whose release is also stimulated by ACTH, can be detected within 2min of capture and handling. There are no previous reports of the effects of very brief (1–5min) periods of stress on plasma aldosterone levels in birds. The present results do suggest that blood sampled from mist-netted wild birds, if procured promptly upon capture, should be representative of the wild condition.

Effect of time of day

Aldosterone concentrations vary with a circadian rhythm in rats on both high- and low-salt diets, peaking towards the end of the daily resting phase (Hilfenhaus, 1976). In contrast, I could detect no difference in aldosterone concentrations between the start and end of the sparrows' active phase.

Wild birds

Wild house sparrows had high aldosterone concentrations. This is consistent with their known diet, which consists almost entirely of plant material (Kalmbach, 1940), a food low in Na^+. These are the first reports of plasma aldosterone levels in any freshly captured wild bird.

Electrical properties of the colon in vitro

In the most general view, the pattern of transport properties that emerges from these studies of house sparrows is similar to that observed in other species, including the chicken (Thomas and Skadhauge, 1982), galah (Skadhauge and Dawson, 1980) and domestic duck (Skadhauge *et al.* 1984). That is, colonic PD and I_{sc} were reduced, and the characteristics of transport were modified, in birds eating a high-sodium diet. However, the data from the house sparrows do offer some novel features and new insights.

Comparison of tissue electrical properties with those of other species

HS and HSS house sparrows had I_{sc} and PD values not significantly different from 0. In contrast, other species have significant I_{sc} (25–265 $\mu A\,cm^{-2}$) and PD (5–15mV) on high-salt diets (Skadhauge and Dawson, 1980; Holtug and Skadhauge, 1982; Skadhauge *et al.* 1984). The near-zero I_{sc} in the house sparrows resulted from approximately half of the birds having lumen-positive PDs on the high-salt diets. Lumen-positive PDs have not been reported previously for avian colon, although the coprodeum of galahs eating a high-salt diet had a short-circuit current of $0\pm5\ \mu A\,cm^{-2}$ (Skadhauge and Dawson, 1980). The tissue resistance of the high-salt house sparrows was very similar to those reported for other species (50–75 Ωcm^2; Skadhauge and Dawson, 1980; Holtug and Skadhauge, 1982; Skadhauge *et al.* 1984). PD and I_{sc} values of house sparrow colon were increased by the low-sodium diet, as were those of the chicken (Holtug and Skadhauge, 1982) and the duck (Skadhauge *et al.* 1984), but not the galah (Skadhauge and Dawson, 1980). There was little effect of diet on tissue resistance in any of the species.

Time course and dose–response curves for colonic acclimation to dietary sodium

Changes in transport characteristics of house sparrow colon occurred within 4h of switching to a high-sodium diet and 1 day of switching to a low-sodium diet, the shortest acclimation times that I studied. This is substantially faster than in the chicken, for which half-times for acclimation are 10–20h for resalination and 25–50h for desalination (Thomas and Skadhauge, 1982). A faster acclimation time might be expected for a smaller animal such as the sparrow, in which turnover of body components (such as sodium) and the pace of the metabolic machinery are accelerated relative to larger animals. The rapid acclimation times for tissue electrical properties, as for plasma aldosterone concentrations, imply that these variables are likely to reflect the recent diet of animals measured just after capture in the field.

Acclimation of the house sparrow lower intestine to high-salt characteristics was apparently complete on the HS diet, with a sodium intake of approximately $36 mequiv Na^+\,kg^{-1}\,day^{-1}$, despite the fact that plasma aldosterone concentrations were 2.5 times as high as in HSS birds. In the chicken, full acclimation to the high-salt condition occurs at a sodium intake of $10 mequiv Na^+\,kg^{-1}\,day^{-1}$ (Clauss and Skadhauge, 1988), at which point the concentration of aldosterone in plasma is minimal (Arnason and Skadhauge, 1991). The two rates of sodium intake are similar when scaled to metabolic mass (i.e. when expressed per mass raised to the 0.75 power; values are $14 mequiv kg^{-0.75}$ per day for the sparrows, $12 mequiv kg^{-0.75}$ per day for the chickens).

This again suggests that the rapid acclimation of the sparrow tissues may reflect the rapid pace of the metabolic machinery.

Response to organic substrates and amiloride

The colon of chickens appears to have two pathways for entry of sodium across the apical membrane. The first, involving co-transport of Na^+ with glucose or amino acids, predominates in birds on a high-sodium diet, whereas the second, involving amiloride-blockable sodium channels, predominates on the low-sodium diet (Clauss and Skadhauge, 1988). Only the second of these pathways is found in the coprodeum. I tested for the presence of both of these pathways in house sparrow lower intestine.

In general, tissues from sparrows on the HS and HSS diets were more responsive to glucose and amino acids than were tissues from LS birds; this pattern is similar to that observed in chickens (Lind *et al.* 1980) and ducks (Skadhauge *et al.* 1984). However, even on the high-sodium diets, the responses of house sparrows were quite variable (Table 4). Variable responses to glucose and amino acids have been observed previously in birds eating low-sodium diets (Skadhauge and Dawson, 1980; Skadhauge *et al.* 1984), but in both of these studies the compounds were consistently stimulatory in birds with high-sodium intakes.

In contrast to its responsiveness to organic substrates, the inhibition of short-circuit current and PD by amiloride was very consistent, regardless of the sodium content of the diet. Full inhibition of electrical activity has been found previously in colonic tissues of both chickens and galahs on low-salt diets, but amiloride only partially inhibits transport in colons of these species on high-sodium diets (Lind *et al.* 1980; Skadhauge and Dawson, 1980). In contrast, chicken coprodeum is fully inhibited by amiloride on both high- and low-salt diets (Lyngdorf-Henrikson *et al.* 1978; Thomas and Skadhauge 1982). In terms of evaluating wild-caught birds, the variable response of the sparrow tissues to organic substrates, as well as the full responsiveness to amiloride on both low- and high-salt diets, would suggest that these characters are not of themselves reliable indicators of hydromineral balance.

In the sparrows, addition of amiloride consistently revealed a mucosa-positive electrical potential. Similar amiloride-induced reversals of tissue polarity have also been reported both for colon (Grubb and Bentley, 1988) and coprodeum (Skadhauge and Dawson, 1980) from birds on low-sodium diets. This suggests that the normal lumen-negative electrical potential in these avian intestines results from a balance between an amiloride-blockable sodium absorption and some additional ion transport. Either cation (e.g. K^+; Lind *et al.* 1980) secretion or anion absorption could produce the lumen-positive potential; the identity of the species actually transported is unknown (Grubb and Bentley, 1988).

Condition of wild birds

The properties of the lower intestine of wild house sparrows were most similar to those of birds maintained in the laboratory on a low-sodium diet. This is consistent with the high levels of aldosterone found in these birds and, as mentioned above, with the known

dietary preferences of this species. However, the I_{sc} was higher, and the tissue resistance lower, than values in the low-salt birds in the laboratory. I do not know whether these differences represent the effects of some other hormone (such as corticosterone, prolactin or antidiuretic hormone, Arnason and Skadhauge, 1991) or of another factor. These are the first reports of *in vitro* electrical properties measured on lower intestine of a freshly caught wild bird.

Overall properties of the house sparrow lower intestine: colon versus *coprodeum*

The distinction between coprodeum and colon in the avian lower intestine is not always clear. For example, Baumel *et al.* (1979, p. 332) noted that 'although this fold (separating colon and coprodeum) has often been illustrated and described in *Gallus*, it is in fact not present in this species'. I could detect no obvious point of transition between colon and cloaca in the dissected house sparrow lower intestine; it appeared uniform in width without any 'grossly bell-shaped enlargement' (Baumel *et al.* 1979, p. 332) at the caudal end. The histological appearance of the lower intestine resembled descriptions of colon for other species, though villus height varied along its length; a typical 'coprodeal' morphology appeared only for the last 1–2mm. Thus, I have referred in this report to the tissues whose electrical properties I examined as colon.

Despite this histological appearance, the overall physiological pattern that emerges from this study is more similar to coprodeum than to colon of other species. These similarities include PD and I_{sc} values not significantly different from zero in HS birds, mucosa-positive PDs in HS birds and sensitivity to amiloride in birds eating both HS and LS diets. These data would seem to disprove the conjecture that 'the amiloride-independent transport induced in colon by Na loading...is presumably general for birds' (Skadhauge and Dawson, 1980, p. R289).

The utility of plasma aldosterone level and in vitro *properties of the lower intestine for the study of wild birds*

The present studies indicate that both the plasma aldosterone concentrations and the electrical properties of the lower intestine may serve as useful indicators of dietary sodium intake in wild small birds (though the higher I_{sc} in wild than in captive birds suggests that other factors may also be involved). Both of these variables change rapidly in response to changing diet. Aldosterone can be measured accurately on fairly small plasma samples and, at least in house sparrows, levels of this hormone are affected by neither dehydration nor brief handling stress. The variable levels of plasma aldosterone and colonic electrical variables among species indicate that laboratory studies will be required to provide an interpretive framework for values collected from wild birds. Nevertheless, these physiological variables can add constructively to the arsenal of measurements used to assess hydromineral balance in wild birds.

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References

ARAD, Z., ARNASON, S. S., CHADWICK, A. AND SKADHAUGE, E.(1985). Osmotic and hormonal responses to heat and dehydration in the fowl. *J. comp. Physiol.* **155**, 227–234.

ARNASON, S. S., RICE, G. E., CHADWICK, A. AND SKADHAUGE, E. (1986). Plasma levels of arginine vasotocin, prolactin, aldosterone and corticosterone during prolonged dehydration in the domestic fowl: effect of dietary NaCl. *J. comp. Physiol.* B **156**, 383–397.

ARNASON, S. S. AND SKADHAUGE, E. (1991). Steady-state sodium absorption and chloride secretion of colon and coprodeum and plasma levels of osmoregulatory hormones in hens in relation to sodium intake. *J. comp. Physiol.* B **161**, 1–14.

BAUMEL, J. J., KING, A. S., LUCAS, A. M., BREAZILE, J. E. AND EVANS, N. E. (eds) (1979). *Nomina Anatomica Avum.* London: Academic Press.

CLAUSS, W., ARNASON, S. S., MUNCK, B. G. AND SKADHAUGE, E. (1984). Aldosterone-induced sodium transport in lower intestine: effects of varying NaCl intake. *Pflügers Arch.* **401**, 354–360.

CLAUSS, W., DANTZER, V. AND SKADHAUGE, E. (1988). A low-salt diet facilitates Cl secretion in hen lower intestine. *J. Membr. Biol.* **102**, 83–96.

CLAUSS, W. AND SKADHAUGE, E. (1988). Modulation of Na and Cl transport by mineralocorticoids. *Comp. Biochem. Physiol.* **90**, 583–589.

DAWSON, A. AND HOWE, P. D. (1983). Plasma corticosterone in wild starlings (*Sturnus vulgaris*) immediately following capture and in relation to body weight during the annual cycle. *Gen. comp. Endocr.* **51**, 303–308.

GOLDSTEIN, D. L. AND BRAUN, E. J. (1986). Lower intestinal modification of ureteral urine in hydrated house sparrows. *Am. J. Physiol.* **250**, R89–R95.

GOLDSTEIN, D. L. AND BRAUN, E. J. (1988). Contributions of the kidneys and intestines to water conservation, and plasma levels of antidiuretic hormone, during dehydration in house sparrows (*Passerdomesticus*). *J. comp. Physiol.* B **158**, 353–361.

GOLDSTEIN, D. L., WILLIAMS, J. B. AND BRAUN, E. J. (1990). Osmoregulation in the field by salt-marsh savannah sparrows *Passerculussandwichensisbeldingi*. *Physiol. Zool.* **63**, 669–682.

GOLDSTEIN, D. L. AND ZAHEDI, A. (1990). Variation in osmoregulatory variables of captive and wild house sparrows (*Passerdomesticus*). *Auk* **107**, 533–538.

GRAY, D. A., HAMMEL, H. T. AND SIMON, E. (1986). Osmoregulatory effects of angiotensin II in a bird with salt glands (*Anasplatyrhynchos*). *J. comp. Physiol.* B **156**, 315–321.

GRUBB, B. R. AND BENTLEY, P. J. (1988). Relationship of transmural electrical variables to the luminal Na concentration in the colon of the fowl (*Gallus domesticus*). *J. comp. Physiol.* B **158**, 19–24.

HARVEY, S., KLANDORF, H., RADKE, W. J. AND FEW, J. D. (1984). Thyroid and adrenal responses of ducks (*Anasplatyrhynchos*) during saline adaptation. *Gen. comp. Endocr.* **55**, 46–53.

HILFENHAUS, M.(1976). Circadian rhythm of the renin–angiotensin–aldosterone system in the rat. *Arch. Tox.* **36**, 305–316.

HOLTUG, K. AND SKADHAUGE, E. (1982). NaCl transport across hen colon: dependence on electro-chemical driving force. *Pflügers Arch.* **394**, 222–225.

JOHNSON, O. W. AND SKADHAUGE, E. (1975). Structural–functional correlations in the kidneys and observations of colon and cloacal morphology in certain Australian birds. *J. Anat.* **120**, 495–505.

KALMBACH, E. R. (1940). Economic status of the English sparrow in the United States. *U.S. Dept. Ag. Bull. 711*.

KLINGBEIL, C. K. (1985). Effects of chronic changes in dietary electrolytes and acute stress on plasma levels of corticosterone and aldosterone in the duck (*Anas platyrhynchos*). *Gen. comp. Endocr.* **58**, 10–19.

KOBAYASHI, H. AND TAKEI, Y. (1982). Mechanisms for induction of drinking with special reference to angiotensin II. *Comp. Biochem. Physiol.* **71**A, 485–494.

LIND, J., MUNCK, B. G., OLSEN, O. AND SKADHAUGE, E. (1980). Effects of sugars, amino acids and inhibitors on electrolyte transport across hen colon at different sodium chloride intakes. *J. Physiol., Lond.* **305**, 315–325.

LYNGDORF-HENRIKSEN, P., MUNCK, B. G. AND SKADHAUGE, E. (1978). Sodium chloride transport across the lower intestine of the chicken: dependence on sodium chloride concentration and effect of inhibitors. *Pflügers Arch.* **378**, 161–165.

RADKE, W. J., ALBASI, C. M. AND HARVEY, S. (1984). Dietary sodium and adrenocortical activity in ducks (*Anasplatyrhynchos*) and chickens (*Gallusdomesticus*). *Gen. comp. Endocr.* **56**, 121–129.

RADKE, W. J., ALBASI, C. M., REES, A. AND HARVEY, S. (1985). Stress and ACTH stimulate aldosterone secretion in the fowl (*Gallusdomesticus*). *Comp. Biochem. Physiol.* A **82**, 285–288.

ROSENBERG, J. AND HURWITZ, S. (1987). Concentration of adrenocortical hormones in relation to cation homeostasis in birds. *Am. J. Physiol.* **253**, R20–R24.

SCHNEIDER, E. G. (1990). In water deprivation, osmolality becomes an important determinant of aldosterone secretion. *News. physiol. Sci.* **5**, 197–201.

SKADHAUGE, E.(1981). *Osmoregulation in Birds.* Berlin, Heidelberg, New York:

SKADHAUGE, E. AND DAWSON, T. J.(1980). *In vitro* studies of sodium transport across the lower intestine of a desert parrot. *Am. J. Physiol.* **239**, R285–R290.

SKADHAUGE, E., MUNCK, B. G. AND RICE, G. E.(1984). Regulation of NaCl and water absorption in duck intestine. In *Lecture Notes on Coastal and Estuarine Studies*, vol. 9, *Osmoregulation in Estuarine and Marine Animals* (ed. A. Pequeux, R. Gilles and L. Bolis), pp. 131–142. New York: Springer-Verlag.

SKADHAUGE, E., THOMAS, D. H., CHADWICK, A. AND JALLAGEAS, M.(1983). Time course of adaptation to low and high NaCl diets in the domestic fowl. Effects on electrolyte excretion and on plasma hormone levels (aldosterone, corticosterone and prolactin). *Pflügers Arch.* **396**, 301–307.

THOMAS, D. H. AND SKADHAUGE, E. (1979). Dietary Na^+ effects on transepithelial transport of NaCl by hen (*Gallus domesticus*) lower intestine (colon and coprodeum) perfused luminally *in vivo*. *Pflügers Arch.* **379**, 229–236.

THOMAS, D. H. AND SKADHAUGE, E. (1982). Time course of adaptation to low and high NaCl diets in the domestic fowl. *Pflügers Arch.* **395**, 165–170.

WINGFIELD, J. C. AND FARNER, D. S. (1978). The endocrinology of a natural breeding population of the white-crowned sparrow (*Zonotrichialeucophryspugetensis*). *Physiol. Zool.* **51**, 188–205.