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ION AND LUMINAL MARKER CONCENTRATIONS IN THE GUT OF SALINE-ACCLIMATED DUCKS[1]

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Abstract. Concentrations of Na^+, K^+, and Cl^- in digestive tract fluid were measured in eight saline-acclimated domestic Pekin ducks, *Anas platyrhynchos,* and the concentration of nonabsorbable luminal marker ^{14}C-polyethylene glycol (^{14}C-PEG, orally loaded in 180 mM NaCl) was measured in five of these birds. The birds were sacrificed and the gut was ligated into sections. All sections were hyponatremic to plasma; [Cl^-] in the proventriculus and ventriculus were equal to and higher than plasma [Cl^-], respectively, and elsewhere [Cl^-] was less than plasma [Cl^-]. The [Na^+]/[K^+] ratio (1.5 or less) was lower than that of birds without salt glands (2–3, Hurwitz et al. 1970). The initial 10-fold dilution of [^{14}C-PEG] in the duodenum and subsequent 80-fold increase in [^{14}C-PEG] in the ileum are consistent with secretion into the duodenum and net absorption of Na^+ and water in the ileum. Rectal (and cecal) [^{14}C-PEG] was 5-fold lower than ileal [^{14}C-PEG], a decrease presumably due to retropulsion of urine into the rectal-cecal complex. These observations suggest sodium-replete ducks continue to reflux urine into the hindgut where NaCl and water uptake have been shown to occur (Skadhauge et al. 1984) and offer indirect evidence that hindgut NaCl reabsorption and extrarenal NaCl secretion may be linked.

Key words: Anas platyrhynchos; *gut ion concentration; gut luminal marker; intestine; saline acclimation; urine refluxing.*

INTRODUCTION

Marine birds secrete NaCl via the salt glands, a capacity absent in birds that lack these glands. Schmidt-Nielsen et al. (1963) suggested marine birds might reabsorb NaCl (and water) in the hindgut and secrete it extrarenally in less water than was reabsorbed with it thereby generating osmotically free water to meet their physiological needs. Some of the reabsorbed Na^+ (Cl^- and water) could derive from urine refluxed into the hindgut (Duke 1989). If such a system is operative in marine birds, it would be advantageous for them to maintain high uptake of NaCl in all gut sections even during high NaCl intake. There is evidence that this is so. Aldosterone is a hormone that promotes Na^+ retention. In birds without salt glands, aldosterone plasma concentration ($[aldo]_{pl}$) is high when dietary Na^+ is low and vice versa. High $[aldo]_{pl}$ facilitates Na^+ uptake from the anterior gut (Hurwitz et al. 1970) and hindgut (Chosniak et al. 1977) of chickens (*Gallus domesticus*) and, when $[aldo]_{pl}$ is low, chickens decrease gut Na^+ uptake, particularly in the hindgut (Clauss et al. 1988). But, in birds with salt glands, dietary Na^+ has little effect on $[aldo]_{pl}$ (Klingbeil 1985; M. R. Hughes and W. N. Holmes, unpubl. data). When Pekin ducklings (*Anas platyrhynchos*) drank saline water, aldosterone mediated Na^+ uptake in the small intestine actually increased (Crocker and Holmes 1971) and, in mature Pekin ducks, high salt intake did not affect Na^+ uptake in the small intestine (Hughes and Roberts 1988) and only slightly reduced it in the hindgut (Skadhauge et al. 1984). In mature Glaucous-winged Gulls (*Larus glaucescens*) saline acclimation did not affect Na^+ uptake in the small intestine (Hughes and Roberts 1988) or rectum (Goldstein et al. 1986). However, the extent to which NaCl retrieval by the hindgut and NaCl secretion by the salt glands are linked is unclear. In birds without salt glands, such as the chicken, the rectum (Rice and Skadhauge 1982a) and especially the ceca (Rice and Skadhauge 1982b) are large and are important sites for Na^+ and water retrieval. These organs are also large in Pekin ducks and duck rectal Na^+ and water transport capacity equal and exceed, respectively, those of the chicken (Goldstein 1989). In Glaucous-winged Gulls both these transport capacities exceed those of chickens

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TABLE 1. Mean plasma and cloacal fluid osmolality and ion concentrations in saline-acclimated Pekin ducks.

	Hct, %	Ion concentrations, mM			Osmolality, mOsmole·kg^{-1}
		Na^+	Cl^-	K^+	
Plasma	39.2 ±1.0	147.5 ±1.0	116.4 ±2.3	3.2 ±1.6	306.9 +0.3
Cloacal fluid		138.0 ±13.5	144.6** ±14.0	16.6 ±3.7	416.6** ±23.1

** $P < 0.01$ comparison of plasma and cloacal fluid values, combined data for ducks given 180 mM NaCl orally ($n = 5$) or not ($n = 3$), since these two groups were not significantly different. Cloacal fluid was collected in three nonloaded ducks and in five ducks during the hour following oral loading with 180 mM NaCl. Blood was obtained at the end of this hour. Data compared by analysis of variance (with pooled variances).

(Goldstein 1989) and, although the rectum (Goldstein et al. 1986) and ceca of gulls are very small, urine does enter the rectum, since uric acid has been observed there (M. R. Hughes, unpubl. observ.).

In his cogent review of intestinal regulation of Na^+ and water Thomas (1991) pointed out how little is known of the osmoregulatory role of the gut in birds with salt glands. Gut ion concentrations have not been measured and there have been no studies using nonabsorbable luminal markers. Also movement of urine into the hindgut has not been documented in birds with salt glands. Data presented here should partly rectify these omissions. Concentrations of ions and (in some ducks) the luminal marker (^{14}C-polyethylene glycol, [^{14}C-PEG], disintegrations per minute [DPM] ml^{-1}) were measured in the fluid from different gut regions in saline-acclimated Pekin ducks.

METHODS AND MATERIALS

Adult Pekin ducks (seven female and one male, mean body mass = 2,950 ± 122 g) were held on 180 mM NaCl for at least six months. This NaCl concentration did not increase plasma osmolality or ion concentrations, but did maintain efficient extrarenal NaCl secretion without dehydrating the birds (Hughes et al. 1989). Each bird was fasted overnight (with free access to saline), weighed, and placed in a foam-lined restrainer in an upright position. Spontaneously-voided cloacal fluid was directed through a funnel into a preweighed vial for 1 hr. A blood sample (1 ml) was taken. Anesthesia was induced by intravenously injecting approximately 1.5 ml (60 mg) sodium pentobarbital (Nembutal, Abbott Laboratories, Montreal, Canada) over about 2 min. About 5 min later the bird was killed without trauma with an intravenous bolus of about 25 ml of air. The intestinal tract was exposed as quickly as possible and the proventriculus, ventriculus, duodenum (two sections), jejunum (two sections), ileum (two sections), both ceca, and rectum were separated by ligatures and the digestive tract was sectioned (Mongin 1976). Jejunum and ileum were assumed to be separated by Meckel's diverticulum. The contents of each segment were gently expressed into 1.5 ml centrifuge tubes that were sealed, frozen overnight, thawed, and centrifuged. Aliquants of the supernatant fluid were analyzed for ion concentrations and osmolality. Samples of the gut contents from the first two birds yielded very little fluid and samples from the third bird yielded almost none. Samples from these three birds were gently heated in a hot water bath and recentrifuged. Boiling increased ion concentration 10.6% ± 2.8% (determined by boiling 13 samples from which adequate supernatant fluid had been available). To assure that sufficient fluid could be obtained for the necessary analytical procedures in the remaining five ducks, immediately following restraint, each was intubated with a bolus of the saline they had been drinking (22.5 ml 180 mM NaCl kg body mass^{-1}). The bolus also contained 1–2 μCi of the luminal marker ^{14}C-PEG. One hour later a blood sample was taken and the birds were anesthetized and killed as above.

All determinations were done in duplicate or triplicate. From each blood sample triplicate Strumia microhematocrit (Hct) tubes were immediately filled and centrifuged simultaneously with the remaining blood for 3 min at 15,600 × g. Plasma, intestinal fluid, and cloacal fluid sodium and potassium concentrations, [Na^+] and [K^+], were determined by lithium internal standard flame photometry (Instrumentation Laboratory, Inc. Model 143); chloride, [Cl^-], by a Buchler digital chloridometer; and osmolality by freezing point depression using an Advanced Osmometer, model 5300. The [^{14}C-PEG] was measured in a Beckman model LS 9000 liquid scintillation counter. Data are given as means ±

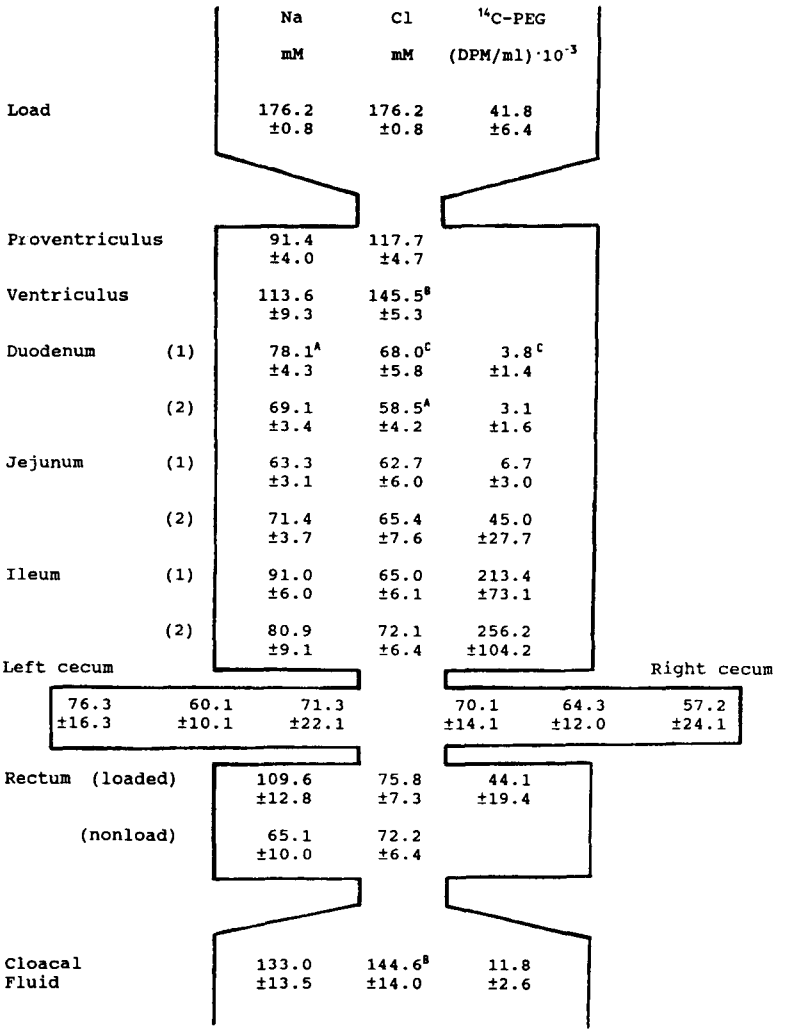

FIGURE 1. Concentrations of Na^+, Cl^- and nonabsorbable ^{14}C-polyethylene glycol (^{14}C-PEG) in sections of the digestive tract. Ion concentrations were determined in eight ducks; five ducks were orally loaded with 180 mM NaCl containing 1–2 μCi ^{14}C-PEG one hour prior to sacrifice. $^{A}P < 0.05$, $^{B}P < 0.01$, and $^{C}P < 0.001$ comparison with immediately preceding section or load (paired t-tests, Bonferroni-adjusted significance values).

standard errors and statistically analyzed using SYSTAT (Wilkinson 1990). Unrelated data were compared by one-way analysis of variance; sequential values for gut sections were compared using paired t-tests with significance values adjusted with Bonferroni's method. Significance was assumed if $P < 0.05$.

RESULTS AND DISCUSSION

One hour after saline-acclimated ducks received an oral load of 180 mM NaCl their Hct and plasma and cloacal fluid ion concentrations were the same as those of ducks that received no oral saline. Therefore, data for both treatments were combined yielding mean values (Table 1) similar to those previously reported for this strain of Pekin ducks following mild saline acclimation (Hughes and Roberts 1988, Hughes et al. 1989). Mongin (1976) reported [Na^+] doubled along the length of the intestinal tract from the duodenum to the ileum in the laying hen, while [Cl^-] decreased significantly, but Hurwitz et al. (1970) did not observe this pattern in the chicken, nor was it seen in the Emu (*Dromaius novaehollan-*

diae; Skadhauge et al. 1991) or the ducks we studied. In all sections (Fig. 1) the gut fluid was hyponatremic to plasma; in the proventriculus and ventriculus [Cl^-] was equal to and higher than plasma [Cl^-], respectively, while [Cl^-] in all other sections was less than that of plasma (Table 1). Rectal fluid [Cl^-] was similar in ducks that received 180 mM NaCl orally and ducks that did not, but ducks given 180 mM NaCl had higher rectal fluid [Na^+] than nonloaded ducks ($P < 0.05$) (Fig. 1). Data for [Na^+] and [Cl^-] of orally-loaded ($n = 5$) and nonloaded ducks ($n = 3$) were combined in Figure 1 for all gut sections except rectum. Birds which did and did not receive saline orally had the same proventricular [K^+] (mM), 38.0 ± 5.2 and 37.3 ± 2.6, respectively, and [K^+] increased equally along the small intestine in both groups, reaching 79.0 ± 4.8 and 79.9 ± 4.7 ($P < 0.01$, 0.02, respectively) at the end of the jejunum. Rectal [K^+] was the same in both groups, 51.9 ± 13.5 and 52.5 ± 14.9, respectively. However, cecal [K^+] was lower in saline-loaded ducks, 33.8 ± 4.1, than in ducks that did not receive saline, 70.6 ± 3.8 ($P < 0.05$). The [Na^+]/[K^+] ratio in the small intestine of the laying hen on high dietary Na^+ range between 2–3 (Hurwitz et al. 1970), while ducks that drank 180 mM NaCl had a lower ratio (1.5 or less) in all sections of the small intestine, even though some birds had received Na^+ orally. Though absolute lengths (cm) of the three sections of the ducks' small intestine (duodenum, 33.6 ± 2.6; jejunum, 76.6 ± 3.8; and ileum 73.9 ± 3.4) were greater than those of the laying hen (Mongin 1976), the two digestive tracts were similarly proportioned.

At sacrifice there was no evidence of ^{14}C-PEG in the plasma, verifying that the luminal marker was restricted to the gut. The [^{14}C-PEG] was quite uniform in the imposed oral loads, but the [^{14}C-PEG] of discrete gut segments varied greatly from one bird to another. As a result, although there were large mathematical differences in [^{14}C-PEG] between sections, only the 10-fold difference between the intubated NaCl solution and fluid in the duodenum was statistically significant (Fig. 1). Such a large dilution of the [^{14}C-PEG] in the duodenum of a fasted bird was unexpected, since water movement induced by the osmotic gradient between the intubated NaCl solution (334 mOsmole kg^{-1}) and plasma (307 mOsmole kg^{-1}) should have been insufficient to account for this large change in [^{14}C-PEG]. Removal of water along the length of the small intestine gradually increased [^{14}C-PEG] 80-fold (Fig. 1). The large increase in [^{14}C-PEG] in the final third of the small intestine emphasizes the excellent water retrieval capacity of this segment and suggests net absorption of Na^+ and water rather than simple recovery of secretory product. The [^{14}C-PEG] in the rectum (and ceca) was much lower than the [^{14}C-PEG] in the ileum ($P < 0.08$). In one bird ileal [^{14}C-PEG] decreased by only one-third, but in the other four ducks the decrease was 5- to 10-fold. Digesta arriving at the rectum from the ileum must have been diluted with refluxed urine (which contained no ^{14}C-PEG) in proportions of one volume of ileal fluid to five volumes of urine. The fluid entering the ceca appears to have been mainly urine. The imposed oral loads of Na^+ and Cl^- could have been excreted renally since they were not large and did not exceed the concentrating capacity of the kidneys. Nonetheless, substantial volumes of urine were moved into the hindgut where Na^+, Cl^-, and water could be reabsorbed for subsequent extrarenal excretion. The modest reabsorptive capacity of the duck rectum (Skadhauge et al. 1984) in conjunction with the large surface area of the rectal and cecal compartments present good possibility for NaCl retrieval. After freshwater ducks had been acclimated to NaCl concentrations that did not exceed their capacity to maintain body mass and plasma osmolality, water flux exceeded (probably mainly due to drinking, Hughes et al. 1991) and glomerular filtration rate and urine flow rate remained at freshwater levels (Holmes et al. 1968; M. R. Hughes, unpubl. data). The present study indicates that saline-acclimated ducks also have a high rate of urine retropulsion into the rectal-cecal complex where Na^+ uptake rate may be slightly depressed relative to freshwater uptake, but solute linked water retrieval is twice as high (Skadhauge et al. 1984).

In summary, this study shows that sodium-replete ducks reflux urine into the hindgut (where the capacity for Na^+ uptake is only slightly decreased by saline acclimation [Skadhauge et al. 1984]) offering indirect evidence that hindgut reabsorption of urinary NaCl (and water) and extrarenal NaCl secretion are linked.

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