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Respiratory and cutaneous water loss of temperate-zone passerine birds

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ABSTRACT

We measured respiratory water loss (RWL) and cutaneous water loss (CWL) of 12 species of passerine birds, all from a temperate environment, and related their CWL to classes of lipids within the stratum corneum (SC). We purposed to gain insight into the generality of patterns of CWL in birds that have been generated mostly from studies on species from deserts, and we addressed the hypothesis that CWL is a passive diffusion process. Despite taxonomic and ecological differences among 12 species of temperate birds, mass-specific RWL and surface-specific CWL were statistically indistinguishable across species. When the birds were dead, CWL was reduced by 16.3% suggesting that CWL is, in part, under physiological control. We found that ceramides, cerebrosides, dioscylceramides, cholesterol, cholesterol sulfate, fatty acid methyl esters, free fatty acid, sterol esters, and triacylglycerol constituted the intercellular lipids of the avian SC. CWL was positively associated with amount of ceramide 3, but this lipid class represented less than 2% of the total SC lipids. Combining direct measurements ($n = 24$) of RWL with indirect estimates ($n = 25$) yielded the equation log RWL (g H_2O/d) $= -0.86 + 0.73$ (log body mass, g).

1. Introduction

To maintain an aqueous internal milieu in the face of a desiccating external environment, terrestrial animals have developed behavioral, morphological, and physiological mechanisms, produced by natural selection, to conserve body water (Bartholomew and Cade, 1963; Addo-Bediako et al., 2001; Lillywhite, 2006). Among the morphological and associated physiological mechanisms that terrestrial endotherms possess to reduce evaporative water losses are structures in the nares called nasal turbinates that are thought to recover water from the exhaled air stream, thereby reducing respiratory water loss (RWL), and a relatively impermeable integument with an attendant lower cutaneous water loss (CWL) (Hillenius 1992; Gorden and Olson, 1995; Tieleman et al., 1999; Tieleman and Williams, 2002). In many species of birds, evaporative water demand is relatively high because they are diurnal, exposed to higher solar radiation loads, higher ambient air temperatures (T_a), and increased wind speeds (Maclean, 1996). In addition, birds have high mass-specific rates of metabolism and as a result high oxygen demand, which influences RWL (Tieleman et al., 1999).

The importance of understanding mechanisms of evaporative water loss in small birds is underscored when one considers that total evaporative water loss (TEWL), the sum of RWL and CWL, is their major avenue of water loss, accounting for up to 83% of total water loss at moderate temperatures (Willoughby, 1968; Bartholomew, 1972; Williams, 1996). At these same temperatures, CWL comprises over 60% of TEWL emphasizing the importance of this variable in the water economy of birds (Wolf and Walsberg, 1996; Tieleman and Williams, 2002; McKechnie and Wolf, 2004; Muñoz-Garcia and Williams, 2005b, 2007).

Most investigators have indirectly estimated RWL by measuring the temperature of exhaled air and minute volume during respiration, and then calculating RWL as a product of minute volume and the saturated water vapor density at the temperature of exhaled air (Withers and Williams, 1990; Tieleman and Williams, 1999; Geist, 2000). CWL has also been indirectly estimated by using whole body plethsesmography (Withers and Williams, 1990), or by evaluating resistance of sites on the skin and calculating whole organism CWL from Fick's law of diffusion and estimates of skin surface area (Michaeli and Pinshow, 2001; Marder et al., 2003; Larcombe et al., 2003). Direct measurements of RWL and CWL of birds are few, mostly from desert species; RWL and CWL has been measured in only four non-desert species of birds (Tieleman and Williams, 2002; Muñoz-Garcia and Williams, 2005b, 2007).

Whether water permeation through the skin is dictated entirely by passive diffusion, or if it is under active physiological control mechanism(s), remains a subject of debate (Chuong et al., 2002; Muñoz-Garcia and Williams, 2005b; Falkenberg and Georgiadis, 2008). Some authors have suggested that CWL is a passive diffusion process (Pinnagoda, 1994; Wilson and Maibach, 1994; Chuong et al., 2002), whereas others have suggested that water transport across the living epidermis can be physiologically altered by changing ion gradients, a process involving active transport (Falkenberg and Georgiadis, 2008), or by altering vascular blood supply to the dermis;

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although general support for this latter idea is lacking for birds other than Columbiforms (Ophir et al., 2004). In part, these different views arise because some authors think about water movement through the entire epidermis (Falkenberg and Georgiadis, 2008), whereas others have focused their attention only on the outer layer of epidermis, the stratum corneum (SC) (Kalia et al., 1996; Kasting et al., 2003). If water movement across skin is under physiological control, one may expect to see differences in the rate of water loss through the skin when the animal is alive compared with values immediately after death. Because ion gradients that are maintained by metabolic activity will be no longer retained, or because blood flow to the dermis will be absent, one would hypothesize that CWL will vary between live and dead individuals with an equivalent hydration state. Comparison of rates of CWL between live versus dead animals should provide insight into the extent to which active or passive processes are involved in water permeation through skin.

In birds and mammals, intercellular lipids in the SC are thought to form the physical barrier to water vapor diffusion (Windsor and Burch, 1944; Menon and Menon, 2000). When lipids in the SC were extracted by various organic solvents, CWL increased more than 200 times in experiments on humans and mice (Sweeney and Downing, 1970; Elias, 1983; Wertz and van den Bergh, 1998). Intercellular lipids in the SC of birds have been identified as mixtures of cholesterol, free fatty acids, ceramides, and cerebrosides, the latter a ceramide molecule with a hexose sugar attached (Menon and Menon, 2000; Muñoz-Garcia and Williams, 2005b, 2007).

The total quantity of lipids in the SC does not appear to determine the rate of water loss through skin, but rather the specific mixtures of lipids in the SC influence CWL (Muñoz-Garcia and Williams, 2005b, 2007; Muñoz-Garica et al., 2008). As evidence for this assertion, Sweeney and Downing (1970) found that the total amount of extracted lipids was independent of the rate of CWL. In addition, Haugen et al. (2003a, b) and Muñoz-Garcia and Williams (2005b) found that total lipids did not correlate with CWL, but rather amount or proportion of classes of lipids such as free fatty acids, ceramides and cerebrosides were tightly associated with differences in CWL.

We measured RWL and CWL of 12 species of temperate-zone passerines, and related their CWL to classes of lipids within the SC. We aimed to gain insight into the generality of patterns of RWL and CWL in birds that have been generated mostly from studies on species from deserts. We addressed the extent to which CWL varies between specific groups of passerine birds with different life-styles ranging from ground-foraging seed-eaters to aerial-foraging insectivores, all from the same temperate environment. Because most of previous studies on evaporative water loss of birds reported indirect estimates of RWL, we tested the consistency of such measurements with our direct measurements of RWL. In addition, we tested the hypothesis that CWL is a passive diffusion process. Our measurements of RWL and CWL represent the largest data sets that have been obtained by direct measurements on these parameters. Our comparison of CWL between live and dead animals represents the first of its kind to tease apart the extent of physiological control on water permeation through skin.

2. Methods and materials

2.1. Capture of birds

Using mist nets, we captured 58 individuals of 12 species of passerine birds that were resident in Ohio, USA. We netted birds beginning April 8 to June 20, 2008. Birds were transported to the lab, and housed in small wire cages for 12–24 h prior to measurements; water and food were provided ad libitum. Before each measurement of RWL and CWL, we weighed the birds with a balance (± 0.1 g) (Mettler Toledo [Columbus, OH], PB3002). We used Meeh's equation (Meeh, 1879) with Rubner's (1883) constant of 10, surface area $(\mathrm{cm}^2) = 10 \cdot (\text{body mass g}^{0.667})$, to calculate skin surface area from body mass (Walsberg and King, 1978). Sex was determined by plumage characteristics or by dissection after measurements.

2.2. Measurement of metabolic rate and evaporative water loss

We measured rates of oxygen consumption, RWL, and CWL using standard flow-through respirometry and hygrometry methods (Gessaman, 1987; Tieleman and Williams, 2002). All measurements were made during daytime, the active phase of the daily cycle for these birds. Birds were fasted 2–3 h prior to measurements to ensure they were in postabsorptive conditions, and then placed either in a 2 L or 5.9 L stainless steel chamber depending on the bird's size. Chambers were rendered airtight with a lid and rubber gasket. We placed chambers in an environmental cabinet in which T_a was controlled with a Sable system temperature controller ([Las Vegas, NV, USA], PELT-5) at 30 ± 0.1 °C. We measured chamber temperature using a 36-gauge thermocouple and a Baily Batt-12 thermocouple reader. Birds stood on a wire mesh platform, over a layer of mineral oil to trap feces, thus eliminating them as a source of water in our evaporative water loss measurements.

We quantified RWL and CWL separately using a mask system following Tieleman and Williams (2002) (Fig.1). Briefly, air was drawn through tubes of Drierite, then into a chamber by two vacuum pumps that were connected to two outlet ports; portmask, and port$_{\text{chamber}}$. We set the flow rate of air through the port$_{\text{mask}}$ 200 mL greater than the port$_{\text{chamber}}$, so that the mask captured all respiratory gases from a bird and also drew in air from the chamber. Flow rates ranged from 300 to 1250 mL/min. The dew point of each outlet air stream was measured by a dew point hygrometer (General Eastern [Woburn, MA, USA], model M4), and recorded by a Cambell Scientific CR-23X data logger (Logan, UT, USA) every minute. Dew point

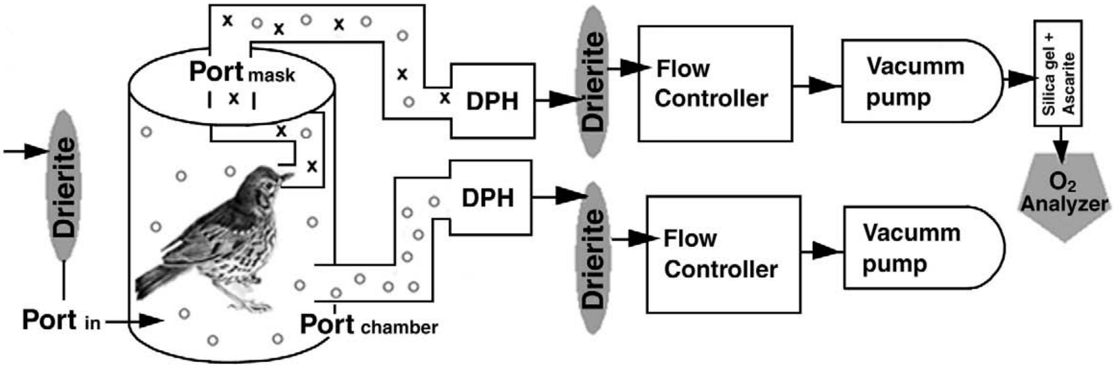

Fig. 1. A diagram illustrating our mask system used to measure RWL and CWL. The symbol "X" indicates water molecules expired from the respiratory passages, whereas the symbol "O" indicates water loss from the skin. DPH denotes dew point hygrometer. Arrows show direction of air flow.