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CUTANEOUS WATER LOSS AND LIPIDS OF THE STRATUM CORNEUM IN DUSKY ANTBIRDS, A LOWLAND TROPICAL BIRD

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Abstract. The stratum corneum, the outer layer of the epidermis, consists of flattened cells embedded in a matrix of lipids, primarily cholesterol, free fatty acids, ceramides, and cerebrosides. The stratum corneum forms a barrier to water vapor diffusion through the skin. In birds, the skin limits excessive water loss at thermoneutral temperatures, but also serves as a vehicle for thermoregulation during episodes of heat stress. We measured total evaporative water loss, cutaneous water loss, and lipids in the stratum corneum in Dusky Antbirds (*Cercomacra tyrannina*), the first such measurements ever made for birds living in tropical rain forests. We predicted that these birds would have high rates of cutaneous water loss because of their need to thermoregulate rather than to conserve water. We found that Dusky Antbirds lose twice as much water through their skin as birds from temperate environments. We also hypothesized that the proportion of cerebrosides in the stratum corneum would increase relative to that of ceramides if Dusky Antbirds use their skin as a thermoregulatory organ. However, we found that Dusky Antbirds did not show different proportions of ceramides and cerebrosides in the stratum corneum than other species of birds. We also found that Dusky Antbirds had low amounts of free fatty acids in their stratum corneum. Overall, our data support the idea that the interactions of the lipids in the stratum corneum may play an important role in determining rates of water vapor diffusion through the skin.

Key words: Cercomacra tyrannina*, cutaneous water loss, Dusky Antbird, lipids, lowland rain forest, Panama, thermoregulation.*

Pérdida de Agua por Evaporación a través de la Piel y Lípidos del Estrato Córneo en *Cercomacra tyrannina*, Ave Tropical de Tierras Bajas

Resumen. El estrato córneo, la capa más externa de la epidermis, está formado por células aplanadas embebidas en una matriz de lípidos, principalmente colesterol, ácidos grasos libres, ceramidas y cerebrósidos. El estrato córneo forma una barrera que evita la difusión del vapor de agua a través de la piel. En aves la piel limita excesivas pérdidas de agua en la zona termoneutral, pero también sirve como vía de termoregulación durante episodios de estrés térmico. Medimos la pérdida total de agua por evaporación, la pérdida de agua por evaporación a través de la piel y los lípidos en el estrato córneo en *Cercomacra tyrannina*, la primera medida de esta naturaleza realizada en aves en selva húmeda tropical. Esperamos encontrar una elevada pérdida de agua por evaporación a través de la piel en esta especie, debido a sus necesidades de termoregulación. Encontramos que *C. tyrannina* pierde el doble de agua a través de la piel comparado con especies de clima temperado. También esperamos encontrar una alta proporción de cerebrósidos en el estrato córneo si estos pájaros usan la piel para termoregular. Encontramos que *C. tyrannina* no presenta unas concentraciones de cerebrósidos y ceramidas en el estrato córneo diferentes de las de otras especies de pájaros. También hallamos que *C. tyrannina* posee una baja cantidad de ácidos grasos libres en el estrato córneo. En conclusion, nuestros resultados apoyan la idea de que las interacciones entre los lípidos del estrato córneo juegan un papel importante en la determinación de las tasas de diffusion del vapor de agua a través de la piel.

INTRODUCTION

In birds, total evaporative water loss (TEWL), the sum of cutaneous water loss (CWL) and respiratory water loss (RWL), is the major avenue of water loss, being five times greater than fecal and urinary water loss in small species (Dawson 1982). Although initially thought to be insignificant (Bartholomew and Cade 1963, Mount 1979), subsequent studies showed that CWL equaled or exceeded RWL,

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at least in some species (Dawson 1982, Webster and King 1987, Wolf and Walsberg 1996, Tieleman and Williams 2002).

The integument in birds prevents excessive rates of evaporative water loss, but it can also be used as an important component of the thermoregulatory apparatus. Some species of birds, especially doves and pigeons, seem to rely on CWL to maintain their body temperature below lethal limits during episodes of heat stress (Marder and Ben-Asher 1983, Wolf and Walsberg 1996, Hoffman and Walsberg 1999). Individual birds can also adjust their CWL rates when acclimated to high temperatures (Marder and Ben-Asher 1983, Wolf and Walsberg 1996, Tieleman and Williams 2002, Haugen et al. 2003). Therefore, the skin is thought to be structured in a manner compatible with its role, thermoregulation or water conservation.

The avian skin is composed of an outer epidermis and an inner vascularized dermis. The epidermis of birds consists of four strata, stratum basale, stratum intermedium, statum transitivum, and statum corneum. Cell proliferation takes place in the stratum basale, adjacent to the dermis; after dividing, cells go through a process of keratinization and lipid accumulation in the stratum intermedium and the stratum transitivum. The outermost layer of the epidermis is the stratum corneum, formed by nonliving flattened cells called corneocytes, embedded in a matrix of intercellular lipids (Lucas and Stettenheim 1972). The main determinant of the resistance to water vapor diffusion through the skin is the lipid composition of the stratum corneum (Blank 1953, Squier et al. 1973, Elias et al. 1981, Blank et al. 1984, Potts and Francoeur 1990, Elias and Menon 1991). The main intercellular lipid classes in the avian stratum corneum are cholesterol, free fatty acids, ceramides, formed by a fatty acid bound to a sphingosine base, and cerebrosides, ceramides bound to a sugar molecule (Menon and Menon 2000, Muñoz-Garcia and Williams 2005).

Evidence suggests that alterations in the lipid composition of the avian stratum corneum are associated with changes in CWL (Haugen et al. 2003, Haugen, Williams et al. 2003, Muñoz-Garcia and Williams 2005, Williams and Tieleman 2005). Birds that live in deserts show a higher proportion of ceramides and a lower proportion of free fatty acids in their stratum corneum than birds living in mesic environments (Haugen et al. 2003, Muñoz-Garcia and Williams 2005). Ceramides would confer a higher resistance for water vapor diffusion through the skin because they provide the main structural element for the formation of bilayers, called lamellae, which prevent water loss. The combination of a high proportion of ceramides and a low proportion of free fatty acids has been hypothesized to create a highly ordered crystalline phase in the intercellular spaces of the stratum corneum, thus reducing CWL (Bouwstra et al. 2003, Haugen et al. 2003). The role of cerebrosides in the stratum corneum has not yet been explored. The polar heads of cerebrosides might attract water molecules, potentially creating water channels (Carruthers and Melchior 1983) that would promote water loss through the skin. Therefore, cerebrosides could potentially serve an important role in thermoregulation. Thus, one might predict that birds living in environments where water conservation is critical would have higher concentrations of ceramides in the stratum corneum and birds living in environments in which water loss through the skin is important for thermoregulation would have higher concentrations of cerebrosides in their stratum corneum. In support of this idea, House Sparrows (*Passer domesticus*) living in the deserts of Saudi Arabia, which experience the need to conserve water and also to thermoregulate, had higher amounts of ceramides and cerebrosides than sparrows living in Ohio (Muñoz-Garcia and Williams 2005).

Birds in lowland tropical rain forests encounter relatively constant temperatures of about 30°C, high humidity, and abundant water all year. In this environment, selection for reduced water loss would be minimal. In this study we measured CWL of Dusky Antbirds (*Cercomacra tyrannina*), and investigated for the first time how CWL is associated with the lipid composition of the stratum corneum in a lowland tropical rain forest bird. We predicted that Dusky Antbirds would have low amounts of ceramides and high amounts of free fatty acids in the stratum corneum, a combination that would result in high CWL. However, tropical birds may need to control their temperature by evaporative cooling after periods of activity. Thus, we hypothesized that Dusky Antbirds would have a high amount of cerebrosides in the stratum corneum to thermoregulate.

METHODS

CAPTURE OF BIRDS

We mist-netted Dusky Antbirds near Gamboa, Panama (9°7′N, 79°42′W) in secondary forest from 17 to 29 May 2005, during the rainy season; average air temperature during this period was 24 ± 2°C. Dusky Antbirds were held during the day in small cages and provided with mealworms. We measured metabolic rate and total evaporative water loss at night, on the same day of capture. Birds were weighed at the beginning and end of measurements with a calibrated Pesola scale. We also measured their tibiotarsus, bill, and head-plus-bill length using dial calipers (±1 mm), and the length of their right wing with a ruler (Svensson 1992). Sex was determined by plumage.

MEASUREMENT OF BASAL METABOLIC RATE AND TOTAL EVAPORATIVE WATER LOSS

We measured oxygen consumption and total evaporative water loss (TEWL) at night using standard flow-through respirometry methods (Gessaman 1987, Tieleman and Williams 2002). We removed food from the cages of birds 2–3 hr prior to measurements to ensure post-absorptive conditions. Dissection of specimens at the end of measurements confirmed that the digestive tract was empty.

Birds were placed in a stainless steel 7.4 L metabolic chamber that had a Lexan® (General Electric Company, Hunterville, North Carolina) lid rendered airtight by a rubber gasket. Chambers were placed in a large coolbox. A Peltier (Pelt-4, International Sable Systems, Las Vegas, Nevada) maintained temperature of the air in the coolbox at 30.0 ± 0.1°C (measured with a 30-gauge thermocouple), within the thermoneutral zone of Dusky Antbirds (JBW, unpubl. data). Birds stood on a wire mesh platform over a layer of mineral oil that trapped feces, eliminating them as a source of water in our measurements.

Compressed air was routed through Drierite® (W. A. Hammond Drierite Co. Ltd., Xenia, Ohio) to remove water and then passed through previously calibrated (Levy 1964) Mykrolis mass flow controllers (Tylan® FC-2900, Celerity Inc., Milpitas, California; 2 standard liters per min), set between 350 and 400 ml min^{-1} standard temperature and pressure (STP), prior to entering the chamber. Exiting air passed through a dew point hygrometer (model 2001-C1-S3, EdgeTech, Marlborough, Massachusetts; factory calibrated in August 2003). A subsample was directed through silica gel, ascarite, and silica gel to remove water and carbon dioxide, and then routed to an oxygen analyzer (S3 A-II, Applied Electrochemistry, Temecula, California). The air stream from the tank was split so that one line could be used as reference air. We validated our ability to measure oxygen consumption by infusing pure oxygen into the chamber with a syringe pump at a known flow rate and simultaneously measuring oxygen concentration of exiting air. The difference between the known influx of oxygen via the syringe pump and the calculated influx of oxygen was <2% ($n = 10$).

After 3 hr of equilibration, we began recording dew-point and oxygen concentration of the inlet and outlet air and the temperature in the chamber with a CR23X data logger (Campbell Scientific, Logan, Utah). For analyses, we used data that showed stable traces of O_2 consumption for at least 10 min. We calculated oxygen consumption with equation 4 of Hill (1972:262) and converted it to kJ day^{-1} using 20.08 J ml^{-1} O_2 (Schmidt-Nielsen 1997). To calculate TEWL (g day^{-1}), we used the equation:

$$\mathrm{TEWL} = (\mathrm{V'_e}\rho_{\mathrm{out}} - \mathrm{V'_i}\rho_{\mathrm{in}})(1.44 \times 10^{-3}),$$

where ρ_{out} and ρ_{in} are absolute humidity (g m^{-3}, STP) of inlet and outlet air, respectively, V'_e is the flow rate (ml min^{-1}) of exiting air, and V'_i is the flow rate (ml min^{-1}) of air entering the chamber (for development of this equation, see Tieleman and Williams [2002]). We assumed a respiratory quotient of 0.71 (King and Farner 1961).

After metabolic measurements were completed, we recorded body temperature by inserting a 36-gauge thermocouple into the cloaca and reading temperature with a Physitemp (Batt-12, Clifton, New Jersey) thermometer; both instruments were calibrated with a thermometer traceable to the National Institute of Standards and Technology.

MEASUREMENT OF CUTANEOUS WATER LOSS

We measured cutaneous water loss with a vaporometer (CWL_{Vap}; Delfin Technologies, Ltd., Kuopio, Finland). Ambient temperature

in the laboratory at the time of measurements averaged 21.6 ± 0.5°C. We took two measurements of CWL from the ventral apteria of birds and two from the dorsal apteria. The recording area of the vaporometer was 16 mm^2, always smaller than the area of the apteria. We calibrated the vaporometer in the laboratory and found an error of ±10%.

SEPARATION AND IDENTIFICATION OF SKIN LIPIDS IN THE STRATUM CORNEUM

We isolated and identified classes of lipids in the stratum corneum of Dusky Antbirds following Muñoz-Garcia and Williams (2005). Briefly, we immersed skin in distilled water at 65°C for 3 min, then peeled the epidermis from the dermis. The epidermis was incubated at 4°C overnight in a solution of 0.5% trypsin in phosphate buffered saline and reimmersed in fresh 0.5% trypsin solution for 3 hr at 38°C, a procedure that separated the stratum corneum from the remaining epidermis (Haugen et al. 2003, Haugen, Williams et al. 2003). Thereafter, we freeze-dried the stratum corneum for 12 hr, and stored it in a test tube at −20°C in an atmosphere of nitrogen. After determining dry mass of the stratum corneum (±0.01 mg), we extracted lipids with a series of mixtures of chloroform:methanol 2:1, 1:1, and 1:2 volume/volume for 2 hr at each step. We added 50 mg L^{-1} of butylated hydroxytoluene to prevent oxidation of lipids (Law et al. 1995).

Classes of lipids were separated using analytical thin layer chromatography on 20 × 20 cm glass plates coated with silicic acid (0.25 mm thick; Adsorbosil-Plus 1, Altech, Deerfield, Illinois; Muñoz-Garcia and Williams 2005). We used nonhydroxy fatty acid ceramides, galactocerebrosides, cholesterol, and a mixture of free fatty acids, all dissolved in 2:1 chloroform:methanol, as standards. Concentrations ranged from 0.30 mg mL^{-1} to 50 mg mL^{-1}. Our samples were composed of redissolved lipids of the stratum corneum from each bird in 200 µl of 2:1 chloroform:methanol containing butylated hydroxytoluene. We pipeted 5 µl of each lipid extract in triplicate onto the preadsorbent area of the plates using a Teflon-tipped Hamilton (Reno, Nevada) syringe. Two solvent systems were used, one for polar lipids, such as ceramides and cerebrosides, and another for nonpolar lipids, i.e., free fatty acids and cholesterol. To separate ceramides and cerebrosides, we developed plates with a mixture of chloroform:methanol:acetic acid (190:9:1) to the top, followed by development with hexane:ethyl ether:acetic acid (70:30:1) run to 12 cm from the bottom, and lastly by development with chloroform:methanol:water (60:40:5) run to 5 cm from the bottom. This procedure yielded five bands of ceramides and four bands of cerebrosides. Cholesterol and free fatty acids were separated using development with hexane to the top of the plate, followed by toluene to the top, and finally hexane:ethyl ether:acetic acid (70:30:1) run to 12 cm from the bottom. We revealed bands of lipids by spraying the plates with a solution of 3% cupric acetate in 8% phosphoric acid, and then placing the plates on a 20 × 20 cm aluminum hotplate slowly raised to 220°C over the course of 2 hr.

We quantified the concentration of lipid classes by photodensitometry using TN-Image (Nelson 2003). Proportions of lipid classes were based on the total mass of the four classes. To validate our ability to measure the quantity of lipids in solution using thin layer chromatography and photodensitometry, we followed the above protocol but used known concentrations of cholesterol as our unknown. The average error, calculated as [(observed − actual/actual) × 100], was −0.27 ± 2.86% ($n = 9$).

STATISTICAL ANALYSES

All statistical tests were performed with SPSS 12.0 (SPSS 2003) with the null hypothesis rejected at $P \leq 0.05$. Averages are reported ± SD. We tested for differences between means in CWL for dorsal and ventral sides measured with the vaporometer using a two-tailed t-test for independent samples. We performed regressions of all the lipid classes against CWL using a general linear model (Zar 1996).

RESULTS

OXYGEN CONSUMPTION AND TOTAL EVAPORATORY WATER LOSS

The body mass of five males and three females averaged 15.5 ± 1.7 g. Using Meeh's equation (Walsberg and King 1978), surface area of Dusky Antbirds was equal to 62.3 ± 4.6 cm^2. Dusky Antbirds consumed oxygen at a rate of 37.99 ± 14.41 mL O_2 hr^{-1}, which converted to heat production of 18.31 ± 6.95 kJ day^{-1} ($n = 8$). TEWL equaled 2.76 ± 1.22 g H_2O day^{-1} (n

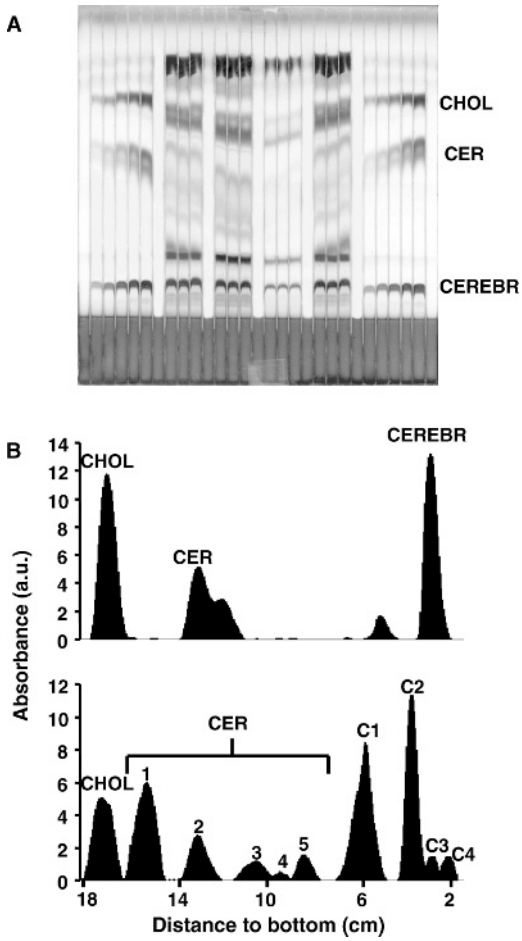

FIGURE 1. (A) Thin layer chromatograph of the polar lipids in the stratum corneum of Dusky Antbirds, and (B) Densitometric profile of lipid standards (upper figure); and cerebrosides, ceramides, and cholesterol from the extracted lipids in the stratum corneum of Dusky Antbirds (bottom figure). CHOL = cholesterol; CER = ceramide; CEREBR = cerebroside; C1–C4 = cerebrosides 1–4; a.u. = arbitrary units.

= 8). Expressed per unit body mass, TEWL was 0.18 ± 0.08 g H_2O g^{-1} day^{-1}.

CUTANEOUS WATER LOSS

We found no differences between rates of CWL_{Vap} from ventral and dorsal regions (t = 0.9, P = 0.35, n = 8), so we combined these data. CWL_{Vap} of Dusky Antbirds calculated for the whole organism averaged 10.30 ± 1.67 g H_2O day^{-1}; when these values were adjusted for differences in surface area, total CWL_{Vap} was 1.55 ± 0.19 g H_2O cm^{-2} day^{-1}.

TABLE 1. Lipid classes and their amounts (mg lipid g^{-1} dry mass) and percentages in the stratum corneum of Dusky Antbirds (n = 8) determined by thin layer chromatography.

Lipid class	Total amount	Percentage
Cholesterol	10.6 ± 7.8	8.0 ± 2.3
Free fatty acids	30.7 ± 6.9	27.3 ± 9.3
Ceramides	37.6 ± 29.6	27.7 ± 7.4
Ceramide 1	6.2 ± 6.1	4.7 ± 4.0
Ceramide 2	4.3 ± 4.9	2.9 ± 1.5
Ceramide 3	5.1 ± 6.1	3.4 ± 1.8
Ceramide 4	5.2 ± 4.3	1.9 ± 2.3
Ceramide 5	16.5 ± 10.8	12.8 ± 3.9
Cerebrosides	46.0 ± 21.0	37.0 ± 5.8
Cerebroside 1	22.9 ± 11.7	18.4 ± 6.0
Cerebroside 2	19.1 ± 7.5	15.8 ± 3.9
Cerebroside 3	2.0 ± 2.0	1.4 ± 0.6
Cerebroside 4	2.0 ± 2.0	1.4 ± 0.6
Total	124.7 ± 56.9	100

Our vaporometer only measured CWL at four points on the skin, but we wanted to estimate CWL of the whole organism. To do so, we needed an estimate of RWL that could be subtracted from our measurement of TEWL. We calculated RWL using equation 10 in Tieleman and Williams (1999:95), and estimated CWL (hereafter CWL_{Est}) by subtracting RWL from TEWL. Average RWL was 1.10 ± 0.08 g H_2O day^{-1}, whereas average CWL_{Est} was 1.66 ± 1.22 g H_2O day^{-1}, or 26.60 ± 19.23 mg H_2O cm^{-2} day^{-1}, similar values to those obtained with the vaporometer.

LIPIDS IN THE STRATUM CORNEUM

Thin layer chromatography revealed distinct bands of lipids corresponding to standards of cholesterol, free fatty acids, ceramides, and cerebrosides. The ceramides that separated into bands were named in order of increasing polarity (ceramide 1–5) as were the cerebrosides (cerebroside 1–4; Fig. 1). Sphingolipids, i.e., ceramides and cerebrosides, were the predominant lipid class in the stratum corneum, and cerebrosides were the most abundant sphingolipid (Table 1).

Because it is thought that changes in the proportions of lipid classes in the stratum corneum can influence the fluidity of the lipid layer and therefore water permeation (Bouwstra et al. 2003, Haugen et al. 2003, Haugen, Williams et al. 2003), we also expressed lipid

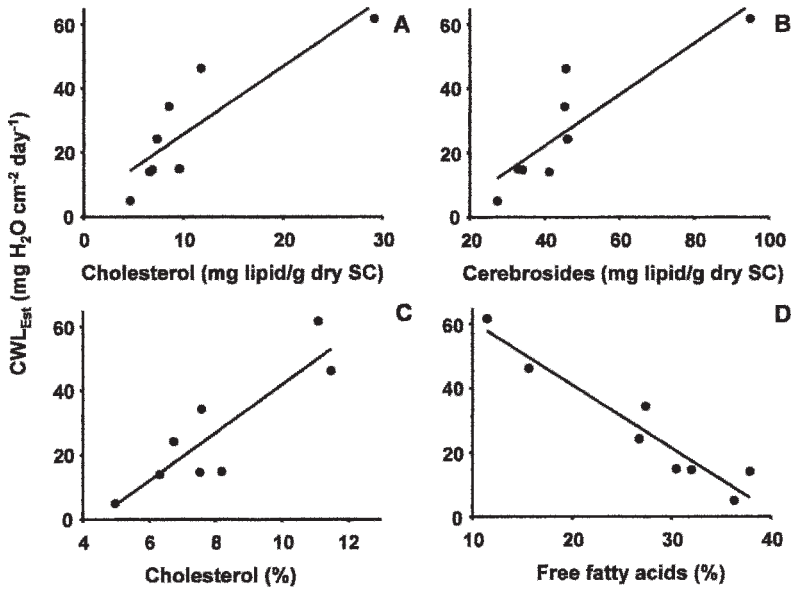

FIGURE 2. Estimated cutaneous water loss (CWL_{Est}) was positively associated with the amount of cholesterol (A), the amount of cerebrosides (B), and the percentage of cholesterol (C), and negatively correlated with the percentage of free fatty acids (D). All regressions were significant ($P < 0.05$).

classes as a percentage of the total lipids extracted (Table 1). Sphingolipids constituted 65% of the lipids in the stratum corneum of Dusky Antbirds.

CWL AND LIPIDS

CWL_{Vap} and CWL_{Est} varied significantly with the quantity of total lipids in the stratum corneum for Dusky Antbirds ($r > 0.71$, $P < 0.05$). However, we noted that one outlier drove the regressions. With this datum removed, regressions were no longer significant ($r < 0.33$, $P = 0.45$).

With the outlier removed, CWL_{Vap} was significantly associated with amount of cholesterol ($r = 0.77$, $P < 0.05$). There was also a significant positive association between CWL_{Est} and amount of cholesterol ($r = 0.86$, $P < 0.01$; Fig. 2A). In addition, CWL_{Est} was significantly positively correlated with the amount of cerebrosides ($r = 0.87$, $P < 0.01$; Fig. 2B) and the percentage of cholesterol ($r = 0.88$, $P < 0.01$, Fig. 2C), and varied negatively with the percentage of free fatty acids ($r = 0.95$, $P < 0.001$, Fig. 2D). CWL_{Est} was positively correlated with the amount of ceramides ($r = 0.74$, $P < 0.03$), but an outlier drove the regression. Without this datum, the regression was not significant ($P > 0.73$). Although the same trends were observed using CWL_{Vap} as our dependent variable, relationships were not significant ($r = 0.71$, $P = 0.07$ for percentage of cholesterol, $r = 0.56$, $P = 0.19$ for percentage of free fatty acids). CWL_{Vap} and CWL_{Est} were not correlated with either the percentage of ceramides or cerebrosides ($P > 0.05$).

DISCUSSION

TEWL of Dusky Antbirds, 2.76 g H_2O day^{-1}, exceeded predictions of an allometric equation for mesic birds by 20% (Williams 1996). Estimated CWL in Dusky Antbirds was 26.60 mg H_2O cm^{-2} day^{-1}, more than twice as much as the expected CWL (11.96 mg H_2O cm^{-2} day^{-1}) using the equation in Muñoz-Garcia and Williams (2005). Therefore, this study provides evidence that Dusky Antbirds have a high TEWL and high CWL rates, consistent with the idea that tropical birds use CWL to thermoregulate.

The total amount of lipids in the stratum corneum of Dusky Antbirds (124.7 mg g^{-1} dry mass) was lower than that for House Sparrows from the desert of Saudi Arabia (408.0 mg g^{-1} dry mass), or sparrows from temperate Ohio (315.0 mg g^{-1} dry mass). A diminution in the

amount of free fatty acids accounted for this difference; Dusky Antbirds had only 15% of the total amount of free fatty acids in their stratum corneum compared with House Sparrows. Lower amounts of lipids in the stratum corneum of Dusky Antbirds apparently resulted in higher rates of CWL.

Our hypothesis was that the proportion of cerebrosides in the stratum corneum should be higher relative to ceramides if the skin is an important vehicle for thermoregulation. The amount of sphingolipids in the stratum corneum of Dusky Antbirds was similar to that of House Sparrows in mesic environments, but lower than that of House Sparrows in the desert. However, the cerebroside:ceramide ratio is similar in Dusky Antbirds and House Sparrows from desert and mesic habitats, about 0.55–0.65 in both species. Therefore, cerebrosides are not more abundant in the stratum corneum of Dusky Antbirds than in House Sparrows from desert or mesic environments. This suggests that other lipids, such as cholesterol and free fatty acids, need to be included in our understanding of how the lipid composition of the stratum corneum affects CWL.

A remarkable feature of the stratum corneum of Dusky Antbirds is the reduced amount of free fatty acids compared to that of House Sparrows. The structural and chemical characteristics of free fatty acids and ceramides contribute to make the skin less permeable. Because of their rod shapes, interactions of these classes of lipids would promote the formation of a highly ordered lattice that would impede water loss through the skin (Wertz 2000, Bouwstra et al. 2003). Ratios of the different classes of lipids in the stratum corneum also seem to be important in the formation of lamellar structures that are responsible for the low CWL rates of mammals (Bouwstra et al. 2003). For example, the stratum corneum in mammals contains an equimolar mixture of ceramides, cholesterol, and free fatty acids. This proportion appears to confer the mammalian stratum corneum with a high resistance to water vapor diffusion. Changes in the ceramide:free fatty acid ratio will disrupt the ordered phase in the stratum corneum, increasing water vapor flow through the skin (Bouwstra et al. 2003). In species of larks and populations of House Sparrows in mesic areas, the increase of free fatty acid content may alter this ratio and prevent formation of the lamellae in the intercellular spaces of the stratum corneum (Haugen et al. 2003, Haugen, Williams et al. 2003, Muñoz-Garcia and Williams 2005). Dusky Antbirds, however, seem to follow the opposite strategy: a marked reduction in the free fatty acid content, that nevertheless will alter the free fatty acid:ceramide ratio and prevent the formation of orthorhombic lattices, producing a less fluid and more permeable domain.

Although cholesterol has been identified as a key factor promoting the stability of the lamellae in the mammalian stratum corneum, it does so only at high concentrations (Norlén 2001). In the range of proportions in which it is found in the stratum corneum of Dusky Antbirds ($<12\%$), cholesterol would disrupt the orthogonal phase, making the stratum corneum more fluid and less permeable.

In conclusion, Dusky Antbirds exhibited a low amount of total lipid, the result of a low amount of free fatty acids in their stratum corneum. However, Dusky Antbirds did not have different proportions of ceramides or cerebrosides when compared to House Sparrows. Overall, our data support the idea that Dusky Antbirds use cutaneous water loss to thermoregulate, and suggest that the interactions of the lipids in the stratum corneum may play an important role in determining the permeability of the skin.

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LITERATURE CITED

BARTHOLOMEW, G. A., AND T. J. CADE. 1963. The water economy of land birds. Auk 80:504–539.

BLANK, I. H. 1953. Further observations on factors which influence the water content of the stratum corneum. Journal of Investigative Dermatology 21:259–269.

BLANK, I. H., A. G. MOLONEY, A. G. EMSLIE, AND I. SIMON. 1984. The diffusion of water across the

stratum-corneum as a function of its water content. Journal of Investigative Dermatology 82:188–192.

BOUWSTRA, J. A., P. L. HONEYWELL-NGUYEN, G. S. GOORIS, AND M. PONEC. 2003. Structure of the skin barrier and its modulation by vesicular formulations. Progress in Lipid Research 42:1–36.

CARRUTHERS, A., AND D. L. MELCHIOR. 1983. Studies of the relationship between water permeability and bilayer physical state. Biochemistry 22:5797–5807.

DAWSON, W. R. 1982. Evaporative losses of water by birds. Comparative and Biochemical Physiology A 71:495–509.

ELIAS, P. M., E. R. COOPER, A. KORC, AND B. E. BROWN. 1981. Percutaneous transport in relation to stratum corneum structure and lipid composition. Journal of Investigative Dermatology 76:297–301.

ELIAS, P. M., AND G. K. MENON. 1991. Structural and lipid biochemical correlates of the epidermal permeability barrier. Advanced Lipid Research 24:1–26.

GESSAMAN, J. A. 1987. Energetics, p. 289–320. *In* B. A. Pendleton, B. A. Millsop, K. W. Cline, and D. M. Bird [EDS.], Raptor management techniques manual. Yale University Press, New Haven, CT.

HAUGEN, M., B. I. TIELEMAN, AND J. B. WILLIAMS. 2003. Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum. Journal of Experimental Biology 206:3581–3588.

HAUGEN, M., J. B. WILLIAMS, P. W. WERTZ, AND B. I. TIELEMAN. 2003. Lipids of the stratum corneum vary with cutaneous water loss among larks along a temperature-moisture gradient. Physiological and Biochemical Zoology 76:907–917.

HILL, R. W. 1972. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. Journal of Applied Physiology 33:261–263.

HOFFMAN, T. C. M., AND G. E. WALSBERG. 1999. Inhibiting ventilatory evaporation produces an adaptive increase in cutaneous evaporation in Mourning Doves *Zenaida macroura*. Journal of Experimental Biology 202:3021–3028.

KING, J. R., AND D. S. FARNER. 1961. Energy metabolism, thermoregulation and body temperature, p. 215–288. *In* A. J. Marshall [ED.], Biology and comparative physiology of birds. Academic Press, New York.

LAW, S., P. W. WERTZ, D. C. SWARTZENDRUBER, AND C. A. SQUIER. 1995. Regional variation in content, composition and organization of porcine epithelial barrier lipids revealed by thin layer chromatography and transmission electron microscopy. Archives in Oral Biology 40:1085–1091.

LEVY, A. 1964. The accuracy of the bubble meter for gas flow measurements. Journal of Scientific Instruments 41:449–453.

LUCAS, A. M., AND P. R. STETTENHEIM. 1972. Avian anatomy. Agricultural Handbook 362. Government Printing Office, Washington, DC.

MARDER, J., AND J. BEN-ASHER. 1983. Cutaneous water evaporation. I. Its significance in heat-stressed birds. Comparative and Biochemical Physiology A 75:425–431.

MENON, G. K., AND J. MENON. 2000. Avian epidermal lipids: functional considerations and relationship to feathering. American Zoology 40:540–552.

MOUNT, L. E. 1979. Adaptation to thermal environment. Edward Arnold, London.

MUÑOZ-GARCIA, A., AND J. B. WILLIAMS. 2005. Cutaneous water loss and lipids of the stratum corneum in House Sparrows, *Passer domesticus*, from arid and mesic environments. Journal of Experimental Biology 208:3689–3700.

NELSON, T. J. [ONLINE]. 2003. TN-Image. Shareware software. <http://entropy.brneurosci.org> (26 March 2006).

NORLÉN, L. 2001. Skin barrier structure and function: the single gel phase model. Journal of Investigative Dermatology 117:830–836.

POTTS, R. O., AND M. L. FRANCOUER. 1990. Lipid biophysics of water loss through the skin. Proceedings of the National Academy of Sciences USA 87:3871–3873.

SCHMIDT-NIELSEN, K. 1997. Animal physiology: adaptation and environment. Cambridge University Press, Cambridge, UK.

SPSS. 2003. SPSS 12.0 for Windows. SPSS, Inc., Chicago.

SQUIER, C. A. 1973. The permeability of keratinized and non-keratinized oral epithelium to horseradish peroxydase. Journal of Ultrastructural Research 43:160–177.

SVENSSON, L. 1992. Identification guide to European passerines. Fingraf AB, Stockholm, Sweden.

TIELEMAN, B. I., AND J. B. WILLIAMS. 1999. The role of hyperthermia in the water economy of desert birds. Physiological and Biochemical Zoology 72:87–100.

TIELEMAN, B. I., AND J. B. WILLIAMS. 2002. Cutaneous and respiratory water loss in larks from arid and mesic environments. Physiological and Biochemical Zoology 75:590–599.

WALSBERG, G. E., AND J. R. KING. 1978. The relationship of the external surface area of birds to skin surface and body mass. Journal of Experimental Biology 76:185–189.

WEBSTER, M. D., AND J. R. KING. 1987. Temperature and humidity dynamics of cutaneous and respiratory evaporation in Pigeons, *Columba livia*. Journal of Comparative Physiology B 57:253–260.

WERTZ, P. W. 2000. Lipids and barrier function of the skin. Acta Dermato-Venereologica 80, Supplement 208:7–11.

WILLIAMS, J. B. 1996. A phylogenetic perspective of evaporative water loss in birds. Auk 113:457–472.

WILLIAMS, J. B., AND B. I. TIELEMAN. 2005. Physiological adaptation in desert birds. BioScience 55:416–426.

WOLF, B. O., AND G. E. WALSBERG. 1996. Respiratory and cutaneous evaporative water loss at high environmental temperatures in a small bird. Journal of Experimental Biology 199:451–457.

ZAR, J. H. 1996. Biostatistical analysis. 3rd ed. Prentice Hall, Englewood Cliffs, NJ.