

Review

Water relations of tetrapod integument

Harvey B. Lillywhite

Department of Zoology, University of Florida, Gainesville, FL 32611-8525, USA

*Author for correspondence (e-mail: hbl@zoo.ufl.edu)

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Summary

The vertebrate integument represents an evolutionary compromise between the needs for mechanical protection and those of sensing the environment and regulating the exchange of materials and energy. Fibrous keratins evolved as a means of strengthening the integument while simultaneously providing a structural support for lipids, which comprise the principal barrier to cutaneous water efflux in terrestrial taxa. Whereas lipids are of fundamental importance to water barriers, the efficacy of these barriers depends in many cases on structural features that enhance or maintain the integrity of function. Amphibians are exceptional among tetrapods in having very little keratin and a thin stratum corneum. Thus, effective lipid barriers that are present in some specialized anurans living in xeric habitats are external to the epidermis, whereas lipid barriers of amniotes exist as a lipid-keratin complex within the stratum corneum. Amphibians prevent desiccation of the epidermis and underlying tissues either by evaporating water from a superficial aqueous film, which must be replenished, or by shielding the stratum corneum with superficial lipids.

Water barrier function in vertebrates generally appears to be relatively fixed, although various species have 'plasticity' to adjust the barrier effectiveness facultatively. While it is clear that both phenotypic plasticity and genetic adaptation can account for covariation between environment and skin resistance to water efflux, studies of the relative importance of these two phenomena are few. Fundamental mechanisms for adjusting the skin water barrier include changes in barrier thickness, composition and physicochemical properties of cutaneous lipids, and/or geometry of the barrier within the epidermis. While cutaneous lipids have been studied extensively in the contexts of disease and cosmetics, relatively little is known about the processes of permeability barrier ontogenesis related to adaptation and environment. Advances in such knowledge have didactic significance for understanding vertebrate evolution as well as practical application to clinical dermatology.

Key words: skin, vertebrate, permeability, lipids, evaporative water loss, phenotypic plasticity.

Introduction

One of the dilemmas in the evolution of multicellular organization was how increasingly larger organisms might continue to sense, interact and exchange with their environment while simultaneously achieving protection from the mechanical, radiative and chemical hazards with which they came into contact. Hence, the integument evolved as a heterogeneous, pluristratified, multimembrane system providing physical protection while simultaneously regulating the exchange of energy and materials between the organism and its surroundings (Chuong et al., 2002). An impressive array of body coverings evolved, with collectively diverse mechanical properties, appendages and multiple diffusion pathways, but with rate-limiting barriers for specific molecules. While integumentary coverings are highly variable in their morphology, they nonetheless exhibit common themes

of adaptation. An important aspect of vertebrate evolution was the orchestration of characters that rendered the skin increasingly less permeable to water as forms invaded and adapted to harsher and drier terrestrial environments.

Understanding water relations of vertebrate skin has been advanced by empirical knowledge and by perspectives based on several sources. These include descriptive studies of morphology, physiological studies of specific transport mechanisms and phenomena, physicochemical and physiological studies of integumentary lipids (thought to comprise the principal barrier to transepidermal water movement) and, generally, comparative investigations related to quantitative measures of water flux across the skin of animals adapted to specific environments. Holistic understanding of vertebrate adaptation to terrestrial environments requires considerable appreciation for the

integration of characters that determine the water relations of integument *per se*, as well as the water budget for the whole organism.

General features of vertebrate integument

The integument is a complex structure comprised of epithelial cells derived from embryonic ectoderm and attached to an underlying fibrous and vascular dermis originating from mesoderm (Fig. 1). Epidermal–dermal interactions are critical for differentiation and specialization of cells. Mitotic activity takes place in the basal layer (stratum germinativum), which proliferates cells that are eventually lost from the animal's surface. All cell types that are present in the epidermis are represented by precursors in the basal layer. Localized cellular proliferation and differentiation produce appendages such as claws, glands, hair or feathers, which together with patterned folds or scales, contribute to integumentary form. Cellular renewal is a further characteristic of vertebrate skin and serves to maintain the integrity of structure and its various functions.

The earliest protovertebrate was likely a small, soft-bodied animal (Berrill, 1955), separated by millions of years from the radiation of early Paleozoic fishes. Subsequent trends to increasing body size required mechanical strengthening of the body surfaces, which, in turn, led to reduced mobility or to serial, patterned folding and the evolution of scales (Maderson, 1972). Patterns of ossified units suggest that Paleozoic amphibians possessed a scaled integument (Cobert, 1955; Kitching, 1957). Thus, it seems probable that the integuments of most modern vertebrates are derived secondarily from ancestral conditions of integument that were in some sense 'scaled' (Maderson, 1972, 2003; Maderson and Alibardi, 2000). Subsequent evolution of appendages such as hair or feathers is best understood by relating them to developmental fields, originally subdivided by embryonic epidermal–dermal cell populations reflecting a scaled integument of the adult (Maderson, 1972; Maderson and Alibardi, 2000). But scales *per se* do not provide strength of structure, nor, as commonly misunderstood, do they provide an effective waterproofing function (Licht and Bennett, 1972; Bennett and Licht, 1975; Roberts and Lillywhite, 1980). Total or near absence of scales is a derived character in many amphibians, mammals and birds (Maderson and Alibardi, 2000).

Keratin is a prevalent structural feature (Table 1) and is formed in epidermis of all living vertebrates by regulated processes involving synthesis of keratin filaments and cellular death. The synthesis of keratin polypeptides is known from epidermal cells of lampreys 450 million years ago (McLaughlin and Dayhoff, 1969). Subsequent evolution involved variable expression of genes to produce specific keratins and keratinized structures, as well as appropriate protective substances besides keratin. For example,

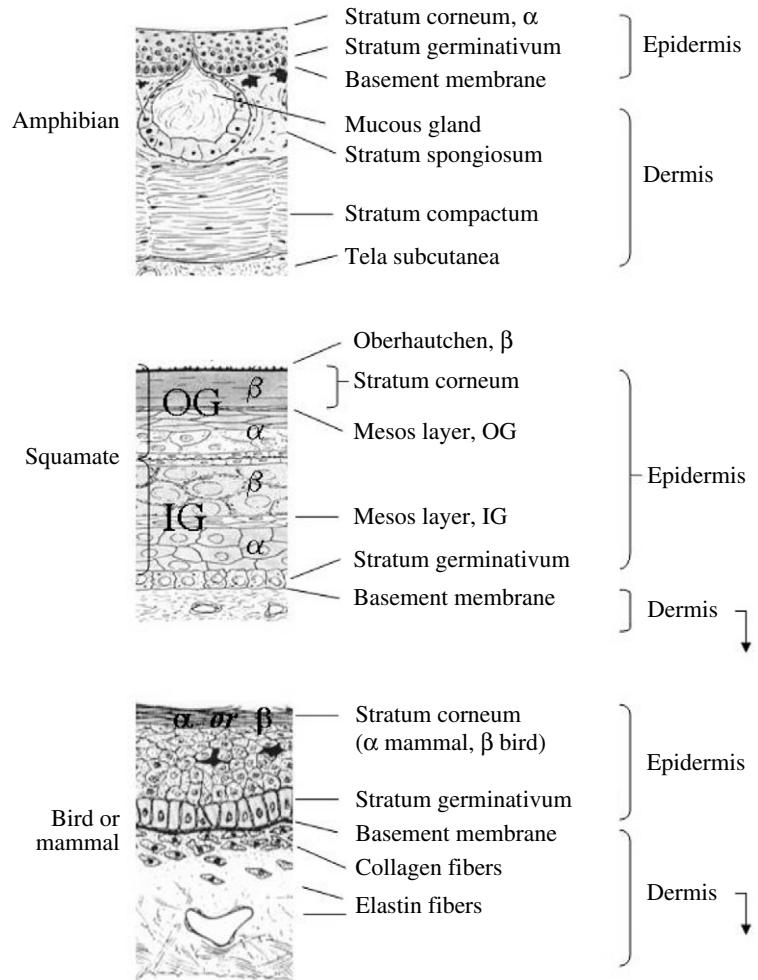


Fig. 1. Generalized features of integument in four major clades of vertebrates. α and β indicate alpha- and beta-type keratins, respectively. OG and IG indicate outer and inner epidermal generations that might be present in a squamate reptile prior to skin shedding. The mesos layer of squamates is a specialized cell type derived from α keratins and contains extracellular, laminated lipids, comprising the principal water barrier in these species.

cyclostomes, most osteichthyans, lissamphibians, and many larval or adult amphibians are protected principally by mucus (Maderson and Alibardi, 2000), and thus the body is covered by a largely non-keratinizing epidermis that suffices for aquatic life (e.g. Mittal and Banerjee, 1980). Additional mechanical protection is provided by a cytoskeleton and a terminal web of keratin filaments in superficial cells. During vertebrate evolution, elaboration of the keratinization process allowed adaptation to amphibious and eventually fully terrestrial life. Protection and movement on terrestrial substrates was facilitated by highly resistant cell envelopes encasing stabilized keratin frameworks in superficial cells. The formation of a corneous cell envelope is absent in fishes but occurs in epidermis of adult amphibians and forms in the periderm and first embryonic layers of amniotes, suggesting a primary role for terrestrial survival (Alibardi, 2003). Formation of a stratum corneum over the body surfaces

evolved in adult amphibians about 350 million years ago (Matoltsy and Bereiter-Hahn, 1986).

Mucus became associated with the filament matrix and was also discharged to fill the extracellular spaces of the stratum corneum. However, the requirement for an efficient permeability barrier to reduce significantly the exchange of water between the body and environment was met by production of neutral and polar lipids as well as mucus to become components of the cellular matrix and intercellular spaces in differentiating epidermal cells. The participation of lipids to form a competent water barrier likely began in reptiles about 300 million years ago (Matoltsy and Bereiter-Hahn, 1986) and has evolved independently in at least four principal lineages: reptiles, birds, mammals (Maderson, 2003; Maderson and Alibardi, 2000; Menon and Menon, 2000) and amphibians (Lillywhite and Mittal, 1999). The composition of Golgi-derived organelles related to barrier function changed during evolution from mucus and glycoproteins (fishes, amphibians) to glycolipids and lipids (amniotes; see below).

Most functions of the epidermis can be considered protective or defensive in all vertebrates. The majority of these functions reside in the stratum corneum, and of these, the protective function of the water permeability barrier is paramount (Chuong et al., 2002). If mammalian skin is damaged or diseased, cutaneous water loss can increase by several orders of magnitude. The rate-limiting process of water diffusion across the epidermis normally resides in the stratum corneum. Accordingly, many cutaneous phenomena, including immune responses, inflammation and hyperplasia, are recruited directly or secondarily to maintain a competent permeability barrier. Thus, mechanisms that normalize the stratum corneum and competent barrier function can abrogate susceptibility to diseases such as psoriasis, atopic and contact dermatitis, which are triggered, sustained or exacerbated by external perturbations (Chuong et al., 2002).

Transepidermal water permeation

Fundamentally, the integument of vertebrates consists of a large number of cell membranes organized essentially in

stratified series with heterogeneous structure and variable resistances to movement of water molecules in both intracellular and extracellular pathways. While there is good understanding of permeation processes in membranes generally, the complexity and diversity of vertebrate skin greatly complicates a generalized analysis. Transepidermal water loss (TEWL) (and cutaneous water loss, CWL), is a passive diffusion process that is not dependent on cellular metabolic energy (Pinnagoda, 1994; Wilson and Maibach, 1994; Chuong et al., 2002). Therefore, limitations are expected to relate to tractable morphological features. Key questions are: (1) what are the pathways of molecular movement across the skin, and (2) which steps in the permeation process are rate-limiting?

Limitations for water transport through the skin can be related to movement through cell membranes and cytosol of cells, diffusion in the cutaneous matrix between the membranes, and passage of molecules across the membrane/environment interface, neglecting unstirred layers or secreted substances at the skin surface (see Feder and Pinder, 1988). Attributes of integument affecting water movement contribute to resistance of the skin to water passage (R_s), and this property combines with resistance of any external boundary layer (R_{bl}) to determine the actual rates of TEWL to ambient air in terrestrial animals. Measurements of R_s (in $s\text{ cm}^{-1}$) range from negligible (near zero) in various aquatic or terrestrial amphibians to several hundred in xerophilic arboreal or cocooned frogs, to over a thousand in desert reptiles, with more moderate values (10–300 $s\text{ cm}^{-1}$) being characteristic of birds and mammals (Table 2). Most measurements of boundary layer resistance are near zero to a few $s\text{ cm}^{-1}$ and thus constitute a negligible fraction of the whole-body R_s in most cases.

The ease of water movement through cell membranes can vary by orders of magnitude depending on the composition and physical state of the structure (Finkelstein, 1978; Carruthers and Melchior, 1983). Membranes that are in a liquid-crystalline state are far less permeable than are those that are fluid. In general, the relative diffusivity of molecules through cutaneous membranes will depend on their solubility

Table 1. *Properties of vertebrate integument that are relevant to integrity of the water permeability barrier. Features apply to the majority of species that have been examined in the listed taxa*

Taxon	General morphology	Keratin	Lamellar granules	Barrier location	Lipid-keratin complex	Facultative
Amphibians	Unscaled, glandular and pliant	α , few layers	Absent	Superficial	No; cocoons?	Yes
Reptiles						
Lepidosaurian	Scaled	α and β multiple layers	Present	Stratum corneum	Yes	Some spp.
Other	Scaled	α and β multiple layers	Present	?	?	?
Birds	Scaled or sculptured with plumage	α and β multiple layers	Present	Stratum corneum	Yes	Some spp.
Mammals	Scaled or sculptured with pelage	α , multiple layers	Present	Stratum corneum	Yes	Some spp.

Table 2. Mean values of skin resistance to evaporative water loss (R_s) reported for various vertebrate species

Taxon	Habitat	Resistance (s cm ⁻¹)	Source
Amphibia: Bufonidae			
<i>Bufo cognatus</i>	Xeric terrestrial–fossorial	1.5–5	1, 2
<i>B. marinus</i>	Mesic terrestrial	1.7	3
<i>B. terrestris</i>	Mesic terrestrial	~0	2
<i>B. valliceps</i>	Mesic terrestrial	~0	5
Amphibia: Hylidae			
<i>Agalychnis annae</i>	Mesic arboreal	10	2
<i>A. callidryas</i>	Mesic arboreal	2.8	1
<i>Cyclorana australis</i> (non-cocooned)	Fossorial–terrestrial	2.4	4
<i>C. australis</i> (cocooned, 3 weeks–6 months)	Fossorial–terrestrial	60–214	4
<i>C. australis</i>	Fossorial–terrestrial	5.4	3
<i>C. longipes</i>	Fossorial–terrestrial	3.5	3
<i>C. maini</i>	Fossorial–terrestrial	2.1	6
<i>C. maini</i> (cocooned, 1–5 months)	Fossorial–terrestrial	114	6
<i>C. maini</i> (cocoon only)	Fossorial–terrestrial	198	6
<i>C. novaehollandiae</i>	Fossorial–terrestrial	4.6	6
<i>C. novaehollandiae</i> (cocooned, 1–5 months)	Fossorial–terrestrial	37	6
<i>C. platycephala</i>	Fossorial	1.8	6
<i>C. platycephala</i> (cocooned, 1–5 months)	Fossorial	41	6
<i>C. platycephala</i> (cocoon only)	Fossorial	67	6
<i>Hyla arenicolor</i>	Terrestrial–riparian	4.2	1
<i>H. cinerea</i> (variable RH)	Mesic arboreal	1.7–3.6	2, 5
<i>H. crucifer</i>	Mesic terrestrial	1.4	2
<i>H. gratiosa</i>	Terrestrial–arboreal	2.2	2
<i>H. gratiosa</i>	Terrestrial–arboreal	3.3	1
<i>H. triangulum</i>	Mesic arboreal	2.2	2
<i>Litoria adelaidensis</i>	Mesic semi-arboreal	6.5	6
<i>L. albobuttatus</i> (= <i>Cyclorana albobuttatus</i>) (hydrated vs cocooned)	Terrestrial–fossorial	3.1–89.4	7
<i>L. albobuttatus</i> (= <i>Cyclorana albobuttatus</i>) (cocoon only)	Terrestrial–fossorial	89*	6
<i>L. aurea</i> (25–35°C)	Semi-aquatic	3.5–6.9	8
<i>L. bicolor</i> (wet season vs dry season)	Arboreal	47.6–79.7	3
<i>L. caerulea</i>	Arboreal	1.8	1
<i>L. caerulea</i>	Arboreal	~6	4
<i>L. caerulea</i> (25–50°C air temperatures)	Arboreal	2.9–8.5	9
<i>L. caerulea</i> (wet season vs dry season)	Arboreal	13.2–15.2	3
<i>L. chloris</i> (25–50°C air temperatures)	Mesic arboreal	2.5–29.6	9
<i>L. coplandi</i>	Terrestrial	9.6	3
<i>L. dahlia</i> (wet season vs dry season)	Aquatic	2.3–2.4	3
<i>L. gilleni</i>	Arboreal	11.5	3
<i>L. gracilentia</i>	Mesic arboreal	118	1
<i>L. inermis</i>	Terrestrial	1.4	3
<i>L. meiriana</i>	Semi-aquatic	0.6	3
<i>L. microbelos</i>	Terrestrial	~0	3
<i>L. moorei</i>	Mesic semi-aquatic	0.5	6
<i>L. nasuta</i> (wet season vs dry season)	Terrestrial	4.4–4.9	3
<i>L. pallida</i> (wet season vs dry season)	Terrestrial	5.4–6.6	3
<i>L. peroni</i> (25–40°C)	Arboreal–terrestrial	8.7–9.7	10
<i>L. rothi</i> (wet season vs dry season)	Arboreal	15.8–16.4	3
<i>L. rubella</i> (variable postures)	Arboreal	1.1–7.3	11
<i>L. rubella</i> (wet season vs dry season)	Arboreal	10.5–14.2	3
<i>L. splendida</i>	Arboreal	10.2	3
<i>L. tornieri</i> (wet season vs dry season)	Terrestrial	3.5–3.9	3
<i>L. wotjulumiensis</i> (wet season vs dry season)	Terrestrial	7.9–8.6	3
<i>L. xanthomera</i> (25–43°C air temperatures)	Mesic arboreal	32–~8	12
<i>Osteopilus dominicensis</i>	Mesic arboreal	1.8	2

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Table 2. *Continued*

Taxon	Habitat	Resistance (s cm ⁻¹)	Source
Amphibia: Hylidae			
<i>O. septentrionalis</i>	Mesic arboreal	1.4–1.9	1, 2
<i>Pachymedusa dacnicolor</i>	Semi-arid arboreal	14	2
<i>Phyllomedusa azurea</i>	Xeric arboreal	242	1
<i>P. hypochondrialis</i>	Xeric arboreal	364	2
<i>P. iherengi</i>	Xeric arboreal	277	2
<i>P. pailoma</i>	Xeric arboreal	336	2
<i>P. sauvagei</i>	Xeric arboreal	206	2
<i>P. sauvagei</i>	Xeric arboreal	300*	13
<i>Pseudacris regilla</i> (= <i>Hyla regilla</i>) (flowing vs still air)	semi-arboreal	2–15	13
<i>Pternohyla fodiens</i> (cocoon only)	Fossorial–terrestrial	414*	6
<i>P. fodiens</i> (cocooned)	Fossorial–terrestrial	457*	13
Amphibia: Hyperoliidae			
<i>Afrixalus fornasinii</i>	Arboreal	4.8	1
<i>A. pygmaeus</i>	Semi-arboreal	5.5	1
<i>A. quadrivittatus</i>	Semi-arboreal	3.2	1
<i>Hyperolius argus</i>	Semi-arboreal	24.5	1
<i>H. kivuensis</i>	Semi-arboreal	73.5	1
<i>H. nasutus</i> (still vs flowing air)	Semi-arboreal	96–257	13
<i>H. pusillus</i>	Semi-arboreal	34.2	1
<i>H. tuberilinguis</i>	Semi-arboreal	29.2	1
<i>Leptopelis bocagei</i> (cocooned)	Fossorial	40*	13
Amphibia: Leptodactylidae			
<i>Lepidobatrachus llanensis</i> (cocooned)	Terrestrial–fossorial	163*	13
<i>L. llanensis</i> (cocoon only)	Terrestrial–fossorial	116*	6
Amphibia: Microhylidae			
<i>Austrochaperina adelphe</i>	Terrestrial	0.1	3
Amphibia: Myobatrachidae			
<i>Crinia bilingua</i>	Terrestrial	0.1	3
<i>C. georgiana</i>	Mesic terrestrial	1.4	6
<i>C. pseudinsignifera</i>	Mesic terrestrial	2.7	6
<i>Heleioporus albopunctatus</i>	Mesic fossorial	1.6	6
<i>H. eyrei</i>	Mesic fossorial	2.5	6
<i>H. inornatus</i>	Semi-aquatic	2.2	6
<i>Limnodynastes convexiusculus</i>	Fossorial	2.7	3
<i>L. fletcheri</i>	Mesic terrestrial	1.5	10
<i>L. ornatus</i>	Fossorial	2.2	6
<i>L. ornatus</i>	Fossorial	3.1	3
<i>L. spenceri</i>	Fossorial	1.4–2.8	6
<i>Neobatrachus aquilonius</i>	Terrestrial–fossorial	0.8	6
<i>Neobatrachus aquilonius</i> (cocooned, 1–5 months)	Terrestrial–fossorial	32	6
<i>N. centralis</i>	Terrestrial–fossorial	2.2	6
<i>N. centralis</i> (cocooned, 1–5 months)	Terrestrial–fossorial	57	6
<i>N. fulvus</i>	Terrestrial–fossorial	2.7	6
<i>N. fulvus</i> (cocooned, 1–5 months)	Terrestrial–fossorial	59	6
<i>N. kunapalari</i>	Terrestrial–fossorial	2.2	6
<i>N. kunapalari</i> (cocooned, 1–5 months)	Terrestrial–fossorial	92	6
<i>N. kunapalari</i> (cocoon)	Terrestrial–fossorial	92	6
<i>N. pelobatoides</i>	Terrestrial–fossorial	1.1	6
<i>N. pelobatoides</i> (cocoon or cocooned, 1–5 months)	Fossorial	41	6
<i>N. sutor</i>	Fossorial	1.1	6
<i>N. sutor</i> (cocooned, 1–5 months)	Fossorial	93	6
<i>N. sutor</i> (cocoon only)	Fossorial	107	6

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Table 2. Continued

Taxon	Habitat	Resistance (s cm ⁻¹)	Source
Amphibia: Myobatrachidae			
<i>N. wilsmorei</i>	Fossorial	2.1	6
<i>N. wilsmorei</i> (cocooned, 1–5 months)	Fossorial	82	6
<i>Notaden melanoscapus</i>	Fossorial	2.0	3
<i>N. nicholli</i>	Xeric fossorial	2.1	6
<i>Pseudophryne guentheri</i>	Mesic terrestrial	1.7	6
<i>Uperoleia lithomoda</i>	Fossorial	~0	3
<i>U. russelli</i>	Terrestrial	1.7	6
<i>U. sp. nov.</i>	Fossorial	~0	3
Amphibia: Pelobatidae			
<i>Scaphiopus couchii</i>	Xeric fossorial	5	2
<i>S. holbrooki</i>	Mesic fossorial	~0	2
Amphibia: Plethodontidae			
<i>Desmognathus ochrophaeus</i>	Mesic terrestrial	0.09	14
Amphibia: Ranidae			
<i>Pyxicephalus adspersus</i> (cocoon only)	Terrestrial–fossorial	175*	6
<i>P. adspersus</i> (cocooned)	Terrestrial–fossorial	190*	13
<i>Rana catesbeiana</i>	Semi-aquatic	~0	2
<i>R. pipiens</i>	Mesic, semi-aquatic	0.05	14
<i>R. pipiens</i>	Mesic, semi-aquatic	1.6	1
<i>R. temporaria</i>	Mesic, semi-aquatic	3	2
Amphibia: Rhacophoridae			
<i>Chiromantis petersi</i>	Xeric arboreal	347	1
<i>C. rufescens</i>	Mesic arboreal	404	1
<i>C. spp.</i>	Xeric arboreal	400–900*	13
<i>Polypedates maculatus</i>	Semi-arboreal	1.9	15
<i>Rhacophorus leucomystax</i>	Mesic arboreal	14	2
Reptilia: Acrochordidae			
<i>Acrochordus granulatus</i>	Aquatic, marine	116	16
Reptilia: Agamidae			
<i>Amphibolurus ornatus</i>	Xeric terrestrial	521*	13
<i>A. pallida</i> (25–35°C air temperatures)	Xeric terrestrial	~175–~660	17
<i>Agama sinita</i> (25–35°C air temperatures)	Xeric terrestrial	~180–738	17
<i>A. savignii</i> (25–35°C air temperatures)	Semi-arid terrestrial	~230–~410	17
<i>A. stellio</i> (5–35°C air temperatures)	Mesic and semi-arid terrestrial	~234–~270	17
Reptilia: Crocodylidae			
<i>Alligator mississippiensis</i> (20–30°C)	Mesic, amphibious	43.3–64	18
Reptilia: Colubridae			
<i>Coluber rubriceps</i> (25–34°C air temperatures)	Mesic terrestrial	~300–~380	19
<i>Elaphe obsoleta</i> (shed epidermis)	Terrestrial	700*	20
<i>Lampropeltis getula</i> (newborn)	Mesic–xeric terrestrial	866	21
<i>Natrix tessellata</i> (25–34°C air temperatures)	Semi-aquatic	117–~190	19
<i>Nerodia rhombifera</i> (shed epidermis)	Semi-aquatic	314*	20
<i>N. sipedon</i> (shed epidermis)	Semi-aquatic	308*	20
<i>Psammophis schokari</i> (25–34°C air temperatures)	Mesic semi-arboreal	~480–~540	19
<i>Psammophis schokari</i> (25–34°C air temperatures)	Xeric semi-arboreal	~700–1088	19
<i>Spalerosophis diadema</i> (25–34°C air temperatures)	Xeric terrestrial	~190–~260	19
Reptilia: Emydidae			
<i>Terrapene carolina</i>	Mesic terrestrial	78	14
Reptilia: Gekkonidae			
<i>Gehyra variegata</i>	Xeric terrestrial	198*	13

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Table 2. *Continued*

Taxon	Habitat	Resistance (s cm ⁻¹)	Source
Reptilia: Iguanidae			
<i>Anolis carolinensis</i>	Mesic arboreal	196	14
<i>A. carolinensis</i> (humid vs dry environment)	Mesic arboreal	~196–296	22
<i>A. cristatellus</i> (8 insular populations)	Mesic–xeric arboreal	29–199	23
<i>A. ernestwilliamsi</i>	Xeric arboreal	168	23
<i>Iguana iguana</i>	Mesic terrestrial	370*	13
<i>I. iguana</i> (shed epidermis)	Mesic terrestrial	108.3*	20
<i>Sauromalus obesus</i>	Xeric terrestrial	1360*	13
<i>Uta stansburiana</i>	Semi-arid, xeric	1080*	13
Reptilia: Scincidae			
<i>Sphenomorphus labillardieri</i>	Mesic terrestrial	248*	13
Reptilia: Trionychidae			
<i>Trionyx spiniferus</i> (15–35°C)	Aquatic	2.98–5.38	24
Reptilia: Viperidae			
<i>Aspis cerastes</i> (shed epidermis)	Xeric terrestrial	788*	20
<i>Cerastes cerastes</i> (25–35°C air temperatures)	Xeric terrestrial	~1200–~1400	25
<i>Crotalus atrox</i>	Xeric terrestrial	1011	16
<i>Echis colorata</i> (25–35°C air temperatures)	Xeric terrestrial	~1300–~1550	25
<i>Pseudocerastes persicus</i> (25–35°C air temperatures)	Xeric terrestrial	1463–1921	25
<i>Vipera palaestinae</i> (25–35°C air temperatures)	Mesic terrestrial	706–878	25
Reptilia: Xantusiidae			
<i>Klauberina riversiana</i> (15–35°C)	Semi-arid	1020–3080	26
<i>Lepidophyma gageae</i> (20–30°C)	Mesic	660–1430	26
<i>Lepidophyma smithii</i> (25–35°C)	Mesic	190–320	26
<i>Xantusia vigilis</i> (25–30°C)	Xeric terrestrial	2150–3310	26
<i>X. henshawi</i> (20–30°C)	Xeric terrestrial	1120–1510	26
Aves: Anatidae			
<i>Anas platyrhynchos</i> (30–35°C air temperatures)	Semi-aquatic	38.5–60.8	27
Aves: Caprimulgidae			
<i>Phalaenoptilus nuttallii</i>	Xeric	125*	27
Aves: Columbidae			
<i>Columba livia</i> (20–52°C air temperatures)	Widespread	9.4–99.8	28
<i>C. livia</i> (30–40°C air temperatures)	Widespread	45–130	27
<i>Streptopelia decaocto</i> (20–52°C air temperatures)	Xeric	16.2–77.1	28
<i>S. risoria</i> (29.4–34.8°C air temperatures)	Xeric	137–250*	27
<i>S. senegalensis</i> (20–52°C air temperatures)	Xeric	25.8–62	28
<i>Zenaida asiatica</i> (35–45°C air temperatures)	Xeric	38–~200	29
<i>Z. macroura</i> (45–50°C air temperatures)	Widespread	24–41	30
Aves: Cuculidae			
<i>Geococcyx californicus</i>	Xeric	139*	27
Aves: Fringillidae			
<i>Poephila castanotis</i>	Xeric	112*	27
Aves: Passeridae			
<i>Zonotrichia leucophrys</i>	Widespread	94*	27
Aves: Phasianidae			
<i>Alectoris chukar</i> (20–45°C air temperatures)	Xeric	80.9–309.1	28
<i>Coturnix chinensis</i>	Open, mesic	199*	27
<i>C. japonica</i> (20–45°C air temperatures)	Open, xeric	77.8–297.1	28
<i>Gallus domesticus</i> (= <i>G. gallus</i>) (30–40°C air temperatures)	Mesic, domesticated	88–101*	27

Table continued on next page.

Table 2. Continued

Taxon	Habitat	Resistance (s cm ⁻¹)	Source
Aves: Ploceidae <i>Ploceus cucullatus</i>	Savannah	123*	27
Aves: Psittacidae <i>Melopsittacus undulatus</i>	Xeric	87*	27
Aves: Pteroclididae <i>Pterocles senegallus</i>	Xeric	15–103	31
Aves: Remizidae <i>Auriparus flaviceps</i>	Xeric	197	32
Mammalia: Hominidae <i>Homo sapiens</i> (human stratum corneum)	Widespread	377*	6
Mammalia: Muridae <i>Mus musculus</i> (30–35°C air temperatures)	Widespread	116–201*	27

Values represent the reciprocal of water vapor conductance and express the water vapor density difference between saturated skin and external environment per unit of evaporative flux.

Note: Approximated values (preceded by ~) are either estimated from graphic data or from data involving assumptions regarding boundary layer resistance, or rounded if close to zero. Variation in reported values may also reflect conditions of temperature and rates of air flow employed during measurements. Data calculated from literature values of CWL are indicated with an asterisk.

¹Withers et al., 1984; ²Wygoda, 1984; ³Young et al., 2005; ⁴Christian and Parry, 1997; ⁵Wygoda, 1988; ⁶Withers, 1998; ⁷Withers and Richards, 1995; ⁸Buttemer et al., 1996; ⁹Buttemer, 1990; ¹⁰Amev and Grigg, 1995; ¹¹Withers, 1995b; ¹²Buttemer and Thomas, 2003; ¹³Withers et al., 1982; ¹⁴Spotila and Berman, 1976; ¹⁵Lillywhite et al., 1997a; ¹⁶Lillywhite and Sanmartino, 1993; ¹⁷Eynan and Dmi'el, 1993; ¹⁸Davis et al., 1980; ¹⁹Lahav and Dmi'el, 1996; ²⁰Lillywhite and Maderson, 1982; ²¹Tu et al., 2002; ²²Kattan and Lillywhite, 1989; ²³Dmi'el et al., 1997; ²⁴Robertson and Smith, 1982; ²⁵Dmi'el, 1998; ²⁶Mautz, 1980; ²⁷Webster et al., 1985; ²⁸Marder and Ben-Asher, 1983; ²⁹McKechnie and Wolf, 2004; ³⁰Hoffman and Walsberg, 1999; ³¹Marder et al., 1986; ³²Wolf and Walsberg, 1996.

and partition coefficients, as well as thermal mobility related inversely to molecular mass.

Cutaneous membranes are mosaic structures composed of lipid bilayers in addition to molecular channels or pores. Therefore, the penetration of water molecules into cutaneous cells will depend, in part, on the fractional membrane area that is occupied by channels and the characteristics of the channels (Finkelstein and Cass, 1968; Finkelstein, 1984). Various proteins that serve as membrane channels for specific ions may also facilitate the concomitant flux of water by means of processes that induce solvent drag and by forming a low resistance pathway between the protein and lipid. Solubility–diffusion mechanisms will depend on the degree of packing and thermal mobility of hydrocarbon chains and on the charge of phospholipid polar groups in the bilayer membrane. In addition to diffusion, water flow across skin may be related to concentration differences of impermeant solutes or a differential of hydrostatic pressure. In contrast to diffusion, bulk osmotic or hydrodynamic flow involves the vectorial movement of an assembly of molecules being driven by an imposed potential. Consequently, various permeability coefficients can differ quantitatively because of the physical nature of the water movement pathway (Schafer and Andreoli, 1972; Finkelstein, 1984).

Water appears to permeate lipid bilayers by interaction with lipid polar head groups rather than a mechanism involving

solubility of water in hydrocarbons (Carruthers and Melchior, 1983). Driven by the transmembrane gradient of water concentration energy, water then diffuses (exchanges) between the various hydration shells of the polar group into the hydrocarbon core (Hauser and Phillips, 1979). Divalent cations potentially displace water molecules by competing for interaction with the negatively charged phospholipid groups (Hauser et al., 1976).

The number of ion channels in many cell membranes may be relatively too few to provide a significant pathway for water movement, and the bulk of transcellular water movement in cutaneous tissues appears to occur by a solubility–diffusion mechanism involving the lipid bilayer pathway (Finkelstein, 1984). However, aqueous pores do comprise a major route for water transport in certain epithelia including the skin of amphibians (Hevesy et al., 1935; Koefoed-Johnsen and Ussing, 1953). Moreover, if there is osmotic flow of water through a significant number of membrane-spanning channels, water transport is increased greatly because osmotic flux of water occurs by laminar or quasilaminar flow (Mauro, 1957). Water flow can also occur at the lipid/protein interface as well as *via* channels formed by protein, and thus water permeability may be governed significantly by the presence of membrane-spanning proteins and their interactions with the lipid bilayer. Diffusional movement of water through lipid bilayers is reduced by

orders of magnitude when proteins are absent (Carruthers and Melchior, 1983).

Cellular junctions and the extracellular diffusion pathway

In addition to the cellular route of water movement across integument, water also can move between the lateral, intercellular spaces. Simple epithelia are often categorized according to the relative permeabilities of the intra- and intercellular pathways. So-called 'leaky epithelia' are ones in which most diffusion occurs between cells, whereas 'tight epithelia' have junctional contacts between cells that impose a high resistance such that most passive diffusion occurs through cell membranes and cytosol. These distinctions may become blurred in consideration of complex, multi-layered barriers such as skin. In the various layers, junctional contacts between cells have various organizations such that cells can adhere, interact and dissipate tensional stresses throughout the tissue.

Intercellular regions of membrane apposition may have junctional features that occlude the intercellular space. The term 'tight junction' has been applied loosely to a broad range of such intimate contacts between plasma membranes, although the term was originally introduced to designate the *zonula occludens* (Farquhar and Palade, 1963). Generally, tight junctions join cells at their apical edges by a continuous belt of fusion with the adjoining plasma membranes. So-called tight epithelia can support large osmotic and ionic gradients and maintain large transepithelial potentials by limiting the diffusion of electrolytes rather than of water (Stachelin, 1974; Madara, 1998).

The sealing elements of tight junctions consist, in part, of diverse fibrils within the membranes. In some tissues the junctional permeability appears related to the number of intramembrane fibers (Claude and Goodenough, 1973), but the packing geometry should be important as well. Sometimes the fibrils are not continuous, and leak pathways might be related to interruptions that occur at more or less regular intervals. Ionic selectivity of junctional zones also suggest the chemical composition of molecules within the junctions are important, as well as the pH of the junction environment. Low pH, for example, weakens intercellular junctions and increases permeation of ions through extracellular spaces (Ferreira and Hill, 1982; Marshall, 1985). The permeability properties of cellular junctions also may depend on the osmotic and hydration status of cells which, in skin, will be determined by the total water barrier properties, their location, and their interaction with blood capillaries and the environment at the skin surface. Changes in cellular volume can alter active transport as well as the structure of intercellular spaces (Erlj and Ussing, 1978). Moreover, the osmotic flow of water within epithelia can induce dilation or collapse of the lateral extracellular spaces, depending on the flow direction, and thereby affect diffusion in a manner that is independent of cellular shrinking or swelling. Such changes in the dimensions of lateral spaces can produce asymmetry of water flow, and

diffusion can become rate-limiting as such spaces collapse (Wright et al., 1972).

Tight junctions restrict the movement of both fluids and solutes between cells. While it is recognized that epithelia are selectively permeable, generally more than 90% of transepithelial ionic conductances appear to be localized in the paracellular pathways of leaky epithelia, whereas less than 10% of the total conductance is attributable to the paracellular route of tighter epithelia such as frog skin (Erlj and Martinez-Palomo, 1978). Some investigators believe that tight junctions also are effective in restricting water movement and estimate that hydraulic conductivity of the junctional pathway can account for only 10% of the entire epithelium (Wright et al., 1972). However, the junctional pathway can account for most of the water movement in leaky epithelia (Levitt, 1981).

The relative importance of paracellular vs transcellular pathways to diffusional water movement, and the significance of tight junctions in the overall resistance to water movement across multi-layered integuments, are unclear. There has been extensive use of amphibian skin and endothelial tissues as model systems for the study of membrane permeability, but little is known about the significance of tight junctions in skin from amniote species representing a broad range of requirements and morphologies. Tight junctions have been identified in skin of all classes of vertebrates, although they are considered to be sparse or absent in the skin of mammals (Matoltsy, 1984). Tight junctions joining cellular walls are present in the stratum corneum of adult anurans and possibly play a role in limiting transcutaneous water movement (Farquhar and Palade, 1965). However, these structures do not impede transcellular water flux and appear to be a general feature of anuran integument without variation among amphibian species from mesic and xeric habitats. Insofar as some anurans evaporate water from the skin at rates equivalent to a free water surface (Adolf, 1932; Spotila and Berman, 1976), it seems that tight junctions are not significant components of adaptation of R_s to desiccating environments. Moreover, skin permeability varies greatly among species that all possess these structures.

Thus, in a very broad sense, there is little evidence to suggest that the presence or nature of tight junctions is a significant determinant of the resistance of integument to transepidermal water flux. However, further research will be important to confirm or qualify this generalization. Recent studies implicate claudin-based cellular junctions to be important for barrier function in mammalian epidermis (Furuse et al., 2002). Claudins are integral membrane proteins that mediate cell-to-cell adhesion and are concentrated in the stratum granulosum of mouse skin. Genetic ablation of claudin-1 in mice induces rapid weight loss, increased TEWL, wrinkled skin and neonatal death, presumably attributable to dehydration. The deficient skin exhibits an impaired water barrier, although the organization of the stratum corneum appears to be normal. Genetic studies have demonstrated that absence of cross-linking molecules essential to organization of the cornified cell envelopes also results in a defective phenotype similar to that

of claudin-1 deficiency (Matsuki et al., 1998). These studies draw attention to the possible importance of correct organization of cellular junctions in the stratum granulosum and of the cornified cell envelopes in the stratum corneum for permeability barrier function in mammalian skin. However, further investigations are required to understand the proposed as well as alternative, complex, and possibly indirect roles for claudins that might relate to permeability barrier function (Bazzoni and Dejana, 2002).

Structural and secreted barriers

Structural barriers

During the evolutionary radiation of vertebrates, a variety of factors related to body size, locomotion, endothermy and especially terrestrial life, necessitated structural and protective reinforcement of the skin (Maderson and Alibardi, 2000; Maderson, 2003). On the one hand, terrestrial locomotion by tetrapods required loose connective tissues in the hypodermis and mechanical decoupling of the skin from underlying muscles. Exceptions are found among animals where skin is directly involved in transmission of locomotory forces, so in birds, for example, skin has extensive attachments to the underlying skeleton. On the other hand, fibrous keratins evolved as heterogeneous complexes of proteins containing sulphhydryl (SH) and disulphide (SS) linkages of cystine. These provided variable protection from physical elements in the environment and formed the principal constituents of dead horny cells in the superficial layers of the epidermis and in derived hair or feathers (Figs 1, 2). Importantly, keratins provided mechanically resistant but relatively lightweight organic material in comparison with calcified dermal shields that were present in some earlier ancestral lineages (Alibardi, 2003).

Evolution of a protective epidermis initially involved α -keratin polypeptides, which are present in all living vertebrates and primitively represented in fish by 70 Å tonofilaments. Alpha keratinogenesis in amniotes became expressed as keratohyalin granules in mammals and keratohyalin-like granules in reptiles and birds (Maderson and Alibardi, 2000; Alibardi, 2001; Maderson, 2003). The capacity for epidermal synthesis of keratin appears to be a shared ancestral (plesiomorphic) feature of vertebrates, and the potential for α -protein synthesis is presumably ubiquitous (Maderson, 1972). In vertebrate embryos, α -keratinogenic cells form before β -keratinogenic cells, supporting the idea that α -keratin characterized the primitive condition in the first amniotes of the Carboniferous (Alibardi, 2003). Genes coding for β -keratin became expressed about 150 million years later than those coding for α -keratin and first appeared in the shells and claws of chelonians (Matoltsy and Bereiter-Hahn, 1986). The strengthening of integument with β -protein synthesis is a derived character shared among sauropsids (synapomorphy). The β -type keratin consists of polypeptide chains having a pleated sheet structure that assembles into an inflexible and inextensible conformation. These occur in the epidermis and scales of all reptiles, including scales, claws, beak and feathers

of birds. Within sauropsid lineages, the synthesis of β -keratin is variably expressed with horizontal or vertical alternation of α and β keratin domains (Baden and Maderson, 1970; Maderson and Alibardi, 2000). However, only α -keratin has been expressed in mammals, with physical strength being enhanced by innovations of orientation and addition of other proteins to the filament matrix.

Various conformations of β -keratin polypeptides played a principal role in the evolution of strong armament among reptiles and made possible the aerial locomotion of birds by means of stiff feathers. Wide variation in structure and mechanical properties are provided by association of keratins with fibrous globular proteins and by mineralization that involves calcium, usually bonded to phospholipid within the keratin complex. In both turtles and crocodylians, β -keratin forms thick, mechanically resistant corneous layers and may be mineralized with calcium. Scales in these animals are tough, dry and relatively non-pliable, except in the skin of the neck, limbs, and tail of chelonians where a softer corneous layer contains only α -keratin. Calcified structures are virtually impermeable to water, depending on thickness. At the other extreme, keratins of amphibians may form thin, transparent membranes, generally only 1–2 cell layers thick.

Desquamation of corneous cells results from wear and is a gradual and slow process. However, in the scales of lepidosaurs, deposition of β -keratin alternates with α -keratin to form a specialized, pluristratified ‘shedding complex’ that facilitates ecdysis (Maderson, 1984, 1985; Maderson et al., 1998). In both lepidosaurian reptiles and amphibians, periodic cellular proliferation (renewal phases) alternates with periods of inactivity (resting phases) and allows the skin to follow seasonal periods of rapid body growth or to restore skin functions such as gas exchange.

Issues of thickness

In very general terms, the permeability of a fibrous structure correlates with length of the diffusion pathway, or thickness, and where hard and soft tissues occur on the body surface, the important sites of exchange are at the softer sites. However, thickness is not an absolute predictor of permeability because the dimension is not independent of the tissue composition (Lillywhite and Maderson, 1982). The thickness of a diffusion barrier contributes to overall R_s across a structure, so overall thickness, and especially the number of keratinized cell layers in series, might have importance for water relations of skin. In this context, the location of blood capillaries determines the ‘effective thickness’ of skin, so vascularization of skin is an important related property.

Generally, in the majority of amniotes the outermost cutaneous capillaries occur within the dermal layers, sometimes just beneath the epidermis or at the dermis-epidermis boundary. However, capillaries penetrate the epidermis in certain fishes and amphibians where their presence is associated with cutaneous gas exchange (Mittal and Datta Munshi, 1971; Guimond and Hutchison, 1973; Hutchison et al., 1976; Toledo and Jared, 1993). The skin of

the pelvic region ('seat patch') of anuran amphibians is especially thin and richly vascularized, features regarded as specializations for water uptake from moist surfaces (Toledo and Jared, 1993). Such pelvic integuments also bear specialized structures, 'verrucae hydrophylicae', which are provided with vascular plexa and are sensitive to peptide hormones that influence permeability (Drewes et al., 1977; Hillyard, 1999). There is some evidence that Angiotensin II increases rates of blood flow through the seat patch of anurans (Slivkoff and Warburton, 2001), but observations also demonstrate that mere water exposure of the seat patch in dehydrated toads rapidly induces large increments of blood flow independent from hormonal influence (Viborg and Hillyard, 2005). These specialized features of integument are important in contexts of water gain ('cutaneous drinking'; Hillyard, 1999) involving ventral integument that is normally not exposed to atmospheric conditions. As in other vascular systems, cutaneous blood vessels are no doubt responsive to neuronal as well as endocrine control.

Studies of anurans suggest that overall skin thickness is related to habitat and is important for water balance in some species (for references, see Toledo and Jared, 1993). However, among amphibians as well as vertebrates generally, the thickness of skin *per se* does not constitute the principal barrier to the diffusion of water. Moreover, the keratinized surfaces of amphibian skin are typically no more than a few cell layers thick, and heavily keratinized integument does not appear to be an adaptive response of amphibian integument.

The periodic production of 'cocoon' in certain amphibian taxa is an exception to these statements. These structures impose considerable resistance to water passage (Table 2) and allow burrowing anurans to establish themselves at shallower depths during drought than do species without cocoons (Lee and Mercer, 1967; Ruibal and Hillman, 1981; McClanahan et al., 1983; Withers, 1998). Cocoons consist of multiple superimposed α -cell layers of stratum corneum resulting from multiple shedding of skin layers during periods of dormancy in drying soils. The phenomenon is present in the anuran families of Bufonidae, Hylidae, Leptodactylidae and Ranidae, as well as in a few urodeles. A cocoon may consist of some 40–60 layers of cornified cells with secreted lipids and proteinaceous materials sandwiched between them (McClanahan et al., 1976; Ruibal and Hillman, 1981; Withers, 1995a; Christian and Parry, 1997).

The presence of osteoderms or 'co-ossified' regions in skin where bone tissue occurs in the dermis is potentially significant for regional enhancement of R_s , and this subject has been given limited attention principally in amphibians (Seibert et al., 1974; Ruibal and Shoemaker, 1984; De Andrade and Abe, 1997; Navas et al., 2002; Jared et al., 2005). The integument of co-ossified regions of skin contains collagen fibres with reduced presence of blood vessels and mucous glands. Co-ossified head regions provide mechanical protection from predators and are used to seal off entries to refugia in plants where frogs seclude themselves (Duellmann and Trueb, 1986). Thickened or mineralized structures in the skin of various

terrestrial amniotes no doubt increase regional R_s of the skin (Seibert et al., 1974), but few studies of regional differences in TEWL have quantified such contributions.

Water permeation in keratins

How do the structural features of keratin influence its resistance to water movement? Generally, structural features that alter the free volume (equivalent to pores or channels) should alter the permeation of water molecules accordingly. Resistance to diffusion is affected by the molecular mass of side chains and tends to increase with cross-linking beyond certain critical levels (Lieberman et al., 1972). However, proteins with bulky, globular side chains may inhibit close packing of molecules and thereby diminish the resistance of a complex membrane. Proteins with generally smaller and non-polar amino acids are able to pack more closely together, thereby permitting formation of many van der Waals forces. The stability of cross-linkages is dependent on a large number of intermolecular forces, including covalent, ionic, and hydrogen bonding in addition to van der Waals attractive forces between non-polar amino acid side chains. All of these act to influence the mobility and free volumes of the structure.

Water itself influences the molecular structure of fibrous protein polymers, and the hydrated status of the skin is an important property. Proteins, in general, bind water very strongly at low relative humidities, and the water content increases in proportion to humidity exposure. As water content increases, it enters between protein molecules and thereby increases the mobility of side chains and the distance between them, potentially reducing the degree of crystallinity. Thus, introduction of water molecules into the polymer structure increases the available free volume as well as the mobility of cross-linkage groups. Dehydration reduces permeability not only by reversing these effects but also by promoting non-covalent bondings between protein chains (Vincent and Hillerton, 1979). Consequently, the permeability of dry protein films can be extremely low (Lieberman et al., 1972), presumably enhancing water resistance in comparatively dry tissue.

Recently it was shown that the 'natural' hydration level of human stratum corneum *in vivo* is about 30–50% of dry mass (Caspers et al., 2001). When the stratum corneum is equilibrated over water, its mass increases to 300–400% of dry mass. At low hydration levels the stratum corneum is relatively brittle and fragile, whereas when water levels exceed a value of around 10–15% of dry mass, the tissue softens markedly. Hydration of the stratum corneum enhances the flux of hydrophilic as well as lipophilic substances (Bouwstra et al., 2003a).

Models of cutaneous water movement are subject to uncertainties related to the phase state of water moving through epidermal tissues and the potential effects of hydration and temperature (Scheuplein and Blank, 1971; Mautz, 1982; Marder and Ben-Asher, 1983). Webster et al. (1985) properly note that if liquid water moves into the superficial layers of the skin, water in the vapor phase might diffuse across a shorter

pathway and, hence, reduced resistance. Studies of human skin have indeed demonstrated that gradients of water exist in the stratum corneum (Warner et al., 1988; Bommannan et al., 1990; Caspers et al., 2001; Bouwstra et al., 2003a). Fourier transform infrared spectroscopy has demonstrated that free water content in stratum corneum is greater in central regions relative to superficial and deeper cell layers at moderate levels of hydration (57%–87%, w/w), whereas at higher levels of hydration (300% w/w) water swells corneocytes in a direction perpendicular to the skin surface except for the deepest cell layers adjacent to the viable epidermis (Bouwstra et al., 2003a). While the mechanism excluding free water from the deeper cell layers of stratum corneum is not understood, it is speculated to play a role in preventing dehydration of the viable epidermis. In relatively dry conditions (18%–26% w/w), only bound water is present in the stratum corneum (Bulgin and Vinson, 1967; Hansen and Yellin, 1972; Bouwstra et al., 2003a).

Extracellular matrices and organelles

Certain extracellular matrices and organelles have been hypothesized to reduce water exchange across the integument, notably in amphibians. Calcium salts and a hydrophilous, amorphous ‘ground substance’ are present in the stratum spongiosum of many amphibian species (Elkan, 1968; Moss, 1972). The so-called ground substance of the calcified dermal layer consists of a polysaccharide gel having glycosaminoglycans usually bonded covalently with proteins. The distribution of this layer among species exhibits little correlation with environment or susceptibility to dehydration, although the layer is generally more developed in dorsal than ventral skin. This layer was suggested to function in water conservation (Elkan, 1968), but the position of the layer and its mere hydrophilic property would appear to render it ineffective (Drewes et al., 1977). Other possible functions, such as a reservoir for mineral homeostasis (Moss, 1972), seem more plausible than a significant role related to water permeability.

Pigmented effector cells that impart color and capacity for physiological color change in vertebrates, have been suggested to influence R_s of amphibian skin. Chromatophore layers, mostly involving silvery iridophores, vary seasonally and appear to correlate with reduced rates of TEWL in some species of frogs exposed to dry conditions (Drewes et al., 1977; Withers et al., 1982; Kobelt and Linsenmair, 1986). However, these frogs remain exposed to sunlight during hot, dry conditions, and the iridophores likely function to increase reflectance of radiation (Kobelt and Linsenmair, 1986). Therefore, the role of iridophores as a causal mechanism for increasing resistance to TEWL is not conclusive. Moreover, the iridophore units usually have gaps between them, and they are overlain by living cells, so they cannot provide complete protection from dehydration of epidermis. Both pigments and structural colors in scales of reptiles, avian feathers and mammalian pelage potentially influence TEWL indirectly by modifying the reflectance and thermal properties of skin.

Secretions and secreted barriers

Fluid secretions can be important to the water relations of skin in a variety of vertebrates, particularly amphibians that lack a fibrous or mineralized covering of significant thickness. The skin of amphibians is glandular and produces three principal categories of secretions: mucus, various toxins, and lipids. A variety of toxic substances – largely alkaloids, peptides and amines – are usually secreted only during defensive interactions with other animals and are not considered further here (see Erspamer, 1994). Lipids are discussed in some detail in the next section. Mucous secretions are predominant and copious in many amphibian species, where they may form a virtually constant film overlying the outer skin layers. These secretions are largely water with varying amounts of proteoglycans and glycoproteins (glycoconjugates), various ions, and often lesser quantities of sugars, amino acids and lipids (Dapson, 1970).

Mucus plays multiple roles in the biology of integument and is especially effective in contexts related to lubrication and keeping the skin hydrated and moist (Lillywhite, 1971, 1975; Mittal and Bannerjee, 1980; Whitear, 1986; Lillywhite et al., 1998). While mucus has been conjectured to limit water movement where it is present in the extracellular spaces of the stratum corneum (Toledo and Jared, 1993), there is no demonstration that this is actually the case. Similarly, externally adhering mucus has been conjectured to reduce evaporative water loss from the skin surfaces (Wygoda, 1988) and to seal small gaps between the body and limbs of frogs (Geise and Linsenmair, 1986; Kobelt and Linsenmair, 1986). In these studies the composition of ‘mucus’ was not determined, so the lipid content is not known. Although mucus is hygroscopic, biophysical studies have shown it is not particularly impermeable, even when air-dried (Machin, 1966, 1972). Further, it was shown by direct measurements *in vivo* that intact mucus covering over the skin of *Litoria dahlia* provided no extra resistance to TEWL (Young et al., 2005). While quantitative comparisons are not available, it seems strikingly clear that any resistance to transepidermal water loss attributable to mucus is miniscule compared with that for lipid secretions (see below).

In terrestrial amphibians, secretion of mucus transfers water to evaporating surfaces that are sculptured to retain the fluid (Lillywhite, 1971). In bullfrogs and several other species of anurans, the rate of mucus secretion is regulated to keep up with increasing evaporative demands related to heliothermy, and thus water evaporates from a regularly replenished mucous film rather than the underlying epidermis (Lillywhite, 1971; Lillywhite and Licht, 1975; Kaul and Shoemaker, 1989; Lillywhite et al., 1998). If frogs are deprived of the external mucus film, the skin dries and is unable to maintain normal levels of evaporative water loss (Lillywhite, 1975). In contrast, anurans without a mucus film (e.g. bufonids) either avoid dehydrating conditions or, in the case of some heliothermic toads, rely on water that is transferred from moist substrates to wet the skin *via* capillary forces that ‘wick’ the liquid across a highly sculptured integument (Lillywhite and Licht, 1974).

Whether the external film overlying the epidermis is mucus or water, the aqueous evaporating film must be replenished by water that is either stored within the animal or is absorbed from a moist substrate. Thus, a mucus film cannot be utilized as a strategy for maintaining a hydrated skin during exposure without water in xeric habitats or during long-term drought.

The importance of mucous secretions in protecting exposed epidermal surfaces from dehydration suggests a fundamental dichotomy of skin organization and water balance (Lillywhite and Maderson, 1988). With few exceptions, either the epidermis is protected from excessive water loss by a lipid and/or fibrous diffusion barrier (some amphibians, reptiles, birds, mammals) (Fig. 2), or the epidermis is covered with a wet film so that evaporation occurs from secreted fluids rather than the epidermis itself (many amphibians). If the epidermis or whole skin dries excessively, its viability diminishes as the dehydrating cell layers compress and stiffen (Lillywhite, 1975). Superficial water films associated with sculptured skin surfaces (channels that act like 'blotting paper') appear to have an important role in protecting skin from dehydration in certain snakes (Lillywhite and SanMartino, 1993) and elephants (Lillywhite and Stein, 1987) as well as amphibians (Lillywhite and Licht, 1974).

Lipids and the evolution of cutaneous permeability barriers

The role of lipids in controlling transepidermal water loss is well established and has evolved numerous times during tetrapod evolution. Moreover, lipids are employed almost universally as a means of waterproofing the skin or body surfaces of terrestrial organisms, including plants, arthropods, some amphibians, reptiles, birds and mammals (Hadley, 1989, 1991). Thus, conferral of resistance to TEWL is fundamentally a matter of molecular rather than mechanical organization (biochemistry *vs* morphology), although these two attributes are intimately interconnected. Indeed, the efficacy of a lipid barrier depends in many cases on structural features that enhance or maintain its integrity and function (Table 1; Figs 1, 2). Understanding the significance and variation in permeability barriers requires first an appreciation for key features related to functional organization and structure of vertebrate integument.

The structural features of vertebrate integument are uniquely different from all other major phyla of animals. Clearly, mechanical strength is enhanced by a layered keratin structure, and the β keratins provide superior strength and rigidity. Importantly, the evolution in basal amniotes of a stratified squamous stratum corneum produced a tough yet flexible epidermis across the body surfaces (Maderson and Alibardi, 2000). Such a stratified corneum conferred an additional important advantage, which is a convenient means to preserve the structural integrity of laminated lipids that might function to impede the transcutaneous efflux of essential body water (Figs 2, 3). The corneous layers provide a framework and sufficiently rigid support for stability of lipid structure without undue movement or mechanical disruption (see below).

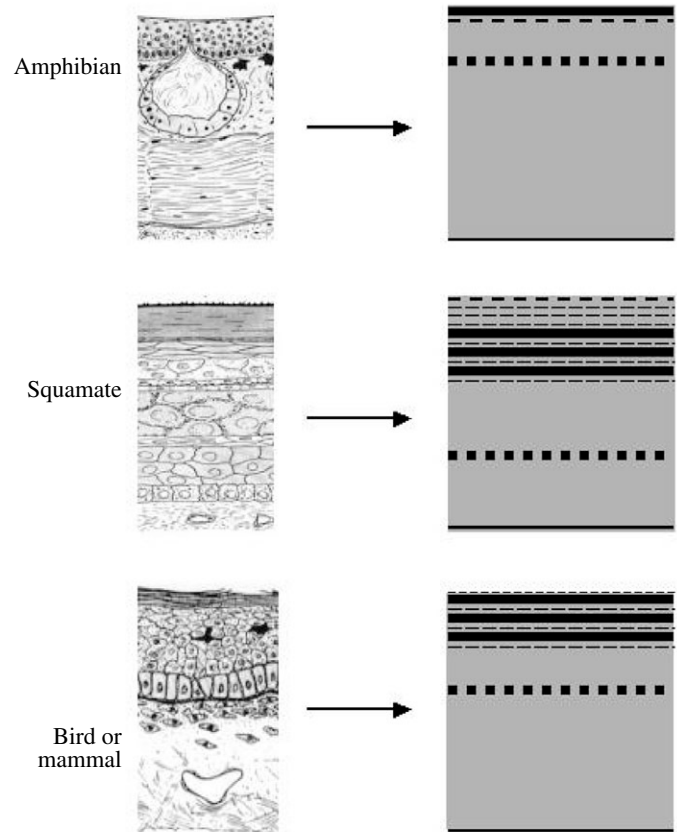


Fig. 2. Schematic illustrations of the water permeability barrier in relation to the stratum corneum of the epidermis in four principal lineages of vertebrates. The images on the left depict the morphology of generalized integument, and drawings to the right illustrate the stylized arrangement of barrier lipids (dark bars) in relation to horny layers of stratum corneum (thin broken lines). The stratum germinativum is represented by the thick broken lines. The features shown are not to scale.

Permeability barriers always contain a complex mixture of lipid molecules. Longer chain-length hydrocarbons tend to comprise a dominant category of lipids in most barriers examined. These tend to melt at higher temperatures and resist water permeation, whereas shorter chain-length molecules reduce the intensity of van der Waals interactions between hydrocarbon molecules and create a more fluid and permeable structure. Relative saturation of hydrocarbons also contributes to a tighter water barrier, whereas unsaturation and methyl branching tend to introduce kinks in molecules and disrupt packing. However, chain length alone is not necessarily a reliable indicator of water permeation because in some systems chain elongation and unsaturation offset each other (Gibbs et al., 1998). Hydrocarbons and wax esters are relatively non-polar, which assists in repelling water. Polar phospholipids and other classes of lipids having intermediate polarity, in addition to branching, might be important in structuring the geometry of a water barrier and providing a degree or specific orientation of fluidity important with respect to potential mechanical distortion or disruption of the barrier structure (Bouwstra et al.,

2000, 2003b). These and other properties of lipids might also play roles that are important in barrier assembly, prevention of degradation by enzymes or microorganisms, thermal properties relevant to thermoregulation, and interactions with non-lipid molecules.

Normal stratum corneum, as studied in mice and humans, demonstrates a markedly acidic pH, ranging from 4.5 to 5.5 at the surface to physiologic pH (≈ 7.4) in hydrated, viable epidermis (Ohman and Vahlquist, 1994; Turner et al., 1998; Fluhr and Elias, 2002). The acidic pH is attributable to influence of key metabolites including urocanic acid, free fatty acids, and a non-energy dependent sodium-proton exchanger (Chuong et al., 2002). The acidic pH appears to regulate several key defensive functions of skin, including antimicrobial and permeability barrier properties, as well as integrity and cohesion of the stratum corneum. The stability of molecular structures is influenced importantly by pH, which also alters the water content of fibrous structures because bonding between chains are replaced by bonding between protein and water (Hackman, 1984). With respect to the permeability barrier, at least two key lipid processing enzymes affecting the barrier lipids within the interstices of stratum corneum are activated at low pH optima and are retarded by exposure to neutral buffers (Uchida et al., 2002; Schmuth et al., 2000). Moreover, the acidic pH also is important for direct influence of lipid-lipid interactions in the lamellar bilayers of the permeability barrier (Bouwstra et al., 1999). Hence, stratum corneum neutralization reduces competence of the permeability barrier lipids (Mauro et al., 1998; Hachem et al., 2003).

Lipid barriers of amphibians

The Amphibia present features, both in evolutionary history and specializations of extant forms, which are transitional between aquatic and terrestrial environments. Numerous species exhibit complex life histories with aquatic larval stages prior to acquisition of terrestrial adult characters, and the adults of extant taxa possess skin that is generally devoid of thick, rigid or heavily keratinized protective armor. Amphibians have retained integument that, in terrestrial as well as aquatic adults, is relatively thin and pliant – lacking extensive development of keratin and retaining permeability properties that allow significant gas exchange across the cutaneous surfaces. While only a small sample of some 4200 amphibian species have been examined in detail, it seems clear that many species of amphibians possess skin of characteristically high permeability and experience relatively high rates of TEWL.

Comparative investigations indicate that arboreal species of frogs tend to have generally higher R_s than do non-arboreal species (Table 2; see Withers et al., 1984; Wygoda, 1984; Young et al., 2005). Selection for increased R_s in arboreal habitats is no doubt related to exposure of animals to increased levels of radiation, temperature and convection relative to ground-dwelling, fossorial or amphibious relatives (e.g., McClanahan and Shoemaker, 1987). Various mechanisms have been suggested to play a role as determinants of R_s in

amphibians, and these have been reviewed previously in attempt to evaluate the relative contributions of characters to the overall R_s (Toledo and Jared, 1993; Lillywhite and Mittal, 1999). As in other tetrapods, lipids appear to be of central importance to comparatively high R_s that has been reported for selected species (Table 2).

The role of cutaneous lipids in water economy is clear in some species, while problematic or elusive in others. Lipids confer exceptionally high resistance in certain tree frogs, which secrete lipids from cutaneous glands to form an external film that is subsequently wiped with complex and stereotyped movements involving all four limbs (Shoemaker et al., 1972). However, lipids that are located within the skin may or may not correlate with R_s (Withers et al., 1984; Toledo and Jared, 1993). Also, lipid mixtures that are produced by general extractions of whole skin undoubtedly include elements of membrane lipids not associated with a water barrier as well as precursor molecules that might be converted to other components if a barrier is present. Lipids that are structured within the dermis are purported to reduce TEWL (Amey and Grigg, 1995), but these cannot act to prevent dehydration of the overlying epidermis. Thus, the more effective lipid barriers among amphibians are those that create a barrier to TEWL at the skin surfaces (Table 1; Fig. 2).

Elaborate wiping of lipids on body surfaces was first described in phyllomedusine frogs, which exhibit very low rates of TEWL (Blaylock et al., 1976). The behavior is associated with a suite of behavioral and physiological characters, including selection of a permanent perch where frogs remain inactive during the day, secretion of lipids from cutaneous glands, wiping behavior, and the assumption of a condition resembling torpor. The secreted lipids are produced in specialized cutaneous glands and are a mixture comprising mostly wax esters, triglycerides, free fatty acids, hydrocarbons and cholesterol (McClanahan et al., 1978). Wax esters are dominant and average about 46 carbons in length. The externally wiped layer is estimated to be about 0.2 μm and 50–100 molecules thick, which is comparable to the epicuticular wax layers of various arthropods. This lipid barrier results in a R_s that is much greater than that demonstrated in typically amphibious anurans (Table 2) and enables frogs to remain exposed to sunlight in hot, dry weather where body temperatures approaching 40°C are tolerated (Shoemaker et al., 1987). The conservation of body water achieved by the high R_s of the waxy layer is complemented by excretion of uric acid, which also conserves water potentially involved in the excretion of nitrogenous wastes (Shoemaker et al., 1972; Shoemaker and Bickler, 1979).

Wiping behavior and secretion of lipids from cutaneous glands have been reported in several species of anurans having variable R_s (generally from about one to several 100 s cm^{-1} ; Table 2). It seems likely that wiping behavior is more widespread among anurans than formerly supposed, and that components or precursors to the full behavior might have evolved in a number of different contexts (Blaylock et al., 1976; Lillywhite et al., 1997a; Barbeau and Lillywhite, 2005).

Wiping behaviors observed in a rhacophorid tree frog, *Polypedates maculatus*, stimulate secretion of lipids from cutaneous mucous glands, which are activated by a tactile neurogenic reflex (Lillywhite et al., 1997a). The lipids are secreted with mucus, and these frogs have a relatively modest R_s (Lillywhite et al., 1997a,b). Both wiping complexity and R_s are variable among six species of hylid tree frogs in Florida (Barbeau and Lillywhite, 2005). The lipids appear to be produced in granular rather than mucous glands and, like *Polypedates*, these frogs are not very waterproof. In all the examples of anurans investigated, elaborate wiping of secreted lipids is associated with arboreal species living in arid, semiarid, or ephemerally arid environments where there is potential for dehydration stress.

The water barrier in amphibian skin is very different from that of amniotes for two fundamental reasons. First, lamellar granules have not been definitively identified in any amphibian, although structures bearing resemblance to these organelles ('small dense granules' and 'tightly packed parallel lamellae') were reportedly seen in the stratum spinosum of *Hyla arborea* (Bani et al., 1985). These structures were possibly misinterpreted, as lamellar granules appear to be uniquely associated with keratinizing epithelia and are not generally recognized in the epidermis of amphibians or fish (Wertz, 1996, 2000; Alibardi, 2003). Secondly, the corneous layers of amphibian epidermis are too sparse to provide an effectively structured lipid-keratin complex, and lipid structures with complex lamellation have not been identified in association with interstices in the thin stratum corneum of any amphibian species. Hence, lipid layers comprising part of

the tela subcutanea have been described in several species of arboreal frogs, but these lipids underlie blood vessels and the frogs are not particularly resistant to cutaneous water loss (Wygoda et al., 1987). Similarly, a lipid band has been demonstrated histochemically in the uppermost aspect of the stratum spongiosum of the arboreal frog *Litoria fallax* (Amey and Grigg, 1995). These lipids appear to be derived from congealed lipid droplets, but there is no reported evidence for structured lamination or mechanical support associated with adjacent cell layers or other epidermal structures. This lipid layer possibly contributes to reduction of evaporative water losses in this species, but the reported rates of TEWL are not exceptionally low.

The limited keratinization of the epidermis of modern amphibians imposes a critical constraint, due possibly to developmental canalization related to aquatic life stages (Lillywhite and Mittal, 1999). Although keratin was presumably present in basal amphibians (Findlay, 1968; Maderson, 1972), neither extensive keratinization nor synthesis of β -type keratins characterizes the skin of modern amphibian lineages. The capacity for extensive synthesis of keratin is present in connection with a few specialized structures such as the cornified protrusions (spade) used in digging by fossorial anurans, so the generalized condition of the skin may be more related to a lack of genetic expression rather than gene deficiencies.

Amphibians thus differ from amniotes insofar as layered complexes of lipid and keratin are absent (Table 1; Figs 1,2). One possible exception might be the laminated structure characteristic of 'cocoon' that occur in various species during dormancy of estivation (Fig. 3A). In the Australian frog *Cyclorana aaustralis*, the composition of both skin secretions and cocoon material was shown to consist of 5–10% neutral lipids and 78–85% proteinaceous material (Christian and Parry, 1997). The lipid fraction of these materials was chemically similar to the secreted lipids that provide an external water barrier in

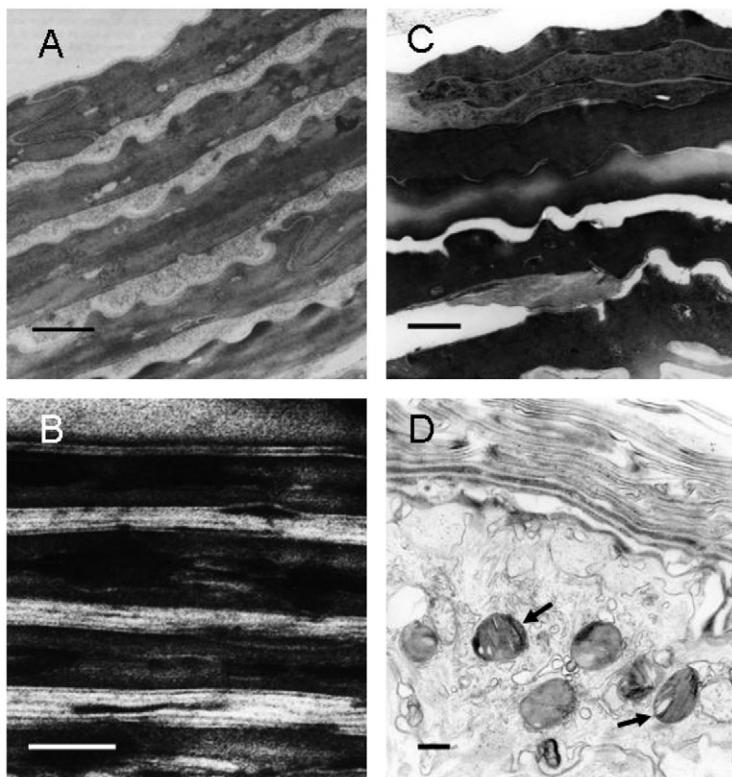


Fig. 3. Electron micrographs showing details of stratum corneum and permeability barrier of terrestrial vertebrates. (A) Section through a portion of cocoon of a burrowing hylid frog, *Pternohyla fodiens*. The layers of squamous epidermal cells are separated by granular extracellular materials in the subcorneal spaces. Scale bar, 500 nm. Reproduced with permission (Ruibal and Hillman, 1981). (B) Section through mesos layer of snake epidermis (*Natrix natrix*), which is the recognized permeability barrier of squamates. Laminated lipids occur between the darker bands of keratin layers. Scale bar, 100 nm. Courtesy of Lukas Landmann. (C) Section through stratum corneum of human skin. Lipids (unstained) occur between the distinct layers of keratin. Scale bar, 200 nm. Courtesy of Gopi Menon. (D) Section through epidermis of a canary, showing nucleated layers as well as stratum corneum (top). Lipids occur between the distinct layers of keratin toward top of figure. Note the multigranular bodies (source of lipids; arrows). Scale bar, 200 nm. Courtesy of Gopi Menon.

phyllomedusine frogs (McClanahan et al., 1978), but it is not clear whether these were extracted from the cell layers of the cocoon or were derived from the interspersed 'mucus.' Further investigations are required to examine the possibility that lipids are secreted in mucus (as in *Polypedates maculatus*: Lillywhite et al., 1997a,b) to provide a layered lipid-keratin complex in amphibian cocoons.

In the case of both secreted cocoons and the extraepidermal lipid layers that are secreted and wiped by arboreal frogs such as *Phyllomedusa* or *Polypedates*, subsequent function depends on immobility of the animal for otherwise the structural integrity of the lipid barrier is disturbed. Thus, amphibians produce cocoons when they are in soil and in a state of torpor, while wiping of lipids by tree frogs occurs characteristically prior to resting in a water-conserving posture while either in seclusion or on perches. In other systems, lipid water barriers occur either on external surfaces of rigid structures (as in plants, feathers and arthropod cuticle) or are contained in layered arrays within corneous tissues (as in reptiles, mammals and birds). With reference to the integument of amniotes, the corneous structure that contains barrier lipids is either comparatively rigid (scales) or somewhat supple (mammalian skin) while resilient to creasing. Importantly, the numerous layers of specialized corneocytes associated with the water barrier region of the stratum corneum of amniotes provide a tough and resilient framework for the intercellular lamellar lipids. On the other hand, the more delicate, pliant nature of amphibian integument and its limited keratinization appear to have constrained the possible evolutionary pathways for waterproofing mechanisms in amphibians (Lillywhite and Mittal, 1999). Due to the limitation of keratinization, in particular, external lipid barriers seem to provide the more effective and practical potential means of waterproofing in amphibians (Fig. 2).

Studies of 'waterproof' frogs have demonstrated that reduction of skin permeability by means of lipids to prevent dehydration also reduces cutaneous gas exchange, which normally contributes importantly to respiration in amphibians (Feder and Burggren, 1985). Thus, under these circumstances of periodic waterproofing, frogs rely increasingly on pulmonary ventilation to maintain arterial blood pH and levels of respiratory gases (Stinner and Shoemaker, 1987). Increased use of the lung also is characteristic in circumstances such as increased temperature or activity when the metabolic demand for oxygen exceeds the passive uptake across the skin (Hutchison et al., 1968). *A priori*, there seems no compelling reason why amphibians could not have evolved more effective lungs and used them more dominantly in gas exchange, similar to reptiles. However, this has not been a directional trend in amphibian evolution, and some have argued that reliance on cutaneous gas exchange is the explanation for retention of a generally permeable integument with minimal keratinization in the stratum corneum. Thus, a permeable and pliant integument might be characteristic of modern amphibians either because these attributes are important for aquatic larval stages, and the integument of metamorphosed adults is genetically

constrained, or the conditions are maintained by natural selection in the adult as well as larval stages of development. Either of these possibilities, of course, does not exclude the other (Lillywhite and Mittal, 1999).

Lipids and the evolution of cutaneous water barriers in terrestrial amniotes

The sources of lipids contributing to the epidermal water barrier of amniotes are lamellar bodies, which are discreet lipid-enriched secretory organelles first observed by Selby (1957) and later described in detail by Odland (1960) and Elias and Friend (1975). These and similar structures are present in amniote epidermis generally and are known synonymously as lamellar bodies (Elias and Friend, 1975), Odland bodies (Odland, 1960), keratinosomes (Wilgram, 1965), cementsomes (Hashimoto, 1971), membrane-coating granules (Matoltsy, 1966), lamellar granules (Breathnach et al., 1973), multigranular bodies (Matoltsy, 1969) or mesos granules (Roth and Jones, 1970; Landmann, 1979). These Golgi-derived organelles originate within the epidermal keratinocytes, and their synthesis is upregulated as the cells progressively differentiate. The lipid contents of these organelles appear to consist of stacks of flattened lipid vesicles (Landmann, 1986, 1988) comprising primarily glycosphingolipids, free sterols and phospholipids, which are precursors of the stratum corneum lipids (Fig. 4). Eventually, the lipid contents of the organelles are secreted into the extracellular domain, where they are further processed into compact lipid bilayers that occlude the extracellular spaces among adjacent and overlapping corneocytes (Fig. 4), a condition that has been likened to a 'bricks-and-mortar' organization (Elias, 1983; Elias and Menon, 1991). It has been proposed that acylglucosylceramides serve as molecular 'rivets' to promote flattening and stacking of lipid vesicles that subsequently fuse edge-to-edge to produce lamellae comprising paired bilayers that are stacked parallel to the skin surface (Engström et al., 2000; Wertz, 2000). These form multiple lamellar sheets with smooth surfaces shown in freeze-fracture studies. In this manner, the extracellular lipids form a continuous domain throughout the stratum corneum and function as the principal barrier to water diffusion (Elias and Friend, 1975; Fig. 2). The lamellar bodies also deliver a cocktail of lipolytic enzymes to the extracellular domain, and these convert the polar lipid precursors into nonpolar products that assemble into lamellar structures surrounding the corneocytes (Freinkel and Traczyk, 1985; Madison et al., 1998; Bouwstra et al., 2003b). Bound lipids that are chemically linked to the corneocyte envelopes are arranged in a crystalline packing and act as a template during formation of the extracellular lamellae (Bouwstra et al., 2003a).

In mammals and lepidosaurian reptiles, the lipid contents of lamellar granules are secreted and exocytosed into the extracellular spaces where they become visible as distinctive lamellae (Landmann, 1980; Elias, 1983; Elias and Menon, 1991; Hou et al., 1991; Menon et al., 1994). In most

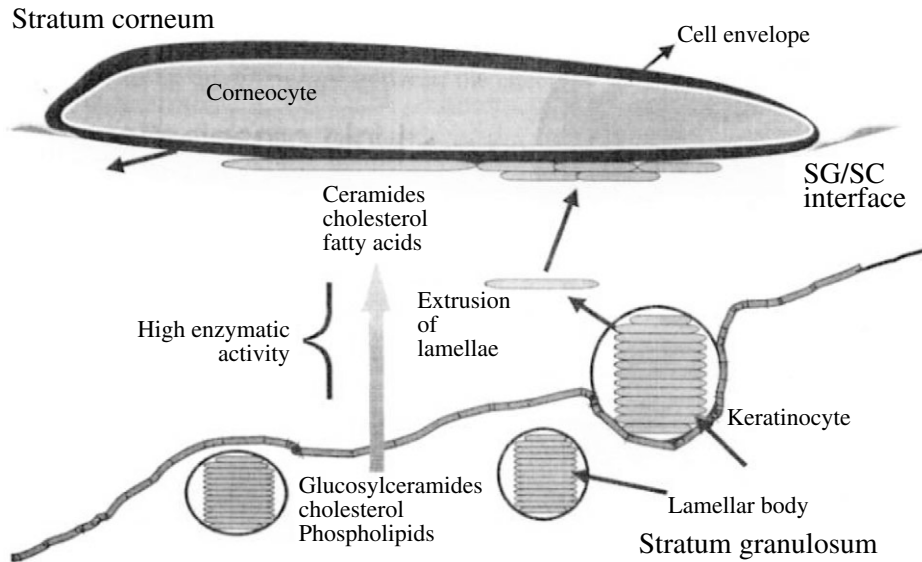


Fig. 4. Schematic illustration of the process involved in formation of intercellular stratum corneum lipids of a mammal following extrusion from lamellar bodies. The lipid content of lamellar bodies is altered in composition and rearranged into long lipid lamellae that fill the extracellular regions in the stratum corneum. Reproduced from Bouwstra et al. (2003b), with permission.

lepidosaurs lamellar granules are limited to the mesos layer of stratum corneum, but they are also present in the α -cells of *Sphenodon* (Maderson et al., 1998; Alibardi, 2003; Alibardi and Maderson, 2003a) and certain snakes (Tu et al., 2002). With reference to squamate integument, lamellar granules are secreted from differentiating α -cells and become organized into lamellar sheets filling the extracellular spaces of a specialized layer known as the mesos layer, which is subjacent to the β -keratin of the stratum corneum. The mesos layer consists of multiple layers of alternating keratin and lamellar lipids, forming a laminated structure sandwiched between the β - and α -keratin of the mature stratum corneum (Figs 1, 2, 3B) (Lillywhite and Maderson, 1982). The lipid sheets of the mesos layer prevent the outward flux of tracer molecules such as lanthanum and comprise the recognized water barrier in squamate reptiles (Landmann et al., 1981; Lillywhite and Maderson, 1982). The lipids concentrated in the reptilian mesos layer, as inferred from studies of shed epidermis, appear to be a complex mixture having a composition resembling that of the mammalian stratum corneum (consisting mostly of cholesterol, free fatty acids, phospholipids and ceramides: Roberts and Lillywhite, 1980, 1983; Burken et al., 1985; Itoh et al., 1990; Elias and Menon, 1991; Weldon, 1996; Ball, 2004). The biochemistry and modulation of these lipids are not well studied in reptiles, but the quantity of lipids in epidermis from a broad range of species correlates generally with the aridity of habitat (Roberts and Lillywhite, 1983).

In avian integument, the lipid contents of epidermis are derived from comparatively large lamellar granules (multigranular bodies; Fig. 3), indeed 3–5 times larger than those of mammals, in addition to large lipid droplets that resemble sebum or oil (Menon et al., 1986). The organelles break down to form large intracellular lipid droplets during terminal differentiation into corneocytes. These fuse with the nascent lipid droplets, and the resulting contents are passed through membrane pores into the extracellular domains of the

stratum corneum (Menon et al., 1991, 1986). However, in the absence of water stress these secreted lipids fail to form bilayered structures, and some of the lamellar bodies are retained within the corneocytes where they remain visible as lamellar sheets when stained with ruthenium tetroxide (Menon and Menon, 2000). Thus, among amniotes there are pronounced differences as well as similarities in epidermal lipogenesis and the structural organization of water barrier lipids within the stratum corneum (Table 1; Figs 2, 3). Of course, the details of lipid composition and organization vary among species, environment and location on the body, albeit such variation has been explored in a very limited number of species.

Critical molar ratios among stratum corneum lipids are crucial for permeability barrier formation and function, most importantly cholesterol, ceramides and free fatty acids. A defective water barrier results if the synthesis of either of these lipids is inhibited, or if the enzymatic processing of the lamellar body derived discs is disturbed (reviewed in Menon and Ghadially, 1997). Ceramides account for as much as 50% of the total stratum corneum lipids in mammals (Raith and Neubert, 2000), and desert birds also have higher proportions of stratum corneum ceramides than free fatty acids (Haugen et al., 2003a,b). Adjustments of lipid ratios to favor ceramides over free fatty acids and sterols have also been shown to correlate with reductions of TEWL in desert larks (Haugen et al., 2003a,b). The comparatively higher ratios of ceramides in stratum corneum allow the lipid lamellae of the permeability barrier to exist in a more highly ordered crystalline phase, which creates a tighter barrier to water vapor diffusion (Velkova and Lafleur, 2002; Bouwstra et al., 2003b). Unlike terrestrial mammals, the stratum corneum of marine mammals retains appreciable amounts of glycolipids, and the bilayers are less tight (Elias et al., 1987). The water relations of skin in marine mammals are in need of further study.

Investigations of mammalian stratum corneum suggest that resistance to water permeation is related to tight, gel-like

packing of hydrocarbon chains and changes in lipid phase behavior related to component ratios and molecular arrangement of cholesterol (Potts and Francoeur, 1990; McIntosh, 2003). It also appears that fluid and crystalline phases of sublattices alternate vertically in repetition with stacked lamellae (Bouwstra et al., 2000). The presence and localization of fluid domains facilitates deformation of the lamellae, which evidently satisfies demands for elasticity as lamellae follow sharp edges of cell boundaries. Models further suggest that water diffusion is limited in directions both perpendicular and parallel to the plane of the lipid bilayers (McIntosh, 2003). Comparable molecular and biophysical details related to barrier function are not yet available for non-mammalian vertebrates. However, it seems clear that evolutionary or phenotypic adjustments in barrier efficacy will be founded in alterations of lipid composition, deposition and organization (Williams and Elias, 1987; Critchley, 1993; Menon and Ghadially, 1997).

The reptilian water barrier is fundamentally similar to that of mammals, but may show more taxonomic variation in addition to differences in morphology and renewal. The majority of studies of the water barrier in mammalian stratum corneum have employed skin from humans, neonatal rodents or hairless mice. Most reptilian studies have employed lepidosaurs, and little is known about the comparatively weaker water barrier of turtles and crocodylians. Overall, however, there is broader ecological and taxonomic coverage of reptiles than of mammals.

In addition to the stratum corneum barrier, glandular lipids are deposited exteriorly to the epidermis in both mammals and birds (Hadley, 1991). Sebaceous glands, unique to mammals, produce an oily secretion called sebum that moves outward along hair shafts to distribute generally non-polar lipids rich in triacylglycerols on epidermal or hair surfaces. In birds, 'preen waxes' from the uropygial gland are spread over feathers to prevent water penetration and ingress of bacteria and fungi. Uropygial secretions contain a complex mixture of lipids in which wax esters usually predominate. Glandular waterproofing lipids are also conjectured to pass onto scale surfaces in certain species of snakes (*Malpolon* spp., *Psammophis* spp.), and self-wiping movements by means of the head and chin perhaps function similarly to body-wiping behaviors described for various arboreal frogs (Branch, 1998; Dunson et al., 1978).

In birds and mammals, plumage and pelage appear to impede significantly the passage of water vapor from skin to atmosphere, although the skin remains the principal barrier to TEWL (Cena and Clark, 1979; Webster et al., 1985). In pigeons, for example, plumage contributes 5–20% of total resistance to water loss through the integument, and the plumage and boundary layer together account for 6–26% of total resistance to water vapor diffusion (Webster et al., 1985). Therefore, adjustments of plumage or pelage and seasonal shedding patterns are potential means of adjusting rates of TEWL.

Plasticity of the water barrier in vertebrate integument

Insofar as the more resistant barriers to TEWL among amphibians appear to be either cocoons or extraepidermal lipid sheets formed by wiping, as discussed above (see also Lillywhite and Mittal, 1999), effective waterproofing is clearly facultative among amphibians. Notwithstanding, there remains the enigma of certain frogs (*Chiromantis* and *Hyperolius*) that have been reported to have low skin resistance, and the mechanism has not yet been resolved (e.g. Drewes et al., 1977; Withers et al., 1982; Kobelt and Linsenmair, 1986). However, we have observed wiping behaviors in *Chiromantis petersi* akin to that of *Phyllomedusa* and *Polypedates* in my laboratory, and both extraepidermal lipids and wiping behaviors have been observed in six species of Florida tree frogs having moderate resistance to TEWL (Barbeau and Lillywhite, 2005). Wiping behaviors are context-dependent, and many persons familiar with various wiping frog species have not witnessed the behavior even though they might have observed the animals closely in other contexts (H.B.L., personal observation). Thus, I venture to predict that wiping, and by inference secretion of cutaneous lipids, will be found to occur in a number of anuran species inhabiting semi- or ephemerally arid environments. The stimulus for renewal of the lipid barrier is not known, whether osmotic and related to skin or body dehydration, or to sensory perception of low ambient humidity.

Aside from wiping, some studies have suggested that R_s in some amphibians varies seasonally (Table 2). While mean values of R_s in various anuran species are consistently higher during the dry season compared with wet season, these are not significantly different from wet season values (Young et al., 2005). Clearly, the possibility of R_s acclimatization beckons further investigation.

Water barrier function in amniotes appears to be relatively fixed and characteristic of species, and the heritability of TEWL has been demonstrated in mammals (Furuyama and Ohara, 1993). However, the capacity of animals to adjust R_s when environmental conditions change is also an important aspect of adaptation, including phenotypic plasticity, acclimatization and developmental plasticity. Although studies on the relative importance of 'plastic' responses and genetic variation of skin permeability properties are few, it seems clear that both plasticity and genetic adaptation can account for covariation between TEWL and environment (Eynan and Dmi'el, 1993; Dmi'el et al., 1997; Tieleman and Williams, 2002).

In reptiles, the barrier can be rapidly restored following trauma (Maderson et al., 1978), and some species have been shown to exhibit plasticity for enhancing R_s under conditions of water stress (Kobayashi et al., 1983; Maderson, 1984; Kattan and Lillywhite, 1989). Following injury to skin, hyperplasia of α -keratin ensues to form a wound epithelium, which, as in mammals (Odland and Ross, 1968), rapidly establishes a barrier to water loss in the disturbed tissue (Maderson, 1985; Alibardi and Maderson, 2003b). Experimental tape-stripping of squamate epidermis results in

α -layer hyperplasia, and in this type of barrier repair, no mesos layers are formed until the next cycle of ecdysis (Maderson et al., 1978). Further ultrastructural investigations of the α layers during the repair response might reveal whether newly formed α cells are secreting lamellar lipids to reseal the barrier-defective areas in advance of a pan-body renewal of epidermis and ecdysis that are required to form the mesos layer.

In the lizard *Anolis carolinensis*, barrier effectiveness is upregulated in response to dry environments, regardless of whether animals are allowed to dehydrate and regardless of whether animals undergo ecdysis (Kattan and Lillywhite, 1989). The changes in water permeability have been attributed to changes in skin lipids, likely in response to local stimuli acting at the integument. Recently it was shown that R_s increases considerably following the first postnatal ecdysis in California king snakes *Lampropeltis getula*, and this can be interpreted as an adaptive adjustment to the transition from the aqueous environment of the embryo to the aerial environment of the newborn (Tu et al., 2002). In the studies of neonatal king snakes, there was found a striking correlation relating a doubling of R_s with the first postnatal ecdysis to a doubling of the thickness and lipid histochemistry of the mesos barrier (Tu et al., 2002). While compositional features of these lipids are not yet known in detail, upregulation of the barrier during early postnatal ontogeny might be principally or entirely a quantitative phenomenon (Fig. 5). Others have also suggested that the principal determinant of barrier efficacy is the lamellar organization of exocytosed lipids in the extracellular domain, independent of the lipid molecular composition (Friberg et al., 1990; Menon et al., 1996).

A priori it would seem that permeability of mammalian and avian integument can be rapidly adjusted to the demands of the environment by virtue of more-or-less continuous exfoliation and renewal of corneal cells. In contrast, episodic synchronized sloughing of the entire epidermis in lepidosaurian reptiles involves periodic renewal of corneal cells interrupted by varying periods of stasis when the epidermis is in a resting stage. Insofar as the mesos barrier layer is completed prior to ecdysis and is underlain by keratinizing α -cells, shedding would seem to be a requirement for altering the efficacy of the

water barrier. Indeed, data for postnatal ecdysis of California king snakes in relation to upregulation of R_s suggest this is the case (Tu et al., 2002).

Few data are available for such responses to radically changing environments, and little is known about the processes underlying permeability barrier ontogenesis in vertebrates. The skin of full-term human and rodent newborns possesses a competent water barrier at birth, with rates of TEWL at least as low as in adults (Williams et al., 1998). Barrier formation begins during late gestation and involves a progressive increase in the thickness of skin layers, formation of a multilayered stratum corneum, secretion of lipid lamellar bodies in the interstices of stratum corneum, and transformation of short lamellar disks into compact, continuous, lamellar units (Aszterbaum et al., 1992). The keratinization and barrier formation in skin coincide with changes in the composition of amniotic fluid, and are thought to be essential for protection from amniotic fluid during late gestation (Hardman et al., 1999; Parmley and Seeds, 1970). Moreover, contact of rat fetal skin with air accelerates barrier formation (Williams et al., 1998). Similar changes occur in snake skin, but at a relatively later time in development. In the context of air exposure, it is of interest that newborn snakes undergo ecdysis within a short period (generally a few days) following birth or hatching (e.g. Greene et al., 2002; Tu et al., 2002).

In snakes, it appears the periderm and embryonic epidermis slough within the egg (Alibardi, 2002), and a permeability barrier (beta and mesos layers) of partial competence is formed within the epidermis prior to hatching (Maderson, 1985). In timing this crudely resembles the barrier formation in mammals (Williams et al., 1998). However, R_s increases at both the first and second postnatal ecdysis, indicating a continued capacity for improvement of barrier function, as shown in lizards. Unlike lizards, improvement of R_s appears to be incremented in association with ecdysis. Measurements indicate a threefold improvement of R_s over two postnatal shedding cycles (Tu et al., 2002).

Recent studies of skin from hatchling snakes include observations on lipid inclusions within the α -layer of epidermis (Tu et al., 2002). These inclusions show lamellar as well as electron-lucent morphologies within the mature α -layers, closely resembling what has been described for avian stratum corneum (Menon and Menon, 2000). In the

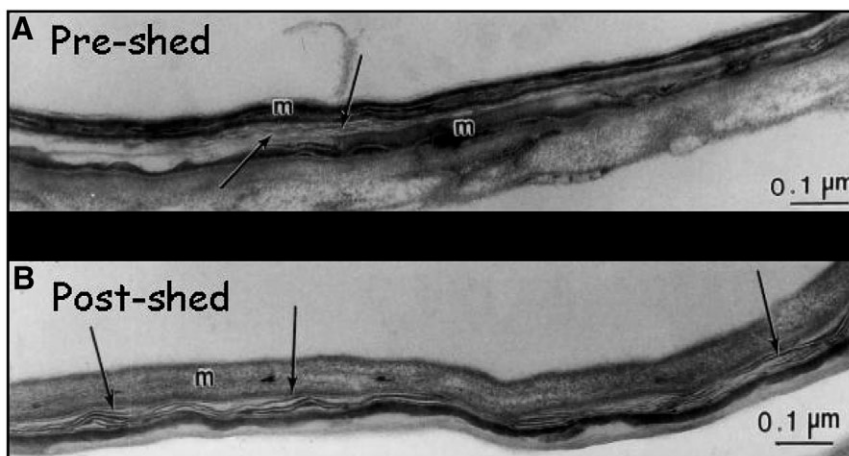


Fig. 5. Electron micrograph showing ultrastructure of epidermis in hatchling king snakes *Lampropeltis getula*, sampled on the day of hatching (A) and 2 days after the first ecdysis (B). The photos feature the mesos layer (m), which pre-shed has disorganized bilayer structures (arrows) but well-organized and continuous bilayers in the extracellular domains of post-shed skin (arrows). The number of bilayers is doubled in the post-shed skin relative to pre-shed. After Tu et al. (2002).

immature α -cells of pre-shed skin, multilamellar bodies and different stages of 'dissolution' of lamellar inclusions into electron-lucent lipids are dominant features, again very similar to what is seen in avian transitional cell layers. These observations point to the intriguing possibility that α -cells themselves might be involved in barrier homeostasis, which has not previously been suggested for ophidian epidermis. The facultative waterproofing ability of avian epidermis (Menon et al., 1996) resides in its capacity to modulate the type of lipids secreted, i.e. non-bilayer, electron-lucent lipids under basal conditions, but lamellar lipid structures under xeric stress, leading to significantly decreased TEWL. Whether snakes and other lepidosaurs can modulate R_s by secreting lamellar lipids from the α layer (previously named cholesterol clefts by Jackson and Sharawy, 1978) has not yet been evaluated, but clearly such a possibility might represent a reserve barrier mechanism. Such a mechanism could explain the upregulation of R_s in dry-acclimated *Anolis* lizards (Kattan and Lillywhite, 1989) and perhaps the variability of R_s observed among hatchling snakes (Tu et al., 2002). There is a trend toward increasing R_s for periods of weeks following hatching in king snakes, although again the largest and statistically significant increments are associated with skin shedding.

The ontogeny of barrier effectiveness demonstrated in snakes differs also from that of altricial species of birds endemic to arid environments. Nestlings of zebra finches *Taenyopygia guttata* have a remarkably tight water barrier that progressively decreases in efficacy as they fledge, evidently allowing evaporative cooling for thermoregulation (Menon and Menon, 2000). However, under conditions of water deficit, adult zebra finches appear to be capable of rapid facultative waterproofing. Within 16 h of water deprivation, TEWL measured in four adult zebra finches decreased by 50%, and the skin barrier efficacy continued to improve until mammal-like values were achieved (Menon et al., 1996). Similarly, TEWL decreased significantly in emus subjected to high ambient temperatures when they were first deprived of water (Maloney and Dawson, 1998). Studies of hoopoe larks, which are permanent residents in extreme arid regions of the Arabian Peninsula, demonstrated rates of TEWL about 30% lower than larks from mesic environments, and these rates decreased significantly when the birds were acclimated to high temperatures (Tieleman and Williams, 2002). However, acclimation of water loss rates was not observed in skylarks and woodlarks from mesic environments in Europe, nor in Dunn's larks from the Arabian Desert. Opposite changes in R_s occur in response to short-term thermal acclimation in other species (i.e. lower R_s in heat-acclimated birds; McKechnie and Wolf, 2004). Thus further investigations are needed to understand patterns of TEWL in response to environmental changes as well as confirm the nature of plasticity mechanisms.

Studies have convincingly demonstrated that TEWL in various vertebrates is closely related to temperature of the skin and generally increases with increasing ambient temperature (e.g. Grice et al., 1971; Mautz, 1982; Marder and Ben-Asher, 1983; Webster et al., 1985; Buttemer and Thomas, 2003). It is

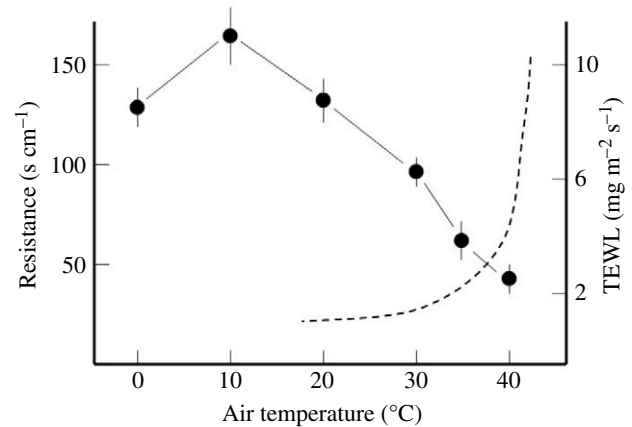


Fig. 6. Whole body resistance to water vapor diffusion (solid circles) and cutaneous evaporation rates (broken line) as a function of ambient temperature in pigeons, *Columba livia*. Data are re-plotted from Webster et al., 1985.

apparent from these and other studies that cutaneous evaporation increases with increased air temperature as a result of both higher skin temperature (Campbell, 1977) and decreased resistance (Fig. 6) to the passive diffusion of water vapor through cutaneous tissues (Webster et al., 1985). Patterns of blood flow changes are no doubt responsible, in part, for these changes in water vapor diffusion through skin. Furthermore, increased hydrostatic pressure in the cutaneous microvasculature of heat-acclimated rock doves *Columba livia* results from adrenergic control of arterial and venous blood flows and elevates water outflow from capillaries leading to elevated TEWL (Ophir et al., 2002). Blood-flow related adjustments in epidermal water supply presumably occur over shorter time scales than do structural changes in cutaneous lipids, and may be responsible for rapid adjustments in TEWL such as those reported for mourning doves by Hoffman and Walsberg (1999). In non-sweating endotherms such as birds, the temperature dependence of changes in R_s and TEWL suggests adaptation for increasing evaporative water loss via the skin during heat stress (McKechnie and Wolf, 2004). Studies of model mixtures of stratum corneum lipids demonstrate their stability as crystalline domains at temperatures below 40°C, whereas transitions to a liquid ordered phase occur at higher temperatures (Velkova and Lafleur, 2002). Therefore, adjustments of R_s over a non-stressful, physiological range of temperature are probably not due to phase changes of lipids (see also McClanahan et al., 1978).

Conclusion

There has been much detailed research concerning various physical and chemical attributes of vertebrate integument, including speculations regarding plausible effects on permeability. While virtually all features of integument can influence water passage to some degree, judgments about

effective barrier properties must consider the geometry and properties of blood capillaries as well as the multiple functions of skin in the species considered. The overall picture of water barriers in a broad range of vertebrate taxa shows a central role for lipids in determining R_s . Clearly, however, assessment of water relations of integument must include appreciation of the integrative features of multiple structures and how water relations of the skin relate to water relations of the whole organism. In this context, it is important to appreciate that effective barriers must protect the skin organ itself from desiccation, as well as serving a role in prevention of water loss from the whole body. Thus, barrier properties that appear most effective in terrestrial taxa involve features associated with epidermis, and usually its outermost aspect, where keratin provides a structural framework for laminated lipids.

Comparatively little is known regarding adaptive adjustments of permeability barriers in the contexts of evolution, phylogeny and environment (Lillywhite, 2004). In most cases we do not understand the environmental signals that activate genetic or phenotypically plastic mechanisms. For example, do facultative adjustments in barrier function involve direct sensory perception of ambient humidity, or do mechanisms depend instead on secondary effects such as internal signals related to dehydration and osmotic changes? Such questions could drive new directions for research following exploratory investigations such as those of Kattan and Lillywhite (1989). Future improvements in understanding the mechanisms and adaptive significance of permeability barriers will benefit from new questions as well as comparative and experimental approaches that include multidisciplinary contributions from genetics, morphology, physiology, ecology and evolutionary biology.

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