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Ultrastructure of Bat (*Pipistrellus kuhlii*) Epidermis with Emphasis on Terminal Differentiation of Corneocytes

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Abstract: The ultrastructure of the skin of air-adapted mammals (bats) is not known. The study at the electron microscope of the skin of the back and the flying membrane of *Pipistrellus kuhlii* showed that the thickness of the epidermis is very low (10 – 12 μm), and that 1 – 2 flat spinosus cells are present beneath the stratum corneum which is formed by very thin corneocytes that resemble those of avian apteric epidermis. The stratum granulosum is discontinuous and few small (less than 0.3 μm large) keratohyalin granules are present. The epidermis is reduced to one flat basal layer in contact with the stratum corneum in many areas of the flying membrane. Transitional corneocytes are almost absent suggesting that the process of cornification is very rapid. In the basement membrane numerous hemidesmosomes are present and form attachment points for the dense dermis underneath. Numerous collagen fibrils directly contact with the hemidesmosomes and the dense lamella of the basement membrane. Sparse elastic fibrils allow the stretching of the epidermis during flight and the rapid folding of the epidermis after flying without damaging the epidermis. Like in avian epidermis, the production of lipids is high in bat keratinocytes, and multilamellar bodies discharge lipids extra- and intra-cellularly. This may compensate the lack of a thick fat layer in the dermis of the flying membrane as lipids may help in thermical insulation against the cooling air currents flowing on the bat skin during flight. Fur hairs are very thin (4 – 7 μm), and they have an elaborated cuticle made of pointed expansions similar in texture with that of the cortex. Cuticle cells form hook-like grasping points that allow to keep hairs stuck together. In this way the pelage remains compact in order to maintain body temperature.

Key words: Bat; Skin; Epidermis; Hairs; Cornification; Ultrastructure

白边油蝠表皮角质化细胞末端分化的超微结构

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摘要: 蝙蝠是一种唯一能够飞行的哺乳动物, 其皮肤的超微结构尚未见报道。在电镜下观察了白边油蝠(*Pipistrellus kuhlii*)背部和翼膜皮肤的超微结构。表皮的厚度较低(10 ~ 12 μm), 角质层下有 1 ~ 2 层的刺细胞, 该刺细胞由相似于鸟类无羽表皮的纤细角化细胞形成。颗粒层不连续且仅有少量小型透明角质颗粒(< 0.3 μm)。在翼膜的若干区域, 表皮简化为一层与角质层相连的基底层。过渡期的角化细胞几乎不存在, 提示其角质化过程非常迅速。基底膜上的无数半桥粒在真皮下面形成密集的附着点。大量胶原纤维直接维系在半桥粒和基底膜的致密层上, 稀疏的弹性纤维使得蝙蝠表皮在飞行时易于伸展、在飞行后易于迅速折叠而不会受到损伤。与鸟类的表皮相似, 蝙蝠角化细胞富有大量的脂质。由于脂质有助于蝙蝠皮肤在飞行中与冷空气流的传热绝缘, 大量脂质的存在可能是为补偿蝙蝠翼膜的真皮缺乏厚的脂肪层。研究还表明, 毛发较薄(4 ~ 7 μm), 并具有与皮层相似的突状物组成的精细表皮, 其表皮细胞形成钩状抓握点使毛发紧紧粘结在一起, 通过这种方式毛皮保持紧凑以恒定体温。

关键词: 蝙蝠; 皮肤; 表皮; 毛发; 角质化; 超微结构
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The origin of the mammalian integument from that of reptilian ancestors (synapsids) required numerous modifications in both the dermis and epidermis (Spearman, 1964, 1966; Findlay, 1970; Maderson, 1972, 2003). The epidermis of basic amniotes of the Carboniferous was either scaled or unscaled, but that only a form of soft keratinization (alpha-keratin) was present while beta-keratin was produced in derived reptiles and birds (Maderson & Alibardi, 2000).

In comparison to the dry and hard epidermis of extant reptiles, the epidermis of mammals is soft, elastic and moisturized, characterized that have been associated to the fine action of mammalian musculature to the skin while the mechanical protection has mostly been taken over by the pelage, together with its role for thermal insulation (Spearman, 1966; Findlay, 1970; Maderson, 1972). The softness of the mammalian epidermis may be in relation to the evolution of a granular layer that produce a soft stratum corneum (orthokeratotic) from a more primitive agranular or even parakeratotic (nucleated or anucleated without keratohyalin) epidermis of the reptilian progenitors (Spearman, 1964, 1966). Parakeratosis in mammalian epidermis has been considered as a reversion to a more primitive form of cornification, possibly present in the first cotylosaurian reptiles from which therapsids and later true mammals derived.

Although numerous morphological, physiological and molecular information are available on the mammalian epidermis, they mainly derived from studies on a limited number of species such as human and mouse (Matoltsy, 1986; Menon et al, 1986; Elias et al, 1987; O'Guin et al, 1987; Fuchs, 1990; Resing & Dale, 1991; Pfeiffer & Jones, 1993; Rawlings et al, 1994; Pfeiffer & Rowntree, 1996; Ishida-Yamamoto et al, 2000; Kalinin et al, 2002).

An extensive survey on the skin of most domestic and many wild mammals showed some histological variation of the epidermis, in relation to the adaptations to different environment (Sokolov, 1982). The study showed that the stratum granulosum was apparently absent (marsupial, pholidota, dermoptera, cetacea, sirenia, arctiodactyla, proboscidea, and microchiroptera), discontinuous (insectivores, carnivores, xenarts) or present with different degrees (lagomorphs, primates, rodents, pinnipeda, and megachiroptera). Among the different skin adaptations (terrestrial, marine, freshwater, borrowing, arboreal and flying) the reduction or even the disappearance of the stratum granulosum have been reported in the epidermis of bats.

The lack of a stratum granulosum may be due to a parakeratotic form of cornification or to the rapid cornification of keratinocytes that does not allow the accumulation of a sufficient amount of profilaggrin to form large keratohyalin granules and a microscopically visible stratum granulosum. Keratohyalin has a brief existence and is unevenly distributed along the epidermis. The fact that the skin of chiroptera is soft and pliable suggests that no parakeratosis is involved but instead suggests that the epidermis of these flying mammals has a rapid turnover (Iversen et al, 1974).

Despite some histological descriptions on bat skin are available (Quay, 1970; Sokolov, 1982), no ultrastructural study has precisely shown the structure of bat epidermis. The main morpho-physiological requirement for the epidermis of bats would be the resistance to exposure to air fluxes that tend to cool down the body temperature and desquamate the epidermal surface. Whether these external factors may have conditioned a unique morphological adaptation of the epidermis is unknown. The evolution of flying mammals may have had a deep impact on skin specializations, producing morphological convergence with the skin of birds (Menon & Menon, 2000). In the latter a large amount of lipids are produced intracellularly and extracellularly under environmental requirements (dry, cool, etc., Menon et al, 1996; Peltonen et al, 1998, 2000). Avian keratinocyets (termed sebokeratinocyets for the production of lipids together small quantity of keratin) are very thin and a stratum granulosum is absent (Alibardi, 2004).

In order to verify possible morphological convergence between the skin of flying vertebrates, the present study presents for the first time the ultrastructure of bat epidermis and hairs.

1 Materials and Methods

Small pieces of the skin (2 mm × 3 mm or larger pieces) were collected from the flying membrane (glabrous), and back (densely hairy) of two individuals of the bat *Pipistrellus kuhlii*, Natterer 1819. The tissues were immediately fixed for about 8 hours in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4, postfixed for 90 min with 2% osmium tetroxide, rinsed in distilled water for about 10 min, immersed in 2% uranyl acetate for 1 h, dehydrated in ethanol, infiltrated in propylene oxide for 1 h, and embedded in Epon resin at 60 ℃ for one day.

Sections of 1 – 3 μm in thickness were obtained using an ultramicrotome (LKB-Nova). For the light mi-

croscopic study, sections were stained with 0.5% toludine blue. From regions of interest of the skin, thin sections (40 – 80 nm thickness) were collected on copper grids, routine stained with uranyl acetate and lead citrate, and observed under a CM-100 Philips electron microscopy operating at 80 kV.

2 Results

The flying membrane (thickness 0.5 – 0.8 mm) presented an irregular, papillate surface, with a flat, toluidinophilic flat epidermis (less then 10 mm thickness) covered by a thick toluidinophobic stratum corneum with a similar thickness to the living, precorneous part (Fig. 1 A). The dense dermis followed the epidermal folds and few fibroblasts were seen among the fibrous extracellular matrix (Fig. 1 B). Among the dense collagen fibrils, the ultrastructural study showed that sparse elastic fibrils were present among the prevalent collagen bundles (Fig. 1 C). These elastic fibrils were mainly composed by electron-dense amorphous component while the microfibrillar component was scarce (Fig. 1 C, inset).

The ultrastructural examination of the epidermis of the back showed long tonofilaments bundles in basal and 1 – 2 suprabasal (spinosus) cells, connected with the granular layer where few keratohyalin granules were visible (Fig. 1 C). Numerous hemidesmosomes were directly connecting the lamella densa of the basement membrane to the numerous collagen fibrils (Figs. 1 C, D). The epidermis of the flying membrane is even thinner than the hairy epidermis of the back, and often only one flat basal cell is present underneath the thick stratum corneum (Fig. 1 E).

The stratum corneum was made of very thin (0.02 – 0.3 μm) and electron-dense corneocytes (more than 10 layers), and only in few areas of the epidermis, transitional cells were present (Fig. 2 A). Small keratohyalin granules were present in some areas but a stratum granulosum was not evenly developed beneath the stratum corneum (Fig. 2 B). Some maturing corneocytes presented in large areas occupied by electron-pale material, in some occasion represented by loose keratin filaments but in other cases contained electron-pale lipids or vesicular bodies (Fig. 2 C). Lamellate bodies were occasionally seen in the upper spinosus and transitional layer but non-lamellar dense bodies were very common in keratinocytes. Vesicles contacting corneous cells or discharging lipid material into the extracellular space among corneocytes were commonly observed (Fig. 2 A, C). Cornified cell membrane of 15 – 20 nm in thickness was present in mature corneocyets (Figs. 1 E, 2 A – C). Mature corneocytes showed the typical alpha-keratin pattern, made by 10 – 12 nm electron-pale filaments (Fig. 2 D).

The hairy epidermis of the back and neck was also very waved and showed a thick stratum corneum (Fig. 3 A). The numerous hairs covering the epidermis were very thin, with a diameter thinner than 8 – 10 μm, and had a developed ring-shaped oblique cuticle (Fig. 3 B). The ultrastructural analysis of hairs gave a value for the hair fiber of 3 – 4 μm, which reached a diameter of 6 – 8 μm including the sectioned spiny-like cuticle (Fig. 3 C).

Most of hairs in our samples were in catagen or telogen (collection done in September), but the outer root sheath showed the typical cuticle roots directly attached to the outer root sheath at the base of the hair bulb (Fig. 3 D). In other hairs, electron-dense remnants of the inner root sheath were seen near the cuticle (Fig. 3 E).

At maturity, cuticle cells were merged to the hair fiber (cortex) and only a thin line separated the two components until they completely merged at the base of the cuticle (Fig. 3 C, F). The compaction of the corneous material in both cuticle and cortex showed a similar alternating pattern of small dense areas within a majority of paler areas.

3 Discussion

The epidermis of bats appears very thin and strong connected with the numerous collagen fibrils to the dense dermis. Elastic fibrils, as reported abundantly in previous histological descriptions of the dermis (Iversen et al, 1974; Sokolov, 1982), are sparsely found among the largely prevalent collagenous network using the electron microscope. They appear to be mostly constituted by the amorphous component while microfibrils are scarce. A more detailed study on bat elastin was not the purpose of the present observations, but would deserve a further analysis.

The strong dermal epidermal connectivity (numerous collagen fibrils directly contacting the basal membrane) is especially seen in the epidermis of the flying membrane. The epidermis results tightly attached to the dermis, a histological detail that may be in relation to the high resistance that this membrane must have for sustaining flight and for folding the membrane in resting position. The dense lamella is directly linked to collagen fibrils, an arrangement seen in other skins where a strong mechanical connection and folds is present in the

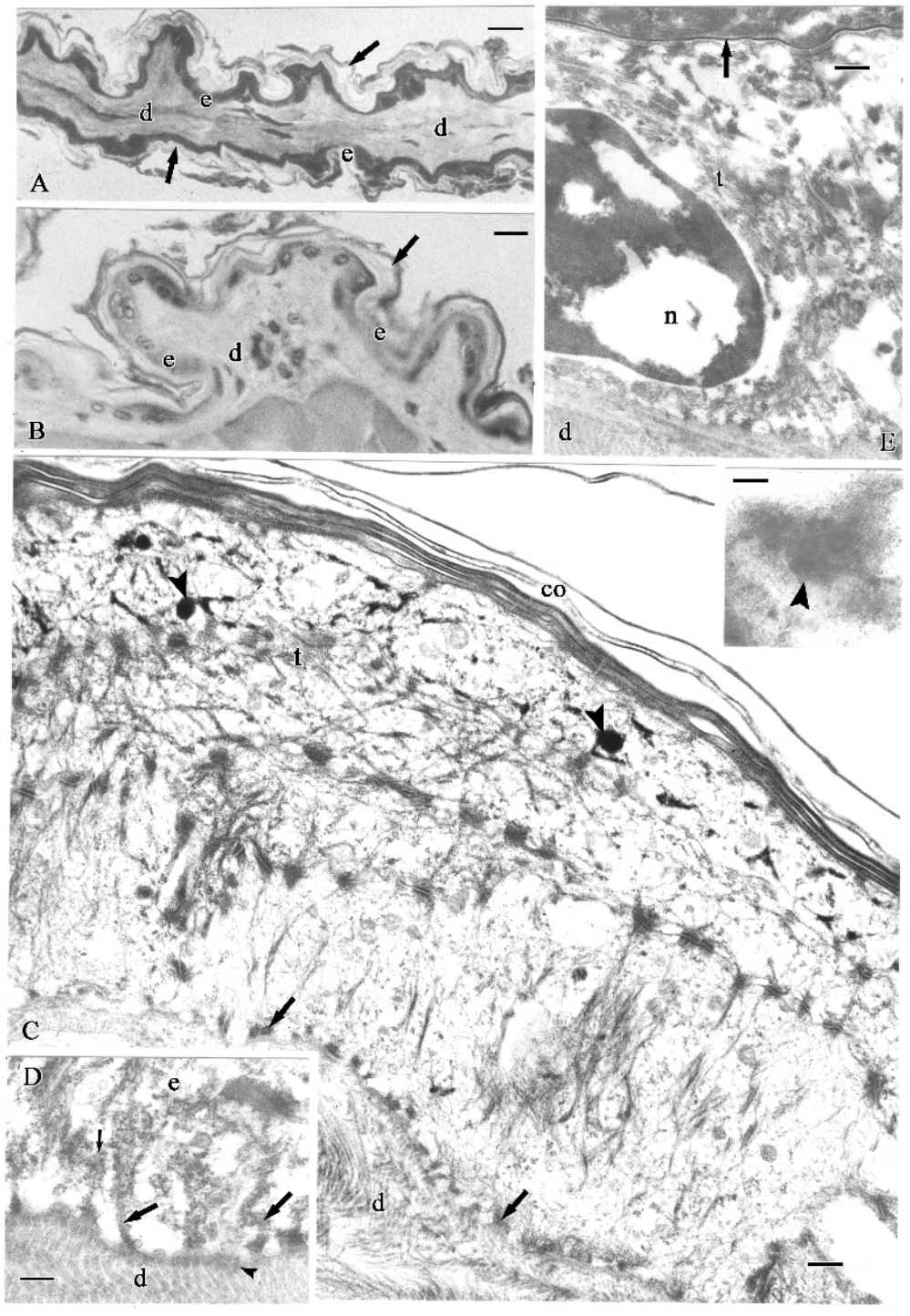

Fig. 1

A, Cross section of flying membrane. The arrow indicates the corneous layer of the undulated epidermis (e). d, dermis. Bar, 10 mm.

B, Detail of folded and flat epidermis (e) and dermis (d) of flying membrane. A thick stratum corneous (arrow) covers the living epidermis. Bar, 10 mm.

C, Ultrastructural general view of epidermis of flying membrane covered with thin corneocytes (co). Arrowheads point to sparse keratohyalin granules. The tonofilaments netwoks (t), in basal cells converge in hemidesmosomes (arrows) linked to the richly-collagenous dermis. Bar, 1 mm.

D, Detail of tonofilaments (arrows) of epidermal basal cells (e) joining to hemidesmosomes in contact with the basal membrane and the numerous collagen fibrils (arrowhead) of the dermis (d). Bar, 250 nm.

E, Detail of single layered epidermis (n, nucleus of the epidermal cell) of a region of the flying membrane. t, tonofilaments. d, dermis. The arrow points to the cornified cell membrane of the first corneocyte. Bar, 500 nm.

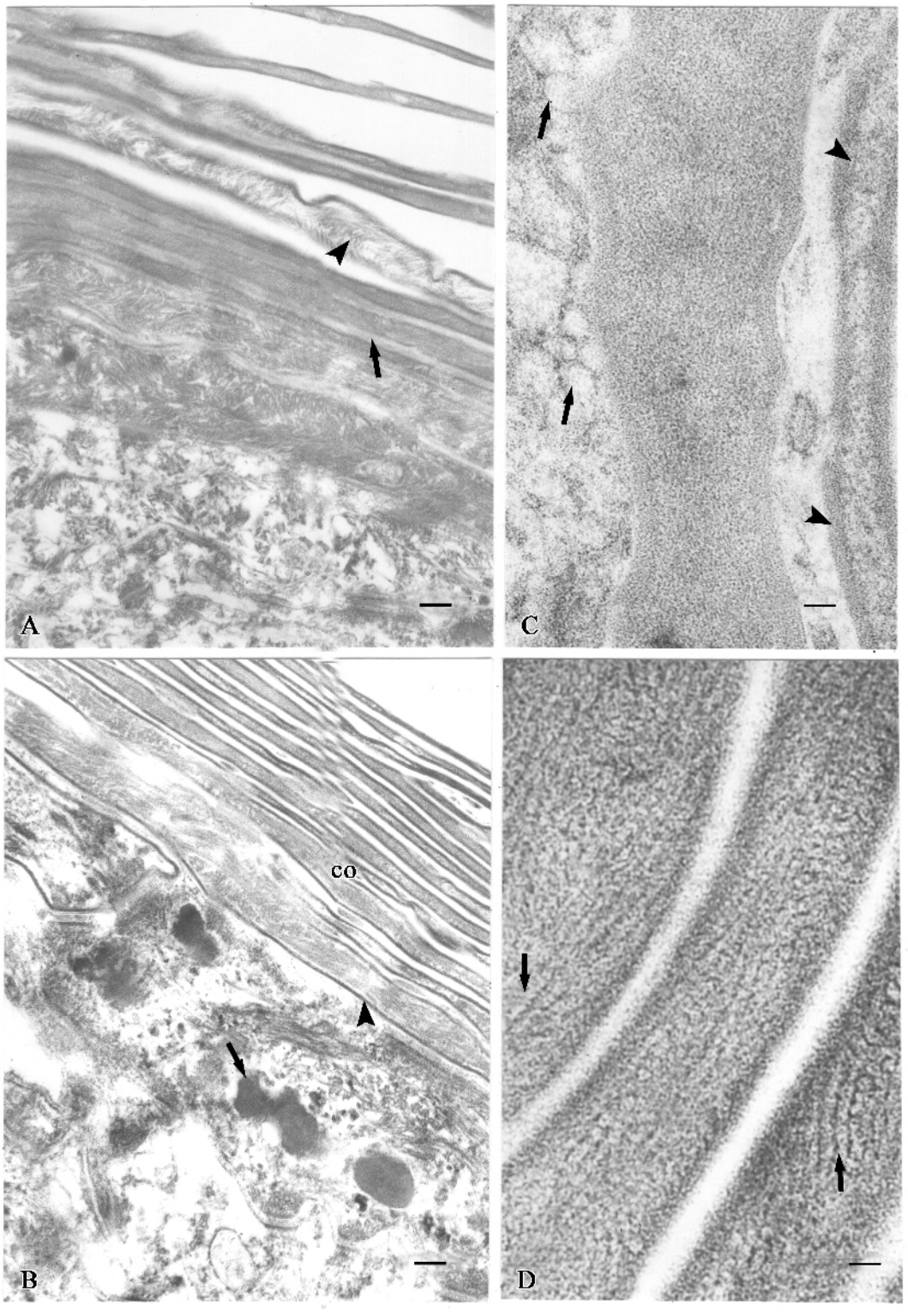

Fig. 2

A, Pre-corneous layer with no keratohyalin granules visible in contact with dense or pale corneocytes (arrowhead). The extracellular space among the lowermost corneocytes (arrow) is occupied by some lipid-like material. Bar, 500 nm.

B, Detail of precorneous cells with keratohyalin granules (arrow) and corneocytes with evident corneous cell envelope (arrowhead). Bar, 250 nm.

C, Detail of lipid-like vesicles (arrows) contacting (or discharging material extracellularly) the first corneocyte. Arrowheads indicate the thickened corneous cell envelope. Bar, 100 nm.

D, High magnification of the keratin pattern (arrows) of corneocytes. Bar, 50 nm.

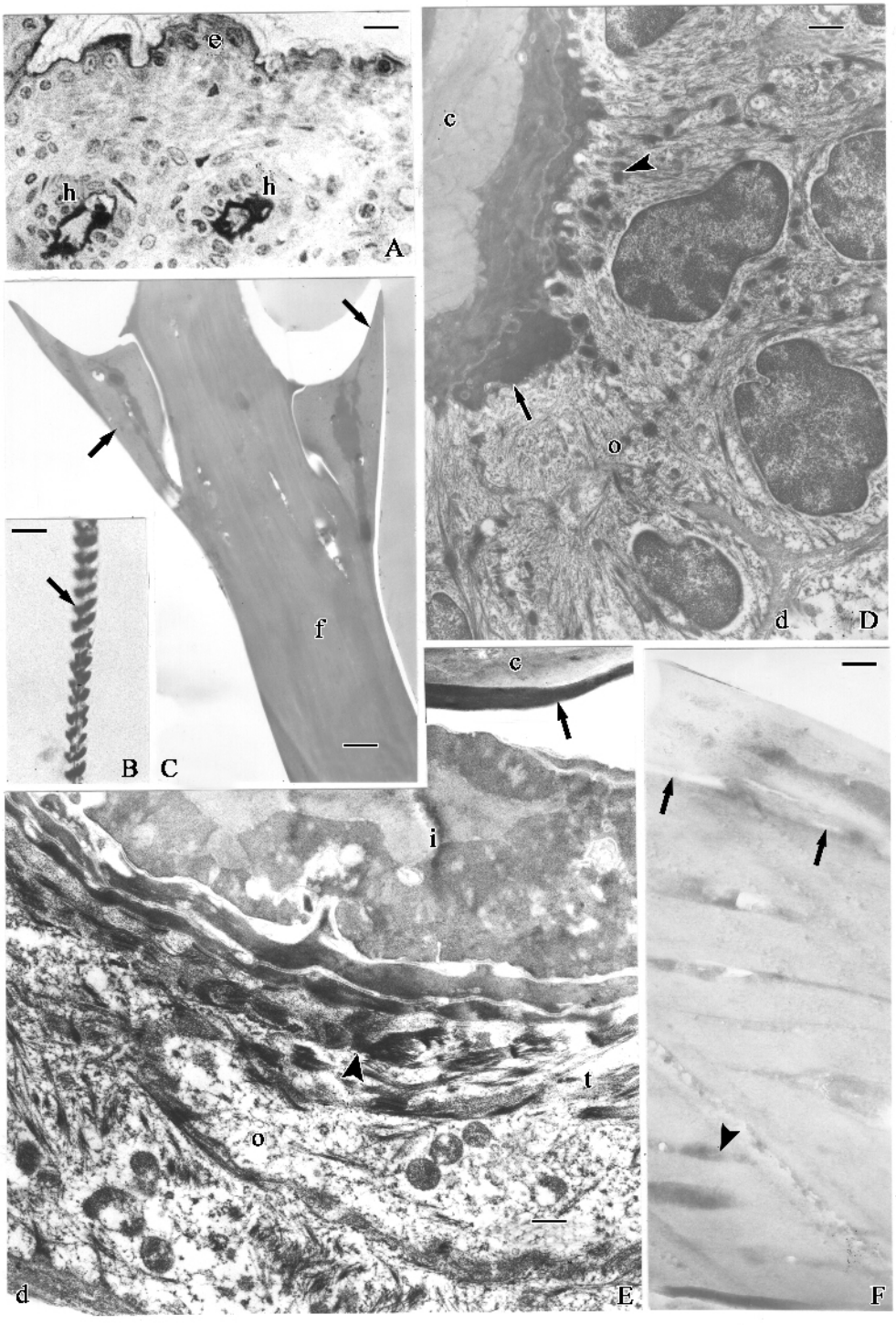

Fig. 3

A, Epidermis of hairy dorsal epidermis featuring a waved epidermis (e) and two sections of hairs (h). Bar, 10 mm.

B, Aspect of hair fiber showing the obliquely-oriented scaled cuticular pattern (arrow). Bar, 10 mm.

C, Ultrastructural detail of pointed sectioned cuticle cells (arrows) lateral to the hair fiber (f). Bar, 2 mm.

D, Extensive keratin tonofilaments network (arrowhead) of portion of cross-sectioned outer root sheath (o) of telogen hair (evidenced by the presence of arrowed cuticle roots). c, cortex. d, dermal (glassy) membrane. Bar, 2 mm.

E, Part of cross-sectioned outer sheath (o) hair with tonofilaments aggregation in the companion layer (arrowhead). A residual cornified cell of the inner root sheath (i) is present. The arrow points to the dense cuticle surrounding the cortex (c) of the hair. Bar, 1 mm.

F, Detail of corneous dense (arrowhead) and pale material of the cortex. Arrows indicate the membrane of a cuticle cell with similar texture of cortical corneous material. 500 nm.

epidermis and dermis (eg. turtle tail skin, Alibardi, 1999). The numerous hemidesmosomal connections ensure that the epidermis follows the flexibility, folding, contraction or stretching of the dermis when the flying membrane is folded or opened for flight. In this way the epidermal cells are not damaged during folding and unfolding, and free from the impact of the air on the flying membrane.

The present ultrastructural survey reveals that bat keratinocytes contain the typical organelles of differentiating mammalian epidermis: small keratohyalin granules, numerous lipid and lamellar granules (Matoltsy, 1986; Menon & Norel, 2002). The most peculiar features of the epidermis are the richness in lipid material, the scarcity of keratohyalin and the thinness of corneocytes (0.02 – 0.1 μm). These characteristics recall those of sebokeratinocyets of bird epidermis (Menon & Menon, 2000).

Lamellar bodies, resembling those of the other mammals (Matoltsy, 1986; Elias et al, 1987; Rawlings et al, 1994), have a similar role for the water-loss barrier in all amniotes (Landmann, 1980). However, without a specific counterstaining (eg rutenium tetroxide, Menon et al, 1986, 1996) the detailed structure of lamellar bodies was not visible in the present study. Lipids are abundant in bat epidermis as that in birds, although lipid droplets are not very common as that in avian sebokeratinocytes. Besides acting in the formation of the water barrier, the presence of a high amount of lipids in bat epidermis may connect with the need to counteract the cooling effect of air on the epidermal surface during flight: this is probably a common problem for both birds and bats. Lipids can produce metabolic heat or form a heat barrier to maintain the surface sufficient warm. The creation of a heat barrier in the epidermis seems to be the case for the epidermis of aquatic mammals (Menon et al, 1986; Elias et al, 1987; Pfeiffer & Jones, 1993; Pfeiffer & Rowntree, 1996).

The present cytological and ultrastructural study has shown that the stratum granulosum is limited or discontinuous in the available epidermal areas of bat skin. Therefore, it is possible that, as in the epidermis of other mammals, also in the epidermis of bats where the stratum granulosum was not observed was due to the submicroscopical dimension of keratohyalin granules (less than 0.5 μm; Alibardi & Maderson, 2003). Since the scarcity of keratohyalin and of a transitional layer has been detected also in different species of bats collected in other periods (Sokolov, 1982), it is unlikely that the present observations are due to a rapid turnover of the epidermis collected in the studied period (September).

Keratohyalin granules, which contain profilaggrin and filaggrin (Resing & Dale, 1991), are dispersed among keratin filaments in the transitional layer and rapidly disappear in the corneous layer where filaggrin is degraded and corneocytes flake away. The matrix material is diminished in external keratinocytes but still produce the alpha-keratin around the electron-pale keratin fibrils (Matoltsy, 1986). The scarcity of keratohyalin suggests that little matrix material is present among keratin filaments, another characteristics in common with avian sebokeratinocyets (Menon & Menon, 2000; Alibardi, 2004). A corneous layer made of numerous layers of thin corneocytes, together with a thin living epidermis made of 1 – 2 suprabasal cells, suggests that the cell turnover in bat epidermis is rapid. In fact, epidermis cells of the African bat *Eidolon helvum* reach the corneous layer in 6 – 9 days in comparison to the 14 – 30 days of that of human (Iversen et al, 1974). It is also likely that the turnover of the thin avian sebokeratinocyets is rapid, less than 10 days (Lavker, 1975; Alibardi, 2004).

Different from the typical mammalian corneocytes that have a spiny or tortuous surface, bat corneocytes have a smooth surface, as avian sebokeratinocytes. Also corneodesmosomes appear limited in number in comparison to other epidermises: this is a further sign of rapid desquamation and turnover of the epidermis.

Bats have very thin hairs (4 – 7 μm) with complex cuticles that enhance the grip one with another (Quay, 1970). The alternating or circular disposition of cuticle cells (Sokolov, 1982) probably increases the cohesion among hairs. This compact pelage may help the animal to maintain the body temperature against the cooling action of air currents met during the nocturnal flight of these mammals. This is obtained by a strong cohesion of numerous thin hairs by a spiny cuticle: this thick fur keeps the skin surface protected.

In conclusion, a thin, lipid-rich epidermis with a thick stratum corneum and a dense pelage may be adaptations to the nocturnal flight forming an anti-cooling surface capable of maintaining a thermal barrier. Also, hairs help to maintain the water-barrier creating a moisturized microenvironment on the surface of the skin.

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