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Review Article

The biology of vernix caseosa

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Synopsis

The biology and physical properties of the uniquely human skin cream 'vernix caseosa' are discussed. This material coats the foetal skin surface during the last trimester of gestation and provides multiple beneficial functions for the foetus and newborn infant. Vernix has a complex structure similar to stratum corneum but lacks lipid lamellae and is more plastic due to the absence of desmosomal constraints. *In utero*, vernix is made in part by foetal sebaceous glands, interacts with pulmonary surfactant, detaches into the amniotic fluid, and is swallowed by the foetus. At the time of birth, vernix has a remarkably constant water content approximating 80%. Postnatally, vernix is simultaneously a cleanser, a moisturizer, an anti-infective, and an anti-oxidant. Vernix facilitates acid mantle development and supports normal bacterial colonization. Its hydrated cellular structure and unusual lipid composition provide a 'best' solution for the needs of the foetus and newborn, not least of which is the attraction of caregivers. Vernix is an important natural biomaterial of potential interest to cosmetic scientists, and other disciplines involved in product development and therapies targeting the complex interface between the stratum corneum and a changing terrestrial environment.

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Résumé

La biologie et les propriétés physiques de la crème de peau exclusivement humaine 'Vernix caseosa « sont discutées. Ce matériau couvre la surface de la peau foetale pendant le dernier trimestre de gestation et remplit des fonctions avantageuses multiples pour le foetus et le nouveau-né. Le Vernix a une structure complexe semblable au stratum corneum, mais manque de lamelles lipidiques et est plus plastique en raison de l'absence de contraintes desmosomales. In utero, le Vernix est constitué en partie par des glandes sébacées foetales, il interagit avec le surfactant pulmonaire, il se détache dans le liquide amniotique et est avalé par le foetus. Au moment de la naissance, le Vernix a une teneur remarquablement constante en eau de l'ordre de 80%. Après la naissance, le Vernix devient simultanément un produit de lavage, un produit hydratant, un anti-infectieux et un anti-oxydant. Le Vernix facilite le développement du manteau acide et soutient la colonisation bactérienne normale. Sa structure cellulaire hydratée et sa composition en lipide inhabituelle en font 'une des meilleures » solutions pour les besoins du foetus et du nouveau-né, à laquelle le personnel soignant n'attache pas la moindre importance. Le Vernix est un biomatériau naturel important potentiellement intéressant pour les scientifiques cosméticiens et pour les autres disciplines impliquées dans le développement de produits et de thérapies visant l'interface complexe entre le stratum corneum et un environnement terrestre changeant.

Introduction

In mammals, birth marks an abrupt transition to a world filled with problems and promise. The human infant must simultaneously cope with potential threats of desiccation, cooling, infection, and trauma while presenting a panoply of sensory cues to attract potential caregivers. The study of the biology of vernix caseosa offers a glimpse into the little explored underwater world of the developing foetus and the controlled morphogenetic cataclysm of birth. This paper focuses on the developing epidermal barrier and summarizes the existing data on the uniquely human skin cream called 'vernix caseosa' (Fig. 1). Recent biochemical and ultrastructural data on vernix are cast within a biological context. Gaps in our knowledge and the evidence supporting various prenatal and postnatal functions of vernix caseosa are explicitly noted. Information regarding vernix structure and function is consolidated in a manner as to facilitate future investigation of this unique biological material. The paper is subdivided into discussion of vernix composition, morphology, water handling properties, physical characterization and biological properties. What emerges from the available data is a coherent and intriguing glimpse of a new area of perinatal biology with relevance to adult skin and cosmetic science.

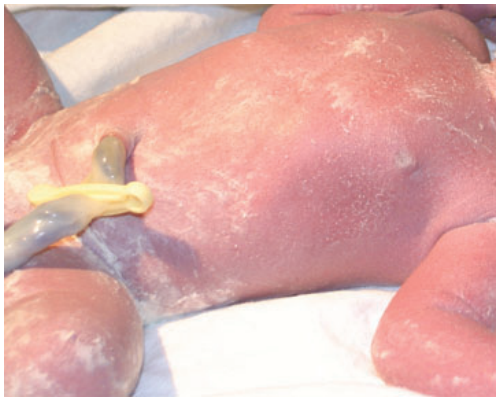

Figure 1 Vernix caseosa on the skin of a full term infant. Vernix covers the skin of the human foetus to varying degrees during the last trimester of gestation. It is more prominent following Caesarian deliveries than after vaginal births [53]. Vernix is absent in very low birth weight premature infants. Prior to birth, it partially detaches into the amniotic fluid under the influence of pulmonary surfactant.

Morphology of vernix

Phase-contrast and standard light microscopy reveal that vernix caseosa is a highly cellular material (Fig. 2). Agorastos *et al.* provided the first systematic characterization of the cells within vernix [1]. These cells are typically polygonal or ovoid in shape, with absent nuclei, although nuclear ghosts are frequently present. The authors also identified acid phosphatase activity within intracytoplasmic granules and within amorphous material deposited between the cellular components. Lanugo hairs, which are frequently entrapped within the vernix material, also exhibit strong, positive acid phosphatase activity, especially in the papillae. More extensive characterization of the corneocytes in vernix caseosa reveals that these foetal cells can be distinguished from corneoctyes found in mature stratum corneum by the lack of desmosomal attachments [2]. Ultrastructural analysis of vernix corneocytes by transmission electron microscopy shows a sparse network of keratin filaments with a little evidence of tonofilament orientation (Fig. 3). The vernix corneocytes are approximately 1–2 μm in thickness and are surrounded by a thickened layer of amorphous lipids lacking the typical lamellar architecture present in stratum corneum [3]. This structural arrangement of corneocytes and lipids imparts a different architecture to vernix compared with the stratum corneum, which constitutes the human permeability barrier during postnatal life. The lack of a lamellar lipid matrix surrounding the foetal corneocytes as well as the infrequent appearance of intercorneocyte desmosomal connections in vernix supports the notion that vernix is a kind of mobile or fluidic stratum corneum possessing a more permeable architecture to the transport of water and other small molecules [3]. Of considerable heuristic importance, is the finding that the outermost layer of the stratum corneum is similarly devoid of lipid lamellae and corneodesmosomes (Fig. 4). The notion that adult stratum corneum reverts in its oldest (outermost) layer to a form characteristic of the foetus (vernix morphology) deserves further consideration.

Cryoscanning electron microscopy coupled with X-ray beam analysis was used to localize the high water content within freshly collected vernix caseosa [4]. Fig. 5 depicts a cryofractured corneocyte in the left panel. In the right panels, the same corneocyte is examined by elemental analysis

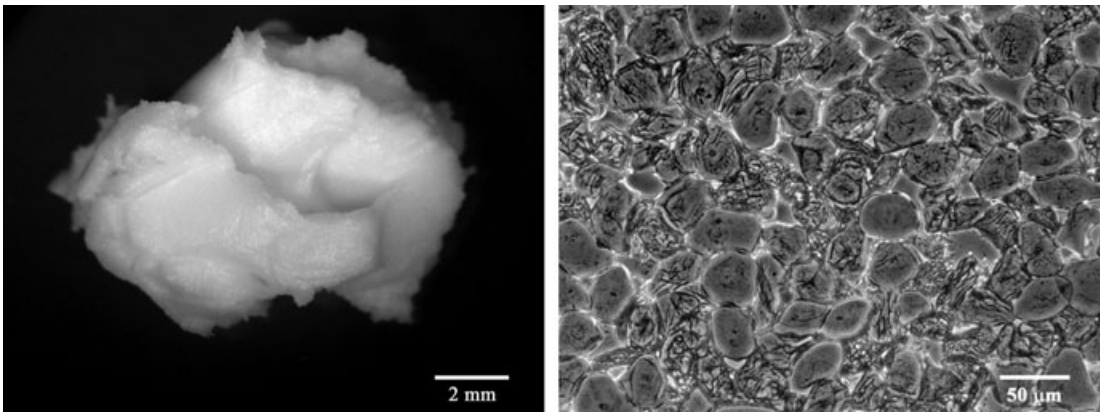

Figure 2 Vernix caseosa. Macroscopically, vernix is a thick, viscous, white paste (left panel). The phase contrast image of native vernix (right panel) reveals a dense packing of foetal corneocytes surrounded by a thin lipid matrix. The cells are heterogenous in size and structure. Many nuclear ghosts are evident. Scale bars are shown in the figures.

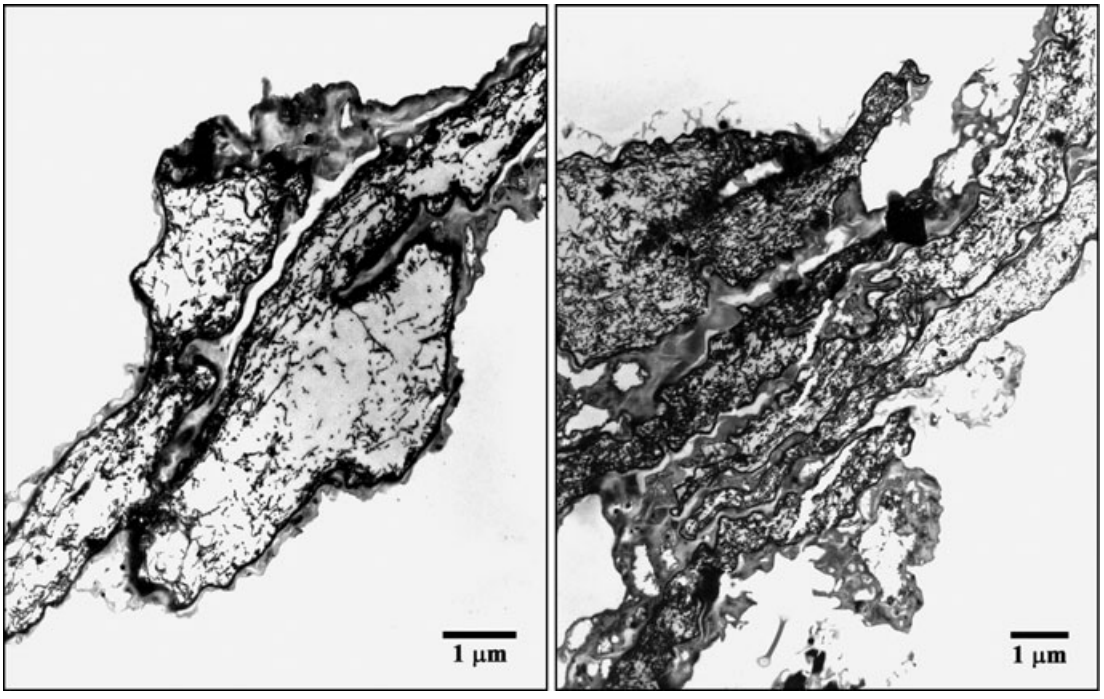

Figure 3 Transmission electron microscopy of vernix caseosa corneocytes. Fresh vernix was obtained at the time of delivery from the skin surface of a normal term infant. Vernix corneocytes often exhibit an irregular form with absence of corneodesmosomal attachments. The curvature of the cornified cell envelope is possibly indicative of malleability. Note the sparse network of tonofilaments (left panel). No corneodesmosomal attachments are observed. The lipid matrix surrounding the corneocytes is generally nonlamellar (right panel).

using X-ray spectra and elemental maps with a dispersive spectrophotometer. This analysis shows the localization of carbon (lipid) in a distribution pattern surrounding the corneocyte and a high level of oxygen (water) relative to carbon within the corneocyte interior. This distribution pattern indicates that the extremely high water content of vernix resides within the corneocyte population. The water content of these corneocytes is a function of the surrounding osmotic environment [5].

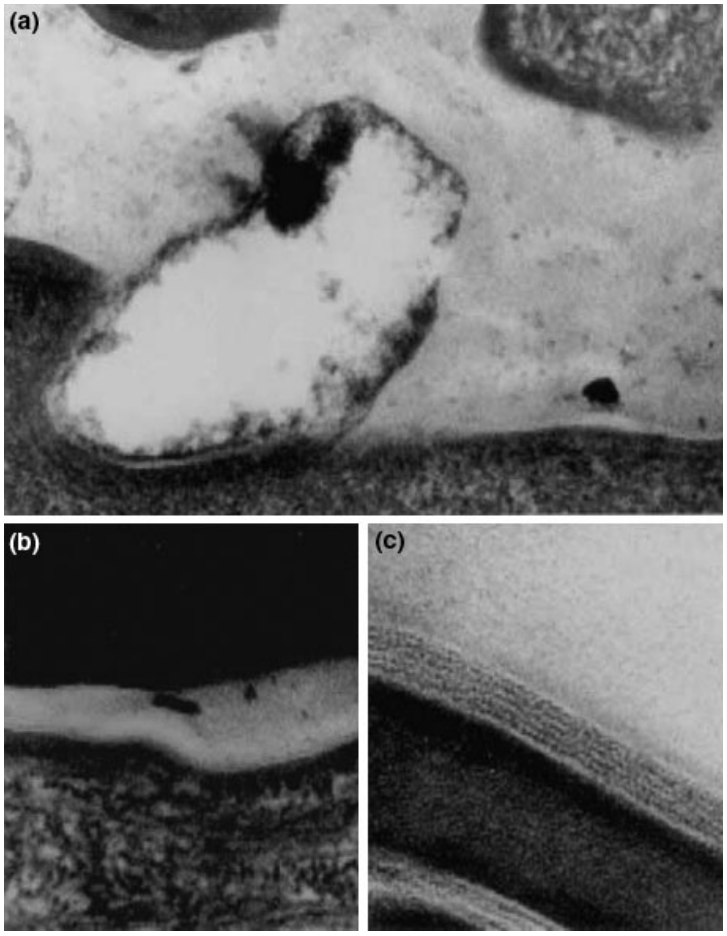

Figure 4 Transmission electron micrographs of serial tape-strippings from individuals with clinically normal skin. Ultrastructural changes in lipid organization towards the surface of the stratum corneum. (a) First strip; absence of bilayers and presence of amorphous lipidic material. (b) Second strip; disruption of lipid lamellae. (c) Third strip; normal lipid lamellae (× 200,000). Modified from Rawlings *et al.* [55].

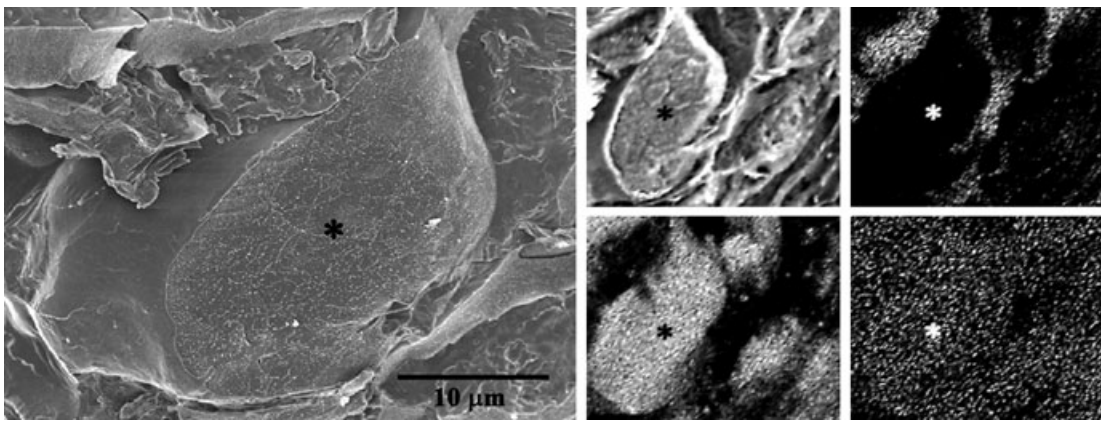

Figure 5 Cryoscanning electron microscopy and elemental analysis of vernix caseosa. These two photomicrographs depict vernix that was examined by cryoscanning electron microscopy and X-ray elemental analysis. The single lipid-covered corneocyte observed in the image on the left is partially fractured and reveals intermediate filaments within the cell. This same corneocyte is imaged throughout the four panels on the right with its position denoted by asterisks. X-ray mapping of oxygen (lower left panel) and carbon (upper right panel) show the distribution of water and lipid, respectively. The X-ray map in the lower right panel reflects the distribution of sulphur and is used as a background element to check for X-ray absorption artefacts due to surface topography effects (reprinted with permission from Pickens *et al.* [4]).

Thus, isolation of vernix corneocytes from fresh, native vernix in 0.5% SDS with heating for 15 min at 55°C followed by exposure overnight to varying osmotic conditions results in significant size change (Fig. 6).

Vernix composition

Early studies on vernix composition focused primarily on characterizing its lipid contents following extraction with organic solvents [6–9]. The total amount of lipid present in vernix is approximately 10% (w/w) [10]. A breakdown of this complex mixture reveals the presence of wax and sterol esters, ceramides, squalene, cholesterol, triglycerides and phospholipids. To our knowledge, no other animal species produces vernix, making this material a uniquely human skin barrier film. Other structures such as the periderm in rodents, however, may play a similar role *in utero* [11, 12]. Animals such as sheep produce lanolin, which also contains wax and sterol esters [13]. However, unlike vernix, this sebaceous secretion has not been reported to contain desquamated corneocytes. *In utero*, vernix progressively coats the infant in a cephalocaudal manner during the last trimester of gestation. The high squalene and wax-ester content in vernix strongly suggests that a significant portion of the lipid content is of sebaceous origin. The putative synthesis of vernix in the pilosebaceous apparatus is highly significant with regard to the initiation of epidermal barrier maturation *in utero*. As shown by earlier studies, stratum corneum formation and the presence of an epidermal permeability barrier begins anatomically in the immediate vicinity of the pilosebaceous apparatus [14–16]. Zouboulis has discussed the progressive development of sebaceous gland function [17]. This model is consistent with a mechanism whereby vernix lipids limit water transport across the developing epidermis thereby facilitating the cornification process.

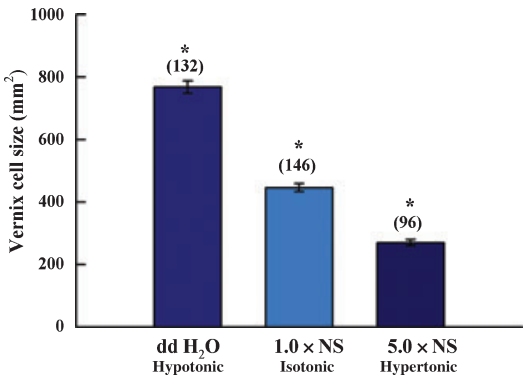

Figure 6 Vernix cell response to osmotic variation. Isolated vernix cells were incubated overnight at room temperature in either deionized water, normal saline or a saline solution with an osmotic content that was five times more concentrated than normal saline. The unstained cells were imaged by phase contrast microscopy and the cell surface areas were recorded. The number of observations is shown in parentheses. One-way ANOVA indicates that each group is significantly different from all other groups ($p < 0.05$) [5].

Analyses of the lipid constituents within vernix has been published by Sumida *et al* [18] and Hoeger *et al* [10]. Vernix contains ceramides and cholesterol as well as triglycerides, wax and sterol esters, squalene and phospholipids. Ceramides and cholesterol are generally considered products of stratum corneum development, whereas triglycerides, wax and sterol esters, squalene and phospholipids are components of sebum. These findings support the concept that the vernix lipid matrix is constituted of both stratum corneum lipids and sebaceous lipids. Sumida *et al.* also synthetically reconstituted this unique lipid matrix and found that the reconstituted vernix lipids were more hygroscopic than reconstituted sebaceous lipids alone [18]. A more extensive analysis of vernix lipids has been completed by Rissmann *et al.* [3]. These authors demonstrate for the first time the presence of lipids bound to vernix corneocytes as well as lower levels of barrier lipids compared with previous reports (see Table 1). Of note, the method by which vernix is obtained at delivery may be an important variable. Scraping the stratum corneum surface, for example, may increase barrier lipid constituents.

The protein constituents of vernix are not as well characterized as the lipid constituents. Recently, however, a plethora of antimicrobial peptides have been identified in vernix including Lysozyme, lactoferrin, human neutrophil peptides 1–3, and secretory leukocyte protease inhibitor, LL-37, cystatin A, UGRP-1 and calgranulin A, B and C [19–23]. The abundance of foetal corneocytes present in vernix has not yet been systematically compared with adult corneocytes for the presence of distinguishing molecular constituents such as involucrin, filaggrin and keratin subtypes. Analysis of free amino acids following chloroform–methanol extraction of lipids and acid hydrolysis of the precipitated residue revealed an abundance

Table 1 A comparison of the lipid components of vernix caseosa, stratum corneum and skin surface (sebaceous) lipids

Lipid class	Vernix Sumida [18]	Vernix Hoeger *et al.* [10]	Vernix Rissmann *et al.* [3]	Stratum corneum lipids [18]	Skin surface lipids [18]
Cholesterol esters and wax esters	38	Not reported	42		23.3
Ceramides	17.9	7.7	4.9	40	
Triglycerides	15.1	Not reported	35.9		41.8
Cholesterol	7.5	12.1	4	25	
Free fatty acids	6.5	6.6	2	25	18
Phospholipids	6.1	4.4	Not reported		1.5
Diols	Not reported		6		
Squalene	4	Not reported	5		12.2
Alkane	–		—		2.8
Cholesterol sulphate	0.3		Not reported	10	

Data are given as percent total lipids.

of asparagine and glutamine [24] (Table 2). The latter is particularly significant insofar as vernix is known to detach from the foetal skin surface before birth and is subsequently swallowed by the foetus [25]. Glutamine is currently under investigation as a trophic factor for the developing foetal gut [26]. Water extraction of amino acids may yield different percentages [27].

The major bulk constituent of vernix is water. Dry weight to wet weight ratios of vernix caseosa and standard topical creams used in the newborn nursery have been compared [4, 28]. It was found that approximately 80–81% (w/w) of vernix is volatile (Fig. 7). Analysis by Karl–Fischer titration revealed that the volatility is due exclusively to water content. Upon exposure to a dry ambient environment, a situation analogous to the transition that vernix encounters at birth, vernix slowly releases its water content. Placement of completely desiccated vernix in normal saline or deionized water results in slow rehydration over a period of

Table 2 Amino acid composition of vernix caseosa

Amino acid	Per cent
Asparagine	34.7
Glutamine	22.7
Proline	14.9
Cysteine	7.9
Alanine	7.4
Leucine	5.3
Valine	3.7
Methionine	3.4

Native vernix was collected at birth from 20 newborns. Following lipid extraction, the specimens were acid hydrolysed and analysed by thin layer chromatography to determine the amino acid composition. The abundance of asparginine and glutamine residues is likely derived from the numerous foetal corneocytes found in vernix.
Data adapted from Baker *et al.* [24].

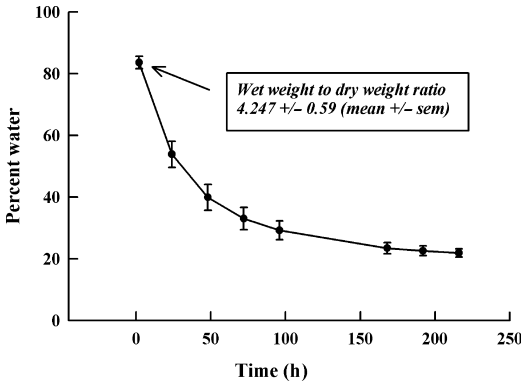

Figure 7 Dehydration kinetics of vernix caseosa. Freshly harvested vernix caseosa was collected from 10 newborn infants. A small aliquot (*c.* 50 mg) from each infant was placed under vacuum at room temperature for a period of 9 days. Weights were recorded daily. After the specimens attained constant weight, the percentage of water in the vernix was back-calculated for each weighing. Several points are worthy of note: (1) the high water content of vernix at birth; and (2) the ability of vernix to retain water for a protracted period of time. Logarithmic transformation of these data indicates that there are two separate water compartments, one relatively fast release compartment and another that releases water much more slowly. Data reported as mean ± SD.

days with greater rehydration rates following exposure to deionized water [2].

The physiological meaning and relevance of the remarkably stable water content of vernix is unclear. The normal environment of vernix *in utero* is amniotic fluid which is tightly regulated in terms of its ionic composition [29]. Studies *ex utero* have demonstrated that vernix corneocytes can swell or contract as a function of a hypoosmotic or hyperosmotic environment [5]. Taken together, the data are consistent with a mechanism wherein vernix participates in osmoregulation. Following birth, the regulation of water at the body surface is key for normal thermoregulation as well as to prevent desiccation and maintain plasticity of the stratum corneum [30]. Of interest, the wet-to-dry weight of fresh, native vernix obtained from term infants at the time of delivery is 4.247 ± 0.59 (Fig. 7). Parenthetically, this number is statistically indistinguishable from the cube of the golden section ratio (Φ) $= 1.618034^3 = 4.236$ [31].

In separate work, the organization of human epidermis has been hypothesized on empirical grounds to manifest phi proportionality [31, 32]. In human epidermis, for example, the ratio of corneocytes to underlying epidermal cells is approximately Φ^3 [31]. Phi, or the golden section ratio, is found in many biological systems and is particularly associated with systems requiring structural stability in the face of change [31, 33]. Its presence in dynamic structurally stable systems such as the epidermis, therefore, is perhaps not surprising, but it has hitherto never been described in association with biomaterials such as vernix.

Physical properties

Understanding of the biological functions of vernix caseosa requires characterization not only of the morphology and composition of vernix, but also its physical properties. Vernix forms the ultimate interfacial film coupling the newborn infant with the extrauterine environment following birth. Prenatally, vernix performs a similar function coupling the developing skin surface of the late gestation foetus with the amniotic fluid. During the third trimester of pregnancy, there is a progressive increase in the turbidity of the amniotic fluid surrounding the foetus [34]. This turbidity has been assayed as an index of foetal lung maturity by various methods ranging from spectrophotometry to visual examination.

In an earlier study, Agorastos *et al.* demonstrated a progressive increase in vernix-related sediment in amniotic fluid with advancing gestational age [35]. These authors suggested that amniotic fluid turbidity resulted from increasing amounts of vernix within the amniotic fluid, although the mechanism underlying this progressive increase was unclear. To investigate this phenomenon and to explore a potential mechanism for the induction of amniotic fluid turbidity, an *in vitro* analysis was performed wherein vernix caseosa was immobilized on a polypropylene substrate and exposed to pulmonary-derived phospholipids at physiologically relevant concentrations; i.e., levels present in late gestation amniotic fluid [25]. Following incubation, the overlying solutions were spectrophotometrically analysed. The exposure of vernix to pulmonary surfactant (Survanta®, Abbott Laboratories, Columbia, OH, U.S.A.) resulted in a dose dependent increase in turbidity indicating emulsification and release of vernix from the substrate surface. This phenomenon was markedly temperature dependent with increased turbidity noted at body temperature (37°C) compared with room temperature (23°C). The response was not observed using a synthetic mixture of phospholipids devoid of surfactant proteins indicating a possible effect of surfactant proteins on the emulsification process.

Other studies were conducted to address the rheological behaviour of vernix caseosa. Tests performed in a controlled stress rheometer indicate that vernix rheological behaviour is characterized by plastic flow with a decrease in viscosity occurring with increasing shear stress (shear thinning) and the presence of a yield value. Calculation of a yield value for vernix was 6.7×10^3 dyne cm^{-2} at 25°C (Wael Youssef, PhD Thesis, University of Cincinnati, Cincinnati, OH, U.S.A.). Vernix plastic rheological behaviour shows clear temperature dependence over the range of 25–40°C with viscosity decreasing with increasing temperature. The addition of pulmonary surfactant (Survanta®) to vernix markedly changes the rheological behaviour of vernix with a precipitous drop in viscosity.

As noted earlier, vernix has the physical appearance of a thick, white, viscous cream, but upon chemical analysis has been found to consist primarily of water (*c.* 80–81%). The water within vernix is not evenly distributed but is primarily present in the form of cellular sponges as indicated by freeze-fracture cryoscanning and X-ray

elemental analysis [4]. At birth, vernix is suddenly exposed to an air environment in which standing water is potentially deleterious to the newborn infant insofar as evaporative heat loss leads to rapid cooling of the body surface. The interaction of vernix with exogenous water was investigated by contact angle measurement with calculation of the critical surface tension of vernix using Zisman plot analysis [36]. The critical surface tension of vernix was approximately 39 dyne cm^{-1} indicating a relatively low energy surface comparable with that of petrolatum (35.8 dyne cm^{-1}). Such a low critical surface tension for vernix is unexpected insofar as the main component of vernix is water, which has a critical surface tension of 72 dyne cm^{-1}. These results are consistent with the proposed physical organization of vernix as a dispersion of water rich corneocytes embedded in a hydrophobic lipid matrix.

Partition of the surface free energy of vernix into its dispersive (non-polar) and non-dispersive (polar) components using Owens/Wendt analyses indicates that vernix is essentially non-polar despite its very high water content. These results compare with the findings of Mavon *et al.* who found that the total surface free energy of forehead skin which is rich in sebaceous lipids, is around 43 dyne cm^{-1} [37]. The authors in that study found that the major part of the surface free energy of forehead skin was non-polar which essentially agrees with the results found for vernix. The overall low surface free energy of vernix supports the notion that at least one of its major functions is protection and 'waterproofing' of the foetus. Postnatally, a water repellent film of vernix on the surface of the newborn skin may be biologically advantageous insofar as heat loss is thereby limited.

The sparse to absent lipid lamellar architecture within vernix, however, suggests that water transport through this material may be markedly increased compared with native stratum corneum. Riesenfeld *et al.* studied the influence of vernix caseosa on water transport through semipermeable membranes under controlled environmental conditions [38]. These authors reported that the evaporation rate over semipermeable membranes covered with vernix caseosa was approximately 50 g m^{-2} h^{-1} at a relative humidity of 50%. This value is comparable with the evaporation rate of a premature infant at 27 weeks gestation studied the first day after birth. In the *in vitro* experiment described, the evaporation rate fell exponentially over the first 3 h to 10 g m^{-2} h^{-1}. In contrast, vernix spread on a semipermeable membrane maintained over a water reservoir, exhibits a much slower decrease in evaporation rate over the 3-h period with values reaching 30 g m^{-2} h^{-1} [28]. Transepidermal water loss in the term newborn infant is approximately 5 g m^{-2} h^{-1} [39]. These data indicate that vernix is much more vapour permeable than native stratum corneum presumably secondary to the non-lamellar structure of the lipid matrix and the high water content of the embedded corneocytes [3]. These results may be relevant insofar as studies of the effect of epidermal barrier films on wound healing of tape stripped adult human skins indicate that barrier films with water vapour permeability in the range of 20–60 g m^{-2} h^{-1} are preferable to either no occlusion or full occlusion [40]. This finding may be relevant to proposed uses of vernix as a prototypical wound healing ointment.

Using a similar *in vitro* system, the penetration of exogenous chymotrypsin was studied through vernix films of controlled thickness as a function of temperature [41]. Chymotrypsin may be present in the amniotic fluid as a function of elimination via the stool (meconium) or as an endogenous enzyme in the stratum corneum associated with desquamation. Vernix effectively blocked chymotrypsin passage while being itself devoid of enzyme activity [41]. Similarly, vernix blocks penetration of exogenous methylene blue (Fig. 8). This is consistent with the earlier findings of Hardmann *et al.* who demonstrated the development of barrier to methylene dye penetration in developing human skin, particularly in the vicinity of the pilosebaceous apparatus [14].

Biological properties

Table 3 lists evidence supporting a plethora of biological functions for vernix casesoa. Understanding such functions must take into account physiological needs of the organism prenatally and at the time of birth. The formation of a permeability barrier under aqueous conditions is difficult to attain under standard skin culture conditions. Raising cultured skin to an air–liquid interface imposes a 'xeric stress' on the skin surface with subsequent cornification [42, 43]. A similar mechanism may be responsible for initiating the rapid postnatal cornification observed in extremely low birth

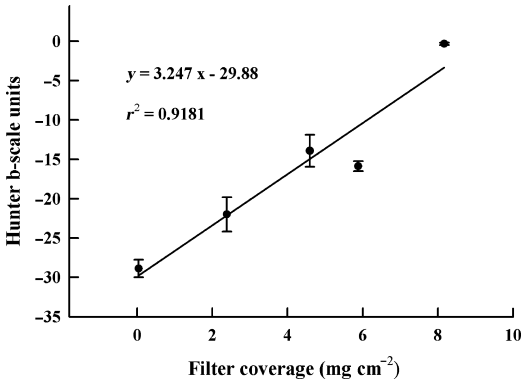

Figure 8 Ability of vernix to block methylene blue penetration. Vernix was applied to nylon filters placed over collagen supports at film thicknesses ranging from 0 to 8 mg cm^{-2}. All filters were exposed to methylene blue dye for 30 min. Hunter b-scale data were collected using a Minolta chromameter to obtain multiple measurements of the underlying collagen surface. The results are reported as means ± SEM. A less negative Hunter b-scale value denotes a more effective barrier.

weight premature infants [11]. Whether or not this mechanism can be ascribed to the intrauterine environment is unclear. A hypothetical working mechanism for the participation of vernix in epidermal barrier maturation is shown in Fig. 9. Recently, Ito, *et al.* reported that human hair follicles synthesize cortisol and exhibit a functional equivalent of the hypothalamic–pituitary–adrenal axis [44]. This significant finding has not been investigated with reference to the control of vernix production and the foetal pilosebaceous apparatus.

Interposition of a hydrophobic vernix film overlying the nascent epidermis, however, would presumably change the water gradient between the skin surface and the surrounding amniotic fluid. The concomitant development of vernix and the underlying stratum corneum markedly change the electrical properties of the foetal skin leading to formation of a high impedance skin surface [45]. Electrical isolation of the foetus *in utero* is presumably an important aspect of developing foetal autonomy.

Hypothetically, vernix participates in controlling water transport across the developing epidermis as well as water content within the stratum corneum at the time of birth. The epidermal barrier is unique in that it appears to have evolved as a mechanism of limiting evaporative water transport in anticipation of terrestrial life while simultaneously using water as its chief plasticizing agent. Water is key. Studies by Denda *et al.*, for example, clearly demonstrate the importance of the transepidermal water gradient as a potential regulator of DNA synthesis and lipid biogenesis [46, 47]. Whether a similar influence of water flux on epidermal biology occurs in the foetus, preterm or term newborn infant is unknown. Spreading of gamma-irradiated vernix onto nylon filters with subsequent superpositioning of the vernix over cultured human skin results in increased glucose consumption and lactate production [48]. These preliminary experiments, however, cannot dissociate direct effects of vernix on the transepidermal water gradient vs. the possibility of a putative growth factor effect. Epidermal growth factor, for example, is

Table 3 Proposed biological functions of vernix

Function	Evidence	References
Waterproofing	Newborn animals exhibit hydrophobic surface, surface free energy of vernix indicates hydrophobic material	[11, 12, 36, 57]
Anti-infective	Contains multiple diverse antimicrobial peptides in particulate form; reports of barrier properties to bacterial passage	[19–23, 59, 60]
Anti-oxidant	Contains alpha-tocopherol and melanin, human sebum high in vitamin E	[51–53, 66]
Moisturization	High water content of corneocytes, *in vivo* and *in vitro* evidence of hydrating ability	[2, 38, 53, 57]
Cleansing	Possesses both hydrophilic and hydrophobic domains, comparable efficacy to commercial cleansers	[36, 64]
Wound healing/ maturation	Increases skin metabolism *in vitro*, high glutamine content, effect on trophic ulcers	[24, 48, 58]
Acid mantle	Facilitates acid mantle production in newborn infants	[53]
Protectant	Barrier film to passage of chymotrypsin and methylene blue (Figure 8), mechanical barrier to bacterial passage	[41, 59]

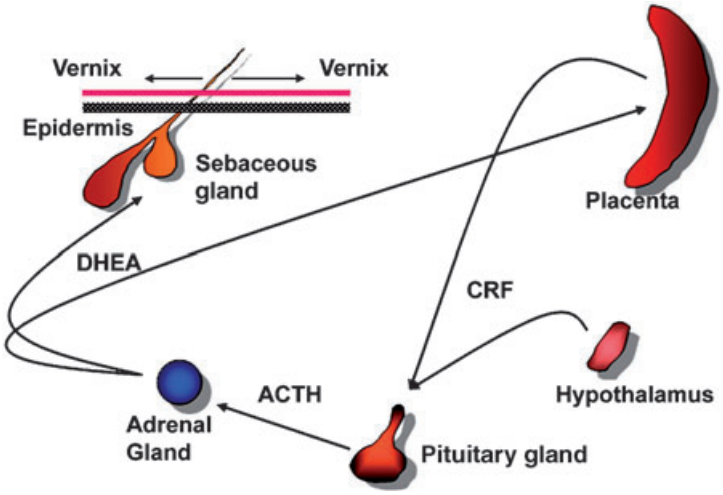

Figure 9 Hypothetical endocrine-based mechanism for vernix production and epidermal barrier maturation. Under this working hypothesis, corticotropic-releasing factors (CRF) from either the placenta or hypothalamus act to initiate adrenocorticotropic hormone (ACTH) release from the pituitary gland. ACTH stimulation of the adrenal cortex promotes synthesis and release of androgenic steroids such as dihydroepiandrosterone (DHEA) which are subsequently converted enzymatically within the sebaceous gland to active androgens. Production of superficial lipid film (sebum) in the immediate vicinity of the hair follicle modulates the transepidermal water gradient with putative effects to facilitate cornification of the underlying epidermis [14]. Desquamation of corneocytes into the overlying lipid matrix results in formation of vernix. The recent finding by Ito *et al.* that human hair follicles synthesize cortisol and exhibit a functional equivalent of the hypothalamic–pituitary–adrenal axis [44] raise interesting questions regarding local vs. systemic control mechanisms in the above schema (modified from Zouboulis *et al.* [17]).

present in high concentrations in urine and amniotic fluid [49]. Its presence in vernix, therefore, would not be unexpected with possible secondary effects on adjacent cellular epithelia. The complex nature of vernix composition makes the ascription of biological effects to single causes difficult.

Other recently reported molecular constituents of vernix suggest potential biological functions relevant to the time of birth. Surfactant protein D, for example, has been measured by ELISA assay in extracts of vernix and immunolocalized to sebaceous glands in human newborn foreskin [25]. Surfactant protein D is a molecule, present in tracheal fluid with responsibility for maintaining airway sterility [50]. This molecule is a member of the broader collectin family and its presence in vernix and sebaceous glands suggests a potentially important role for this molecule on the skin surface. Recent reports of additional antimicrobial peptides arranged in particulate granules within vernix support a primary function in the control of infection [19–23].

In addition to entry into an environment teeming with microorganisms, birth also marks a time of high oxidative stress. Thiele *et al.* reported that human skin exhibits antioxidant properties with high levels of alpha tocopherol in human stratum corneum and sebum [51, 52]. Alpha tocopherol is also present in vernix [53]. In contrast to term infants, very low birth weight preterm infants do not exhibit sebaceous gland hyperplasia and have a little to no vernix at birth. These preterm infants would, hypothetically, be deficient in both vernix and related molecules in addition to possessing an incompetent stratum corneum with impaired barrier function.

A similar situation occurs in association with the finding that pulmonary surfactant results in detachment of vernix under *in vitro* conditions (Fig. 10). The increasing concentrations of pulmonary surfactant in amniotic fluid suggest a possible initiating mechanism for the induction of amniotic fluid turbidity *in vivo* [25]. This mechanism is highly attractive as a means of 'cross-talk' between different epithelial surfaces during the third trimester of pregnancy. The developing lung secretes increasing concentrations of pulmonary surfactant into the amniotic fluid with effects to emulsify and detach superficial vernix. The foetus subsequently swallows the detached vernix

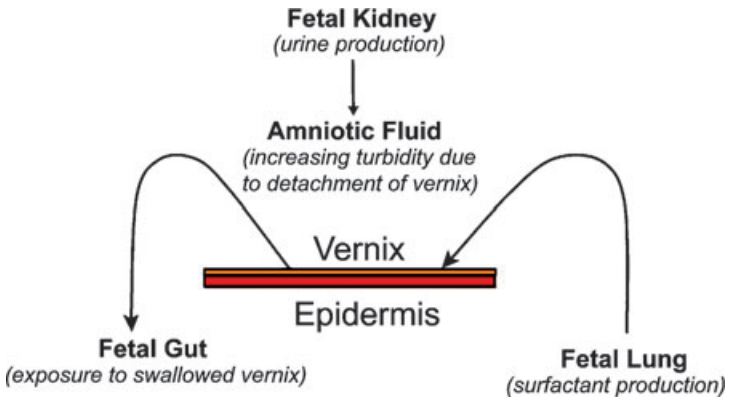

Figure 10 Proposed mechanism for surfactant-mediated vernix detachment. During the last trimester of gestation, the foetal lung produces and secretes increasing amounts of pulmonary surfactant into the amniotic fluid. Concomitantly, vernix on the skin surface builds up and detaches into the surrounding milieu resulting in increasing amniotic fluid turbidity. Recent data indicates a role for pulmonary surfactant to emulsify vernix and aide in the detachment mechanism [25]. Vernix within the amniotic fluid is subsequently swallowed by the foetus with potential effects on the foetal foregut and/or systemic absorption of vernix components (modified from Hoath *et al.* [65]).

resulting in potential trophic effects on the developing foregut. Amniotic fluid, in turn, is produced in large part by the developing kidney and is known to contain high concentrations of potent growth factors such as EGF. Such interactions among incipient environmental interfaces before birth have yet to be investigated.

Other proposed biological functions for vernix at the time of birth include a role for vernix in temperature control. Early clinical reports attributed the development of subnormal temperatures in newly born premature infants to the removal of vernix at birth [54]. At present, vernix is often wiped off the skin and discarded. The high water content in vernix supports a potentially deleterious effect of vernix to increase evaporative heat loss. Washing the skin surface after birth reportedly reduces evaporative heat loss compared with the surface of newborns in which vernix is left *in situ* [38].

The fact that vernix is hydrophobic supports a potential role of vernix in repelling exogenous water. In contrast, the high water content of vernix may have the seemingly paradoxical effect of maintaining stratum corneum moisturization with slow controlled drying after birth. In newborn rodents, a specialized cell layer called the periderm results in a highly hydrophobic skin interface which functions to decrease evaporative heat loss after birth [12]. Slow drying of the stratum corneum is necessary for filaggrin proteolysis with resultant production of small hydrophilic molecules known collectively as natural moisturizing factors (NMF) [55]. Scott *et al.* determined that NMF production is optimal at relative humidities between 80% and 95% [56]. Hypothetically, the high water content of vernix provides this optimal high humidity microenvironment. Saijo and Tagami reported that the term infant exhibits marked drying of the skin surface with impaired stratum corneum water holding capacity during the first few days after birth [57]. The poor water sorption properties of the stratum corneum and the desquamation commonly observed in term infants may result in part from a combination of rapid drying, particularly under radiant warmers, and exposure to harsh surfactants during the bathing process. It is possible that vernix left in place at birth will diminish such activities [53]. Further studies need to be conducted to evaluate this possibility.

Review of the older literature contains sporadic reports of vernix as a potential wound healing ointment [58]. Other reports describe mechanical barrier properties of vernix with respect to bacterial invasion [59]. Direct anti-infective action or possible roles of vernix to impede or guide bacterial colonization at birth are equivocal [24, 60]. Vernix on the foetal skin surface is presumably transferred to the mother's perineum during the birth process. It is not unreasonable, therefore, to anticipate that vernix may have a beneficial effect on epidermal wound healing with potential application as a therapeutic barrier cream in very low birth weight infants. Recent studies evaluating the

use of Aquaphor (Beiersdorf, Inc., Norwark, CT, USA) a topical emollient have shown increased rates of nosocomial infection secondary to staphylococcus epidermidis in extremely low birth weight infants [61]. The application of physiological barrier creams based on vernix caseosa as a prototype have yet to be explored but evidence is mounting that emollient creams are beneficial in reducing infections in newborn populations, particularly in developing countries [62, 63].

Finally, recent data indicate that vernix functions as an endogenous skin cleanser (Fig. 11). In experiments performed using human skin soiled with fine carbon particles, vernix had comparable efficacy to standard commercial skin cleansers [64]. The concept that the human infant enters the world covered with a material possessing endogenous cleansing capabilities is intriguing. Such a material, unlike soaps, would be composed of physiologically relevant lipids that would seamlessly integrate with the skin surface and pores. Any residue remaining after cleaning would presumably have a beneficial effect in the form of antioxidation, moisturization, and infection control. In this view, vernix functions, in large part, in an analogous manner to the self-cleaning properties of the stratum corneum in which desquamation results in a continual dynamic renewal and 'cleaning' of the organism–environmental interface (Table 4).

Summary and relevance to cosmetic science

Understanding the physical chemistry and biology of vernix caseosa pose a significant challenge to the cosmetic scientist. Structurally, similar to the stratum corneum, which it intimately covers, vernix lacks lipid lamellae and desmosomal contacts. Uniquely human, vernix is multifunctional: a skin cleanser, moisturizer, anti-infective and anti-oxidant which works in both aqueous and non-aqueous environments. *In utero*, its rheological properties are modified by extracutaneous secretions such as pulmonary surfactant. Detached vernix is swallowed by the foetus. Vernix contributes to the electrical isolation of the foetus and has osmoregulatory capability. At the time of birth, its

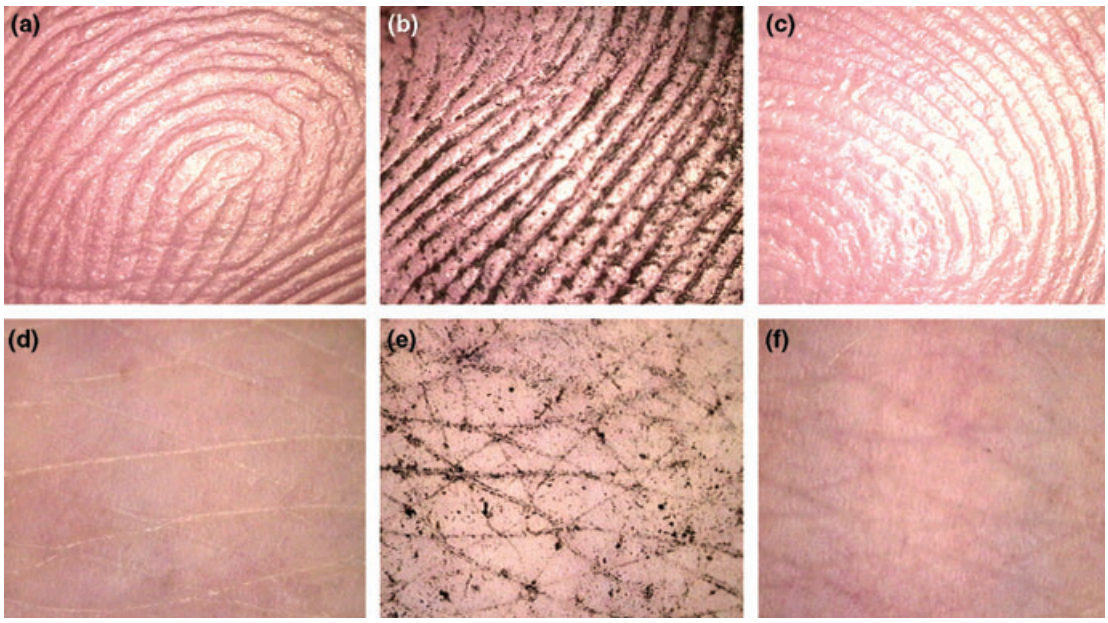

Figure 11 Qualitative assessment of cleansing capacity of vernix. A skin surface analyser was used to obtain digital images of the fingerpad by surface illumination (a–c) and the volar forearm by transdermal illumination (d–f) at 30 × magnification. The specular reflectance observed in the surface-illuminated images is eliminated in the transdermal illuminated images due to back-scattering of the light source. Both techniques demonstrate the efficacy of topically applied vernix as an effective skin cleanser removing residual carbon particles from soiled skin (b, e) with cleaning of skin furrows, crevices and pores (c, f). Presoiled skin images are also shown (a, d). Quantification of residual carbon particles from skin images is described by Moraille *et al.* [64].

Table 4 Overview of skin cleansing mechanisms [64]

Endogenous	Exogenous
Amniotic fluid circulation	Water submersion (bathing)
Detachment of vernix	Commercial surfactant application (e.g. soaps)
Stratum corneum desquamation	Mechanical disruption (towelling, brushing)

water content precisely matches the cube of the golden section ratio. Following tactile spreading, polygonal vernix corneocytes orient parallel to the skin surface. The hydrophilic (intracorneocyte) and hydrophobic (external lipid) domains of vernix contain a plethora of biologically active, small molecules in a complex, structured array. Cleansing studies support ready entry of applied vernix into surface pores such as hair follicles. Vernix has a non-greasy feel and its physical properties hypothetically contribute to the panoply of sensory cues which attract caregivers to the skin of the newborn. The possibility that vernix contains pheromones, like mother's milk, is open to investigation. Vernix facilitates acid mantle formation and presumably contributes to optimal bacterial colonization of newborn skin after birth. A useful heuristic idea is that the oldest (outermost) layer of terminally differentiated stratum corneum reverts, before desquamation, to a morphological structure characteristic of the foetal skin surface, i.e. to the form of vernix caseosa lacking lipid lamellae and corneodesmosomes. If so, attention to the function and structure of vernix offers an accessible experimental material with relevance outside the newborn period for understanding the complex identity of body and environment.

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