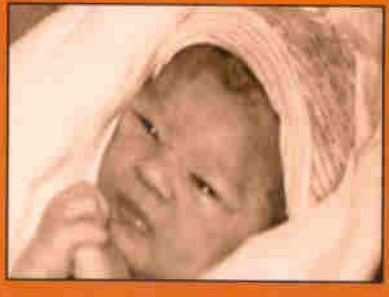
Neonatal Skin

Structure and Function

Second Edition, Revised and Expanded



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Second Edition, Revised and Expanded

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Preface to the Second Edition

Over two decades have passed since publication of the first edition of *Neonatal Skin: Structure and Function*. During this time, sweeping changes have occurred in all aspects of healthcare, from the molecular diagnosis of disease to the advent of the Internet. In this climate of explosive change, the second edition of *Neonatal Skin* seeks to illuminate a topic both scientifically complex and aesthetically compelling—the skin of the newborn human infant. It is our belief that this topic deserves careful study and quantitative attention. It is toward this goal that this book was written.

The picture that emerges from the study of newborn skin is unexpectedly complex. The migration of melanocytes into the epidermis, the participation of the pilosebaceous apparatus in the development of the epidermal permeability barrier, and the production of a complex proteolipid skin "cream," the vernix caseosa, all occur in utero. These fetal developments presage the abrupt transition to extrauterine life marked by birth. In mammals, few events are as transformative as the moment of birth. Certainly, no organ is more suddenly exposed to the exigencies and extremes of the external environment than the skin. Postnatally, the skin surface becomes rapidly colonized with selected microorganisms. It undergoes a complex process of surface acidification and interacts with a variety of environmental agents, including cleansers, drugs, and adhesives. Temperature control becomes a new and vital function requiring not only a barrier to transepidermal water loss but also an active system of eccrine sweating and vasomotor control. Not least in the rich panoply of functions subserved by the skin of the newborn is its ability to evoke emotional attachment and physical connectivity in adults. Aesthetically, newborn skin serves to beckon caregivers, thereby providing an apparent teleological reason for its being.

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This edition of *Neonatal Skin* seeks to summarize and interweave these multiple functions and their cutaneous support structures into a coherent scientific pattern. Our guiding belief is that the study and story of newborn skin are only now beginning to be articulated. The true functions of skin are at the highest level of biological organization (1). It is common knowledge, for example, that the evolutionary features which most clearly distinguish humans from other primates are, first and foremost, a large and versatile central nervous system and, second, a vulnerable and relatively hairless skin surface (2). The brain and the epidermis share a common ectodermal origin, and the skin and the brain are inextricably linked in everyday life at the level of perception. We anticipate growing interest in these complex areas of skin structure and function. As Aristotle is purported to have said, "He who sees things from their beginnings will have the clearest view of them." It is in this spirit that the authors have collaborated to produce this second edition.

Steven B. Hoath
Howard I. Maibach

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Preface to the First Edition

Neonatal human skin is taken very much for granted. It is so soft, so smooth, and so perfect! Unfortunately, until recently, there has been little systematic study of its structure and function. This volume summarizes previous experience as well as considerable new information.

The book starts with a complete description of the histology and ultrastructure of neonatal skin. Next, Drs. Green and Pochi delineate the structure and function of neonatal skin appendages: the eccrine and sebaceous glands. Noninvasive techniques utilized to determine aspects of skin function in the neonate are discussed in Chapters 4–9. The techniques include determination of transepidermal water loss, carbon dioxide emission rates, and oxygen diffusion. These chapters define not only the relevant physiology, but current information on the advantages and limitations of today's technology.

The next two chapters review knowledge of the quantitative aspects of percutaneous penetration in relation to current data in adults. Neonatal cutaneous microbiology with regard to normal and diseased skin is then summarized by Drs. Leyden, Aly, and associates.

The cutaneous dermatotoxicology section includes diaper dermatitis, allergic contact dermatitis in children, and the complex factors involved in diaper and plastic occlusion. In the final chapter, a concise overview of neonatal cutaneous disorders is provided.

Hopefully, this summary will stimulate further investigation. There is much to learn.

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1
Skin Structural Development

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I. INTRODUCTION

The structural development of human skin has been intensely studied and documented, first at the light, then the electron microscopy level (1). These early observations have now been correlated with molecular localization data, primarily using immunohistochemical techniques. The outcome has been an extremely well-defined understanding of structural development in human skin, which underpins the analysis of cutaneous disease. This structural framework can be used to incorporate the flood of new data from species-specific genome projects, expression analyzes using microarrays, and an abundance of functional data from animal models.

At present there is heavy reliance on the mouse model for analysis of skin development, because it can be genetically manipulated and because of restrictions on access to human fetal tissue. Important goals in human skin development research are to identify new structural and molecular markers and to use existing markers to compare precisely developing human and mouse skin (both temporally and regionally) so that murine data can be evaluated in human. Further development and authentication of human embryonic/fetal experimental culture models will serve the unique needs of the human structural developmental biologists, both experimentally and as a source of material for analysis of the human skin cells' transcriptome and proteome.

The aims of this chapter are to (a) summarize the very large body of data on human skin structural development, (b) compare these data to structural development in the mouse, and (c) highlight the areas of human

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skin development where questions are raised or which remain unexplored, usually in the light of data from animal experimental models.

II. DEVELOPMENT OF HUMAN SKIN

Skin development involves juxtaposition of tissues of mainly mesodermal/neural crest (body dermis/head dermis) and ectodermal (epidermal) origin. Experimental work from animals and human culture models shows that interaction between the two tissues is necessary for differentiation of epidermis to form the protective barrier residing in the stratum corneum and formation of skin appendages—hair follicles, glands, and nails. The structure of adult human skin is shown in Figure 1.

A single-layered epithelium covers the human embryo from gastrulation, and a cuboidal ectoderm overlaying an undifferentiated mesenchyme is present by 5 weeks of development (2). Subsequent skin development has been classified according to well-defined embryonic/fetal developmental stages (reviewed in Ref. 1) (Table 1; Fig. 2). These stages comprise:

1. The embryonic period (5–8 weeks)
2. The embryonic/fetal transition period (9–10 weeks)
3. The early fetal period (11–14 weeks)
4. The mid-fetal period (15–20 weeks)
5. The late fetal period (20 weeks to birth)

It is during the late fetal period that skin becomes functional, i.e., develops a protective barrier residing in the stratum corneum. Maturation of the stratum corneum is important for the health of the preterm infant, and it is expected that as morbidity and mortality rates fall due to improvements in treatment for lung immaturity, the status of preterm skin will become an increasingly important issue in treatment of the premature infant. A long-term goal in this field will be to develop an understanding of skin maturation sufficient to allow pharmacological manipulation of barrier function as now practiced for augmentation of preterm lung function.

Several authors have globally assessed human skin development using parameters such as epidermal height (4) and proliferative activity (5,6). These data permit interesting correlation of skin activity with fetal growth and environmental change. For example, the major increase in epidermal thickness is reported during weeks 5–13 (4), the period of organogenesis when rates of fetal size growth are slow (3). In contrast, epidermal height remains essentially unchanged during weeks 14–21, when there is substantial increase in size and skin surface area of the fetus, despite increase in cell

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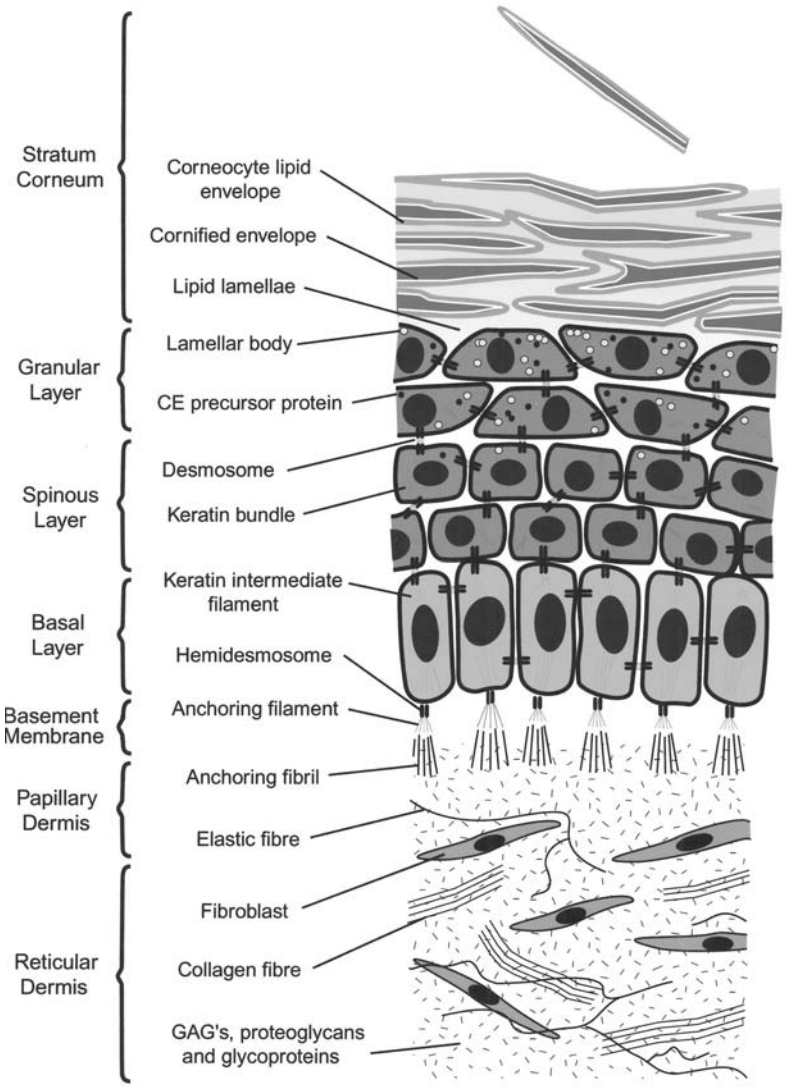

Figure 1 The structure of adult skin. Skin is composed of three separate layers: dermis, basement membrane, and epidermis. The epidermis can be subdivided into four further strata, each representing a specific stage during terminal differentiation.

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Table 1 Stages of Human Skin Development with Mouse Comparisons

Human skin development			Mouse skin development	
Stage	EGA (weeks)	Characteristics[a]	EGA[b] (days)	Characteristics[c]
Embryonic	5–8 weeks	Two-layered epidermis (basal layer and periderm)	8–11 days	Regional stratification to produce periderm
Embryonic/ fetal transition	9–10 weeks	Formation of an epidermal intermediate layer	12–14 days	Hair germ formation, stratification to form intermediate layer
		Induction of differentiation specific keratins (K1 and K10) in suprabasal epidermal cells	14–15 days	Induction of differentiation-specific keratins (K1 and K10)
		Dermal development		
Early fetal	11–14 weeks	Epidermal terminal differentiation, periderm forms surface "blebs"	15–16 days	Epidermal terminal differentiation
Mid-fetal	15–20 weeks	Epidermal terminal differentiation, periderm regression		
Late fetal	20–40 weeks	Stratum corneum formation, barrier function,	16 days	Stratum corneum formation, barrier function
		Periderm disaggregation	17 days	Periderm disaggregation
	40 weeks	Birth	20 days	Birth

EGA, estimated gestational age.

[a]From Refs. 11, 13, 24, 38.

[b]Murine estimated gestational age or time since detection of the vaginal plug (day 0.5).

[c]From Refs. 19, 35, 72, 73.

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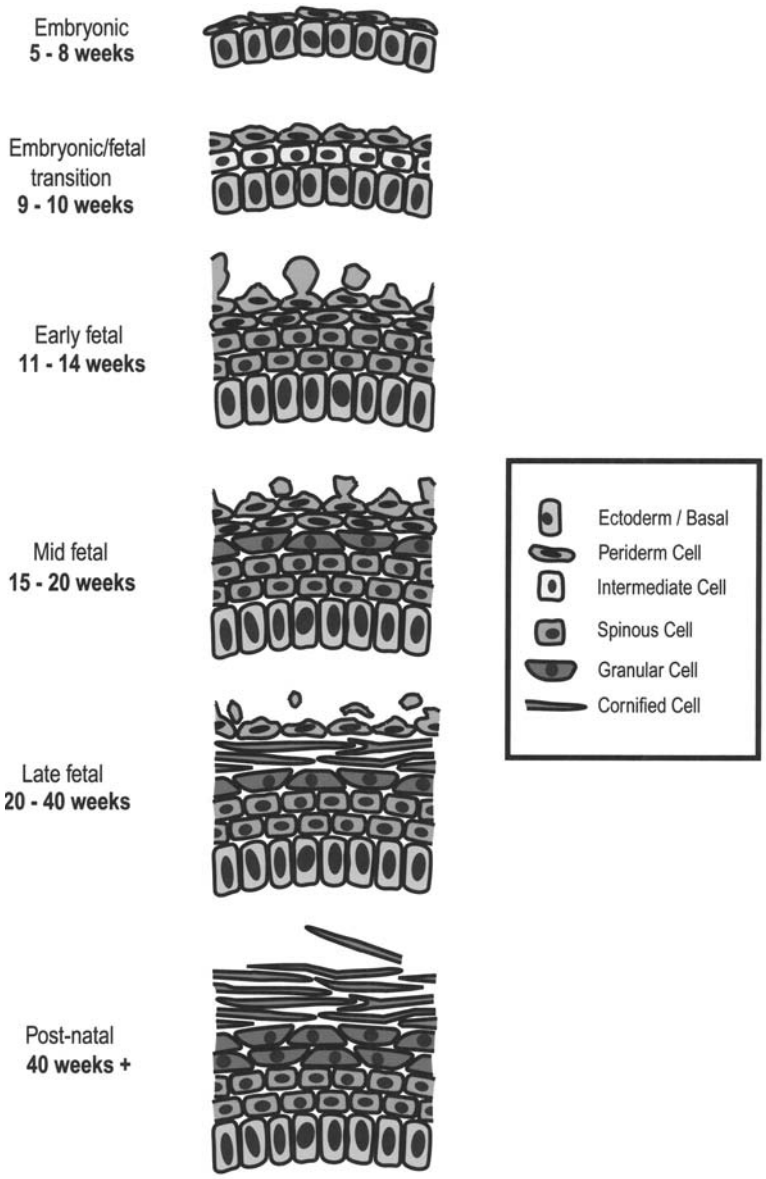

Figure 2 Schematic diagram of the six key stages of epidermal differentiation and periderm development. Epidermis develops from a single layer of undifferentiated ectoderm (5–8 weeks) to a multilayered stratified differentiated epithelia (40 weeks). Onset of terminal differentiation (the process where cells enter a defined programme of events culminating in formation of a functional stratum corneum) is triggered during the embryonic/fetal transition. In tandem, the periderm undergoes intense proliferation (11–14 weeks) and forms characteristic blebs and microvilli thought to be functionally important. This is followed by regression and eventual disaggregation (20–40 weeks). (Adapted from Ref. 42.)

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layers during this period (4). This period is marked by a decrease in the proliferative activity of epidermal cells (6). Foster et al. propose that an increase in surface area is achieved substantially by change in keratinocyte size and shape during this period.

The period of fetal growth deceleration (weeks 22–40) correlates with rigid stratum corneum formation (7,8). From this point expansion of skin is restricted due to reduced ability of stratum corneum to expand horizontally. Subsequent horizontal growth must originate via basal layer expansion and upward migration. It is possible that the requirement for rapid surface area increase preempts stratum corneum formation during earlier development.

It is tempting to speculate as to why the barrier forms so early in humans, even though this inhibits subsequent epidermal expansion. During mouse, rat, and rabbit fetal development, stratum corneum formation is delayed until several days before birth (9). One interesting theory is that the skin barrier activity is necessary early during human fetal development for protection from increasing levels of urine in amniotic fluid (10).

III. PERIDERM—THE EMBRYONIC SKIN LAYER OF UNKNOWN FUNCTION

The periderm is a transient epidermal layer that forms during the embryonic period (7,11) and is shed before birth, in conjunction with stratum corneum formation. The role of periderm is mostly unknown as mutants lacking periderm have not been identified. However, periderm forms the interface between the embryo and amniotic fluid, and during much of gestation its morphology, which consists of surface microvilli and ''bleb''-like protrusions, which increase surface area (Table 1; Fig. 2), suggests an interactive role with amniotic fluid. This has led to speculation that periderm nourishes the underlying ectoderm prior to vascularisation or has an excretory role (11–13). A role as an embryonic barrier is also probable and supported by the finding that periderm cells are sealed by tight junctions (14,15).

During much of development periderm cells express keratins normally associated with stressed or migratory epidermal cells (keratins 6/16/17) (16). In mice, migration and displacement of periderm cells is important in tissue fusions such as eyelid closure, thought analogous to migration of keratin 6/16/17–positive epidermal cells during wound healing (17). The increased migratory/displacement capacity of surface periderm cells may be important for major epidermal expansions and rearrangements during human development.

Periderm forms when surface ectodermal cells are still single-layered and undergoes a series of defined changes prior to regression

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and disaggregation (11,13) (Fig. 2). Initially, periderm cells proliferate, then later withdraw from the cell cycle, flatten, and increase in surface area. The cells are covered in microvilli but, in addition, develop surface blebs or protrusions which later crenulate (13) (Fig. 2). These latter changes result in further increased surface area, fueling speculation that the cells are interacting with amniotic fluid (13). Finally, the periderm cells regress, a process involving nuclear degradation and subsequent disaggregation from the fetal surface in conjunction with keratinization (13) (Fig. 2) (see below).

IV. EMBRYONIC PERIOD (POSTGASTRULATION EMBRYO TO 8 WEEKS)

During the embryonic period, the epidermis becomes two-layered, consisting of a proliferative basal layer and outer surface of proliferative periderm cells. Epidermal keratinocytes express keratins characteristic of simple epithelia (keratins 8, 18, and 19) (18). Stratification could result from cell migration from the basal layer or basal cell division. However, stratification is difficult to observe and analyze in human embryos as it occurs so early (embryos as early 36 days EGA have already stratified to form the periderm layer) (13). In contrast, periderm induction can easily be observed between 9 and 11 days in mouse embryos (Table 1) where periderm formation is highly regional (19).

During the early embryonic period there is no appreciable dermis and no distinction between dermis and underlying mesenchyme. Morphological development of human dermis has been thoroughly analyzed and reviewed by Smith et al (20). These authors used morphological criteria to divide dermal development during the embryonic period into two stages. Initially, the subepidermal surface is cellular and devoid of detectable fibrous material. The second stage, beginning at 2 months, is associated with the definition of the dermal-subdermal boundary by vascular development (20).

During these early cellular stages of dermal development, deposition of protein at the basal lamina separating epidermis and dermis can be detected. Type IV collagen and laminin are present by 5 weeks (21,22), indicating maturation of the basal lamina to a basement membrane. However, there are no hemidesmosomes; i.e., the structures that anchor keratinocytes to the basement membrane. The first traces of anchoring fibrils (type VII collagen fibrils which anchor epidermis to dermis) (Fig. 1) appear at 7–8 weeks (23).

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V. EMBRYONIC FETAL TRANSITION (9–10 WEEKS, 60–70 DAYS)

The embryonic-fetal transition period in humans was described first on the basis of major morphological and biochemical change (24,25). This period is significant during epidermal development in molecular terms and signifies a switch in developmental programmes to enter terminal differentiation. Terminal differentiation initiated here will eventually result in formation of the protective stratum corneum.

Embryonic ectoderm stratifies to form an intermediate layer at approximately 60 days EGA (24), followed by induction of keratins associated with differentiating keratinocytes (keratin 1 and keratin 10) (25,26). The intermediate layer is initially proliferative (6) and fundamentally dissimilar to adult suprabasal keratinoctyes.

During the embryonic-fetal transition period, there is associated maturation of the epidermal-dermal junction (basement membrane) (Fig. 1) and accelerated deposition of fibrous material and matrix in dermis (20,22,23). Anchoring filaments become more abundant (23) and hemidesmosomes are also now detected (23,27).

Hemidesmosomal integrin receptors, alpha 6 and beta 4 (Fig. 1), have been peri-cellularly expressed but following stratification become associated with hemidesmosomes at the basement membrane (27). Other epidermal adhesive structures, the desmosome and adherens junction, connect keratinocytes to each other and are located laterally and apically at sites of cell-cell contacts in mature keratinocytes (Fig. 1). Before the embryonic-fetal transition, protein components of desmosomes and adherens junctions are also located peri-cellularly (28). Relocation of these proteins to cell-cell contacts on the lateral and apical membranes accompanies the embryonic-fetal transition, and it has been proposed that polarization of basal keratinocytes into apical and basal compartments awaits maturation of the basement membrane (28).

It is during the embryonic-fetal transition period that nonkeratinocyte epidermal cells are detected (23). Epidermal melanocytes are derived from dorsal neural crest cells and migrate into the epidermis, then later to hair follicles. Melanocytes are reported at the embryonic-fetal transition period (23) [though they have been detected earlier (13)], as are Langerhan cells (epidermal antigen-presenting/ dendritic cells) that migrate to skin from the fetal thymus and/or bone marrow.

An additional nonkeratinocyte cell of the epidermis is the Merkel cell. Merkel cells are neurosecretory cells of the epidermal basal layer and dermis. They function as mechanoreceptors, enclosing the endings of slowly adapting type 1 afferent fibers (reviewed in Ref. 29). The origin of Merkel cells (epidermal or neural crest) was for many years the subject of debate,

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with the epidermal origin hypothesis being now generally accepted due to identification of keratins and desmosomes in Merkel cells [keratin 20 can distinguish epidermal Merkel cells from keratinocytes (30)] and developmental studies showing that Merkel cells appear first in the epidermis during development, then are later detected in dermis (30,31). Merkel cells have been detected as early as week 8 of human development (32,33).

The murine equivalent of the human embryonic-fetal transition occurs over 2–3 developmental days (Table 1) with strong similarities to human development. Murine epidermis undergoes similar stratification to form a proliferative, intermediate layer which expresses basal cell-specific keratins up to embryonic day 14, again indicating that this intermediate layer differs from a true spinous cell (19). Stratification in mouse is achieved through cell division, rather than migration (34). Expression of differentiation-specific keratins is delayed until embryonic day 15 (19,35) (Table 1). Stratification and keratin induction are highly regional in the mouse embryo, and similar regional stratification has been reported in the human infant (11), complicating the comparison of data from different studies.

The human embryonic-fetal transition period may be modeled by induction of keratinocyte terminal differentiation during culture of isolated adult skin keratinocytes, through either calcium induction or serum withdrawal. Regulation of keratinocyte terminal differentiation is an area of very intense interest (reviewed in Refs. 36,37) and it is probable that molecular signaling pathways identified in adult keratinocyte culture models will be relevant to the molecular changes during the embryonic-fetal transition at 9–10 weeks of human development.

VI. EARLY FETAL PERIOD (11–14 WEEKS, 70–100 DAYS)

The most conspicuous feature of the early fetal period in humans is the appearance of hair germs at 12 weeks, reported to occur in a cephalocaudal direction (38,39).

The mouse does not provide an ideal model for either the early or late fetal period, both of which are extended in the human and truncated in the mouse (Table 1). In addition, murine follicle development, which closely resembles human follicle development (reviewed in Ref. 40), occurs relatively earlier (Table 1), demonstrating that follicular development can be uncoupled from interfollicular development in mammals. This uncoupling has later developmental consequences (see below, stratum corneum formation).

At the epidermal-dermal junction there are abundant hemidesmosomes and anchoring fibrils (22). The density of cell-cell junctional com-

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plexes (desmosomes, adherens junctions, and gap junctions) increases (28).This is a period of dermal maturation marked by increasing fibrous and matrix deposition by dermal cells (22), and by the end of this period (100 days) adipocytes are present in the dermis (41).

VII. MID-FETAL PERIOD (15–20 WEEKS)

The mid-fetal period is marked by increased epidermal stratification and further maturation of hair follicles and additional appendages such a sweat glands and nails. Again, this period is truncated in the mouse. During the lengthy early and mid-fetal periods in human, changes in periderm morphology are among the most noticeable in human skin development (13) (Table 1; Fig. 2).

The epidermis has true spinous layers rather than an embryonic intermediate layer and a granular layer (Fig. 2). At about 18 weeks the first sign of stratum corneum can be detected in the head region associated with the more mature hair follicles of the head (38,39). Stratum corneum formation is also accelerated over the nail bed, another type of epidermal appendage (42). Association of keratinization with hair follicle development does not occur in the mouse, except when hair follicle development is precocious, such as in the murine whisker pad and in association with nails (8). This presumably reflects the relatively late development of body hair in the mouse.

VIII. LATE FETAL PERIOD (20–40 WEEKS)

A. Stratum Corneum Formation (Keratinization)

Human stratum corneum forms between 20 and 24 weeks (8,13,42). Stratum corneum formation is regional, being accelerated on the scalp, face, and plantar epidermis of foot (sole region) (42). Association of keratinization with hair follicles in human means that there is regional acceleration of stratum corneum formation in association with the accelerated follicles of the head (38).

In mouse (and additional mammals) stratum corneum induction is not linked with hair follicle formation, due to the relatively late development of follicles (8). A new type of regional stratum corneum development occurs, associated with skin initiation regions and apparent moving fronts of epidermal terminal differentiation (9). This type of regional stratum corneum formation is conserved in the human fetus (Fig. 3), although the pattern is complicated by follicle-associated keratinization.

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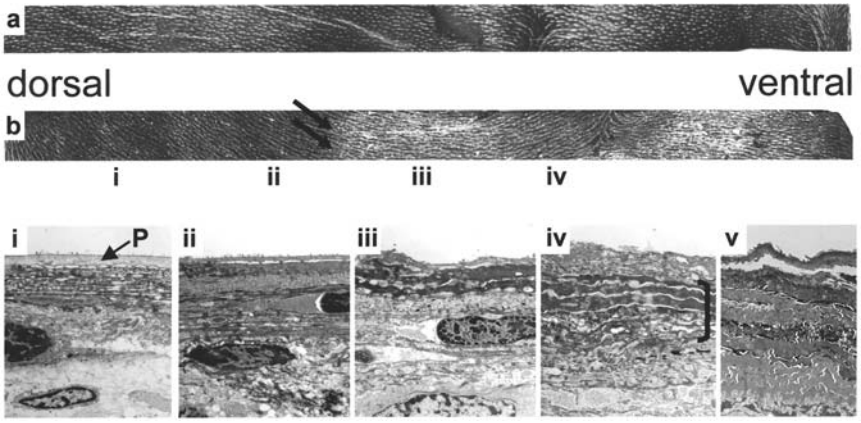

Figure 3 The epidermal permeability barrier is induced regionally in the human infant between 20 and 24 weeks. (a) At 19 weeks a strip of skin from the torso of a preterm infant lacks barrier function (indicated by a blue result in the dye permeability assay). (b) However, 2–3 weeks later distinct barrier positive regions appear (white areas) and are propagated around the body as a ventral to dorsal wave (arrows). (i–iv) Specific ultrastructural changes accompany barrier induction. (i,ii) Immature epidermis, prior to barrier formation, retains periderm (P) attached to the outer epidermal surface. (iii,iv) Interfollicular epidermis from regions with barrier has prominent stratum corneum (bracket) but has yet to achieve the thickness of adult (v.) (Adapted from Ref. 8.)

A useful model of stratum corneum structure, the ''bricks and mortar'' model, was proposed by Elias in 1983 (43). The ''bricks'' represent flattened anucleate corneocytes (the results of keratinocyte terminal differentiation) with a 15 nm thick cornified envelope and outer ceramide capsule or corneocyte lipid envelope (reviewed in Ref. 44), while the ''mortar'' consists of a heterogeneous mixture of predominantly nonpolar lipid, arranged to form a complex lamellar bilayer structure (reviewed in Ref. 45). The high degree of interdigitation and sheet-like nonpolar intercellular lipid make mature stratum corneum virtually impenetrable to water molecules and presumably confers much of the barrier qualities of the stratum corneum.

Stratum corneum is formed from multiple precursor proteins synthesized from week 18 onwards. In the mouse, key cornified envelope precursors are initially sequestered in keratohyalin granules (46), although these structures are far less prominent and significantly smaller in human epidermis (47). In human, most precursors are diffusely expressed throughout the cell (Fig. 1). Cornified envelope construction initiates adjacent to the plasma membrane and involves sequential transglutaminase-mediated crosslinking of protein and lipid components.

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The precise timing and sequence of envelope precursor incorporation has been the subject of intense study over recent years, mostly using murine and human keratinocyte culture models (reviewed in Ref. 48). The strong conservation between models suggests that the results are applicable to the fetal human. Briefly, as intercellular calcium levels rise, envelope scaffold proteins (envoplakin, periplakin, and involucrin) move to the plasma membrane and are cross-linked via membrane-bound transglutaminase 1 enzyme. These and other minor proteins form the initial envelope scaffold, a continuous cross-linked layer. In tandem with initial scaffold formation, cross-bridging proteins (e.g., small proline rich region proteins) in the cytoplasm become cross-linked by the cytoplasmically localized transglutaminase 3 enzyme to from small oligomers. These subsequently bind cornified envelope precursor molecules (such as loricrin) and are incorporated into the envelope (reviewed in Ref. 48). Relatively late in envelope formation the keratinocyte matrix protein, filaggrin, is proteolytically processed, binds keratin filaments internally, and attaches to the outer envelope surface (49). A final family of cornified envelope precursors are expressed in epidermis just prior to barrier formation [XP5/LEP proteins (50,51)]. These late incorporated proteins may confer subtle changes in envelope properties and barrier quality.

In parallel with cornified envelope formation, extrusion of extracellular lipid and deposition of a lipid-bound envelope are key steps in stratum corneum formation. These lipids derive from abundant lamellar bodies synthesized in early granular layer keratinocytes. The contents of lamellar bodies, nonpolar lipids and lipid-processing enzymes, are extruded into the extracellular space (52) and rearranged into the lamellar sheets characteristic of mature stratum corneum. The lipid capsule forms around the extracellular (or inner) surface of the mature cornified envelope. Once again transglutaminase 1 crosslinks the lipid capsule, a key step in forming a water tight barrier (53,54).

B. Skin Barrier Formation (20–34 Weeks)

An assumption based on early tape-stripping experiments, which removed part or all of the stratum corneum followed by barrier activity assessment, has been that all barrier activity resides in the stratum corneum. This view is bolstered by gene knockout mice where knockout of transglutaminase 1 (55) or transcription factors that regulate cornified envelope proteins and lipids (56) results in loss of barrier function leading to neonatal death. However, a recent mouse knockout of tight junction component claudin-1 suggests that granular layer tight junctions are essential for barrier activity (57). In both fetal humans and mice tight junctions appear in periderm cells, then by the

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time of stratum corneum development and periderm disaggregation [22 weeks over most of the body (42)], tight junctions appear in the upper granular layers (14,15).

Other epidermal adhesive complexes, the desmosomes, may have a role in barrier function. Mice with altered desmosomal cadherins (the adhesive proteins of the desmosome) can display barrier defects (58,59).

In situ permeability assays, which measure an extremely early stage in barrier formation, show that barrier forms regionally in the human infant between 20 and 24 weeks gestation (8) (Fig. 3). The barrier appears either at the sites of hair follicle formation (in association with stratum corneum formation) or at initiation sites and then propagates outwards to cover the entire body. In the human infant, the barrier forms first around the head, face, and neck at 19–20 weeks, then several weeks later over the abdomen, followed by the back (8). Barrier formation correlates with an epidermal gradient of differentiation (Fig. 3).

Interestingly, lipid extrusion and cornified envelope development, i.e., initiation of stratum corneum formation, actually precede barrier formation. Initial barrier formation correlates with several changes in the upper epidermal cell layer (8) (Fig. 3). These upper layer keratinocytes adopt a flattened electron-dense phenotype, forming a single stratum corneum precursor layer exactly at the site of barrier initiation. Formation of additional stratum corneum layers will contribute to further barrier acquisition.

Subsequent barrier maturation can be monitored using an evaporimeter (60) to directly quantify transepidermal water loss (TEWL). Evaporimeter studies report a gradual maturation in skin barrier between 26 and 32 weeks (61–63). Barrier levels necessary for postnatal survival are reached several weeks before birth (64).

This feature of late gestation epidermis produces marked regional differences in the barrier function of preterm infant skin, which should be taken into account when managing treatment.

C. Periderm Disaggregation

Periderm undergoes terminal differentiation, including a type of ''keratinization,'' in tandem with underlying embryonic epidermis prior to disaggregation (termed ''regression''). The regressing cells form cornified envelopes and express other markers of terminal differentiation (65,66). If the periderm's role is interactive or protective, then it will be redundant when the underlying epidermis forms its protective barrier. Interestingly, periderm regression coincides with formation of a functional barrier by the underlying epidermis at approximately 22–25 weeks (8,13). In mice, peri-

derm dissociates in a similar pattern to the pattern of barrier formation over the surface of the animal (8), suggesting a link between stratum corneum maturation and periderm release and supporting the idea that periderm had an interactive or protective role prior to epidermal barrier formation.

IX. CONCLUSION

The wealth of detailed descriptive data on human development provides a resource for exploitation of new gene expression and functional data arising from genome projects and animal models, provided that human development can be equated with animal or culture experimental models. In this review, emphasis has been placed on comparing human development with the mouse, using the rationale that the mouse, because of its susceptibility to genetic manipulation and similar physiology and biochemistry to human, is the most likely model for advancing human developmental research. This view was also taken because of the likely success of the large-scale mutagenesis programs currently underway in the mouse model (67,68) and the probability that they can yield mutant phenotypes relevant to specific stages of skin development.

This comparison, however, shows that while the mouse will provide a good, accessible model for the embryonic period and embryonic-fetal transition, the acceleration of late gestation development in the mouse does not equate well temporally with the extended period of epidermal terminal differentiation in the human. Preterm mice cannot survive and provide a good model for barrier maturation in the very preterm infant. In addition, we highlight in this review how the differences in synchronization of hair follicle formation and interfollicular terminal differentiation in human and mouse affects the nature of epidermal differentiation and barrier formation. Despite these difficulties mouse models are providing a rich resource for understanding developmental change in the human.

The differences between human and mouse mean that human culture models may, in part, provide the answer for researchers hoping to advance basic skin biology. Although acquisition of material is restricted by ethical considerations, particularly for the important late gestation period, there has been considerable advance in generation of developmentally authentic organ cultures for human embryonic-fetal skin (26,27,69,70).

In this chapter we highlight the regional nature of skin development in human and mouse. Regional development [e.g., hair follicle formation (38,39), onset of keratinization (11,71)] was well established prior to discovery in the animal models (9). Regional modes of developmental change

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probably reflect a basic developmental process and are important in their own right, but regional change also complicates comparison of developmental status between laboratories and also between mutant or diseased individuals and healthy controls. We conclude that rather than relying simply on estimated gestational age when making skin developmental comparisons, the way forward will be to compare samples for both morphological and biochemical markers, taking into account the anatomical region from which the samples derive. Human skin development research should now reap the benefits of many years of painstaking and meticulous morphological analysis.

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2
Microbiology

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I. INTRODUCTION

The skin is a multipurpose protective barrier for the developing neonate. Among the principal functions served by this barrier is prevention of infection. The dynamic balance between cutaneous colonization and infection is incompletely understood, but involves interplay between mechanical, biochemical, physiological, and immunological properties of the skin and characteristics of the microorganism such as ability to attach, survive, and replicate in the local environment and to express virulence factors necessary for causing infection (Table 1) (1). In the neonatal period, this balance of factors assumes particular importance, as is highlighted by the increased susceptibility of preterm very low birth weight (VLBW) infants to invasive infections due to organisms of typically low pathogenicity, through a cutaneous portal of entry (2,3).

Approximately one fourth of all VLBW infants weighing less than 1500 g in the United States who survive beyond the third day of life have at least one episode of sepsis (4). In developing countries the incidence of sepsis and death from sepsis in premature infants is even higher; septicemia is the single most important cause of death, accounting for up to 50% of preterm neonatal mortality (5,6). Globally, it is estimated that approxi-

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Table 1 Primary Factors Regulating Infection of Human Skin

- Skin Factors
  - Stratum corneum characteristics
    - Xeric environment
    - Acidic pH
    - Mechanical barrier: cell envelope
    - Antibacterial properties (see below)
    - Constant turnover, corneocyte shedding
  - Skin trauma: disruption of stratum corneum barrier, exposure of ligands for bacterial attachment
  - Presence and balance of commensal flora
  - Production of antimicrobial substances (epidermis and dermis)
    - Cationic antimicrobial peptides [e.g., LL-37, human β-defensin-2, secretory leukocyte protease inhibitor (SLPI; antileukoprotease), skin-derived antileukoprotease (SKALP; elafin)]
    - Epidermal lipids (e.g., free fatty acids, phospholipids, glycosphingolipids, such as spingosine, sphinganine, hydroxysphinganine)
    - Granzyme B
    - Fas ligand proteins
    - Hydrogen peroxide
    - Nitric oxide
  - Immunological barrier
    - Production of primary epidermal cytokines (e.g., TNF-α, Il-1α,IL-6, IL-8, IL-12)
      - Inhibition of group A streptococcal adherence (TNF-α, IL-1)
      - Chemotaxis, immunoregulation
    - Langerhans cells: antigen presentation; elaboration of cytokines, cellular adhesion molecules, chemotactic factors
    - Keratinocytes: elaboration of cytokines, cellular adhesion molecules, chemotactic factors, internalization and killing of bacteria
    - Cationic antimicrobial peptides (epidermal and dermal)
- Host Factors
  - Skin barrier and local tissue compromise
    - Chronic dermatoses
    - Skin trauma
    - Foreign body
    - Peripheral vascular disease
    - Host exposure to antibiotics (changes skin flora)
  - Systemic immune compromise
    - Corticosteroid therapy
    - Immunodeficiency disease (particularly involving neutrophils)
    - Malnutrition
    - Diabetes mellitus

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Table 1 Continued

Environmental Factors
Humidity
Temperature
Bacterial Factors
Inoculum size
Growth phase and propensity
Attachment
Virulence factor expression
Synergism with other bacteria

Source: Modified from Ref. 1.

mately 350,000 neonates die yearly from serious invasive bacterial infections (6); approximately half of these deaths occur in the first week of life when epidermal barrier function is most highly compromised.

This chapter will review the development of the neonatal microbiological milieu and its implications in health and disease.

II. SKIN COLONIZATION

Colonization of newborn skin begins upon first exposure to the xeric extrauterine environment. The skin of babies born by Caesarian section, for example, is typically sterile if the amniotic membranes were intact prior to onset of labor. Infants born vaginally, however, become colonized during descent through the birth canal (7) or the process may even begin in utero, following bacterial ascent beyond prematurely ruptured membranes, or, on rare occasions, following penetration of organisms such as *Candida albicans* and group B streptococcus through intact amniotic membranes (8,9). *Staphylococcus epidermidis*, one of 13 species of coagulase-negative staphylococci, is the most common vaginal organism just before birth, is ubiquitous in the environment, and takes up residence immediately as the predominant organism on the skin of most neonates (10–16). Other organisms, such as *Malassezia* spp. and *Proprionibacteria*, soon follow as the skin barrier matures (17). Under hygienic conditions, the resident flora resembles that of adults after the first few weeks of life (Table 2) (18). These skin-colonizing flora typically are low in virulence, stable in number and are infrequent skin pathogens. Commensal bacterial flora normally play a protective role (19), and recent evidence suggests that certain of these organisms (e.g., *S. epidermidis*) are capable of upregulating keratinocyte expression of

Table 2 Skin-Colonizing Flora

Micrococcaceae
Coagulase-negative staphylococci
Staphylococcus epidermidis
Staphylococcus hominis
Staphylococcus saprophyticus (perineum)
Staphylococcus capitis (sebum-rich areas)
Staphylococcus auricularis (ear canal)
Peptococcus spp.
Micrococcus spp.
Diphtheroides
Corynebacterium (moist intertriginous area)
Corynebacterium jeikeium (multidrug resistant)
Brevibacterium (toe webs)
Propionibacterium (hair follicles, sebaceous glands)
Gram-negative rods
Acinetobacter (moist intertriginous areas, perineum)
Rarely *Klebsiella*, *Enterobacter*, *Proteus*
Yeast
Malassezia spp.

Source: Modified from Ref. 18.

cationic antimicrobial peptides (e.g., human β-defensin-2) and may play a role in priming the skin for challenge with pathogens (20–22).

Before the normal flora becomes established, however, neonates are at risk for colonization and infection by pathogens, most notably *Streptococcus pyogenes* and *Staphylococcus aureus*, as well as gram-negative bacilli (23). *S. aureus* generally is not present on the skin of healthy newborns in developed countries, but neonates may readily become colonized in neonatal wards, in situations of poor hygiene and in association with eczematous dermatitis (24–36). Alterations in the ecology of the resident flora due to changes in temperature or humidity or by antibiotic administration may also be associated with colonization and/or infection of the skin, especially by *S. aureus* and *S. pyogenes* (19).

Skin colonization has been shown to predispose to neonatal staphylococcal infections and may lead to nursery outbreaks. Superficial skin lesions in the newborn nursery should also serve as a harbinger for a potential infection control problem and prompt immediate measures to isolate affected infants, identify the infectious source, and treat the infection. The attendant's hands and nares are particularly important sources for coloniza-

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tion of neonatal skin. As a result, hand-washing is the single most effective way to prevent skin colonization and avert nursery outbreaks.

III. MECHANISMS OF BACTERIAL ATTACHMENT AND CUTANEOUS INFECTION

The factors governing colonization and the events leading to infection of skin are manifold, complex, and poorly understood (37–40) (Table 1).

A. Vernix

At birth, vernix caseosa appears to provide insulation and protective functions, possibly including enhancement of epidermal barrier function and development; protection from trauma and hypothermia; and promotion of wound healing, which may result in decreased risk for infection. Vernix does not appear to have intrinsic antibacterial properties (41–44), however, and its effect on bacterial colonization of the skin is not known. Thus, it may act principally as a mechanical barrier and may promote skin barrier integrity by optimizing hydration of the stratum corneum (41,42,45,46). Thus, the best practice is to leave vernix on the skin and allow it to slough off naturally (47,48). Extension of this principle has led to investigations of the impact of topical therapy with skin barrier-enhancing formulations on colonization and risk of infections in preterm infants (2,49–52) (see Sec. VI.D).

B. Role of Cutaneous Injury

In healthy term neonates, pathogenic organisms encounter a high degree of resistance to colonization and cause infection only in the presence of a disrupted skin barrier, although the cutaneous injury may be imperceptible. The mechanism by which cutaneous injury predisposes to infection is not known. Injury of cultured keratinocytes does not alter adherence (39), suggesting that injury to corneocytes rather than keratinocytes likely is of primary importance in facilitating bacterial attachment, perhaps by exposing ligands used by bacterial adhesions for attachment. Adherence of group A streptococci to keratinocytes increases with keratinocyte differentiation, perhaps due to increased expression of ligands for attachment on upper epidermal keratinocytes (37). These ligands may only be accessible, however, following disruption of the stratum corneum. This phenomenon may also explain the subcorneal localization of streptococcal

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impetigo to the most highly differentiated layers of epidermal keratinocytes (37,53).

C. Bacterial Adherence and Infection

Once the cutaneous barrier has been bridged and bacterial attachment has occurred, a combination of bacterial virulence factor expression and host immunological factors determines the extent of bacterial multiplication and subsequent infection, as well as the clinical manifestations of the infection. Proper modulation of bacterial virulence factor expression may be critical for establishment of infection. The presence of hyaluronic acid capsule on the surface of *S. pyogenes*, for example, impedes attachment to keratinocytes (40) and phagocytosis by neutrophils (54,55) and is associated with tissue invasiveness (55–57). Inhibition of capsule production, on the other hand, is associated with markedly increased adherence to keratinocytes (40). Thus, for attachment to occur, it appears that hyaluronic acid capsule expression must first be downregulated, as occurs when *S. pyogenes* enters stationary phase growth (58). Then, once a nidus of infection has been established, the bacteria must be capable of logarithmic phase growth and upregulation of hyaluronic capsule expression, which enables the bacteria produced to evade both attachment on keratinocytes and neutrophil defenses while expressing other virulence factors that facilitate penetration of host tissue. In this way the invasive bacteria may remain in and penetrate through extracellular spaces, since internalization of bacteria by keratinocytes following attachment leads to bacterial death (37,59). Expression of other cell-surface proteins (e.g., group A streptococcal M-protein) (60,61) as well as elaboration of extracellular products (e.g., group A streptococcal secreted cysteine protease, also known as streptococcal pyrogenic exotoxin B, SpeB) (62) has also been associated with tropism for infection of the skin. Identification of the molecular mechanisms of attachment and the factors that facilitate initial bacterial replication in the skin will provide the foundation for development of novel preventative and, possibly, curative therapies.

D. Cutaneous Host Defense

Once pathogenic bacteria have adhered to the skin, they must overcome several avenues of host defense before infection can develop (Table 1). Intact, overlapping cells of the stratum corneum provide the first and foremost mechanism of defense. In addition to its role as a mechanical barrier, the dry, acidic environment of the stratum corneum is inhospitable for bacterial growth. Breakdown products of the stratum corneum, including free fatty acids, polar lipids, and glycosphingolipids, have antibacterial

activity (63). Many of the resident flora, particularly the lipophilic corynebacteria, release lipases and thus contribute to defense by liberating fatty acids from the triglycerides of sebum (64). The acid mantle thus created favors growth of Propionibacteria, which in turn produce propionic acid; this compound has relatively more antimicrobial activity against transient organisms such as *S. aureus* and *S. pyogenes* than against resident flora. Cationic antimicrobial peptides elaborated in the skin exert direct antimicrobial effects on the bacterial cytoplasmic membrane and also link innate and adaptive (specific, antigen-dependent) cutaneous immune responses. Recent reports demonstrate the functional importance of the cathelicidin family of antimicrobial peptides, including human LL-37, in innate cutaneous immunity against group A streptococcus (65–67). Another cationic antimicrobial peptide of potential importance is human β-defensin-2; the ability of commensal bacterial species such as *S. epidermidis* to induce its expression on the one hand and tolerate its antimicrobial activity on the other may enable the commensal to regulate cutaneous flora as well as prime the epidermis for challenge with pathogens which are relatively more susceptible to the antibacterial effects of the peptides (21,22,68). The skin's immune system, including antigen presentation by epidermal Langerhans cells and cytokine production by keratinocytes, plays a key role in defense against cutaneous infection (38,69,70). Cytokine production in the skin [i.e., tumor necrosis factor (TNF)-α, interleukin (IL)-1] may serve directly in antimicrobial defense, for example, by inhibiting adherence of *S. pyogenes* (38), in addition to activating inflammatory immune defenses (69,70). Moreover, internalization of bacteria by keratinocytes, leading to bacterial death and containment of infection, may be a previously unrecognized form of epidermal defense (37,59).

E. Cutaneous Signs of Infection

Clinical presentation of cutaneous perinatal and neonatal bacterial infections, their propensity for development of systemic sequelae, and treatment consideration are complicated by the developmental stage of the infant when infection is acquired [i.e., early (first or second trimester) or late (third trimester) in gestation, early (first few days) or late (2–8 weeks) in postnatal life] and the manner in which inoculation occurs (i.e., congenitally, at the time of birth via an infected mother, or postnatally).

Signs of infection in the skin may develop as a direct result of bacterial factors (e.g., cytotoxicity), the immunological response to the presence of the bacteria (71,72), or both. Streptolysin O from *S. pyogenes*, for example, has cell- and tissue-destructive activity (73). Certain strains of *S. aureus* and *S. pyogenes* are capable of elaborating exotoxins from a site of infection and

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causing disease directly (e.g., proteolytic activity of staphylococcal epidermolytic toxin on desmoglein I within desmosomes) (74) or through release of other biologically active mediators such as cytokines. In scarlet fever, exotoxins cause the rash by exerting cytotoxicity as well as stimulating Arthus and delayed hypersensitivity skin reactions. Streptococcal pyrogenic exotoxins A (SpeA) and/or B (SpeB), as well as certain M-protein fragments of *S. pyogenes*, and the toxic shock syndrome toxin-1 of *S. aureus* also have the ability to act as superantigens and interact simultaneously with the major histocompatibility complex class II antigen on antigen-presenting cells and specific Vβ regions of T-cell receptors, leading to massive synthesis and release of cytokines (75). In hosts lacking toxin-neutralizing antibiodies, production of cytokines may mediate systemic signs of toxicity seen in toxic shock syndrome, and TNF may mediate, at least in part, the rapid, massive tissue destruction seen in streptococcal necrotizing fasciitis (76,77). A central feature of the pathology in necrotizing fasciitis, as in many other necrotizing soft tissue infections, is vascular injury and thrombosis of arteries and veins passing through the fascia (78). Possible mechanisms for the vascular injury leading to tissue ischemia and necrosis in streptococcal nectotizing fasciitis are direct cytolytic factors released from bacteria, immune-mediated vascular damage due to the inflammatory infiltrate surrounding the blood vessels, and/or noninflammatory intravascular coagulation.

F. Bacterial Synergism

Some skin infections appear to be caused by two or more organisms that act synergistically. Synergism occurs when a mixture of organisms causes a more severe infection than the sum of the damage caused by each of the organisms acting alone. Synergism does not appear to be a factor in most superficial skin infections. In many instances of necrotizing soft tissue infection, however, synergism is operative (79–81) and may involve a variety of anaerobic, aerobic, and facultative bacteria. Mechanisms of synergy are not well understood but may involve such factors as mutual protection from phagocytosis and intracellular killing; promotion of bacterial capsule formation; production of essential growth factors or energy sources; and utilization of oxygen by facultative bacteria, lowering host tissue oxidation-reduction potential and facilitating growth of anaerobes (82,83).

IV. IMPACT OF PREMATURITY

The stratum corneum or outer skin layer achieves functional maturity by 32–34 weeks estimated gestational age (10,24,84–88). A developmentally

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immature and functionally incompetent epidermal barrier, a large surface area to body mass ratio, absence of vernix caseosa, and developmental defects in systemic immune function, and, possibly, in innate cutaneous defenses, place preterm infants, particularly those less than 34 weeks gestational age, at relatively high risk for cutaneous and invasive bacterial and fungal infection (84–89). Mechanical disruption of fragile premature skin during handling and medical procedures coupled with disordered cutaneous immunoregulatory function in association with barrier perturbation (84–87) may add further to the morbidity and susceptibility to infection associated with an immature epidermal barrier.

Invasion of opportunistic fungi, including *Candida albicans* and *Aspergillus* spp., through the epidermis of preterm VLBW infants illustrates the tenuous nature of their skin barrier (3,9). In preterm VLBW infants in developed countries, *S. epidermidis* is both the major cutaneous colonizer and the major agent of sepsis (90). While presence of an indwelling catheter is the major risk factor for coagulase-negative sepsis, bacterial penetration might also occur through the immature epidermal barrier at other sites of skin injury, although this is unproven. This is particularly plausible in developing countries, where the most frequent isolates from the skin (27,28,91, 92) and blood (6,93–99) of septic neonates are often relatively virulent organisms. Moreover, entry into, and in some cases transcytosis through epithelial cells has been demonstrated for several gram-negative and gram-positive bacteria (37,59,100–107).

V. DIAGNOSIS OF SKIN INFECTION

Cultures taken from the surface of a wound or site of infection may yield organisms that are colonizing or infecting the skin. The likelihood that an organism recovered from cultures of skin swabs is playing a pathogenic role is increased, however, if the organism is found on both Gram stain, or special stains in the case of fungi, as well as culture, particularly if the culture shows pure growth of a single organism. Chances of identifying the true pathogen(s) in culture are enhanced if exudate is obtained directly from the source of suppuration, by fine-needle aspiration, or by biopsy. Specimens from abscesses and subcutaneous tissue infections should be cultured for both aerobic and anaerobic organisms. Specimens should be cultured on blood agar, chocolate agar, and/or MacConkey's agar to identify the full range of pyogenic, fastidious, and gram-negative enteric organisms that may infect the skin and subcutaneous tissues. Isolation of anaerobic organisms can be accomplished by use of anaerobic media such

as Brucella agar supplemented with vitamin K_1 and hemin, and brain heart infusion broth or thioglycolate broth.

Diagnosis of some skin infections is aided by observing characteristic histopathological findings (e.g., bullous impetigo). Additional studies on biopsy material, such as immunofluorescence testing and electron microscopy, may also be useful in certain situations, particularly in excluding noninfectious diseases that may closely mimic a given skin infection (108).

In some cases, proper diagnosis and treatment mandates that one define the depth of the infection. For example, in the case of soft tissue infection, magnetic resonance imaging may be useful as an adjunctive to surgery in distinguishing cellulitis from necrotizing fasciitis or myositis (109).

Antigen detection or immunological tests have little utility in the management of skin infections. Antideoxyribonuclease B titers, however, may aid in confirming previous infection with *S. pyogenes* and may be useful in reaching a diagnosis of impetigo-associated poststreptococcal glomerulonephritis (110).

VI. CARE PRACTICES THAT IMPACT THE CUTANEOUS FLORA

Despite the importance of maintaining and promoting skin integrity and barrier function, few guidelines have been published regarding optimal care of neonatal skin in order to reduce the risk of skin and invasive infections (47,48,111). Aspects of care that are particularly important include caregiver hygiene, especially hand-washing; routine bathing and skin care to prevent skin irritation and injury; care of the umbilical cord stump; and preparation of the skin prior to invasive procedures that have the potential to introduce infectious organisms into the skin or deeper tissues and the bloodstream.

A. Caregiver Hygiene

The hygienic practices of caregivers are particularly important, as attendants' hands are the most common source of colonization of neonatal skin. Hand-washing with a mild, alkaline to neutral pH, nonantimicrobial skin cleanser can help prevent colonization and nosocomial transmission (111). Chlorhexidine is the preferred agent for bacterial decontamination of hands, although 10% povodone-iodine is advantageous for elimination of *Candida* spp. (112). Gowning does not appear to alter colonization or infection rates (113).

Like bacteria, *Candida* species also transiently colonize the hands of health care workers (114). *Candida* species rapidly colonize the skin and

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mucous membranes of about 60% of critically ill neonates and can progress to invasive infection (115). Fungal disease can occur in the absence of colonization of neonatal skin, however, suggesting that other factors also play a role in neonatal candidiasis (116).

B. Bathing

Bathing newborns has many potential hygienic and cultural benefits and, therefore, is a routine practice in most nurseries. Nevertheless, bathing newborns after birth is unnecessary and may be harmful (117–119), particularly in the first few hours after birth when dramatic physiological changes are occurring and the risk of hypothermia is accentuated. Delayed bathing of newborns beyond 6 hours of life is recommended by the World Health Organization, especially in high-risk settings such as encountered in many developing countries (120,121).

Bathing may compromise skin defense by promoting removal of vernix, particularly in preterm infants in whom the layer is underdeveloped and scant to absent to begin with. Bathing with soap generally is unnecessary, given the low level of sebaceous gland output of the neonate, and may increase bacterial counts on the skin (122,123). Bathing with soap, moreover, may also be detrimental by inducing changes in skin pH. The pH of the skin surface of both term and VLBW preterm infants is alkaline at birth (e.g., 6.5–7.5) and declines rapidly over the first week of life and more gradually during the remainder of the first month of life to reach values comparable to those of adults (e.g., pH 4.0–5.5) (124,125). Washing with an alkaline soap (pH 10) increases skin pH (e.g., 2.5 pH units) and requires an hour or more in term infants and up to several days in preterm infants to return to baseline values (126–128). In contrast, use of a neutral detergent changes skin pH insignificantly and only briefly (i.e., less than 1 hour). Rise in skin pH may be associated with qualitative and quantitative changes in the cutaneous flora, as skin antibacterial effect is optimal at pH values below 5.0 (124), and with increased risk for diaper dermatitis (129). Soaps may also cause irritation by removal of epidermal barrier lipids (130); the degree to which they are irritating is a function of alkalinity and length and frequency of use. ''Baby soaps'' may offer some advantages, as they generally lack antimicrobials, fragrances, or abrasives. There are no reports, however, comparing the relative tendency of different baby care products to dry or irritate the skin or their impact on skin pH in neonates.

Despite the potential harmful effects of bathing, routinely bathing neonates with mild soap has not been shown to significantly impact rates of skin colonization or infection. Meduras randomized 141 infants to receive either a water bath or mild pH soap with water following birth and found no

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difference between the groups in either rates of colonization of the anterior fontanelle or umbilicus in the subsequent 24 hours or infection (131). Franck et al. also found no difference in skin flora or incidence of infection in preterm infants bathed every other day compared to every fourth day (132). Neither is the skin flora altered during bathing with sterile water (133,134). This practice may be of benefit in some instances such as when the infant is excessively soiled or perhaps following vaginal delivery to an HIV-infected mother, although the impact of bathing in this later instance has not been reported. Moreover, immersion bathing provides tactile stimulation, may be soothing to the newborn, and adds hydration to the skin (119,135,136). Thus, bathing with water alone may be considered for stable preterm infants once the umbilical cord is removed or umbilical lines discontinued; it may be safe even with an umbilical clamp in place (119). The technique used for bathing, i.e., immersion vs. washing without immersion, does not appear to impact bacterial colonization rates or infections of newborn skin (137,138).

C. Maternal Vaginal Cleansing

Maternal vaginal cleansing with 0.25% chlorhexidine gluconate in conjunction with whole-body bathing of the neonate has been examined as a strategy to reduce vertical transmission of human immunodeficiency virus (HIV) and colonization and infection with bacterial agents of early neonatal sepsis. The practice has shown promise for reduction of HIV transmission, although impact was observed only in women whose membranes were ruptured for more than 4 hours before delivery (139), and for the reduction of serious maternal and neonatal infections (140). Other studies have demonstrated that vaginal cleansing with chlorhexidine reduces colonization and subsequent development of early-onset sepsis due to group B streptococcus (141) and a wide range of vaginally acquired pathogens (142). Some hospitals in developing countries are beginning to advocate routine use of chlorhexidine vaginal cleansing, although further research is warranted on indications for and impact of this practice, as well as the contribution, safety, and impact of newborn skin cleansing.

D. Topical Therapy to Enhance Skin Barrier Function

Since the stratum corneum of newborn skin is relatively dry and has reduced water-holding capacity compared to that of older infants or adults (143), hydration of the stratum corneum promotes its integrity and optimizes its function as a barrier. While stratum corneum hydration may initially be provided for by the presence of vernix, certain emollients may also serve

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to improve skin condition and minimize skin injury in VLBW preterm infants and act as a mechanical and semi-permeable barrier (2,50,144). The role of topical skin therapy in augmentation of antimicrobial defense is currently under investigation (49).

Interest in use of emollients in routine care of neonates increased following the demonstration that application of Aquaphor® twice daily for the first 2 weeks of life to the skin of premature infants less than 33 weeks gestational age significantly reduced the number of episodes of clinical deterioration consistent with sepsis (9 vs. 37) and the incidence of positive blood and cerebrospinal fluid (CSF) cultures (3.3% vs. 26.7%) compared with control infants (2). In another study, Aquaphor therapy in neonates weighing less than 1500 g decreased the nosocomial bloodstream infection rate to 5.4 /1000 patient days, compared with a rate of 12.7/1000 patient days during the preceding 16 months (51). More recently, however, a multicenter trial showed that Aquaphor therapy increased relative risk (RR = 1.54) for sepsis with coagulase-negative staphylococci among neonates weighing 501–749 g, although no effects were seen in neonates weighing 750–1000 g (52). Furthermore, a recent case-control study suggested that extremely preterm infants weighing less than 1000 g who were treated with topical petrolatum ointment were at increased risk for *Candida* infections (145). Thus, caution is recommended in use of currently available formulations for topical therapy in neonatal care units, especially for extremely preterm infants whose fragile skin may be easily injured during applications of the emollient. However, newer agents formulated with a mixture of barrier-enhancing ingredients (e.g., lanolin, glycerin, physiological lipids, inert hydrocarbons) (146), an optimal physiological balance of epidermal lipids (3:1:1:1 molar ratio of cholesterol, ceramide, palmitate, and linoleate) (146,147), and/or fatty acid ligands of peroxisome proliferator–activated receptor-α (PPARα), which have the capacity to accelerate barrier ontogenesis (148,149), may provide new avenues for augmentation of skin barrier function and providing protection from infections through a cutaneous portal of entry. Topical therapeutics examined to date for protection against infections in preterm infants have not had direct antimicrobial effects and have not altered cutaneous flora (2,49,50,144). Thus, incorporation of ingredients that have antimicrobial activity and that are safe for application to the highly permeable skin of preterm infants (e.g., cationic antimicrobial peptide derivatives) may also prove beneficial.

In developing country settings, where infections are due primarily to more virulent gram-positive (i.e., *S. aureus*, *S. pyogenes*, *Streptococcus pneumoniae*) and gram-negative pathogens (99), mechanisms of transcutaneous sepsis may be different than in developed country settings and may

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be more readily amenable to topical therapy. In developed country settings where the predominant mechanism for entry of coagulase-negative staphylococci, the major agents of sepsis, is via the tip of transcutaneously placed catheters (see Sec. VI.F) (150), topical therapy with emollients may be inconsequential. In developing countries, however, where indwelling intravascular devices are seldom used, and where levels of environmental contamination and skin colonization are much higher, it is more plausible that infections occur via cutaneous sites amenable to topical therapy, such as microscopic sites of skin barrier compromise due to injury or maturational or nutritional underdevelopment. Preliminary data on use of high-linoleate, low-oleate vegetable oils to improve skin barrier function in such settings is promising (49) and is being further investigated.

E. Umbilical Cord Care

Exposed necrotic tissue of the umbilical stump is readily colonized and infected by pathogenic bacteria, which subsequently may access the systemic circulation and cause sepsis. It is widely recognized that hygienic umbilical cord care is capable of reducing umbilical colonization, cord infection, and neonatal tetanus and sepsis (111,151,152). The role of antiseptic cord care, however, in reducing infections in neonates is less clear, due in large part to inadequacies in study design and size. At a minimum, it is recommended that the diaper remain folded and away from the cord stump to facilitate drying, that application of emollients to the stump be avoided, and that the stump be kept clean and dry, using soap and water to clean a visibly soiled cord (151). Data on whether use of antiseptics on the umbilical cord stump reduces cord infections or neonatal sepsis are conflicting, however (153–157), and prospective, controlled trials with sufficient power and appropriate outcome measures are lacking. On the other hand, use of dry cord care or alcohol alone has been associated in some cases with increased rates of infection (47,153,158,159), suggesting that these regimens may be inadequate. Among potential antiseptics for cord care, in general, chlorhexidine appears to have a superior record of safety and efficacy for reducing cord colonization with the major agents of omphalitis (111). Chlorhexidine in a liquid formulation, however, has delayed cord separation in some neonates (155).

Identification of the most efficacious agent for reducing omphalitis and sepsis when applied to the umbilical cord stump of newborns in the nursery awaits definitive evidence. Until then, chlorhexidine may provide the most effective antisepsis with the fewest potential side effects (159), although dry cord care without use of antiseptics is now favored in some neonatal care

centers. No data are available on the most effective method of cord antisepsis at home.

F. Skin Preparation Prior to Puncture

Heavy skin colonization and the presence of an indwelling intravascular catheter are major risk factors for systemic infection with coagulase-negative staphylococci in preterm infants (90). Bacteremia presumably results primarily from colonization of the catheter tip at the time of its insertion through the skin (160) and is particularly prevalent in preterm infants less than 32 weeks gestational age (161). The best choice for broad-spectrum sterilization of the skin prior to invasive procedures appears to be chlorhexidine (170). Chlorhexidine (0.5%) was superior to 10% povidone-iodine in reducing the risk of peripheral catheter colonization in neonates (161). Furthermore, there was a greater increase in colonization risk with duration of catheter placement for povidone-iodine compared to chlorhexidine. The improved protection from chlorhexidine relative to other antiseptics for catheters in place for longer periods of time is particularly advantageous in neonates in whom frequent catheter rotation is impractical. Greater reduction in skin colony counts with chlorhexidine was achieved with use of two consecutive 10-second exposures or a single 30-second exposure compared to a single 10-second wipe (160). The superiority of chlorhexidine may be due, at least in part, to residues on the skin which prolong its half-life. No toxic systemic effects have been attributed to chlorhexidine alone, although systemic absorption has occurred in preterm infants when alcohol was applied concurrently, sometimes as a vehicle, suggesting that chlorhexidine is best used alone. Anaphylaxis or sensitization is rare, and there is potential for corneal damage due to pluronic additives.

Other means of skin care have shown promise for preventing catheter-related episodes of sepsis. Weekly application of a chlorhexidine gluconate–impregnated dressing was as effective as 10% povidone-iodine in combination with several dressing changes each week (162). Addition of 25μg/mL of vancomycin to total pareteral nutrition solution decreased the rate of catheter colonization from 40 to 22% and dropped the rate of catheter-related sepsis from 15% to 0, while decreasing the need for catheter reinsertion and speeding the recovery of birth weight in treated infants (163).

VII. CONCLUSION

Bacterial colonization of the integument begins at birth. Organisms such as coagulase-negative staphylococci and diptheroids establish a lifelong com-

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mensual residence, while others organisms may transiently attach, replicate, and ultimately infect the skin. A balance of microbiological, host, and environmental factors determines these two divergent bacterial fates. Rational skin care practices should continue to evolve in lockstep with bacteriological and immunological advances aimed at maintaining the integrity of the skin barrier, including the population of commensal flora, while preventing and combating challenge from agents of skin infection.

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3
Acid Mantle

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I. INTRODUCTION

Neonatal skin must adapt rapidly from an aqueous to a terrestrial environment at birth. The development of an acidic stratum corneum (SC), required for the normal processing of SC lipid to form a competent epidermal barrier, is an essential feature of this adjustment. Babies develop an acidic SC, the so-called ''acid mantle,'' within 4 weeks of birth. Both the etiology of the SC acidity and the mechanisms through which it produces a normal epidermal barrier have been subjects of controversy. Proposed functions of the acid mantle include (a) the facilitation of enzymatic processing of lipids to form competent lipid bilayers that provide the epidermal permeability barrier, (b) regulation of enzymes that control desquamation and cohesion, and (c) antimicrobial defense. Proposed extracellular etiologies include (a) exogenous substances such as lactic acid from eccrine glands, breakdown products from sebaceous gland derived free fatty acids, and metabolic byproducts of bacteria, (b) endogenous metabolic products such as *trans*urocanic acid or free fatty acids, and (c) H^+ generated from cellular antiporters such as the Na^+/H^+. This article will review studies of stratum corneum pH in adults and infants. Although normal skin morphology coexists with an alkaline or neutral surface pH in newborn infants, alkaline skin pH is found in pathological states later in infancy, suggesting that, after the immediate postnatal period, infant skin resembles adult skin in its mechanisms of barrier maintenance and repair. The SC pH gradient probably is generated by multiple factors and probably fulfills multiple roles.

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II. HISTORY AND BACKGROUND

The history of SC pH studies also represents the history of the technology available to study this tissue. SC pH measurement presents several methodological problems: (a) it is a multilayered, heterogeneous tissue, (b) SC is not easily reproduced in vitro, and (c) it possesses an extraordinary pH-buffering capacity. Furthermore, while pH itself is a measure of H^+ ions in an aqueous environment, the SC extracellular environment is almost entirely lipid, leading to some confusion about what a pH measurement in the SC actually represents. Finally, even though the skin surface pH has been known to be acidic for more than a century (1), the existence of a pH gradient within the stratum corneum is a recent finding. Thus, it has been difficult to measure accurately the SC pH and even more difficult to correlate measurements with specific mechanistic processes.

In 1923 Sharlit and Scheer (2) measured surface pH of normal skin using a colorimetric method and found the average surface pH of adult skin measured to be 5.5. In 1928 Schade and Marchionini (3) introduced a new method, the *Glockenelektrode*. They found that skin surface pH varied from 3.0 to 5.0 using this method and ascribed this variation to sweat. Levin and Silvers (4) reported that axillae and interdigital areas were more alkaline, using a microquinhydrone electrode. The study of skin pH advanced into our current era largely through the work of Irvin Blank. Starting in 1938, he undertook the systematic study of skin surface pH, developing a H^+ electrode virtually identical in principle to the flat electrode used for pH measurements today (5). In addition to confirming earlier studies of adult skin surface pH, he measured skin surface pH in children aged 3–14 years (6). He found that skin surface pH varied from 4.2 to 6.0 in children. Prepubertal girls displayed a wider variation in skin surface pH and also had, on average, more alkaline surface pH than boys. Adult males had more acidic surface pH values than prepubertal boys, while there was no difference in skin surface pH between adult women and prepubertal girls. Blank also noted that "unbuffered solutions of varying pH quickly assumed the pH of the surface of the skin," the first recognition of the innate buffering system of the SC. These findings have been replicated and expanded in subsequent years, with minor surface skin pH variations depending on gender, race, age, and body location reported (7–12).

Ohman and Vahlquist first reported the SC pH gradient in 1994. Experiments using tape-stripping with flat electrode pH measurement demonstrated consistently that the pH of the upper epidermis is similar to that of other viable tissues, about 7.0–7.4, while there is a rapid acidification of the SC with the major change occurring in the lower third of the stratum

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corneum (13). In total, the pH changes from approximately 7.0 in the upper epidermis to 4.5–5.0 in the outer stratum corneum, a remarkable change of at least 2 pH units in less than 100 μ*m* of tissue. Skin pH appears to be regulated in a manner largely independent of blood pH, because in hemodialysis patients SC pH is alkaline even when they are acidemic (14).

The first measurements of infant skin pH were performed by Taddei in 1935 (15), who found that surface skin pH at birth measured 6.5. In 1957 Behrendt and Green (16) found that abdomen skin surface pH in term infants at 1–48 hours measured 6.8, at 3–6 days pH measured 5.6, and at 7–30 days pH stabilized at values approximating children, 4.9. Behrendt and Green (16) further proposed that the relatively alkaline pH measured at 1–48 hours was due to the vernix caseosa, which they measured at pH 7.4. More recent studies agree with these findings in term infants; however, studies have expanded to measure skin pH and barrier function in premature infants as well (see below).

III. pH OF NEWBORN SKIN

The most commonly used in vivo measure of epidermal barrier function is transepidermal water loss (TEWL). TEWL is approximately equal in term infants and children (17,18). In contrast, TEWL is up to 15 times higher in 25 to 30-week-gestation infants (19,20), indicating a severely perturbed barrier. The epidermal permeability barrier of preterm infants normalizes rapidly over the first weeks of postnatal life. Before the barrier has formed, however, preterm infants are at higher risk for sepsis originating in the skin (21–24), increased loss of fluids, and percutaneous absorption of toxic agents (25), in a manner analogous to burn patients. While nonpermeable dressings such as diapers can cause skin maceration and worsen the epidermal barrier (26), semipermeable dressings can lessen skin breakdown and improve barrier function in premature infants (27–29).

The skin surface pH of both term infants and premature infants is less acidic than that of children and adults, but acidifies rapidly in the first 2 weeks (Fig. 1) with a similar time course in both term and premature infants (30, 31). Premature boys are noted to have more alkaline pH values (31) and more impaired barrier function relative to girls of the same gestational age. However, it is not clear whether a relatively neutral surface pH causes the impaired barrier formation seen in premature infants, is a result of the impaired barrier formation seen in infants, or simply coexists, unrelated, to the impaired barrier formation seen in premature infants. Several possibilities exist:

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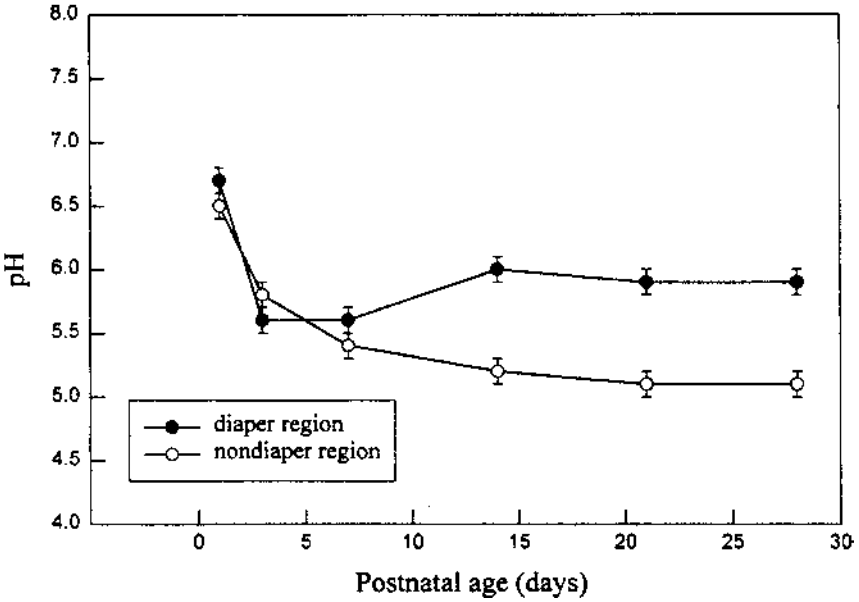

Figure 1 Development of acidic skin surface pH in newborn infants. Surface skin pH measured from nonoccluded skin initially is neutral but acidifies over the first 4 weeks of life. In contrast, surface skin pH from occluded skin remains more neutral. Skin pH was measured using a flat electrode (Courage and Khazaka pH meter, model 900, Cologne, Germany) from nonoccluded (abdominal) or occluded (diaper area) skin of male and female infants, gestational age 37–42 weeks ($p < 0.05$). (From Ref. 30.)

1. Acidification of the SC pH and decreased TEWL develop in parallel, but some other factor (e.g., incomplete formation of the SC, altered lipid production or processing, androgenic impairment of barrier formation) may be responsible for both pathological findings in premature infants. This situation may be analogous to another regulator of barrier recovery, the Ca^{2+} gradient in the epidermis, whose formation follows and depends on the formation of a competent barrier (32). If true, this would imply that SC pH is less important in the prenatal formation of the epidermal barrier than it is in the postnatal maintenance of the barrier.
2. The skin surface pH in newborn infants may not reflect the pH of the stratum corneum, but instead might reflect the pH of the amniotic fluid (pH 7.15) or the vernix (pH 7.4).

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3. The baseline TEWL might be misleading, since recording TEWL of unperturbed skin may not reflect the actual ability of the epidermal permeability barrier to recover from an acute insult. Measuring barrier recovery is a dynamic assessment of barrier function, similar to the use of a treadmill exam to detect cardiac dysfunction. Thus, even though resting TEWL values are normal, infants with alkaline SC may not respond optimally to the normal wear and tear of terrestrial life. This situation would be analogous to that seen in aged skin, which exhibits lower resting TEWL values, suggesting a better barrier, but is unable to recover form barrier perturbation as effectively as younger skin (33), demonstrating impaired barrier competence.

Unfortunately, it is not known whether a SC pH gradient exists, nor is it known whether barrier function is completely normal, at rest or recovering from an acute insult, in term or preterm infants. Thus, resolution of this question awaits further investigation.

IV. pH OF INFANT SKIN

As mentioned previously, skin surface pH rapidly becomes more acidic in the first week of postnatal life, then undergoes a more gradual decrease until it reaches child/adult values by the end of the first month. However, this SC acidification is blunted in the diaper area, even in areas not often exposed to feces (30). Moreover, skin surface pH is higher and barrier function is worse in children with atopic dermatitis (34). In contrast with the confusing data regarding pH in newborn infants, elevated skin surface pH clearly is associated with an increased incidence of contact dermatitis (particularly diaper dermatitis) and atopic dermatitis (26,34,35). Further, acidic hot spring bathing has been reported to improve atopic dermatitis in teenagers/young adults (36). Finally, a relatively alkaline pH also is seen in inherited diseases such as X-linked recessive and ichthyosis vulgaris (37), suggesting that SC pH is important in the pathogenesis of inherited as well as acquired diseases.

V. PROPOSED ETIOLOGIES OF THE SC pH GRADIENT

Both exogenous and endogenous mechanisms have been proposed to explain the development of the acidic SC pH. Most early investigators proposed exogenous mechanisms, including lactic acid from eccrine sweat, free fatty acids generated from metabolism of sebaceous gland lipids, and

byproducts of bacterial metabolism from colonizing bacteria. However, recent findings that (a) appendage-free and bacteria-free mouse SC is nevertheless acidic (unpublished data) and (b) the stratum corneum will buffer a small amount of almost any externally applied substance (38) have rendered these putative mechanisms less likely. Instead, more recent efforts have focused on endogenous mechanisms, including urocanic acid (39), H^+ transport by Na^+/ H^+ antiporters or H^+ ATPases (40–42), and concentration of H^+ by progressive desiccation of the upper layers of the SC (Table 1).

VI. PROPOSED FUNCTIONS OF THE SC pH GRADIENT

Shifts in pH may be important in regulating several processes in SC differentiation in both health and disease (Table 2). Clearly, an acidic pH is essential for the postsecretory lipid processing required for barrier repair (43). The SC enzymes β-glucocerebrosidase and acidic sphingomyelinase are activated at an acidic pH, and inhibition of these enzymes, either pharmacologically or as seen in the inherited disease Type II Gaucher's (44) or Niemann-Pick disease (45), produces identical defects in lipid processing. Moreover, experimental acidification of the SC with lactic acid enhances ceramide synthesis (46). These findings suggest that one of the SC pH gradient's primary functions is to activate the pH-sensitive enzymes required for normal SC lipid processing.

In addition, SC enzymes responsible for conversion of phospholipids to free fatty acids (sPLA2) and desquamation/cohesion (cathepsin D, chy-

Table 1 Possible Etiological Factors Giving Rise to the Acid Mantle

Exogenous factors
Lactic acid from eccrine sweat
Free fatty acids from metabolism of sebaceous gland products
Metabolic by-products of bacteria
Endogenous factors
Urocanic acid
Lactic acid from cell metabolism
Free fatty acids generated from phospholipid breakdown
H^+ from lysosomes/lamellar bodies
H^+ from Na^+/H^+ antiporter or H^+ ATPases
Dehydration of the SC

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Table 2 Proposed Functions of the Stratum Corneum pH Gradient

Extracellular lipid processing via pH-sensitive enzymes
β-glucocerebrosidase
Acidic sphingomyelinase
Acyl CoA: cholesterol acyltransferase
Acyl CoA: retinol transferase
Desquamation/Cell cohesion via pH-sensitive enzymes
Cathepsin D
Chymotryptic serine protease
Tryptic serine protease
Antimicrobial defense
Optimal stacking and organization of lipids

motryptic serine protease, tryptic serine protease) are pH-dependent, some with acid, and others with neutral to alkaline optima. Clinically, desquamation increases when exogenous acids are applied (47), a property that has been exploited by many therapeutic and cosmetic preparations. Thus, different enzymes could be activated in healthy (acidic pH) vs. pathological (neutral pH) states, perhaps causing either normal desquamation or the pathological scaling seen in skin conditions associated with abnormally neutral or alkaline surface pH, such as irritant contact or atopic dermatitis or certain ichthyoses.

An acidic SC pH also may be important in defense against microbial invasion. Early studies demonstrated that experimental maneuvers that resulted in less acidic skin pH resulted in increased numbers of organisms on the skin, especially gram-negative bacteria (48) and proprionibacteria (49). More recently, skin candida infections were shown to be more inflammatory when the SC was buffered to pH 6.0 vs. 4.5, suggesting that pH also may mediate the immune reaction to infection (50). Finally, human β-defensin-1, an endogenous antimicrobial peptide found in skin (51), may be pH dependent, as it is released from carrier macromolcules in the blood by acidic conditions (52). Increased invasive bacterial and fungal infections (22) are a major cause of morbidity in very low birth weight infants, suggesting that these experimental findings may have clinical significance.

Lastly, an acidic SC pH changes the biophysical properties of lipid in the SC. When the lipid components of the SC—ceramides, free fatty acids, and cholesterol—are mixed independent of the SC corneocytes, lipid interactions and lipid phase behavior change markedly as pH is varied from acid to alkaline values (53,54). These properties more closely resemble those

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found in vivo when the pH is acidic, suggesting that an acidic pH also is required for normal lipid interactions in the SC.

VII. CONCLUSION

Neonatal skin must accomplish two essential goals. Immediately after birth, it must develop a competent barrier to prevent fluid loss and microbial invasion. It is not clear what role the SC pH gradient plays in this initial process, since in term infants baseline TEWL is low but surface pH is neutral. Further experiments measuring the pH gradient across the SC and assessing the ability of term newborn skin to recover from barrier perturbation will be required to elucidate the role of pH in perinatal barrier competence. Following the perinatal period, newborn and pediatric skin must maintain its integrity and respond to external insults. Failure to accomplish these later tasks is associated with various forms of dermatitis. In these infants, the pH gradient functions in a similar fashion as in adults. Impaired pH gradients are associated with increases in irritant contact dermatitis (especially diaper dermatitis), atopic dermatitis, inherited diseases such as ichthyosis vulgaris, and increases in bacterial numbers on the skin.

The pH gradient is likely to be generated from a number of sources and is also likely to control a number of processes essential for the formation of a competent epidermal permeability barrier. Elucidation of the mechanisms of pH gradient formation and function should allow formulation of more effective approaches to ameliorating diseases caused or worsened by barrier dysfunction in neonates and children.

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4
Sebaceous Glands

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I. INTRODUCTION

Sebaceous glands are found in the skin of all mammals except whales and porpoises (1). One of their functions is to excrete sebum, a mixture of relatively nonpolar lipids, most of which are synthesized de novo by the glands (2). In furred mammals, sebum coats the fur as a hydrophobic protection against overwetting and for heat insulation (3). The composition of sebum is remarkably species specific (2,4,5). In addition, specialized sebaceous-like structures, such as the preputial glands of rodents, are responsible, in many species, primarily for the release of pheromores used for territorial marking and sexual attraction (6,7). In humans, however, these sebaceous gland functions appear to have limited importance. Although sebum is the major ingredient of human skin surface lipids, the latter form no barrier, and sebum has been considered not to have antibacterial or antifungal properties in vivo (8). On the other hand, increased sebum excretion is a major etiological factor involved in the development of acne (9). Hormones control sebaceous gland size and activity, however, the acne patient is not considered to be an androgen mismatch (9). Therefore, Kligman has suggested that the sebaceous gland is a vestigial organ of human development, a "living fossil with a past but no future" (8).

The development of experimental models for in vitro sebaceous gland cell research (10–12) has led to the identification of several, unknown or disregarded functions of sebaceous glands (Table 1) introducing a major role of this organ in skin homeostasis (13,14).

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Table 1 Current Aspects of Sebaceous Gland Functions

Production of sebum
Regulation of the cutaneous steroidogenesis (13–15)
Regulation of local androgen synthesis (14)
Production of vernix caseosa (16)
Exhibition of pro- and anti-inflammatory properties (13,17,18)
Synthesis of specific lipids with antimicrobial/antifungal activity (19)
Possible involvement in hair growth and differentiation (13,20)
Substantial involvement in skin aging processes (16,21)
Delivery of antioxidants to the skin (22)

II. DEVELOPMENT AND STRUCTURE

The development of the sebaceous glands is closely related to the differentiation of hair follicles and epidermis (5,23,24). At the tenth to twelfth weeks of fetal life, a stratum intermedium becomes apparent, and at about the same time developing hair germs are quite distinct. In the following weeks, the follicles extend downwards into the dermis and the rudiments of the sebaceous glands apppear on the posterior surfaces of the hair pegs. By 13–15 weeks the glands are clearly distinguishable arising in a cephalocaudal sequence from hair follicles. The cells in the sebaceous anlagen are identical to those in the basal layer of the epidermis and the piliary canal.

The cells at first contain glycogen. Glucogen lingers at the periphery of the gland, but is quickly lost at the center, where lipid drops are visible at 17 weeks (25,26). The future common excretory duct, around which the acini of the sebaceous gland attach, begins as a solid cord. The cells composing the cord are filled with sebum and they eventually lose their integrity, rupture, and form a channel that establishes the first pilosebaceous canal. New acini result from buds on the peripheral sebaceous duct wall. The cell organization of the neonatal sebaceous acini consists of undifferentiated, differentiating, and mature sebaceous cells (27) (Fig. 1).

The human sebaceous gland is a multiacinar, holocrine-secreting tissue that occurs in all areas of the skin, except for the palms and soles, and only sparsely on the dorsal surfaces of the hand and foot (28). The average transit time of sebaceous cells from formation to discharge has been calculated as 7.4 days in the human gland (29), with 4–7 days in undifferentiated and 14–25 days in differentiated lipid-producing cells (30). Within any one glandular unit, the acini vary in differentiation and maturity (31). The synthesis and discharge of the lipids contained in the sebaceous cells require more than a week. Following involution after birth, the size of sebaceous glands

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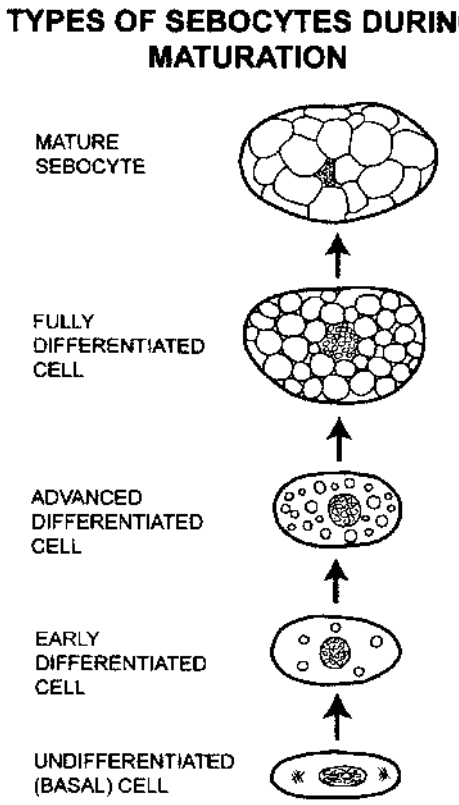

Figure 1 Maturation steps of sebaceous gland cells. Small undifferentiated cells with high proliferation potential, almost no cytoplasmic lipids, and a low cytoplasm: nucleus ratio develop gradually to markedly larger cells with low proliferation rates, abundant cytoplasmic lipids, and a high cytoplasm:nucleus ratio.

increases with age. The mean size increases from 0.2 ± 0.5 to 0.4 ± 2.1 mm^2. The sebaceous cells of prepupertal and hypogonadal males are qualitively similar to those of normal adults, even though the glands are smaller (32). Generally, the number of sebaceous glands remains approximately the same throughout life, whereas their size tends to change with age (33). The turnover of the sebaceous glands in the aged is slowed down compared to that in young adults (34).

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III. FUNCTION

The sebaceous glands are functional from their formation: sebum is the first demonstrable glandular product of the human body. The development and function before birth and in the neonatal period appear to be regulated by maternal androgens and by endogeneous steroid synthesis by the fetus, but also by other ''morphogens,'' such as growth factors, cell adhesion molecules, extracellular matrix proteins, intracellular signaling molecules (β-catenin and LEF-1), other hormones, cytokines, enzymes and retinoids, that play a regulatory role in development (24). Hydroxysteroid dehydrogenases, which activate and inactivate androgens (14), are present after 16 weeks of fetal life (35,36).

A. Vernix Caseosa

Vernix caseosa is a naturally occurring biofilm, consisting of lipids (37) and cellular elements (38). It can be accurately described as a combination of fetal corneocytes surrounded by a matrix of sebaceous and epidermal lipids instead of its traditional view as a thick lipid paste. Vernix is uniquely human, although hydrophobic surface films due to differentiation of the periderm appear to serve a similar function in perinatal rodents (39,40). During the last trimester of gestation, vernix progressively coats the fetus (41,42). Its production coincides in utero with terminal differentiation of the epidermis and formation of the stratum corneum (43,44).

Phase contrast microscopy of vernix caseosa showed multiple cellular elements with nucleic ''ghosts'' embedded in a putative lipid matrix. Transmission electron microscopy revealed flattened structures approximately 1–2 μm in thickness with distinct cellular envelopes indicative of differentiated corneocytes (45). In this context, the corneocytes in vernix would act as ''sinks'' to interdict water moving across the fetal skin, whereas the sebacous lipids would provide a hydrophobic barrier. These results are consistent with the novel view of vernix as a ''mobile'' or ''fluid'' phase stratum corneum lacking rigid desmosomal connections (46).

A comparative study of adrenal morphology between normal fetuses and those with anencephaly or congenital adrenal hyperplasia has shown a close dependency of fetal adrenal growth and development upon fetal pituitary function from an early age, mediated primarily through adrenocorticotropin (ACTH) throughout gestation (47). The fetal adrenal cortex produces cortisol and DHEA sulfate early in gestation (6–12 weeks) (48). During the period between 15 and 27 weeks of gestation, mean adrenal weight of normal fetuses showed a sixfold linear increase, while in anencephalic fetuses of similar gestation age, adrenal weight was below the

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normal range and did not show a rise. ACTH regulates the steroidogenic activity of the fetal zone, which is the primary source of DHEA sulfate (29,48). In mid- to late gestation (25–30 weeks), the transitional zone, which early in pregnancy is functionally identical to the fetal zone, is the likely site of glucocorticoid synthesis. The circulating DHEA sulfate is converted to DHEA by blood monocytes (49), and DHEA can be further converted into androstenedione and the tissue potent androgen testosterone in skin by sebocytes only, since only sebocytes express 3β-hydroxysteroid dehydrogenase-Δ^{5-4} isomerase (13,14). Therefore, the sebaceous glands are likely to be the major skin target of fetal androgens. Indeed, the glands reach a peak of activity in the third trimester (3), and several investigations have shown that sebaceous glands are abundant during late gestation. Their secretion forms a significant portion of the vernix caseosa, the variably adherent fetal skin surface film consisting of lipids, epidermal cells, water, and other substances from the amniotic fluid (50) (Fig. 2). The vernix lipids resemble sebum in their content of fatty acids, squalene, and wax esters, but they also contain lamellar lipids, such as sterols and sterol esters (51). Stewart et al. (52) also noted a higher ratio of wax esters to cholesterol + sterol esters (WE/CH + CE) in vernix caseosa from male fetuses compared with female fetuses, although indivi-

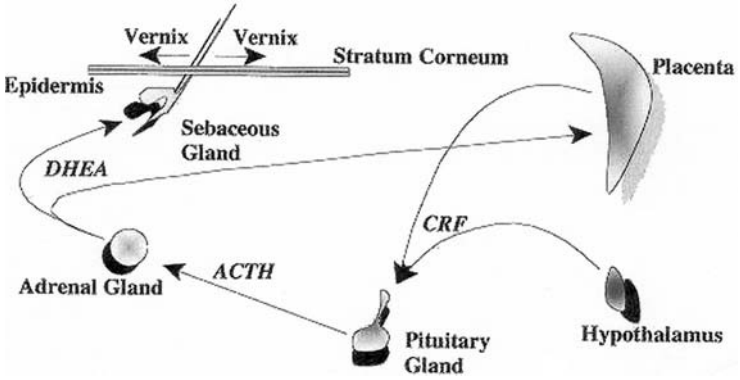

Figure 2 Proposed mechanism for endocrine control of vernix caseosa production. The placenta regulates adrenocorticotrophin (ACTH) secretion in the pituitary gland through corticotropin-releasing factor (CRF). ACTH stimulates the production and secretion of adrenal androgens, especially dehydroepiandrosterone (DHEA), which can only be converted to the tissue-active testosterone by sebaceous cells. The high intracellular tissue-active androgen levels in sebaceous cells lead to the synthesis of sebaceous lipids, a major component of vernix caseosa. These events are prominent at the third trimester of pregnancy.

dual values showed wide variations and there was overlap between the sexes. Sterol esters are mainly molecules with very long-chain fatty acids (more than 19 carbons) (51). Both saturated and monounsaturated very long-chain fatty acids are present in vernix, with the saturated fatty acids being mostly iso- or anteiso-branched, whereas the monounsaturated fatty acids are mostly straight-chained.

Pickens et al. (45) suggested that antenatal sebaceous secretion may act synergistically with the developing epidermis to promote terminal differentiation and establishment of a rudimentary stratum corneum. It is likely that prior to epidermal cornification, sebaceous lipids cover the skin surface forming a hydrophobic barrier between skin and the aqueous uterine compartment. The skin of very preterm infants is missing this protective mantle of barrier lipids (53). In utero, a hydrophobic film of vernix caseosa may modulate water activity at the skin surface and thereby promote cornification and/or protect the developing epidermal barrier from the deleterious effects of extended and excessive water exposure.

B. Postnatal Activity

A strong increase in sebum excretion occurs a few hours after birth, which peaks during the first week (29,54). Maternal and neonatal sebum excretion rates have been reported to show direct correlation (54). This correlation is lost in the following weeks and is independent from breast-feeding. At this time the sebum level per unit skin surface is in the same range as in young adults (29), and the sequence of sebaceous transformation seems identical to that in postnatal life. These events suggest an important role of the hormonal environment of the mother on the sebaceous gland of the newborn and indicate that androgenic stimulus for sebum secretion occurs before birth through the placenta (29,54). The excretion then slowly subsides. Females display a different pattern of sebum excretion than males. Directly after birth the levels in females are lower than in males, but a large increase takes place between the days 3 and 6, followed by a fall, bringing the levels below that of the males. At 6 months the levels are low in both sexes. Very low levels, approximately 10 $\mu g/cm^2$ and often below 0.5 $\mu g/cm^2$, remain constant from 6 months to the prepubertal period (55). A new rise takes place at about 9 years (56) with adrenarche and continues up to 17 years, when the adult level is reached (55). It has been suggested that the endocrine environment of the neonate correlates and may influence the sebaceous gland development in puberty (54).

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IV. SEBACEOUS LIPIDS

Human sebaceous glands secrete a lipid mixture containing squalene and wax esters as well as cholesterol esters, triglycerides, and possibly some free cholesterol (2,5,56). It has been previously suggested that bacterial hydrolases convert some of the triglycerides to free fatty acids on the skin surface (57,58), but there is current evidence that sebaceous glands are able to synthesize considerable amounts of free fatty acids (11–14). The fatty acids of the ester lipids include species with chain branching or with unusual double-bond positions. The alcohol moieties of the wax esters contain unusual chain types similar to those of the fatty acids. Genetic and hormonal factors cause individual differences in sebaceous lipid composition. Genetic factors also seem to influence the proportions of the various types of branched-chain fatty acids. Sebaceous lipids are responsible for skin surface lipids at most areas of the human body where casual surface lipids are greater than 100 μg/cm (2); in the forehead 150–300 μg/cm lipids can be recovered (8). Lower trunk lipids make an exception with 5–10 μg/cm (2) recovered lipids, which is the rate of lipids originating from epidermal keratinocytes (7). As a consequence, squalene and wax esters cannot be identified at the latter areas.

The extremely low lipid-secretion rates that are typical of children around 6 years of age (51) correlate well with low levels of gonodal and adrenal androgens (59). Androgen stimulation of the glands causes an increase in lipid synthesis and its composition, as it becomes particularly evident when the sebum of prepubertal children is compared with that of young adults (52). Skin surface levels of cholesterol and wax esters in the newborn are indistinguishable from the levels seen at puberty, another period when there is a marked increase in sebaceous activity. In addition, the effect of androgens on sebaceous cell proliferation and differentiation is dependent on the origin of the sebaceous glands from different skin areas. Facial sebaceous glands, for example, are more sensitive to androgens (60). Higher concentrations of linoleate in sebum may protect young children from comedonal acne by preventing the development of an essential fatty acid deficiency in the follicular epithelium (52).

A number of studies have confirmed changes in lipid composition of sebum associated with age or with sebaceous gland activity (2,56,61,62). One difference is that prepubertal lipids exhibit significantly lower WE/CH + CE than adult lipids (56,61), because of the low sebaceous gland activity in prepubertal children and the resulting low levels of wax esters. Secretion of cholesterol and cholesterol esters, on the other hand, remains relatively constant. The ratio of WE/CH + CE can be as little as 0.1 in prepubertal children, whereas it is between 2 and 10 in most adults. In 4- to 6-year-old

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children with the lowest androgen secretion and inactive sebaceous glands, the composition of the secreted lipid is not typically sebaceous (61). Instead, it consists largely of cholesterol esters, which appear to come from the recycling of the cholesterol released when sebaceous cell membranes break down (51). Free cholesterol, which is also a major component of skin surface lipids at this age, is mainly of epidermal origin (50). The fatty acid composition of skin surface wax esters, cholesterol esters, triglycerides, and free fatty acids also changes between childhood and adulthood. In children, these lipids contain larger proportions of palmitate (16:0), stearate (18:0), oleate (18:1D9), and linoleate (18:2Δ9,12), i.e., those fatty acids that are present in serum. Except for palmitate, these fatty acids are minor components of adult skin surface lipids (51). The changeover occurs during adolescence, when the proportion of sebaceous fatty acids, including palimitoleic acid (16:1D6), which is the active antimicrobial fatty acid in human skin sebum (19), increases.

Another age-related difference is that, in children, skin surface lipids tend to contain different proportions of branched-chain fatty acids than adults (63,64). Specifically, they tend to have a high proportion of terminally methyl-branched (iso- and anteiso-) fatty acids and very few methyl-branched fatty acids. With increasing WE/CH + CE ratio, the proportion of terminally branched fatty acids increases. However, there is considerable individual variation in the proportion of iso-branched fatty acids. Because of this variation, which appears to have a genetic basis (65), some adults retain a fairly high proportion of iso-branched fatty acids, whereas others have almost none. In addition, Stewart and Downing (66) reported that cholesterol esters with long branched-chain fatty acids, which are a prominent feature of vernix caseosa, are a minor component of adult lipids.

Dividing sebaceous cells construct cellular membranes from cholesterol and fatty acids, which they either acquire from serum or are able to synthesize de novo (67,68). During differentiation, when synthesis of sebaceous lipids occurs, cholesterol is no longer produced and the fatty acids synthesized tend to be different from those in the circulation (50). With holocrine secretion, cellular membranes are broken and their phospholipids are hydrolyzed. Some of the phospholipid fatty acids are reesterified with cholesterol and the remainder recycle into sebaceous wax esters or triglycerides.

The reactivation of the sebaceous glands with adrenarche (age 7–10 years) leads to transition in the composition of skin-surface lipids towards the adult pattern (50,69). Squalene, wax esters, and triglycerides begin to predominate over cholesterol and cholesterol esters with correlated changes in the WE/CH + CE ratio. Additionally, an increase in the quantity of secreted sebum can be detected. The serum levels of DHEA sulfate also

begin to increase. These adrenal-driven changes occur in many children before the appearance of any physical signs of puberty (52).

V. HORMONAL CONTROL

There is no doubt that androgens play the major role in controlling sebaceous gland activity, but many hormones are directly or indirectly involved. Three components of sebocyte function are controlled by complex endocrinological mechanisms, namely differentiation, proliferation, and lipid synthesis.

A. Androgens

Androgens regulate the sebaceous gland function through binding to nuclear androgen receptors (AR) (14). AR have been detected by immunohistochemistry in sebaceous glands, eccrine glands, and the mesenchymal cells of the hair follicle, whereas the highest AR density in the human skin has been demonstrated in the sebaceous glands (70–72). The distribution of AR in human skin is consistent with known androgen targets and is obvious in androgen-dependent skin diseases, i.e., acne, seborrhea, androgenetic alopecia, hirsutism, and SAHA (seborrhea, acne, hirsutism, and androgenic alopecia) syndrome (24,73). In sebaceous glands, AR were identified in basal and differentiating sebocytes, which indicates that androgens are involved in the regulation of cell proliferation and lipogenesis (70,71). On the other hand, the skin, and especially the sebaceous gland, is an important site of formation of active androgens (13,14). All enzymes required for transformation of the adrenal precursors (DHEA sulfate and DHEA) are localized in the skin (13–15,74) (Fig. 3). DHEA sulfate is transformed locally—in addition to its systemic transformation (49)—to DHEA by the widely distributed steroid sulfatase (75). DHEA is metabolized to androstenedione and testosterone by 3β-hydroxysteroid dehydrogenase-Δ (5–4) isomerase and 17β-hydroxysteroid dehydrogenase, which have been localized to the sebaceous glands (14,74). The intracellular conversion of testosterone to 5α-dihydrotestosterone (DHT), the most potent androgen in tissue, by 5α-reductase is known to stimulate sebum secretion (24). Two types of 5α-reductase have been isolated from human tissues (76). The type I isoform is the predominant type expressed in human skin and could be localized in sebaceous glands, sweat glands, and in the epidermis (77), whereas its highest activity is found in sebaceous glands with a maximum in sebaceous glands of facial skin and scalp (78, 79).

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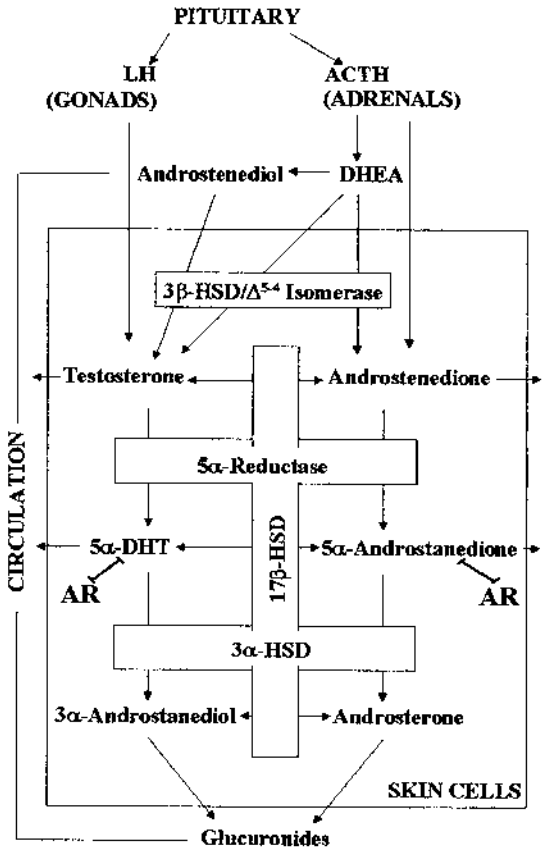

Figure 3 Intracellular androgen metabolism. LH, luteinizing hormone; ACTH, adrenocorticotrophin; DHEA, dehydroepiandrosterone; HSD, hyxdroxysteroid dehydrogenase; DHT, dihydrotestosterone; AR, androgen receptor.

Conversion of adrenal precursors to tissue active androgens is involved in the full development of the sebaceous glands during intrauterine life and adrenarche, whereas gonadal androgens are primarily involved in puberty (24). Androgens administered during fetal life seem to have a long-lasting effect on sebaceous gland activity, as demonstrated in female rats after exposure to androgens in the first week of fetal life (5). The animals exhibited an increased sebum secretion that persisted into adult life.

Terminal differentiation of cultured preputial rat cells in vitro, which exhibit sebocyte-like differentiation, has been shown to be stimulated by combining DHT and peroxisome proliferator–activated receptor (PPAR)

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ligands (80). PPARs regulate multiple lipid metabolism genes in mitochondria, peroxisomes, and microsomes (81), all of which are prominent in the cytoplasm of human sebocytes (11,14).

B. Estrogens

Estrogens exhibit an inhibitory effect on excessive sebaceous gland activity; on the other hand, low estrogen levels, such as in menopause, are associated with reduced sebaceous gland activity (13,21,82,83). These facts indicate a regulating role of estrogens in sebaceous gland homeostasis. The down-regulatory effect of estrogens on activated sebaceous glands can possibly be explaned by an inhibition of gonadotropin secretion or by enhancement of testosterone binding to its binding globulin (5). Estrogens administered during early endocrine environment do not seem to have any long-lasting effect on the activity of the sebaceous glands. Early ovarectomy did not exert changes on the sebum production in adult rats (84).

C. Progesterone

The possible effects of progesterone on the activity of sebaceous glands remain controversial. A stimulation of sebum secretion was seen in female and castrated rats but not in intact males (85). The degree of stimulation was related to the time points of gonadectomy and was higher when this was carried out before puberty. In contrast, in intact males sebum secretion was inhibited by progesterone. These results suggest that the activity of progesterone on sebaceous glands is likely to be dependent on sex and changes in the early endocrine environment (5).

D. Glucocorticoids

In patients with adrenal insufficiency, sebum secretion is decreased and remains decreased even after substitution with glucocorticoids. A possible mechanism of glucocorticoid action could be through the resulting decreased serum levels of adrenal androgens (86,87). Corticosteroids, topically applied in humans, were shown to significantly decrease sebum excretion (88). In vitro studies detected a dose-dependent direct stimulatory effect of hydrocortisone on the proliferation of human sebocytes (11). Cortisol was found to be necessary for the differentiation of rat preputial cells and, additionally, seemed be essential for an optimal cell differentiative response to growth hormone (GH) and insulin-like growth factor-I (IGF-I) (24). The controversial data between in vivo and in vitro studies are probably due to complex endocrinological interactions in vivo, which are still not fully understood.

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E. Thyroid Hormones

Thyroid hormones also seem to play a role in controlling the activity of the sebaceous gland. In hypophysectomized rats, stimulation of sebum by thyroid hormones has been demonstrated (89). Recently, the thyroid hormone receptor $\beta 1$ has been localized in human sebaceous glands (90,91).

F. Insulin

Very little is known about the effect of insulin on sebaceous glands. In diabetic rats, a decreased sebum production was found (84). Dose-dependent stimulation of sebocyte proliferation and differentiation by high doses of insulin has been shown in vitro (11,92). In HAIR-AN syndrome, a variant of SAHA syndrome with hyperinsulinemia (73), seborrhea is prominent.

G. GH and IGFs

The pituitary has an overriding role in controlling the activity of the sebaceous glands. Pituitary hormones probably act through complex direct or indirect mechanisms. Increased serum GH in acromegaly is associated with high levels of sebum secretion (92). In hypopituitary rats, the preputial glands were atrophic and could only be restored to normal size after administration of testosterone and GH but not after testosterone alone (93). In addition, GH supports testosterone-induced sebum production, as detected in animal studies (94). GH receptor, but not IGF-I receptor, has been localized in human sebaceous glands (95). Using rat preputial cells in vitro, Deplewski et al. (96) have shown that GH stimulates differentiation but does not influence proliferation and, in addition, that GH augments the effect of DHT on cell differentiation. In contrast, IGF-I exerts its major effect on proliferation. Therefore, GH can act directly after binding to its receptor and indirectly through IGF production and seems to be a trophic factor for human sebocytes (97,98).

H. Prolactin

A clinical indication for the influence of prolactin on sebaceous glands is the known seborrhea in hyperprolactinemic women. The effect of prolactin is mediated indirectly through increased production of adrenal androgens (99). Neither prolactin synthesis nor prolactin receptors have been identified on human sebaceous glands.

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I. Gonadotropic Hormones

The gonadotropic hormones indirectly control the activity of sebaceous glands by stimulating the secretion of gonadal hormones. As expected, in castrated and hypophysectomized rats no direct effect of the gonadotropic hormones on sebaceous glands could be detected (100).

J. Thyroid-Stimulating Hormone

Thyroid-stimulating hormone has no direct effect on the sebaceous gland in vivo or in vitro (11,101).

K. ACTH and Propiomelanocortin Peptides

Corticotrophin-releasing hormone (CRH) enhances the production and secretion of the propiomelanocortin peptides α-melanocyte stimulating hormone (α-MSH), ACTH, and β-endorphin in several skin compartments but not in the sebaceous glands (13). The latter express CRH, which promotes lipogenesis, while CRH receptor 1 is down-regulated by testosterone in a negative feedback manner (101a). ACTH activates the steroidogenic acute regulatory protein and melanocortin receptors, inducing thereby the production and secretion of cortisol, a powerful natural anti-inflammatory factor that counteracts the effect of stress signals and buffers tissue damage. Melanocortin receptors 1 and 5, which present affinity for α-MSH and ACTH, and the μ-opiate receptors that bind with high-affinity β-endorphin are present in human sebocytes (18,102,103). Although α-MSH does not influence sebocyte proliferation (11), it was shown to reduce interleukin-8 synthesis in interleukin-1–stimulated human sebocytes (18).

L. Parathormone-Related Protein

The possible role of parathormone-related protein (PTHrP) in regulating the function of the sebaceous gland has been demonstrated by the hypoplastic sebaceous glands in PTHrP knockout mice (104). In contrast, mice with overexpression of PTHrP have hyperplastic sebaceous glands. The importance of PTHrP for the development of sebaceous glands in humans remains unknown.

VI. PROTECTIVE ROLE OF SEBUM

Vernix caseosa covers the fetus during the last trimester of pregnancy and has a presumptive waterproofing function for the fetus in utero as a hydrophobic barrier (105). Control of the water gradient across the epidermis is

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also a key factor in regulating lipid and DNA synthesis. Therefore, fetal mammals possess mechanisms regulating the transepidermal water gradient in an aqueous intrauterine environment. Very low birth-weight preterm infants with diminished vernix caseosa and stratum corneum development have an ineffective epidermal barrier (53). The consequences of poor epidermal barrier development are high transepidermal water loss leading to hypothermia and difficulties in fluid balance, accidental percutaneous absorption, and toxicity and skin trauma leading to infection (106). The surface film of sebum has a positive influence on skin moisturization. Topical application of vernix to human skin results in immediate increase in baseline surface hydration, moisture accumulation, and water-holding capacity (107). Whereas vernix initially loses water rapidly when exposed to air, complete desiccation at room temperature and humidity can take days to weeks. It is likely that the lipid film within vernix consists of two separate compartments of water. The rapidly released water may be associated with polar lipids in vernix, whereas the slowly released water is contained within fetal corneocytes. The slow rates of dehydration and rehydration are consistent with the histological finding that vernix contains water-saturable corneocytes surrounded by lipids that are resistant to aqueous diffusion (45). In human adult skin, extensive water exposure leads to disruption of corneocytes and stratum corneum lipid lamellae (108). In the fetus prior to cornification, sebaceous lipids cover the skin surface and establish a hydrophobic barrier between it and the aqueous uterine compartment.

Vernix caseosa has been reported to possess antibacterial properties; inhibition of *Staphylococcus aureus* and *Klebsiella* growth on nutrient agar by vernix has been observed (109). This is probably due to a high asparagine content or elevated lipid components. Because of this protective role, the maintenance of the vernix layer as long as possible on the newborn skin has been recommended until spontaneous drying occurs.

On the other hand, the postnatal development of sebaceous secretion affects skin water binding and adhesive interactions in the newborn (105). Skin surface lipids maintain their protective mechanisms, where sebum lipids play a major role (110). Current in vitro studies have shown that total sebum lipids may cause a reduction in growth of gram-positive (*Staphylococcus aureus*, *Streptococcus salivarius*) and anaerobic bacteria (*Fusobacterium nucleatum*), while they are ineffective against the most gram-negative bacteria (19). Among the sebum fatty acids, palmitoleic acid was the most effective antibacterial one and also prevented the adhesion of *Candida albicans* to porcine stratum corneum.

Sebum is also able to change the surface properties of preterm and term infant skin (105). It has been suggested that the lipase activity of the

resident skin microflora liberates fatty acids from the triglycerides of sebum. However, it is now evident that sebocytes are able to synthesize fatty acids without the influence of bacteria (11–14). These free fatty acids may participate in acid mantle formation on the skin surface and provide antibacterial properties against *S. aureus* and streptococcal species. On the other hand, these changes may facilitate growth of *Propionibacterium* species. Differences in sebum and sweat secretions may lead to changes in skin condition. Such synergisms and their effects on skin microflora require further investigation.

Sebaceous gland secretion is a relevant physiological pathway for the delivery of vitamin E (α- and γ-tocopherol forms), a natural antioxidant, to sebaceous gland-rich skin, especially on the face (22). Vitamin E is a significant constituent of human sebum, and a close correlation exists between squalene and α-tocopherol levels in sebum. Whereas UVA and UVB exposure to skin surface lipids induce oxidation products (111), vitamin E may exhibit its antioxidative properties for skin protection against lipid peroxidation. Therefore, children between 6 months and 7–8 years in age with low levels of sebaceous lipids on their skin surface may need the exogenous application of vitamin E to increase their local antioxidative capacity.

Besides protection, the secretion of apocrine and sebaceous glands plays a thermoregulatory role. There is a temperature dependency of sebum effects. At high temperature, sebum acts as a surfactant for eccrine secretion. It emulsifies sweat, encourages the formation of a sweat sheet, and discourages the formation and loss of sweat drops from the skin. At lower temperature, sebum repels rain from the hair and the skin.

Finally, former investigations have reported that skin surface lipids have a mosquito-repellent effect in vitro (112).

VII. ALTERATIONS IN DISEASE

A. Atopic Dermatitis

Early reports suggested an association between dryness of the skin in atopic dermatitis and reduced sebaceous gland activity (3). More recent studies, however, have shown that sebaceous lipids are not reduced in patients with atopic dermatitis compared with those of healthy controls (113,114). These investigations confirmed previous data of surface lipid levels measured in the forehead area which have shown no difference between individuals with atopic dermatitis and normal subjects (115). Altered enzyme activities relevant to the synthesis of lipids have been identified, which may account for alterations in epidermal lipid fractions (114). A decreased amount and/or structural alterations of endogenous ceramide 1 derived from epidermal

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keratinocytes may be the major etiological factor for the impaired water barrier function of the skin in these patients.

On the other hand, in consitutionally dry skin, a decreased proportion of neutral lipids (sterol esters, triglycerides) coupled to increased amounts of free fatty acids were found to be associated with the severity of dry skin (116).

B. Seborrheic Dermatitis

It is likely that seborrheic dermatitis is also not related to sebum secretion rates (116,117). Therefore, "dermatitis of the sebaceous areas" may be a more accurate term.

C. Psoriasis

In a histological study of the pilosebaceous units of the scalp in nonpustular patch- and plaque-stage psoriasis, sebaceous gland atrophy was a frequent concomitant in psoriatic lesions, with probable downsizing of the hair follicles and thinner hair shafts (108). On the other hand, proliferation cell rates in sebaceous glands, matrices, and external root sheaths were very similar in patients with psoriasis of the scalp and in controls with normal scalp skin in another study (119).

D. Acne Vulgaris

While high levels of sebum linoleate found in young children may protect them from comedonal acne (51), high levels of DHEA immediately after birth and up to 6 months postnatally as well as with the adrenarche may be responsible for acne infantum and prepubertal acne. These types of acne often present with inflammatory lesions because DHEA exhibits a pro-inflammatory activity opposing the anti-inflammatory action of glucocorticoids (120) (Fig. 4). Acne neonatorum, which is present at birth or appears 2–4 weeks after birth, is not uncommon, with a prevalence of approximately 20% in newborn (121). It usually presents with mostly mild facial lesions is self-limited, and shows a male predominance (4.5–5:1) (121–124). Histological findings from babies with acne neonatorum showed hyperplasic sebaceous glands with keratin-plugged orifices (121).

Acne in childhood has been suggested to be strongly associated with the development of severe acne during adolescence (54,121). On the other hand, high sebum-production rates are a predisposing factor associated with the formation of primary acne lesions in adolescence (11).

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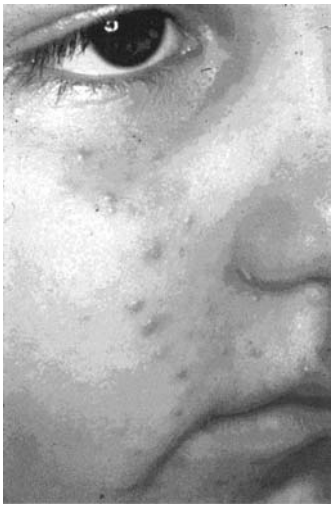

Figure 4 Inflammatory acne infantum.

E. Congenital Disorders

1. Familial Sebaceous Gland Hyperplasia (OMIM 601700)

Sebaceous gland hyperplasia occurs frequently, particularly in men past middle age (21). A few cases of sebaceous gland hyperplasia have been described in younger age groups. Boonchai and Leenutaphong (125) described a Thai family with premature sebaceous hyperplasia in five consecutive generations. Weisshaar et al. (126) described a family with familial nevoid sebaceous gland hyperplasia in three consecutive generations. The pedigree suggested autosomal dominant inheritance with incomplete penetrance. The disorder presents as solitary or disseminated, elevated, soft, yellow papules with central umbilication on the face. A premature form has its appearance during puberty or just afterwards, male predominance, and excessive sebaceous secretion. Most cases are sporadic (Fig. 5).

2. Nevus Sebaceus of Jadassohn (OMIM 163200)

Nothing is known about a possible genetic basis of this familial disorder that presents with alopecia with absent or primitive hair follicles and numerous small hypoplastic sebaceous glands. At puberty the lesions become verrucous with hyperplastic sebaceous glands (127). In late stages benign or malignant tumors develop. Kousseff (128) proposed that Jadassohn nevus phakomatosis, as he called the disorder, is, like other phakomatoses, a

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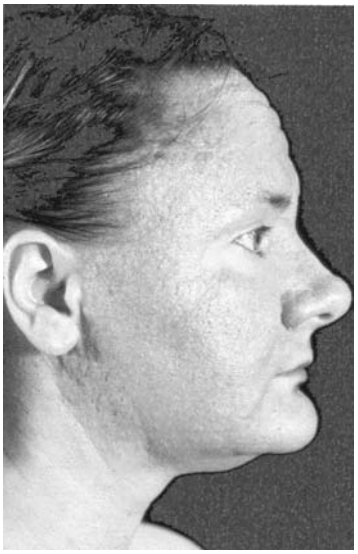

Figure 5 Familial sebaceous gland hyperplasia in a middle-aged woman. The face is mainly involved. The daughter of the patient is also affected, but to a lesser extent.

paracrine growth regulation disorder or paracrinopathy, i.e., dysregulation of paracrine growth and of transforming growth factors at cellular and extracellular matrix levels, leading to localized over- and undergrowth anomalies.

3. Sebaceous Nevus Syndrome with Hemimegalencephaly (OMIM 601359)

The sebaceous nevus syndrome consists of sebaceous nevus of the face or scalp associated with ipsilateral defects of the brain, eye, and connective tissue (129). It may be caused by mosaic mutation of a gene, which would be lethal if expressed in all cells.

4. Organoid Nevus Phakomatosis (OMIM 165630)

The features are ocular anomalies including coloboma, cerebral defects such as mental retardation or seizures, and a systematized linear sebaceous nevus following the lines of Blaschko (Schimmelpenning-Feuerstein-Mims syndrome) (130). All cases are sporadic. Happle (131) suggested that this situation is best explained by a dominant lethal gene arising as a somatic

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mutation in the early embryo or a gametic half-chromatid mutation and surviving by mosaicism.

5. Steatocystoma Multiplex (OMIM 184500)

Steatocystoma multiplex is caused by mutations in the keratin-17 gene (KRT17; gene map locus 17q12-q21), which also has been found to be mutated in cases of pachyonychia congenita of the Jackson-Lawler type (132). In typical cases the patient may exhibit 100–2000 round or oval cystic tumors widely distributed on the back, anterior trunk, arms, scrotum, and thighs. Sebaceous cysts presenting mainly as wens of the scalp were reported by Stephens (133) in a large number of individuals in five generations in a dominant pedigree pattern.

6. Adenomatous Polyposis of the Colon (OMIM 175100)

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder that typically presents with colorectal cancer in early adult life secondary to extensive adenomatous polyps of the colon. Polyps also develop in the upper gastrointestinal tract, and malignancies may occur in other sites, including the brain and the thyroid (134). Helpful diagnostic features include pigmented retinal lesions known as congenital hypertrophy of the retinal pigment, jaw cysts, sebaceous cysts, and osteomata. The APC gene at 5q21 is mutant in FAP (135,136) (gene map locus 5q21-q22). Gardner syndrome, colonic polyposis with extrabowel tumors, especially osteomas with associated fibromas, sebaceous or epidermoid cysts on the back, and a rather characteristic retinal lesion, is now known to be a phenotypic variant of FAP caused by mutation in the APC gene. FAP behaves as an autosomal dominant trait with almost complete penetrance but striking variation in expression.

7. Muir-Torre Syndrome (OMIM 158320)

The Muir-Torre syndrome (137) is part of the Lynch cancer family syndrome II (OMIM 114400), which has been related to mutation in the MSH2 gene located on 2p (gene map locus 2p22-p21, 3p21.3). Mutations in the MLH1 gene, located on 3p, also cause the syndrome. It represents the association of sebaceous skin tumors with internal malignancy. Gastrointestinal cancers are the most common internal malignancies (61%), followed by genitourinary cancer (22%). The sebaceous tumors are relatively uncommon or rare types: sebaceous adenoma, sebaceous epithelioma, basal cell epithelioma with sebaceous differentiation, and sebaceous carcinoma. Rothenberg et al. (138) described two cases suggesting

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that a single sebaceous adenoma can be the sole tip-off to the presence of colon cancer in the Muir-Torre syndrome. Sebaceous tumors appear before the internal malignancy in 22%, concurrently in 12%, and after the internal malignancy in 56% of patients (139). In 16% of patients, a temporal relationship of the two features was not reported. Rodenas et al. (140) reported a family in which multiple members had the Muir-Torre syndrome and hyperlipidemia. Approximately 15% of female patients with Muir-Torre syndrome develop endometrial cancer (141).

8. Ulerythema Ophryogenes/Keratosis Pilaris (OMIM 604093)

Zouboulis et al. described a patient with ulerythema ophryogenes and keratosis pilaris with monosomy 18p with missing sebaceous glands or sebaceous gland anlagen at the areas involved (142,143). Since the first report, three further cases have been identified (143); the derivative chromosome 18 was detected as a dicentric with breakpoints in p11.2 on both the Y chromosome and chromosome 18 (144). The patient had another normal Y chromosome. Involvement of a laminin gene has been postulated (143). Interestingly, in keratosis follicularis spinulosa decalvans (ichthyosis follicularis or Siemens syndrome), pilosebaceous follicles are dystrophic and sebaceous glands are also absent (145).

ACKNOWLEDGMENT

This chapter is dedicated to the lifelong work of Prof. Albert M. Kligman, M.D.

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5
Neonatal Pigmentation

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I. BASIS OF SKIN COLORATION

Skin color is determined by the composite of various chromophores within the skin including oxyhemoglobin (red), deoxyhemoglobin (blue), melanin (red, brown, or black), and carotene (orange). Of these various chromophores, melanin is the principal determinant of skin coloration (1).

Melanocytes residing within the basal layer of the epidermis synthesize melanin in membrane-bound organelles known as melanosomes. Melanosomes are transferred from melanocytes to surrounding keratinocytes, which retain their load of melanin as they migrate from the basilar layer to the stratum corneum (Fig. 1). The total quantity of melanin within the epidermis determines the color of the skin. Greater concentrations of epidermal melanin result in darker skin color.

Ethnic differences in skin color are not a function of the population density (melanocytes/mm^2) of epidermal melanocytes (1,2). Individuals from all ethnic groups, exhibiting a wide range of skin colors, have a remarkably similar number of epidermal melanocytes (3). The differences in ethnic skin color are dependent on the quantity and type of melanin within the epidermis (Fig. 1) (1–6). The quantity of melanin is determined by the size and number of melanosomes transferred from melanocytes into the surrounding keratinocytes. In dark-skinned individuals, there are numerous, large (> 1 μm) melanosomes replete with eumelanin. The epidermis is engorged with melanin (7). In contrast, light-skinned individuals

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have melanosomes that are smaller, less heavily pigmented, and fewer in number (7). Lightly pigmented skin has a relative paucity of melanin within the epidermis (Fig. 1).

There are two chemically distinct forms of melanin, eumelanin and pheomelanin. Eumelanin is either black or brown in color and is derived exclusively from the amino acid tyrosine (Fig. 2). Pheomelanin is reddish-orange in color and is synthesized from a combination of tyrosine and cysteine (Fig. 2). All humans regardless of skin color have a combination of both types of melanin, but those with very dark skin have a preponderance of eumelanin and only a minimum of pheomelanin. Persons of Celtic and northern European descent have mostly pheomelanin in the skin and smaller amounts of eumelanin (8).

The population density (melanocytes/mm^2) in adult skin varies significantly at different sites of the integument. Melanocytes are more numerous in sun-exposed compared to sun-protected skin. Facial skin of adults have an average of 1200–1800 melanocytes/mm^2 compared to truncal skin, where the density averages 800–1000 melanocytes/mm^2 (9–11). In adults the highest concentration of melanocytes are present in the skin of the male genitalia and anal canal, where the density can reach 2000 cells/mm^2 (12). Regional variations are not a feature of the skin of the fetus or neonate, in which the population density of melanocytes seems to be similar in all areas of skin (13,14).

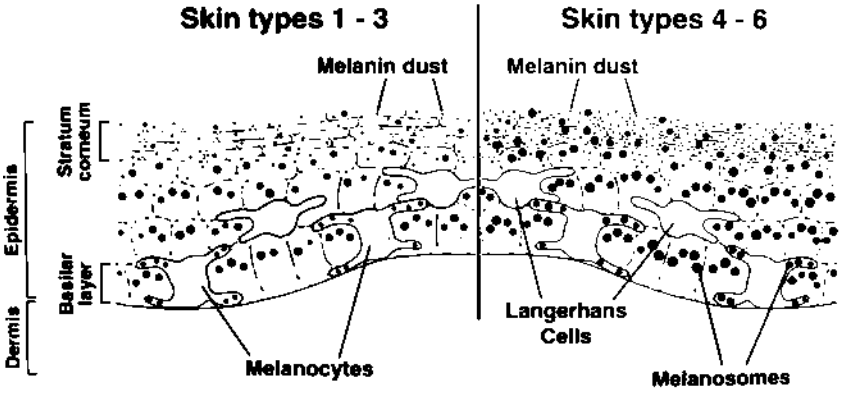

Figure 1 Schematic drawing of the epidermis illustrating three cell types: keratinocytes, melanocytes, and Langerhans cells. The left side of the drawing represents light skin and has fewer melanosomes and less melanin than the darker skin on the right.

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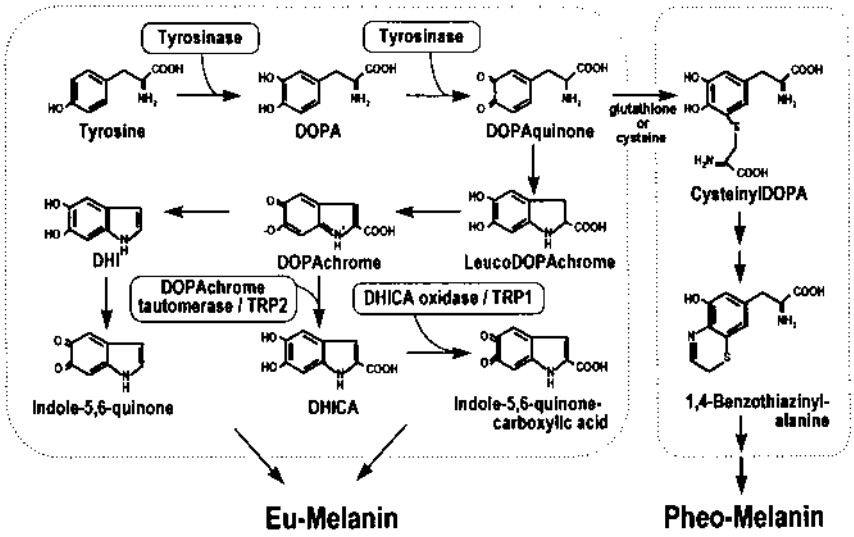

Figure 2 Schematic diagram showing the synthesis of melanin and the importance of the enzyme tyrosinase. Eumelanin is derived exclusively from tyrosine, pheomelanin from a combination of tyrosine and cysteine. Note that two other enzymes, TRP-1 and TRP-2, are involved in the synthetic pathway. DHI, 5,6-dihydroxyindole; DHICA, 5,6-dihydroxyindole-2-carboxylic acid; TRP, tyrosinase-related protein; DOPA, 3,4-dihydroxyphenylalanine.

II. FETAL PIGMENT SYSTEM

The origin of melanocytes from the neural crest has been well established in various species of animals, including humans (13–15). Studies conducted with human embryos have confirmed that melanocytes arise from the surface ectoderm and neural folds (13,14). The neural crest forms first in the cephalad end of the embryo at around 22–23 days estimated gestational age (EGA) and is detectable later at the caudal end, around 24–27 days EGA (13,14).

The embryological timetable for the development of the cutaneous melanocyte system was determined with two classical histochemical techniques, silver and DOPA stains (16). The silver stain takes advantage of the propensity of premelanosomes to bind silver salts and produces a black stain. The DOPA reaction is an indicator of the presence of an active tyrosinase enzyme, an enzyme found only in melanocytes. The inherent limitation of both techniques is that they identify only relatively mature melanocytes. They do not identify melanoblasts (i.e., melanocyte precur-

sors) because these immature melanocytes do not form melanosomes or synthesize tyrosinase.

HMB-45 is an antibody raised against melanoma cells that specifically reacts with most melanoma cells, junctional nevus cells, as well as fetal melanoblasts and neonatal melanocytes (13,14). It does not attach to adult human melanocytes or other nonmelanocytic cells. In more recent studies on human fetal skin, investigators have utilized the HMB-45 monoclonal antibody. The data from these studies expanded our knowledge of the fetal development of the cutaneous pigmentary system.

The initial event in melanocyte differentiation begins with the formation and migration of melanocytes from the neural crest, an event that is detectable around 42–50 days EGA (16). Melanoblasts migrate in the fetal dermis as they move from the neural crest toward the epidermis. By histochemical silver and DOPA stains, small numbers ($< 100/mm^2$) of melanocytes were identifiable in the fetal dermis of the scalp, nape of the neck, and sacrum at around 70–80 days (gestational age) (16). One week later melanocytes were detectable in the skin of most parts of the body, and the population density increased rapidly to 300–400 melanocytes/mm^2 by weeks 13–14. Melanocytes were not detectable with silver stains in the dermis during the second half of fetal development except on the scalp, sacrum, and the dorsum of the hands and feet (16). The fate of these dermal melanocytes has not been determined since the population density of melanocytes in the epidermis detected by silver stains remained constant during the second half of gestation. Dermal melanocytes can persist in some sites and are manifested in infants as sacral spots or in adults as blue nevi.

Using HMB-45 antibodies, Holbrook detected melanoblasts in the dermis before 40 days EGA (13,14). By 40–50 days EGA, high numbers ($\sim$ 1040 melanoblasts/mm^2) of melanoblasts were identified within the basilar layer of the epidermis (13,14). Although the previous studies using histochemical silver techniques identified a second wave of melanocyte influx into the skin occurring at 84–100 days EGA, the HMB-45 studies have failed to substantiate this finding (13,14,16). By the end of the first trimester, Holbrook observed that the population density of epidermal melanoblasts was higher than that in post-natal skin, reaching as high as 2100–2300 melanocytes/mm^2 (13,14). At the end of the first trimester, melanocytes are present within the follicles of developing hair germs (13,14). Melanin synthesis, by epidermal melanocytes, can be detected first by electron microscopy at 70 days EGA and by DOPA staining at 84–110 days EGA (13, 14). Melanocyte transfer of pigment to keratinocytes, however, does not begin until 5 months EGA (16). As fetal gestation progresses into the third trimester, the number of melanocytes/mm^2 decreases from 2300 (late first trimester) to about 800/mm^2 by the time of birth.

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III. NEONATAL PIGMENTATION—PREMATURE AND TERM INFANTS

It seems probable that pigmentary phenomena in infants of all ethnic backgrounds are similar but more easily observed in those of dark-skinned parentage. Clinical studies on infants of parents with ethnic dark skin, therefore, are more informative than observations of infants of fair-skinned parents.

The development of skin coloration is a factor that has been used routinely by pediatricians to estimate gestational age in the neonate (17). However, there are scant data in the medical literature related to the normal process of pigmentation in the neonate, especially in the premature infant.

The pigmentary system is active in various parts of the body of the embryo long before birth (1). Melanin is formed within the retinal pigment epithelium of the eyes by week 6 of gestation. By the middle of the second trimester, small quantities of melanin are transferred from melanocytes into surrounding epidermal keratinocytes. Hair is deeply pigmented in premature African babies as early as 20 weeks gestation. The dark hair of premature infants often falls out and is replaced by lighter hair after birth.

Premature infants in weeks 22–24 can exhibit sacral spots (1). Some infants exhibit increased pigmentation on the acral parts of the fingers and toes (Fig. 3), the areola (Fig. 4), the axilla (Fig. 5), and genitalia (Figs. 6, 7). The epidermal pigmentation is most marked in premature infants whose parents are deeply pigmented (18). In a study done in South Africa, 79% of black preterm infants had increased pigmentation on the genitalia and 27% had deeply pigmented skin in the perineum (18). Increased pigmentation was also reported in the proximal nail folds and terminal phalanges (18). A similar pattern of acral pigmentation associated with increased pigmentation of the external ear has also been described in black term infants belonging to the African Xhosa tribe (19). Interestingly, Xhosa infants whose parents are darkly pigmented may be born with a very lightly pigmented skin color. Acral and ear hyperpigmentation are typically present in these lightly pigmented infants and is regarded as a confirmation of their Xhosa ethnic origin (19). Not all infants with pigmented parents have acral pigmentation. The acral pigmentation disappears in some of these infants by 40 weeks gestational age and persists in others. Premature infants have been noted to have café au lait spots (18).

Skin color of premature and term infants has been assessed by reflectometry. Results from preterm and term infants (25–44 weeks) revealed several interesting findings. Preterm infants at 25 weeks gestation have little to no visible epidermal pigmentation. Their skin is markedly erythematous. White and black infants exhibit similar patterns of skin reflectance prior to

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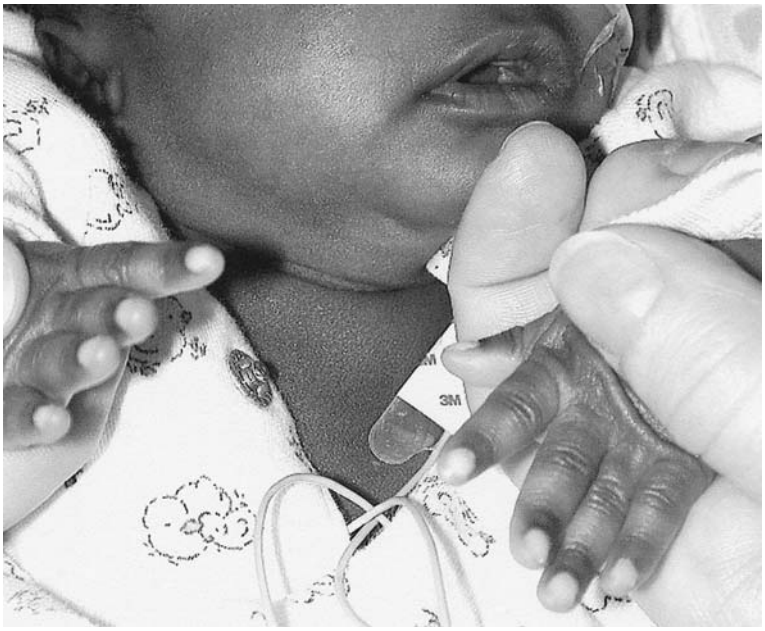

Figure 3 Premature black female, 30 weeks gestation, with increased pigmentation of the distal portions of the fingertips.

32 weeks of age, after which the skin of black infants become darker. Caucasian children retain their lighter skin color. The divergence in skin coloration between black and white infants observed after 32 weeks gestational age may possibly be explained by the differences in melanocyte activity (17). Results of studies have confirmed the presence of equal numbers of melanocytes in black and white newborn foreskins, but other racial differences in the function of the cutaneous pigmentary system have been identified (20). Microscopic studies on the skin from American black fetuses have shown that melanosome transfer from melanocytes to keratinocytes begins around 20 weeks gestational age (17). Melanin production and transfer to keratinocytes accelerates from the 28th week until birth (16,21). Active melanin synthesis and transfer to keratinocytes also occurs in white fetuses but apparently takes place at a slower rate and possibly later in development than it does in blacks (17). Results of studies performed on neonatal foreskins at the time of circumcision have shown that black skin has two to three times the tyrosinase activity as white skin (Fig. 8) (22). At term birth infants of ethnically dark parents have some visible melanin in the skin. In general all neonates are significantly lighter than adults. Previous studies showed

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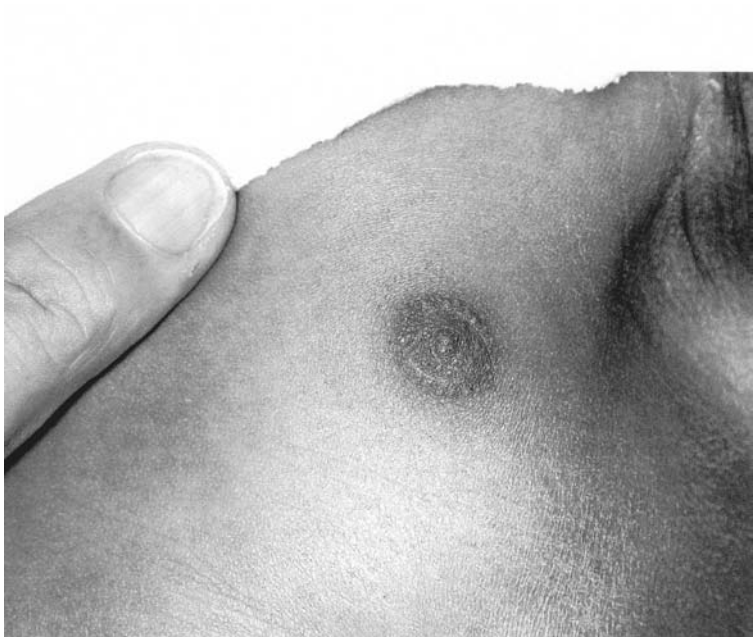

Figure 4 Term black female with pigmented areolae.

that full-term newborn New Guinea indigenes were lighter in complexion than adults but were able to reach adult levels of pigmentation by 6 months of age (17).

The second observation that comes from studies by reflectometry is that, after 35 weeks of gestation, white females are slightly lighter skinned than white males (17). The differences in reflectance were not statistically significant. However, studies on skin color in children and adults seem to indicate that the female is slightly lighter than the male during pre- and postnatal life (1).

IV. PIGMENTATION REGULATION

The factors that regulate neonatal pigmentation are poorly understood. From studies in adults, multiple mechanisms involving both hormonal and environmental stimuli are likely to be involved. Recent work, however, has been focused on the role of α-MSH during fetal development (23). In addition to its role in stimulating pigment synthesis, α-MSH has also been

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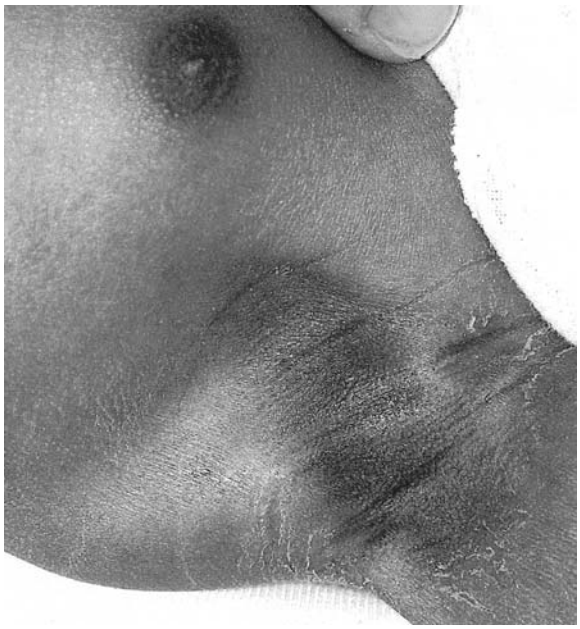

Figure 5 Term black female with increased pigmentation of the axilla and areola.

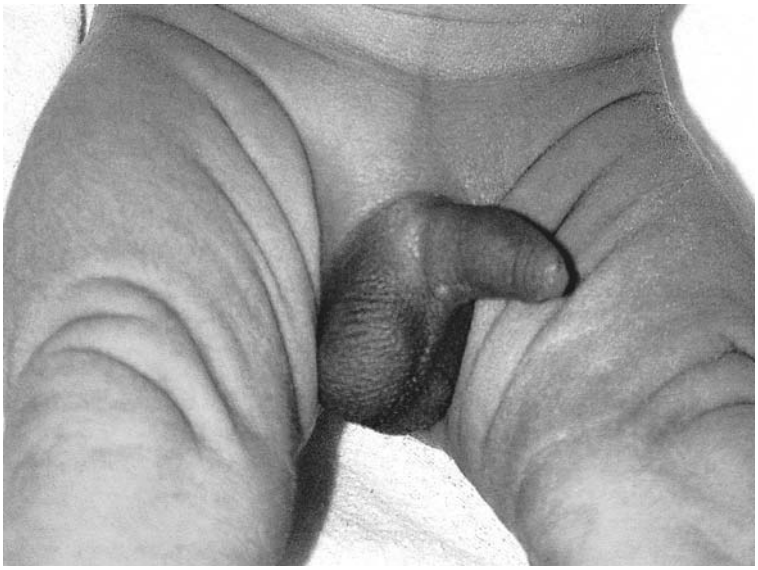

Figure 6 Term black male with marked scrotal and some penile hyperpigmentation.

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Figure 7 Term black female with hyperpigmentation of the labia and linea nigra less easily visualized in this black and white photograph.

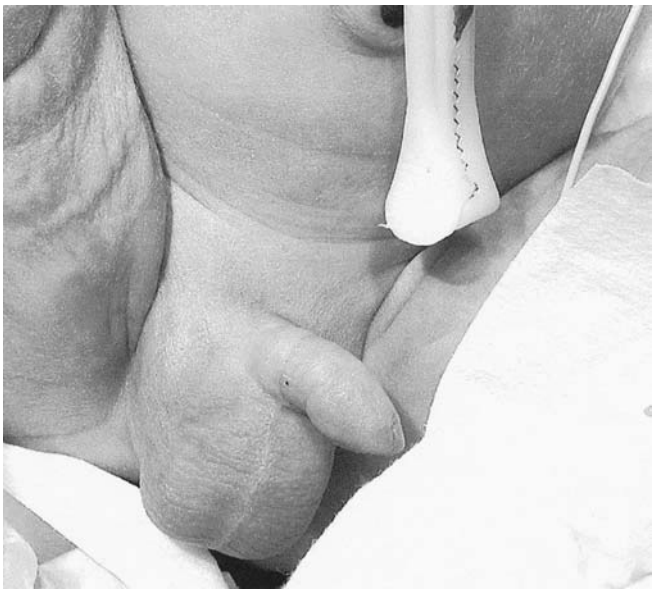

Figure 8 Premature Caucasian male, 34 weeks gestation, with no increased genital pigmentation. The tyrosinase activity of Caucasian melanocytes is less than that found in foreskin melanocytes of African children.

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implicated in a variety of inflammatory, immune, and endocrine functions (24–42). Studies obtained from both fetuses and term infants have demonstrated high levels of circulating α-MSH during the first and second trimesters as well as immediately after birth (43,44). The presence of high levels of α-MSH at birth may explain, in part, the mechanism of increased pigment production in the post-partum period.

V. THE KERATINOCYTE LANGERHANS MELANOCYTE COMPLEX

As previously described, epidermal melanocytes residing in the basal layer of the epidermis transfer melanin to surrounding keratinocytes (Fig. 1). Microscopic analysis has shown that there are approximately 36 keratinocytes for every epidermal melanocyte. This organizational division of a single melanocyte with a cohort of keratinocytes to which it transfers melanin has been termed the epidermal melanin unit by Fitzpatrick and coworkers (45,46). The concept of the epidermal melanin unit has been central to our present understanding of how the melanocyte interacts with the other cells that inhabit the epidermis.

Although the epidermal melanin unit has enjoyed popularity as a unifying model for melanocyte interactions within the skin, this concept is inadequate to account for much of the newer data on interactions between various epidermal cells. In addition to keratinocytes and melanocytes, there are other resident cells within the epidermis, specifically Langerhans cells (47–49). When the epidermal melanin unit was first proposed, the function of Langerhans cell was unknown, and in fact they were regarded as effete melanocytes. Subsequent work has established that Langerhans cells are bone marrow-derived immune cells that function as antigen-presenting cells within the epidermis and dermis. There are 53 epidermal cells for each Langerhans cell. Thus, there appear to be constant ratios maintained among melanocytes, Langerhans cells, and keratinocytes.

Numerous studies have confirmed the role of the Langerhans cell as an important mediator in a variety of immune and inflammatory reactions such as contact hypersensitivity and skin graft rejection. Many of the same molecules that affect the function of melanocytes, such as interleukin-1 and interleukin-6, leukotrienes, and prostanoids, are also capable of altering the function of Langerhans cells (24,25,27,31,34,39,40,50–56). An absence of epidermal melanocytes such as occurs in the disease vitiligo is associated with an alteration in the epidermal immune response to a variety of allergens and irritants (47,48,57–59). Moreover, Langerhans cells exhibit a muted response to injections of interferon-gamma in depigmented compared to normal skin in patients with vitiligo (60). α-MSH is a peptide hormone

that is believed to be the principal factor in the stimulation of melanogenesis. However, in addition to its role in pigmentation, α-MSH has been shown to function as a modulator of both the inflammatory and immune systems (24–42). These and other data suggest a link between the melanocyte and Langerhans cell systems. In view of these findings it would seem reasonable to incorporate the Langerhans cell into the existing concept of the epidermal melanin unit. Such a unit might be called the keratinocyte-Langerhans-melanocyte (KLM) complex (1).

VI. MELANOGENESIS

The rate of melanin formation is dependent on the activity of the enzyme tyrosinase (Fig. 2). Tyrosine, which serves as the primary substrate for the enzyme tyrosinase, is the principal precursor of eumelanin (61). At one time tyrosinase was thought to be the only enzyme involved in melanin formation. Tyrosinase oxidizes the amino acid tyrosine in a two-step process, first to DOPA and then to dopaquinone (Fig. 2). Dopaquinone was once thought to spontaneously convert into melanin. This, however, has proved to be an oversimplification (61). Subsequent research has identified two additional enzymes and numerous other factors involved in the synthesis of eumelanin (Figs. 2,9). Mutations in the tyrosinase gene are the cause of oculocutaneous albinism type I (OCA-1) (42).

TRP-1 (tyrosinase-related protein 1) and TRP-2 (tyrosinase-related protein 2; dopachrome tautomerase) (Figs. 2,9) are the two other enzymes involved in the synthesis of eumelanin. Stabilin is a mouse protein coded for by the silver locus and is thought to function as a DHICA polymerase (Fig. 9) (61).

Mutations can occur in any of the enzymes and other molecules that regulate synthesis of melanin. Disorders that limit melanin production are termed oculocutaneous albinism (OCA) because there is a deficiency of pigmentation in the skin, hair, and eyes. Albinism is defined as a genetic disorder in which the synthesis of melanin is retarded, causing abnormalities in the development of the ocular system and poor visual acuity. In addition, the skin is lighter than expected based on parental heritage. There are three defined types of OCA. OCA-1 is caused by mutations in the tyrosinase gene and is termed tyrosinase-related albinism (62). The activity of the enzyme can be zero or partial. Accordingly the skin and hair of those with OCA-1 can vary from totally white (no tyrosinase activity from either parental gene) to moderately pigmented. The latter have partial tyrosinase activity.

Mutations in the p protein (chromosome 15) are associated with oculocutaneous albinism type 2 as well as several other rare conditions such as

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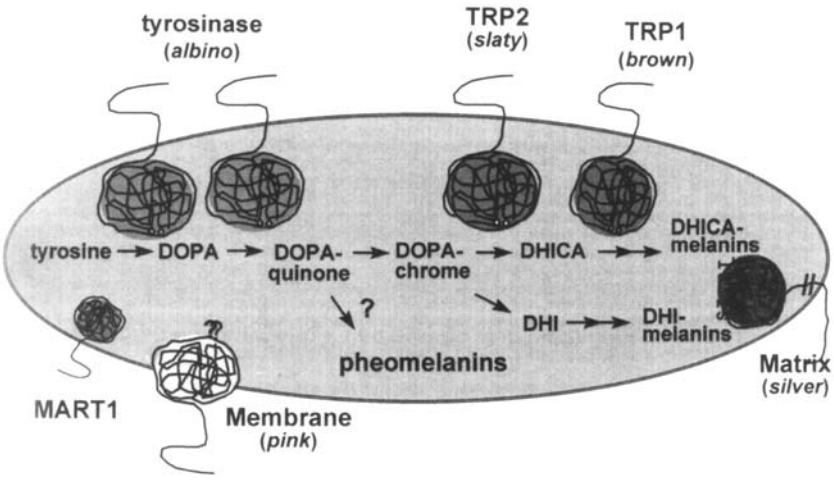

Figure 9 Schematic drawing of a melanosome showing the synthetic pathway for the melanin and some membrane factors such as the p protein necessary for normal production of melanin. Absence of dysfunction of the p protein is responsible for OCA 2 and Angelman and Prader-Willi syndrome. TRP, tyrosinase-related protein; DOPA, 3,4-dihydroxyphenylalanine; DHI, 5,6-dihydroxyindole; DHICA, 5,6-dihydroxyindole-2-carboxylic acid; MART, melanoma antigen recognized by T cells. (Courtesy of Vincent Hearing, Ph.D., Bethesda, MD.)

the Angelman and Prader-Willi syndromes (62). The function of the p protein has not been definitively identified but might regulate protein trafficking to melanosomes (63). Mutations of the p gene are responsible for OCA-2, the most common form of albinism found in Africa and South and Central America. In these continents albinism is 10 times more common than in western countries.

The third defined type of albinism, OCA-3, is related to a defect in the protein TRP-1. This enzyme is involved in conversion of DHICA to brown melanin. Only two children have been described with this genetic defect (64).

VII. PIGMENT GENES AND ABNORMALITIES OF PIGMENTATION

Multiple genes and proteins involved in melanocyte development and melanin synthesis have been identified (15,61,62,65,66). In fact, many of the genes that control various aspects of melanocyte development such as differentiation, migration, and division are the same genes that regulate similar functions in other cell types (15,61,62,65).

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Genetic defects affecting the pigmentary system are frequently associated with defects in other organ systems. Commonly co-affected organs include the eye, ear, the central nervous system, bone marrow, and gastrointestinal tract. It is interesting to note that some of these co-affected tissues such as the ears and eyes are sites where extracutaneous melanocytes are normally present and are known to be essential for normal function of these organs.

Genetic disorders affecting melanocytes can be classified by the nature of the defect. These defects can include alterations in melanocyte migration, melanin production, melanosome formation and transfer, and genetic mosaicism. A summary of some of these disorders, their corresponding gene defects. Salient clinical features are shown in Table 1.

Table 1 Genetic Defects Pigmentary Disorders

Disorder	Gene defect or chromosome	Clinical manifestations
Piebaldism	c-kit	Ventral depigmentation, white forelock
Waardenburg syndrome types 1 and 3 (piebaldism with deafness)	PAX-3	Ventral depigmentation, white forelock, unilateral or bilateral sensorineural deafness, heterochromia irides, other somatic defects
Waardenburg syndrome type 2 (piebaldism with deafness)	MITF-M	Same as types 1 and 3
Waardenburg syndrome type 4 (piebaldism with megacolon)	EDNRB, SOX 10	Depigmentation associated with megacolon
Urticaria pigmentosum	c-kit, stem cell factor	Pigmented macules that urticate with rubbing
OCA-1	Tyrosinase	White to light blond hair, gray or blue eyes, nystagmus, myopia, strabismus (European albinism)

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Table 1 Continued

Disorder	Gene defect or chromosome	Clinical manifestations
OCA-2	p gene	Reddish hair and skin. Lentigines, ocular abnormalities (African albinism); Angelman and Prader-Willi syndromes
OCA-3	TRP-1	Brown hair and skin, minimal ocular abnormalities
Phenylketonuria	Phenylalanine hydroxylase gene	Light skin and hair, mental retardation
MSH receptor defect (MC1R)	16q2	Red hair, blue eyes, increased susceptibility to skin cancer, some obesity syndromes
Chediak-Higashi syndrome	LYST	Hypopigmentation of skin and hair, numerous bacterial infections, early death, large abnormal granules in myeloid cells and melanocytes
Hermansky-Pudlak syndrome types 1 and 2	Type 1 HPS-1 Type 2 AP-3	Hypopigmentation associated with bleeding diathesis, pulmonary fibrosis, colitis, ceroid deposition
Griscelli syndrome	RAB 27, MYO5A	Silvery hair, light skin, immune defects with no natural killer cells, melanocytes filled with melanosomes
Eladjalde syndrome	Unknown	Hypopigmentation associated with cerebellar dysfunction
Hypomelanosis of Ito	Xp11	Swirls of hypopigmented skin, may be associated with somatic defects and mental retardation

VIII. CONCLUSION

Our overall understanding of pigment biology has advanced greatly in the past few years. The processes governing pigmentation in adults have been clarified, although more work remains to be completed (67). Developmentally, significant challenges remain in understanding the embryogenesis of the pigmentary system and the role of the pigmentary system in normal and abnormal fetal development. The genetic basis of pigmentary diseases, for example, is an increasingly exciting area of active research. Postnatally, adaptation to life after birth requires functional interaction of cutaneous melanocytes with other resident epidermal cells. Research on the physiology of such interactions; e.g., the regulation of pigment transfer from melanocytes to keratinocytes, is still in its infancy.

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6
Eccrine Sweating in the Newborn

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I. INTRODUCTION

Sweating is the body's most important mechanism for losing heat. Sweat is produced in response to overheating, and its evaporation from the skin surface results in cooling. The newborn baby is prone to thermal challenge, but this is usually in the form of cold stress. Heat production, a function of mass, is low. Heat loss, a function of surface area, is high. The newborn has a low mass-to-surface area ratio, so keeping warm is the major difficulty, not keeping cool. Congenital inability to sweat because of a deficiency of sweat glands is a feature of the condition hypohidrotic ectodermal dysplasia. Affected individuals are not usually prone to overheating in the newborn period—this becomes a problem in infancy and childhood. Hence, sweating in the newborn is a subject of interest rather than of any major importance. However, one form of sweating occurs in response to arousal and pain rather than temperature. This palmar-plantar or emotional sweating is a vestigial process in the human but is of particular interest because it provides an objective tool for quantifying a newborn infant's response to procedural pain.

The subject of eccrine sweating was reviewed in considerable detail by Green in 1982 (1). In particular, the developmental physiology of sweating in the newborn as revealed by chemical stimulation was explored at some length. In the present chapter more recent research will be reviewed, with some reference to this earlier classical work. The reader who is interested in the mechanisms of sweating and the maturation in physiological function is urged to read the original chapter. Current views of parents and doctors do

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not allow physiological research in the newborn that adds to knowledge but that has no demonstrable benefit to the infants studied if it involves more than minimal interference with the infant. Work of this type is therefore unlikely to be repeated.

II. MECHANISM OF SWEATING

The eccrine sweat gland consists of two portions: a coiled secretory portion in the dermis and a straighter duct leading through the epidermis and emerging as a pore on the surface (2). Secretory cells produce sweat in the proximal part of the gland. This is initially an ultrafiltrate of plasma with a high electrolyte content, but similar cells in the more distal part of the gland reabsorb both electrolytes and to a lesser extent water (2). The sweat therefore becomes progressively more dilute, with eventually a fifth of the electrolyte content of plasma. It is expelled at least in part by contraction of myoepithelial cells that line the gland. Evaporation of 1 mL of sweat from the skin surface removes about 560 calories of heat from the body.

Eccrine sweat glands are innervated by nerve fibers, which are cholinergic, despite belonging anatomically to the sympathetic nervous system. They are stimulated in response to temperature change sensed directly or indirectly by the hypothalamus. They may also be stimulated by circulating catecholamines or by injection of acetylcholine, nicotine, or adrenaline directly into the dermis. Pilocarpine, an acetylcholine analogue, can cause localized sweating if it is applied to the skin surface and a small electrical current is passed through it. This technique of pilocarpine iontophoresis is the basis of the sweat test for cystic fibrosis.

III. DEVELOPMENT OF THE SWEAT GLANDS

Eccrine sweat glands are derived from the epidermis (3). They develop in the early second trimester of pregnancy by downward growth of epidermal cells into the dermis. At about 16 weeks they differentiate into a coiled portion and a duct, with the two cell types—secretory and myoepithelial—becoming apparent (4). Canalisation of the gland is complete by 22 weeks. Glands form first on the palms and soles, then the forehead, followed by the trunk and finally the limbs. Innervation of the glands parallels their formation. It is claimed that there are between 2 and 4 million sweat glands in the human (2). The density is 10 times greater on the sole and palm than the back. Since no new glands are formed after birth, a newborn has its full complement of glands. Since surface area is several times higher in the adult, the density of

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sweat pores (number per unit area of skin) is much greater in the newborn and greater still in the preterm infant.

IV. MEASUREMENT OF SWEATING

Thermal and emotional sweating can both be measured in the newborn using a variety of techniques. Thermal sweating can be measured from the whole body or from a localized area of skin.

Whole body sweating is measured in a metabolic chamber. The baby is placed in a calorimeter, and the water added to the surrounding air is directly measured by its absorption by an anhydrous agent as the ambient temperature is increased (5). Alternatively, the baby can be weighed on an accurate and sensitive balance. Indirect measurement of whole body sweating can be made by measuring evaporative heat loss itself by gradient layer calorimetry (6). All the methods measure transepidermal and respiratory water loss as well, so estimates of these need to be subtracted from the total water loss to obtain a value for water loss by sweating.

Localized sweating may be visible as droplets on the skin, particularly on the forehead (Fig. 1). Its visibility depends on the rate of evaporation

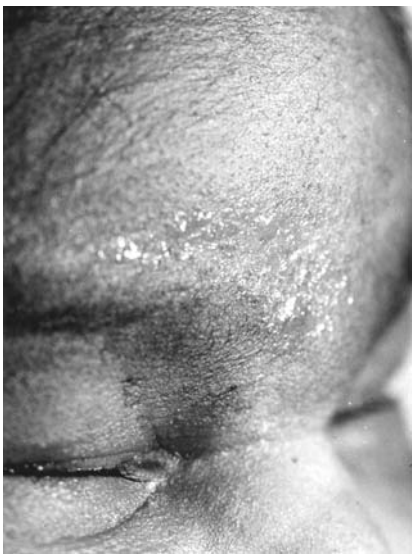

Figure 1 Visible sweating on the forehead of a full-term infant, well wrapped and nursed in a cot in a warm room.

from the surface, which in turn depends on ambient humidity. In a dry atmosphere sweat evaporates quickly and is not visible. Thus inspection of the skin is not a reliable measure of sweating. It can be measured from a small area of skin in a number of ways such as a colorimetric method, a capsule, or a skin evaporimeter. The commonest colorimetric method uses starch and iodine (Wada's method (7)). Iodine is painted on the skin, and sweating is revealed by covering the area with paper impregnated with starch. In the presence of water emerging from a sweat pore, the iodine turns the starch dark blue. Alternatively, the starch and iodine can be incorporated together in the same paper (8). The density of blue dots is a measure of the number of active sweat glands and therefore of sweating itself. This method does not quantify the amount of sweat produced. The capsule method requires an area of flat skin such as the forearm or thigh. Sweating adds water to the air in the capsule, which is then circulated through an absorber to be measured (9). The skin evaporimeter (ServoMed Inc., Sweden) is particularly suitable for use in the newborn of any size or gestation (10). It can be used to measure sweating from multiple areas of skin. The small probe contains two water vapor pressure sensors. The water vapor pressure gradient close to the skin is measured, and from this skin water loss is computed. Since the probe is small and only rests on the skin, the skin evaporimeter provides a good method for studying sweating in small infants. Neither of these methods distinguishes sweating from transepidermal water loss (TEWL), but sweating is easily recognized as a sudden sharp rise in water loss from the same skin site when the ambient temperature is increased (Fig. 2).

Emotional sweating from the palm or the sole can be measured directly with a skin evaporimeter (11) but is usually estimated indirectly by measuring skin conductance or resistance. The presence of water within the sweat ducts and, to a lesser extent, the hydration of the stratum corneum lowers the electrical resistance of the skin and raises its electrical conductance. Conductance is more commonly measured, usually by applying a small alternating current at low voltage to the skin and measuring conductance with a meter (12) (Fig. 3) The technique has been widely used in psychological research for many years.

V. THERMAL SWEATING IN THE TERM NEWBORN

It is very unlikely that infants sweat in utero, even though their core temperature is 0.5°C above maternal temperature and the mother may be febrile. During immersion in water or in a very humid environment, the outer stratum corneum is maximally hydrated and swells. This results in narrow-

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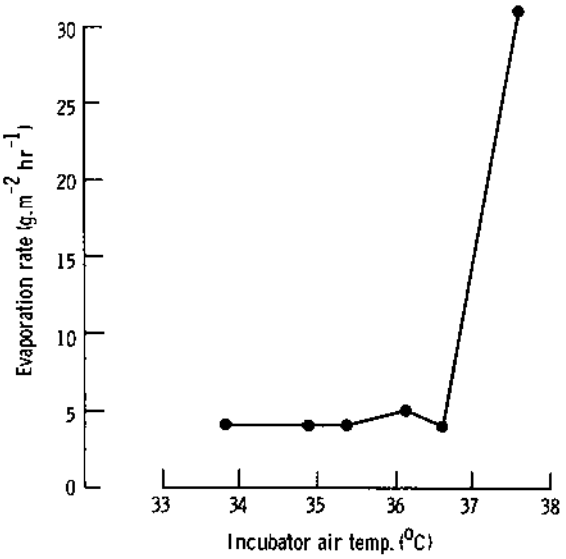

Figure 2 The rate of evaporation of water from the forehead of a newborn infant, gestation 38 weeks, age 3 days, nursed naked in a closed incubator. Measurement was made using a skin evaporimeter. The rate of evaporation is low, representing passive transepidermal water loss only, until the air temperature is increased from 36.6 to 37.6°C. There is then an abrupt, sixfold rise in evaporation rate, signifying the onset of sweating at this site. (From Ref. 20.)

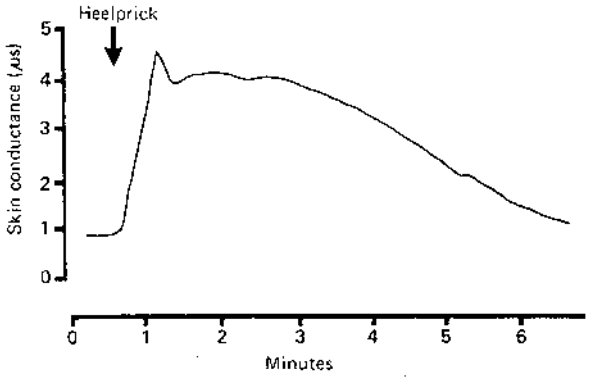

Figure 3 Skin conductance measured from the sole of the foot showing a marked rise in response to a heel prick on the other side (μs, microsiemens). Note that the peak value is reached at about a minute after the heel prick—this is characteristic of the change in skin conductance caused by arousal in the newborn. (From Ref. 12.)

ing of the terminal portion of the sweat ducts, occlusion by solid matter derived from the duct lining and therefore inability to sweat, a phenomenon known as hidromeiosis. This has been reviewed by Kerslake (13).

Most full-term infants possess the ability to sweat in response to a heat challenge from the day of birth (14–22). Progressive increases in ambient temperature within a closed incubator result in an increase in skin and core temperatures with narrowing of the peripheral/abdominal skin and the skin/core temperature gradients (19,23) (Fig. 4). When the core temperature is between 37 and 38°C, sweating can be detected. The incubator air temperature needed to induce sweating is 34–38°C in the first 4 days of life and falls to 33–34.5°C from day 6, probably reflecting an increasing metabolic rate. Similar results have been obtained using whole body calorimetry to measure total water loss (5) or evaporative heat loss (18) or ventilated capsules to measure local sweating (17). The degree of thermal sweating in the mature newborn is weak compared with the heat-acclimatized adult, who can lose

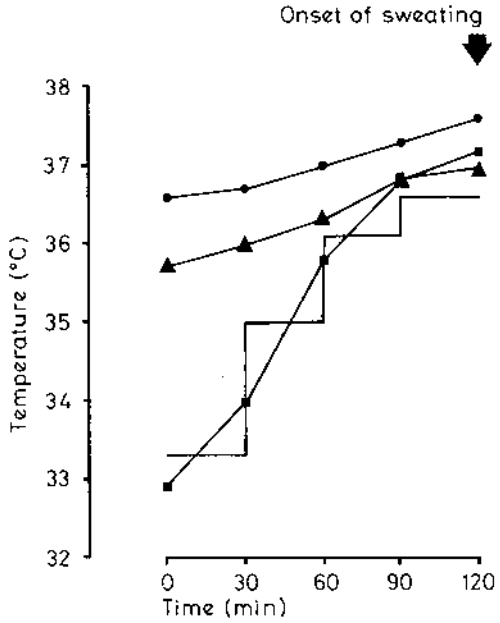

Figure 4 The effect of stepwise increases in the incubator temperature (—) on body temperatures (●, rectal; ▲, abdominal; ■, hand) of a naked, term infant, birthweight 2.95 kg, age 21 hours. The arrowhead indicates the onset of sweating. Note the reduction in skin/rectal and peripheral skin/central skin gradients as the ambient temperature increases. (From Ref. 19.)

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up to 1.5 L of water an hour in a hot environment (13). Since the density of sweat glands is much less in the adult, it follows that the amount of sweat produced by a thermally challenged adult gland is several times greater than that of a newborn gland. Sweating in the newborn can sometimes be detected when the infant in a warm environment becomes active or cries (20). When the infant settles again, the sweating may reduce in intensity and distribution or disappear without any manipulation of the thermal environment. Thermal sweating may be suppressed by drinking a cold glucose solution (21,22).

VI. SITES

If sweating occurs it is always detected on the forehead, either alone or at the same time as at other sites. In some infants it occurs on the forehead alone but spreads to other sites in a caudal direction when the ambient temperature is raised still further. The number of sites where sweating can be detected is gestation related, being significantly greater at 40–42 weeks than at 37–39 weeks. No thermal sweating occurs from the palms or soles. The intensity of sweating is much greater on the forehead than at other skin sites and is also gestation related, being significantly greater at 40–42 weeks than at 37–39 weeks. Rates of 50–120 $g/m^2/h$ can be reached in the most mature infants. Why is the forehead preeminent in newborn sweating? It is the site where sweat glands first appear in fetal life, so they are likely to be more mature. It is also the site of greatest density of glands apart from the palms and soles. After the forehead, the chest and upper arm are sites where sweating occurs frequently and at a higher intensity (Fig. 5).

VII. CHEMICALLY INDUCED SWEATING IN THE TERM NEWBORN

Sweating in response to the intradermal injection of drugs can be induced in term infants soon after birth using acetylcholine (17,25), adrenaline (8), nicotine (26), neostigmine (8), and pilocarpine (14), confirming successful maturation of the sweat glands.

VIII. THERMAL SWEATING IN THE PRETERM NEWBORN

Infants below 36 weeks gestation do not demonstrate a sweat response to a high ambient temperature on the first day of life, even if their core temperature reaches 38°C [5,14,17,20). However, the ability to sweat

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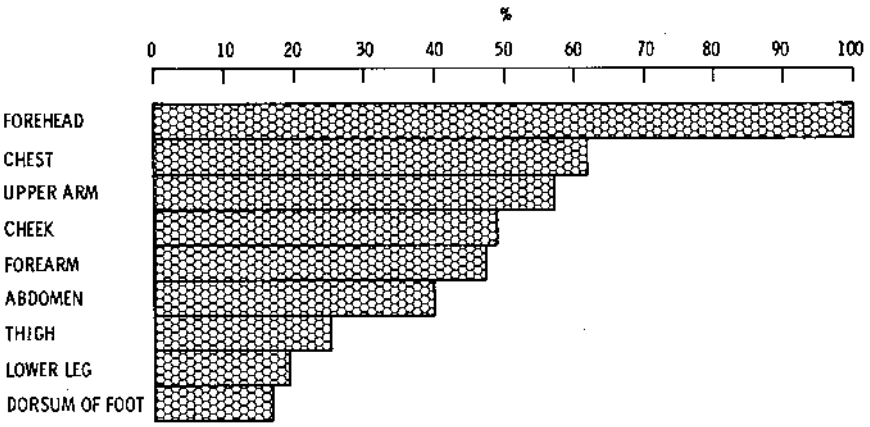

Figure 5 Sites involved in the sweating response expressed as a percentage of the total number of babies able to sweat. Thus all babies who were able to sweat did so from the forehead, but only 18% demonstrated sweating from the dorsum of the foot. Thermal sweating was measured by skin evaporimeter in 85 term and preterm infants. (From Ref. 20.)

appears within a few days so that by 13 days, an infant of 29 weeks can sweat in response to a heat challenge (20) (Fig. 6). More immature infants have never been studied. Like TEWL, maturation of thermal sweating is accelerated by birth, presumably the effect of exposure to air after prolonged immersion in water. Unlike TEWL, however, the sweat response in the preterm infant is still immature at 2 weeks of age. Sweating occurs at fewer sites (Fig. 7), at a lower intensity (Fig. 8), and at higher ambient and core temperatures than in the term infant (20). It is therefore less effective as a defense against overheating, but since the latter is uncommon it is rarely required.

IX. CHEMICALLY INDUCED SWEATING IN THE PRETERM NEWBORN

Sweating in response to an intradermal injection of acetylcholine, adrenaline, nicotine, or neostigmine is similarly impaired or absent in preterm infants but appears with postnatal maturation. It is difficult to make comparisons with thermal sweating because the thigh or the forearm is the usual site chosen for study of chemically induced sweating, not the forehead, chest, or upper arm, where thermal sweating is first seen. However,

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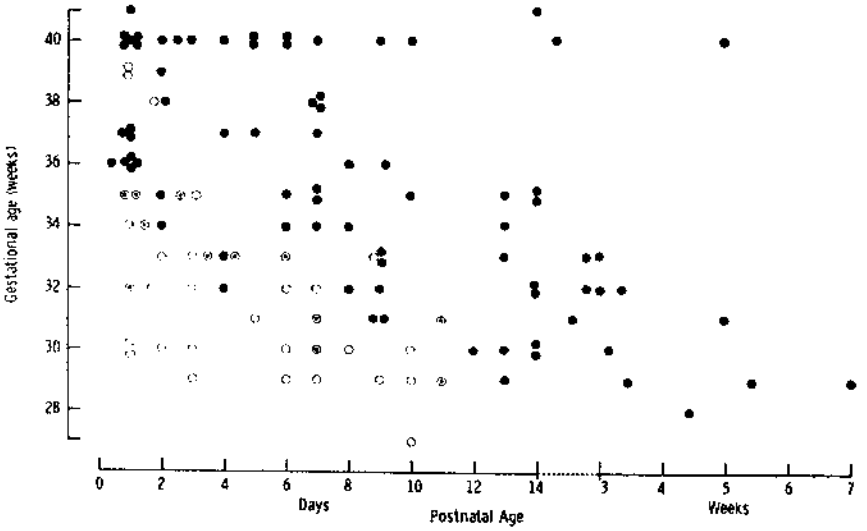

Figure 6 Development of the ability to sweat in response to thermal stress ●, infants in whom sweating was detected; ○, infants in whom sweating was not detected although their rectal temperature reached 37.9°C; ⊙, infants in whom sweating was only detected when rectal temperature reached 37.9°C and there was the additional stimulus of vigorous crying). (From Ref. 20.)

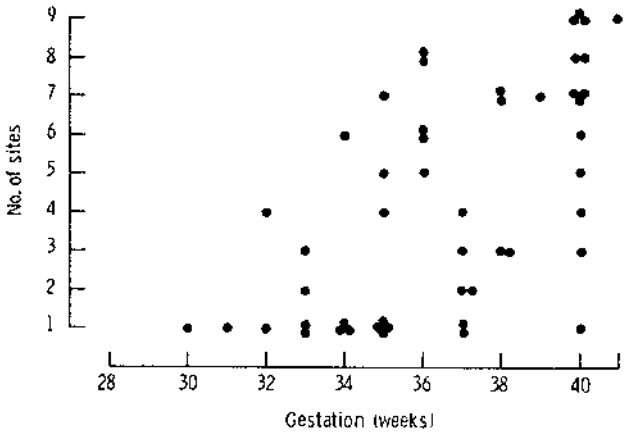

Figure 7 Number of sites where thermal sweating was detected in the first week of life in relation to gestation, measured at the nine sites described in Figure 5. (From Ref. 20.)

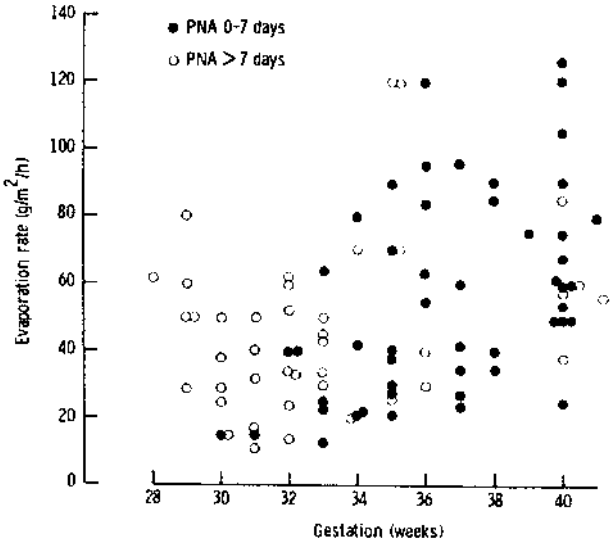

Figure 8 Maximally detected rate of thermal sweating from the forehead in relation to gestation (●, infants age 0–7 days; ○, infants >7 days old). The mean maximal rate detected was significantly higher in infants at 37 weeks or more when compared with those below 34 weeks ($p < 0.0005$). (From Ref. 20.)

if the two types of sweating are examined at the same site in the same infant, it is clear that chemically induced sweating is more advanced in preterm infants than is thermally induced sweating (17,27). In other words, if sweating occurs in response to thermal stimulation, it will always occur in response to chemical stimulation. Fewer infants who sweat in response to chemical stimulation will also sweat in response to thermal stimulation This suggests that the lack of a sweat response in preterm infants is a result of neurological rather than glandular immaturity. There is further evidence that this is the case. Mature infants born with major central nervous system (CNS) defects do not sweat in response to thermal or chemical stimulation below the level of the lesion but do so above the lesion (17,28). Infants born prematurely to mothers who are opiate addicts show spontaneous, generalized sweating in response to withdrawal of the drug, even as early as 32 weeks gestation (1,29). They also show an increased sweat response to intradermal acetylcholine, adrenaline and nicotine compared with healthy preterm controls. This suggests activation of the sweating mechanism in spite of prematurity, the result of CNS stimulation induced by the withdrawal.

X. COMPOSITION OF SWEAT IN THE NEWBORN

Composition of sweat is determined by the sweat test. Localized sweating is induced by pilocarpine iontophoresis, absorbed on to a filter paper, weighed, and then analyzed for electrolyte content. The amount of sweat obtained in a term newborn is less than in an infant, but the electrolyte contents of sodium, potassium, and chloride are the same as in an infant or child. No information is available about the electrolyte content of sweat in preterm infants who are just acquiring the ability to sweat.

XI. EMOTIONAL (PALMAR/PLANTAR) SWEATING

Water loss from the palms and soles is high and unresponsive to changes in ambient temperature. The epidermis is very thick at these sites, so the high water loss is not the result of passive transepidermal water loss. It is the result of active sweating, which is under the control of the sympathetic nervous system via cholinergic fibers, as is sweating elsewhere. Unlike thermal sweating, the stimulus is arousal, in the form of pain, fear, excitement, or concentration rather than temperature. Emotional sweating performs no function in the human. It is considered to be the vestigial remains of the fight-or-flight reflex in mammals, where increased moistness of the paws might increase friction with the ground and therefore help the animal to flee from danger more quickly and securely (30). Emotional sweating is a well-used tool in psychological research and forms the basis of the lie detector. It increases with mental concentration, wakefulness, fear, anger, and excitement. It decreases with contentment, relaxation, and sleep.

XII. EMOTIONAL SWEATING IN THE NEWBORN

In the term newborn, palmar water loss increases with state of arousal from birth. It is lowest during deep, non–rapid eye movement sleep, although the water loss is still mainly a result of active sweating rather than passive TEWL. It is increases by a factor of three during crying and vigorous movement (31) (Fig. 9). Over the first few weeks of life, emotional sweating becomes more marked. Values during deep sleep are higher and the increase during maximal arousal is fivefold (Fig. 9). Similar observations have been made using skin conductance measurement rather than water loss (12).

In the preterm newborn (< 37 weeks) in the first week of life, palmar and plantar water loss during deep sleep is lower and the increase with maximal arousal is small or absent when measured by evaporimeter (31)

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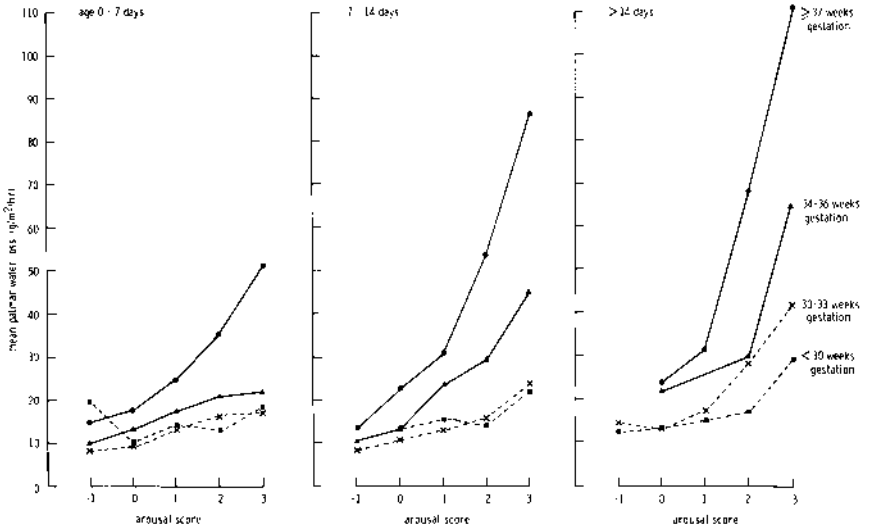

Figure 9 Mean palmar water loss at different states of arousal, demonstrating the effect of both gestation and postnatal age. Measurements were made by skin evaporimeter on 124 infants, gestation 25–41 weeks, age 15 hours to 9 weeks. Arousal is measured on a scale from −1 (deep, non-REM sleep) to 3 (vigorous crying). The increase in palmar water loss with arousal (emotional sweating) is influence by gestation and postnatal age. (From Ref. 31.)

(Fig. 9) or standard skin conductance (12). With increasing postnatal age, this matures (31). Palmar water loss and skin conductance during maximum arousal increase markedly. However, birth does not appear to accelerate maturation of emotional sweating as it does with thermal sweating (Fig. 6) and TEWL. Emotional sweating becomes apparent at around a corrected age (gestational plus postnatal age) of 36 weeks, regardless of gestational age at birth (31) (Fig. 10). This therefore limits its use as a tool in the examination of the response to an acute painful procedure in the immediate newborn period, since most such procedures are performed in the preterm. A term baby shows an abrupt increase in palmar water loss when subjected to heel prick for example (31) (Fig. 11). Similarly, there is an abrupt rise in skin conductance (12). This has been used as a method of comparing different methods of obtaining blood from the heel (32). A preterm infant shows no or minimal response, even though the usual behavioral changes occur (12,31) (Fig. 11). Lack of response is a result of immaturity of emotional sweating, not inability to react to a painful stimulus since the same behavioral changes are seen.

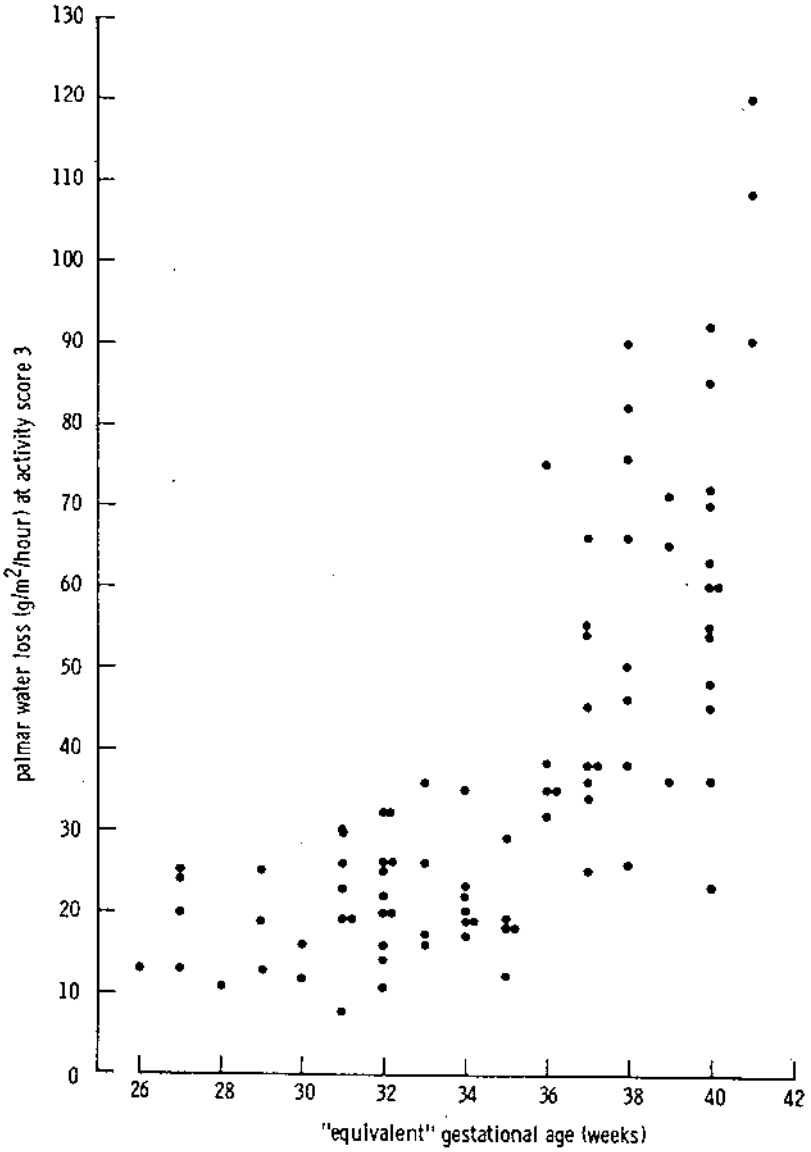

Figure 10 Palmar water loss in the same group of infants (Fig. 9) showing the values at maximal arousal (3 on the scale) displayed according to "equivalent " gestational age (gestation plus postnatal age). Values are 10–30 $g/m^2/h$ until the equivalent gestational age of 36 weeks is reached. Values then increase abruptly to 30–100 $g/m^2/h$. (From Ref. 34.)

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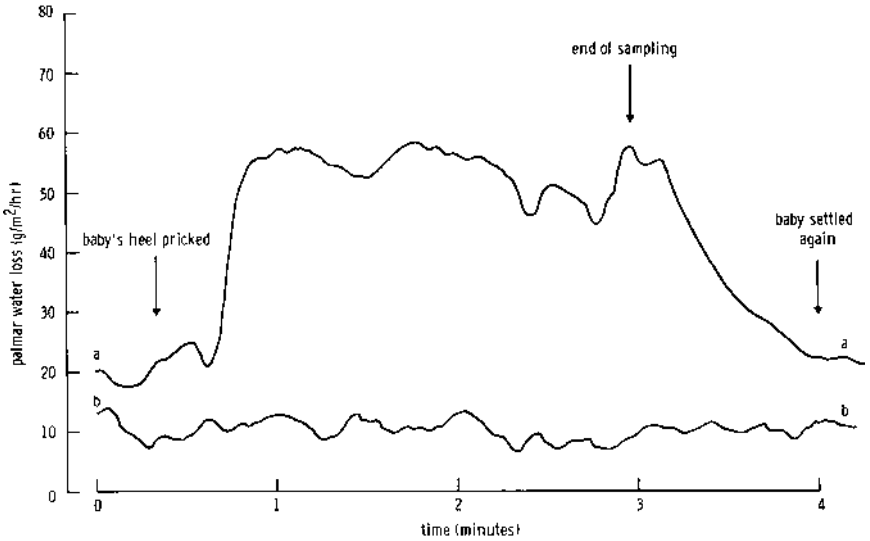

Figure 11 Continuous recording of palmar water loss during heelprick for routine blood sampling, (a) in a term baby aged 6 days and (b) in a baby at 35 weeks gestation aged 4 days. Both were asleep at first, cried vigorously during the procedure, and then settled afterwards. Although the behavioral responses were similar, only the term infant shows an increase in palmar water loss from emotional sweating. (From Ref. 31.)

Recently, a particularly sensitive method of measuring skin conductance in the newborn has been described. It records the number and amplitude of spontaneous waves of skin conductance as well as baseline skin conductance (33). It has demonstrated that baseline skin conductance does rise in response to arousal, even in preterm infants in the early newborn period. Furthermore, the number and amplitude of the waves increase as well, even in infants born as early as 29 weeks gestation (33). Such infants have no measurable increase in palmar water loss, suggesting that there is some production of sweat within the palmar glands but without any loss from the sweat pores. It therefore appears to be a promising tool for the quantitative measurement of arousal in response to painful stimuli in the newborn. Such a tool might be more objective and simpler to use in neonatal pain research than the currently used behavioral scores.

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XIII. SUMMARY

Eccrine sweating, in response to both thermal and emotional stimuli, is present, although not fully developed, in the term infant. In the preterm infant, thermal sweating is absent at birth but usually appears within 2 weeks, albeit weakly. The apparent sweat defect of the preterm infant is thought to be the result of neural rather than glandular maturity. Emotional sweating as measured by conventional techniques is absent at birth in the preterm infant but appears at a corrected age of 36 weeks gestation. Recently, a more sensitive method suggests that it is in fact detectable as early as 29 weeks, making it a potentially useful tool in neonatal pain research.

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7

The Cutaneous Vasculature in Normal and Wounded Neonatal Skin

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I. INTRODUCTION

The definition of neo-natal has less to do with size, weight, or age and more to do with being born. The advent of the "test tube" baby and the survival of increasingly premature infants have added emphasis to some developmental events that previously occurred only in utero. This chapter on the development and maturation in the neonate of the cutaneous vasculature of the skin, therefore, addresses the period throughout gestation and subsequent newborn adaptation. It embraces the subcutaneous adipose tissue as well as the dermis, the lymphatic system as well as the blood vascular system.

The blood supply and lymphatic drainage of organs ensure nutrition and removal of waste products when simple diffusion through the connective tissue embracing the cells is inadequate. This process involves developing preferential channels or low-resistance pathways through the connective tissue and within the evolving capillary bed. It is a process that favors fast intercellular chemical communication while local growth of the organ proceeds apace. The eventual complex interplay between central organs—gastrointestinal, renal, nervous, cardiovascular, or endocrine—and the peripherally situated skin requires delivery of hormones, growth factors, respiratory gases, and nutrients from a distance and also removal of waste

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products from the periphery, as well as mechanisms for assessment of the potential immunogenicity of such products.

To begin with, the genetic code ensures development of the capillary and lymphatic bed. The extent to which the functioning needs of the peripheral tissues, such as epithelium, are involved in initiating this development is unclear, but that they become a rich source of growth factors has become recognized in recent years. At certain sites, and at early times during

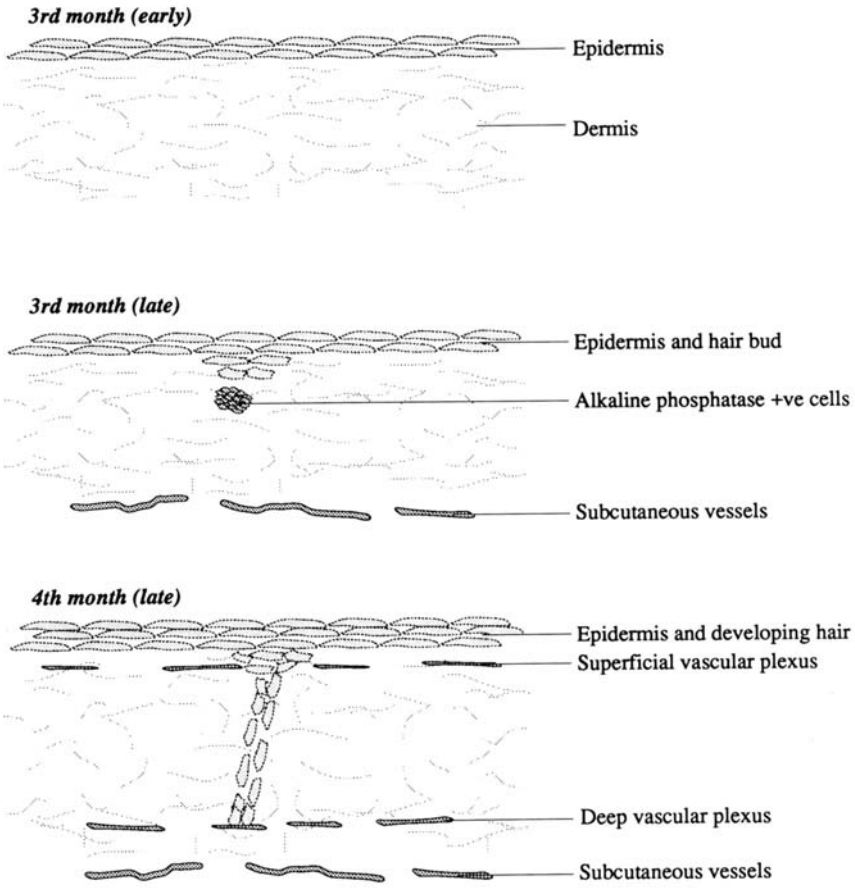

Figure 1 The epidermis throughout life is a stimulus to its blood supply. Alkaline phosphatase positive cells appear subepidermally towards the end of the 3rd month in utero in association with a budding of epithelium, which will become a hair. By the 4th month, the alkaline phosphatase positive cells have become a superficial vascular plexus.

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development, such as during budding from the epidermis of the early hair follicle, one can observe nests of blood vessel precursors close to the budding epithelium using alkaline phosphatase as an early chemical marker of vessel formation (1) (Fig. 1). At an early stage, a fairly well-developed blood vascular system with some preferential channel formation can be observed in isolated in vitro small mammalian limbs, such as the mouse (2). These findings occur without any link to the cardiac pump, supporting the concept that early angiogenesis does not require flow or blood pressure. The growth of the fetal limb occurs in isolation from a blood supply until a certain size is reached. It is questionable whether any of the functions of blood supply that normally are required in adult tissues play any part in this early in vitro system.

II. THE REQUIREMENT FOR BLOOD SUPPLY AND LYMPHATIC DRAINAGE

The functions of the skin only develop fully after birth, and they do so abruptly as new environmental stimuli modulate genetically determined organisation. The cutaneous vasculature develops to serve these functions of the skin, which are to grow, to regenerate and repair, to protect, to thermoregulate, to sense the environment, and, after birth, to display. The most basic requirement of the blood supply of the skin is to meet the metabolic needs of growth and repair. There has long been a controversy as to whether the relatively rich blood supply that has been noted in the adult exceeds the metabolic needs of the resting epidermis (3–5), which requires oxygen, especially when its cells are migrating and multiplying. Most of the processes of differentiation and barrier formation, however, are anaerobic. In contrast, infiltrating cells, such as occur during repair and regeneration and especially in inflammation, require more oxygen. Injury, with a resulting repair response, requires an instant increase of blood supply, and this is effected by axon reflex stimulation from the nerve endings. This is perceptible in the neonate, but it is not nearly as well developed as in the child and adult. When a more prolonged increase in blood supply for wound healing is required in the adult, a new organ, granulation tissue, has to be made (6). In the fetus, granulation tissue is not part of the wound healing response. Fetal vasculature is enough to support the more rapid turnover and movement of the cells that characterize the growing skin in utero. However, although the vasculature in utero is sufficient, blood supply is nevertheless hypoxic with an arterial pO_2 of 20–25 mmHg (7). The epithelium and its blood supply in utero is presumably adapted to a state of low arterial oxygenation. This adaptation includes a molecular isoform of hemoglobin that has a higher

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affinity for oxygen in the fetus compared to the adult. A current hypothesis of relevance is that hypoxia is a necessary condition for the production and activation of vascular growth factors (8). Furthermore, in a more normoxic environment, some growth factors may be destroyed due to free radical production.

While wounds in utero do not result in granulation tissue formation, there is an unexplained change at birth that allows a localized variant of granulation tissue to develop within the first 2 days of postnatal life. It is the strawberry mark or nevus in which rapid proliferation of vasculature similar to granulation tissue is a common birthmark first appearing in the neonate (9). These transient nevi are often preceded by prolonged blanching at the site, so there may be an ischemic precursor state. Such a degree of vascularization is only otherwise seen in wounds that are made close to term, and such nevi only rarely appear before birth. Such vascularization is also quite common in children in response to localized injury forming a pyogenic granuloma. Molecular knowledge generated by studies of angiogenesis must now be applied to clinical observation and to explain pathology. Thus the finding in children with hemangiomata of a truncated protein on chromosome 7q, which interacts with a member of the RAS family of GTPases, points to a failure of the RAPIA signal transduction pathway in angiogenesis (10).

III. THERMOREGULATION

Burton believed that the rich vasculature of the skin is needed for thermoregulation (3). But thermoregulation depends more on sweating, clothing, and huddling together than on diversion of blood supply. In utero, the fetus is maintained in a constant thermal environment of 37°C. At birth, the newborn may be exposed to cold or excessive heat. The water barrier, particularly in preterm infants, is not yet fully established (11), and evaporative water loss is potentially a major source of dehydration, electrolyte imbalance, and cooling. Infants born at 23–25 weeks may take up to 4 weeks to develop barrier function, but at 30–32 weeks barrier function is much better developed (12). Others such as Giusti et al. (13) believe the barrier remains immature with respect to hydration and pH well into infancy. Harpin and Rutter (14) found that babies born at 36 weeks do not sweat but are capable of such as early as 2 weeks postnatally. Sweating is probably the most effective mechanism of heat loss from the surface and is already present at birth. Contemporary theory suggests that evaporation by sweating requires that it is carefully conserved. Droplets of sweat must not simply be lost into the environment but must be spread by

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the sebum or vernix caseosa. Furthermore, since the brain is a major source of heat production, cooling of the head requires that sweat is held by the scalp hair and eyebrows (15), the only site where hair growth is already profuse at birth, especially in the black child (16). In about 25% of white children, the baby is bald (1), whereas in Ghana, 100% of babies are born with hair (17). Perhaps the genetic need for heat conservation in cold climates replaced the need for evaporation in hot climates with the result that the hair loss that occurs towards the end of term in most white children contrasts with the rich complement of anagen hairs in the newborn black infant.

The contribution of blood supply to the control of body temperature and to sweating requires autonomic nervous system control, which is not well established at birth (18) and may not fully compensate for the demands of exposure. Infants often exhibit marked vasconstriction of cool distal extremities (acrocyanosis). The newborn may also exhibit wide fluctuation in the red blood cell content (hematocrit). Another variable determining blood flow is blood volume, which often is a function of how much blood is transferred to or from the placenta at birth (19). Factors, such as increased hematocrit and changes in blood composition, as well as low blood pressure, all contribute to the effect of cooling. Cooling makes blood even more viscous and slow flowing.

Other cutaneous features relating to temperature control include the unique development of brown fat, especially in the interscapular area. This tissue plays an important role in heat production in the newborn infant. The development of brown fat requires a rich blood supply for transport of fatty acids and a poorly developed lymphatic system that retains local lipid (20). In addition to specialized tissues for heat production, the newborn adapts its posture so that it is flexed when cool and extended when heated (Fig. 2). Consequently, from the moment of birth, the skin is cooled on extensor sites more than on flexural surfaces. Swaddling, cuddling to the breast, or lying in a poorly maintained incubator are external influences that moderate thermoregulation. The extensor surfaces may experience environmental cooling and pressures that were not felt in utero. The adipose tissue acquires new roles of insulation and pressure transduction, especially on the palms, soles, buttocks, and upper trunk over the shoulders.

IV. PROTECTION

A major function of the skin is protection. The epidermal barrier, generally designated by the stratum corneum, not only prevents excessive loss of fluid from the body, but also prevents absorption of noxious agents from the

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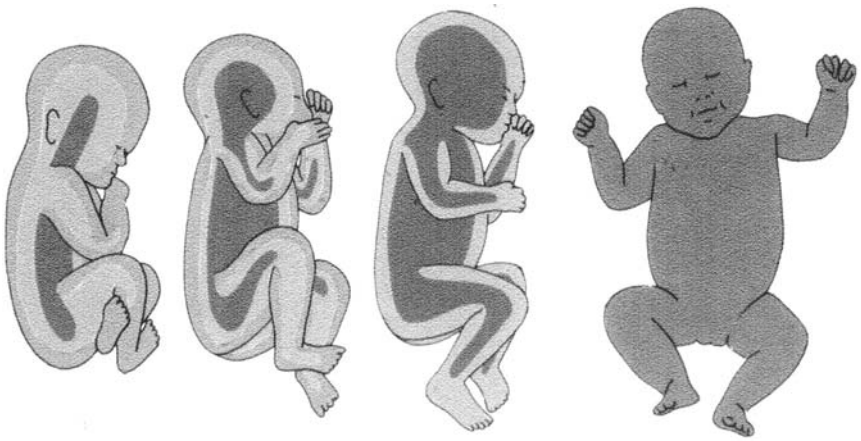

Figure 2 The newborn adapts its posture so that it is flexed when cold and extended when overheated. This influences the rate of maturation of the blood supply of the skin. Adaptation to support thermoregulation is more fully developed in exposed sites and slow to develop in the flexures. Flexion of the limbs is the more normal position in the first few weeks of life. (Courtesy of E Lamont Gregory, unpublished, with permission.)

environment. This barrier is not well developed at birth in premature infants, and agents applied to the skin can be absorbed. These include antiseptics and agents used to control bacterial and parasitic infections such as boric acid and hexachlorophene (21). In addition to the protective barrier function supplied by the stratum corneum, there also is a role played by Langerhans cells and melanocytes.

Langerhans cells form a network below the stratum corneum responsible for the detection and recognition of antigens (22). However, a more primitive function of interest to earlier investigators and worthy of renewed interest may be to provide the acid phosphatase that accelerates differentiation of the keratinocytes, which provide the cornified layer that is a barrier to bacteria. It is most active at birth and is also necessary for the separation of the eyelids (23), which does not take place until the Langerhans cells have found their position in the upper epidermis (J. D. Boyd, personal communication). Presumably, Langerhans cells leave the blood vessels through some specific cytokine stimulus. They are brought in by the blood supply, and they are taken away by the lymphatics. The exact mechanisms whereby Langerhans cells migrate to and from the epidermis have not been fully worked out (24).

In contrast to Langerhans cells, which reside in the midepidermis, melanocytes are found mostly in the basal layers of the epidermis. In

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some amphibians, however, they form a protective layer around blood vessels as they do in the stratum vasculosum of the organ of Corti in the inner ear of the human (25). Their final positioning in the epidermis occurs later, possibly in preparation for exposure to ultraviolet (UV) rays after birth. Prenatally, their putative role is protective and inhibitory in the dermis, dealing with other sources of free radicals derived from the blood supply and protecting free radical sensitive cytokines such as vascular endothelial growth factor (VEGF).

For the epidermis to be an effective protective layer against mechanical stresses, it has to be pliable and elastic. This is dependent upon its water content (5). Throughout childhood and adult life, the call for moisturization is prominent. In the infant and the elderly, the call is more frequently for the surface of the skin of the buttocks to be kept dry (26). In fact, it is a fine balance that requires control of the wetness or dryness of the external environment. In utero, the primitive epidermis is in constant contact with the amniotic fluid, and mechanisms such as vernix formation may be important in "water-proofing" the fetus. After birth, the water content of the stratum corneum derives in part from tissue sources. Water in the upper dermis is maintained by hyaluronan, which has a marked capacity to swell when hydrated. Water may also be partly held in place by contacts with other components of the ground substance such as chondroitin and dermatan sulfate, with attachment to collagen fibers and to elastin also acting as a means of restricting expansion. The length and flexibility of the different forms of collagen, which are changing at the time of birth, may be important in determining the extent of expansion of the upper dermis. At the same time, elastin seems to be laid down as an attachment to the epidermis alternately with fibrillin (27). Elastic tissue has several important roles to play other than resisting distortion. It envelops normal lymphatics in the dermis and assists their response to cutaneous movement and attaches them to the somewhat distant epidermis (Fig. 3). The elastin fiber with its vitronectin coat (28) may be a low-resistance pathway into the lymphatic.

In the adult, invasion of the dermis by bacteria quickly attracts neutrophils, and their elastases rapidly destroy this pathway (29) and prevent systemic infection. Fortunately, for the first few days after birth, bacteria do not thrive on the skin unless the skin is breached. The umbilical cord stump, the conjunctiva, and the site of ritual circumcision are vulnerable sites and go unprotected because the neutrophil in the newborn has less selectin on its surface and the predominant white cell response to epithelial invasion or blistering is eosinophilic as in pemphigus gestatationis or erythema toxicum.

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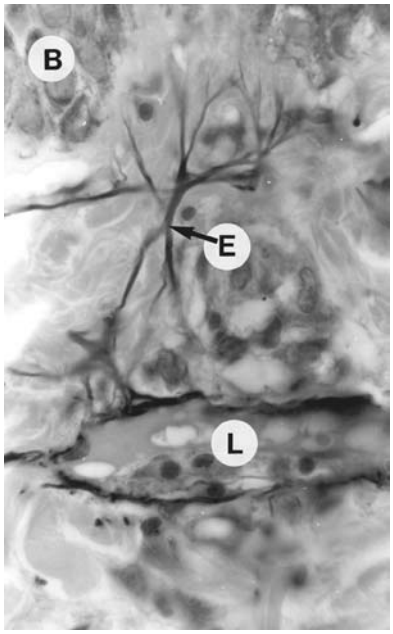

Figure 3 The lymphatic (L) lies at some distance from the basal layer of the epidermis (B) but it is connected by elastin fibers (E), which embrace it and aid its responsiveness to movement so essential for its function. The elastin fibers are hydrophobic and coated in vitronectin and may act as a low-resistance pathway or guide for the passage of materials from the epidermis to the lymphatic.

V. DISPLAY AND COMMUNICATION

The skin does not see or smell, but it shares a common bond with the eye and nose as a platform for perception. The odor and appearance of the skin are important communication factors. Sweating is primarily a function utilized for thermoregulation, but it also expresses emotion, as does flushing and pallor. Warmth and touch are key elements in the mutual bonding of mother and infant from the moment of birth. Failure of normal blood vascular or lymphatic development may result in a deleterious appearance and lead to a poor bonding experience. The skin is that part of the body that is first observed, and it instantly evokes strong emotions, ranging from love to fear.

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VI. STRUCTURE OF THE UPPER DERMAL VASCULATURE

In utero, the vasculature is at first laid out in one or more horizontally disposed layers. One layer is subcutaneous and the other lies just beneath the epidermis (1). When the fetus is small, these two layers lie fairly close together. During the development of the mature neonate, the distance between the systems increases and communicating vessels then lie diagonally, with the distance between them in the deep dermis increasing rapidly during the neonatal period. Mature skin has relatively little regional variation in diffusion distance in the upper dermis for either blood vessels or lymphatics. However, as the skin develops in infancy, much larger distances develop between vessels in the deeper dermis, especially the arterioles of the elongating lower limb. More importantly, there is a papillary system of budding vessels towards the epidermis, which indent the undulating surface of epidermis.

In earlier studies, Ryan (30) emphasized the spectrum between atrophy and hypertrophy of the vascular system closely related to the behavior of the epidermis. In adult elderly skin, atrophy results in a somewhat sparse horizontal plexus of vessels lying somewhat away from a rather thin epidermis (31). In the fetus and at birth, the vasculature is also a horizontal plexus (Fig. 4a), much denser than in the adult and lying close to the epidermis so that there is no failure to meet its metabolic needs during its growth phase. Wound repair and diseases such as psoriasis with a high turnover rate seem to be correlated with the development of an undulating epidermis with growth of capillary loops into the papilla and closer to the epidermis. For fluid exchange, the somewhat glomerular tortuosity and countercurrent exchange in the shape of a hairpin loop may be an advantage. While this may have much to do with metabolic needs, it also probably has much to do with the provision of water, especially when the barrier function is defective as it is in repair and in diseases like psoriasis.

Several investigators have observed that the capillaries of the skin change in morphology during the first few weeks of life. The changes that occur in the blood supply of the upper dermis have been reviewed and studied by Perera and colleagues using surface stereomicroscopy, and the following observations have been made (32):

1. With the exception of the palms, soles, and nail beds, the skin at birth has almost no papillary loops.
2. At birth, the skin demonstrates a disorderly capillary network; the capillaries that make up the network are more prominent in the skin creases, but this disorderly pattern is seen in all areas of the skin.

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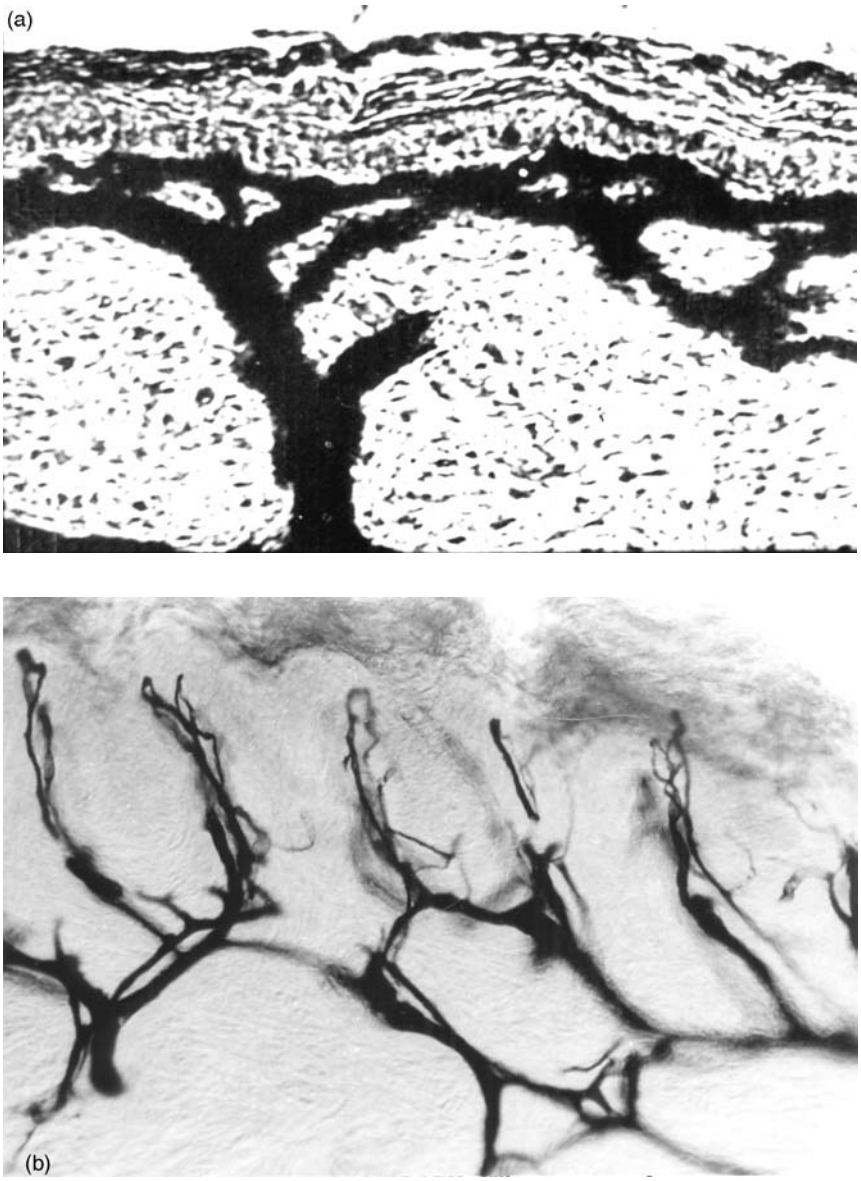

Figure 4 (a) The developing vasculature of the newborn is at first a rich plexus of vessels horizontally disposed and lying close to the overlying epithelium. (b) The mature vasculature of the skin is a system of perpendicular hairpin-shaped loops projecting towards the surface of the skin within the papilla and therefore closely surrounded by the rete ridges of the epidermis.

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3. By the end of the first week of life, the capillary network loses some of its haphazard appearance and assumes a more orderly network pattern. Papillary loops begin to appear as small superficial dilatations or buds in the second week.
4. Clearly defined loops (in at least one area of the skin) are not seen until about the fourth to fifth week of life but all areas of the skin have such loops in babies 14–17 weeks of age (Fig. 4b).
5. The development of order with a distinct horizontal plexus is a gradual process. It is first apparent during the second week of life but is not characteristic of all areas until the fourteenth to seventeenth week, when tissue fluid from the papillary vessels begins to obscure it.
6. The development of an orderly subpapillary plexus and papillary loops is delayed in skin creases.

In a review of the development of the cutaneous circulation (33), Ryan described how the microvasculature of the skin continues to develop during the first 3 months of life and, in particular, how some areas mature faster than others. Some studies suggest that cooling of the skin encourages maturation of the vasculature and slows down the rather haphazard and uninhibited proliferation of the vasculature that has occurred up to the time of birth in the upper dermis. Using pigskin as a model, Ingram and Weaver (34) demonstrated that long-term cooling depresses the development of skin vasculature. There are a number of factors responsible for this response. Foremost, however, are the effects of vasoconstriction, increased blood viscosity, and reduced metabolic demand. The blood of the term newborn infant normally exhibits a high hematocrit, decreased red cell deformability, and a reduced oxygen tension. The mean arterial pressure is only about 53 mmHg (35). Furthermore, in an area such as the proliferating upper dermis and epidermis, demands for oxygen and other nutrients are high, and the permeability of the vessels is appropriately increased. Together with the decreased plasma colloidal osmotic pressure characteristic of the newborn, it is likely that transudation of fluid from the vessels is increased further with cold stress, and this elevates an already high capillary hematocrit.

Ryan (33) refers to evidence that chronic cooling produces redistribution of blood through less cooled areas as a result of stasis within the capillary. Kulka (36) suggested that the crucial factor seemed to be a critical impairment of venous drainage with predisposition to stasis in the venules within the cooled area. Stasis is especially likely to occur where the venular bed is plexiform. With chronic cooling, Kulka noted a predictable sequence of alterations in the microcirculation. Arteriolar and venular spasm occurred first, followed by venular and venous relaxation, and then venular

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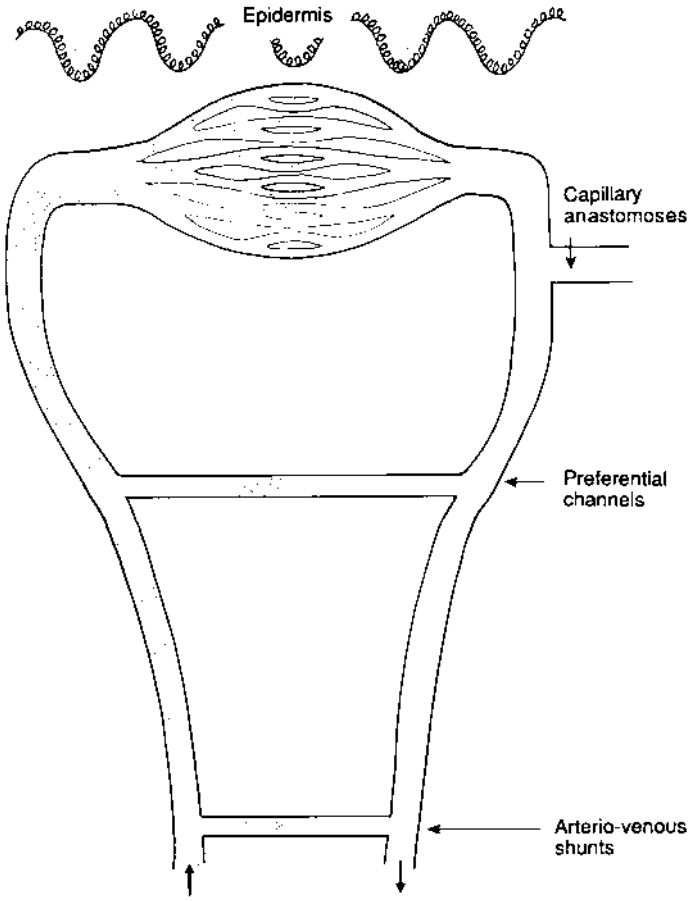

Figure 5 The blood supply of the skin is a system of well-defined and controlled preferential channels that mature rapidly in the neonate. Flow through the most superficial and complex capillary bed may be compromised by cooling or inflammatory stimuli causing, for example, white cell adherence. Blood flow can be maintained by passage through deeper preferential channels. Arteriovenous shunts controlled by the nervous system can serve as fast-flowing diversions assisting thermoregulation.

leakage. He considered the venular dilatation to be a response to arteriolar constriction. Ultimately, a critical slowing of blood flow occurs. Plasma leakage contributes to hemoconcentration and eventual cessation of local circulation. With the progression of stasis, the circulation becomes increasingly confined to thoroughfare channels and bypasses the venular capillary

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plexus (Fig. 5). It is conceivable that, in this way, arteriovenous anastomoses, ultimately of the glomus type, develop. There is some evidence (37) that such anastomoses are not present at birth but that they develop in areas of the body exposed to cold. This could be a direct result of the hypertrophy of deeper, communicating vessels, taking a larger proportion of redistributed blood as more superficial vessels become obstructed by stasis. Studies of the effect of hypothermia on the skin blood flow in dogs (38) have indicated that gross stasis occurs in the superficial capillaries concurrently with opening up of deeper anastomoses. Although Perera and coworkers (32) suggested that the maturation of the microcirculation might depend on how long and to what degree it was exposed to cooling, the study by Syme and Riley (39) suggested that a mature pattern depended more on the weight of the child, rather than on the time that had elapsed since birth. In their study it was apparent that, in the majority of infants, capillary loops developed at or very soon after birth and that there was no particular time during the first 21 days of life when loops always appeared. They pointed out, however, that capillary loops were rarely absent after the infant reached a weight of 5.5 pounds (2.5 kg).

In earlier writings on the development of the vasculature of the dermis, it was recognized that the epidermis, in some way or other, provides a major stimulus for angiogenesis (40). Only in the last decades has it been demonstrated that the epidermis is a rich factory of cytokines such as vascular endothelial growth factor (VEGF), interleukin-1 (IL-1), tumor necrosis factor (TNF) and others (41). Vascular endothelial growth factor made by the epidermis is of the greatest importance for the development of blood vessels and lymphatics. It has many isoforms, and the vasculature has more than one receptor (42). The VEGF-A, also known as vascular permeability factor, is many thousand times more effective as an inducer of permeability than histamine (43). It has been observed in the adult that conditions that lead to increased tortuosity of the upper dermal vasculature, such as wound repair, psoriasis, and lipodermatosclerosis, a condition induced by venous hypertension, are able to induce increased production of VEGF by the epidermis (44). Furthermore, recent studies of living skin equivalents have shown that topical application of a substance such as 2% lactate can induce the production by the epithelium of more VEGF (45). Others have suggested that ischemia, hypoxia, or lactate can stimulate VEGF production (46). The author believes that rapid production of a vascular permeability factor and its leakage into the upper dermis produces an outpouring of fluid from the upper dermal vessels that leads to a rapid expansion of the upper dermis (Fig. 6). Such expansion places the papillary vessels under stretch and can in itself be a mechanical factor transducing biochemical signals and determining the metabolic behavior of the tissues. Such a mechanism may be influ-

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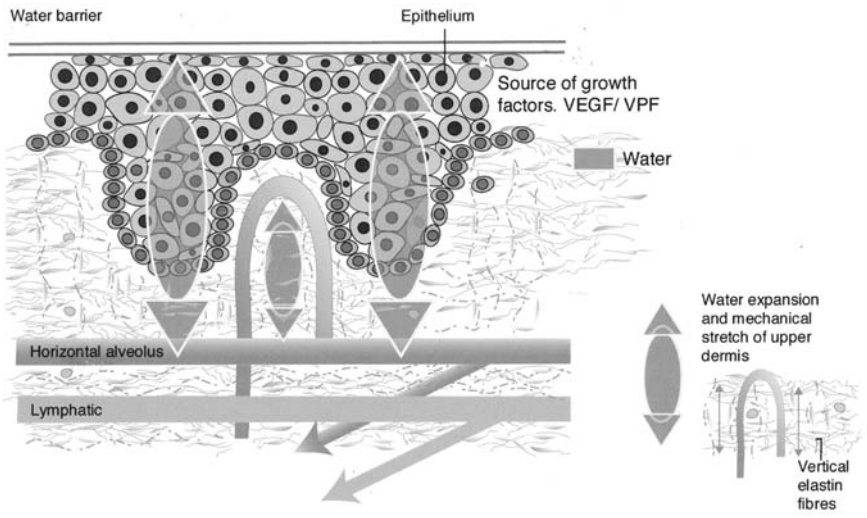

Figure 6 The release of VEGF causes an overwhelming increase in permeability, which expanded the upper dermis and sets into effect a response to mechanical stretch to which the endothelial cell is particularly responsive.

enced by water loss from the surface. No mechanical stimulus postulated to expand the upper dermis would have optimal effects until the surface is watertight. The flexures may be less watertight than exposed skin, which may be another factor determining slow expansion of the upper dermis in the flexures. A competent epidermal barrier is not established until 30 weeks in utero, but transepidermal water loss is also contained by the vernix caseosa, the lipids of which provide some barrier function (47).

How the vascular bed responds to VEGF depends on the amount of VEGF produced, the nature of its isoform, and whether it is a vascular permeability factor and also on the receptors on the endothelium. This concept should be considered against the background of the ground substance and its capacity to hold water or to expand as well as the influence of the type of collagen fiber and the elastin in holding the connective tissue in place. Attachment of such fibers to cells such as the basal layers of the epithelium or fibroblasts or endothelial cells while undergoing mechanical stretch can induce important metabolic activities through stereochemical distortions of cell membrane enzymes such as protein kinase C (48). Furthermore, the distortions that result from expansion contribute to the folding of the epidermis and its direction of growth. Selective intermittent strengthening of the elastin fiber

and its attachment to the basement lamina may also play a part in the remolding of the epidermis wherever it is subjected to repetitive stresses such as in the hand and foot.

The recent surge in information is a consequence of new technology such as the transgenic and knockout mouse, together with the development of biological models in which defects are detected when injury and repair increases the demand on the biological control systems within the tissues. The mechanical transduction of biochemical signals is nowhere more obvious than on cell membranes; a good example is the effect on the endothelial cell of shear induced by blood flow (49). Its effects are modified by other biochemical influences such as the presence of oxygen or its free radicals. After birth the vasculature is less hypoxic, and it experiences higher rates of shear. As blood flow and pressure increase, vasculogenic factors that are flow dependent play a role in converting the slow-flowing venular bed into a faster flowing arteriolar system. This takes place in the gradually widening middle and deep dermis as smooth muscle cells and fibroblasts are recruited. Other parts of the bed show apoptosis at sites where blood flux is reduced as they are bypassed by faster flowing arteriovenous (A-V) shunts.

The neonatal period is a time of accelerated maturation of some specialized epithelial tissues such as the eye and breast. These are well-studied, small, metabolically demanding organs that burn oxygen and easily become hypoxic. In these organs vasculogenic factors are produced that accelerate maturation of the vascular bed, some of which, such as angiopoetin 2, are shear-flow dependent (50). They toughen the vessel wall where flow is rapid and make it less leaky. Overexpression of VEGF-A leads to highly permeable vessels in the elongated papillae. The characteristic high permeability of new vessels gives direction to the forces of interstitial fluid flow, thereby opening up spaces in the ground substance. The breast is a particularly interesting epithelial organ because it is a model for adipose tissue. Accelerated epithelial growth stimulates the release of hypoxic inducable factor (HIF-1α), and adipose cells develop when and where this is lacking (51).

In the neonatal period, angiogenesis is less required, as opposed to vasculogenesis, which is needed for vascular bed maturation. In vitro factors such as vascular endothelial cadherin (VAC) stop endothelial growth at confluence. In vivo they determine single layer capillary tube formation (52). VAC knockout mice do not survive beyond the early stages of gestation. In the neonate, VAC expression plays some part in the maturation of white cell endothelial interaction after a stage of prominent eosinophil transmigration into the skin (53). The cytokines consist of many players; another member of the orchestra is platelet endothelial cell adhesion molecule

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(PECAM-1). In PECAM-1 knockout mice, white cells are trapped at the level of the basement membrane in the newborn (54). PECAM-1, through tyrosine phosphorylase, acts in conjunction with fibroblast growth factor (FGF) on the cell membrane and modulates the biochemical transduction of the attachment or unlinking of actin filaments in response to adherence grip and stick. The transmission of signals from the nucleus through acting filaments to the cell membrane can be extended still further into the surrounding tissues by attachments to collagen. Collagen III is a short fiber encouraged by the presence of hyaluronan (HA). Increasing size postnatally requires lengthening and replacement by collagen I. It has been observed that the fetal wound fluid in the lamb is rich in HA, and its production is prolonged compared to that in the adult ewe (55). Scarring following wounding and repair is one consequence of the replacement of collagen III by collagen I. To ensure control of the amount of hyaluronan, hyaluronidase is bought into play by the infiltration of the tissues by mast cells especially in the elongated papilla. Mast cell proteases play a part in angiogenesis possibly through hyaluronan, fibrin, and an effect on the adhesion processes underlying grip and stick (6).

In wound healing, the ground substance is initially composed of fibrin and its related compounds, which are quickly replaced by HA and collagen III. Later, in wounded mature tissues, this is converted into granulation tissue. The role of fibrin and permeability in promoting angiogenesis was postulated earlier by Ryan (56) and later, as a function of VEGF, by Dvorak et al. (57).

Thus, in summary, the cutaneous vasculature is multifunctional and serves to transport metabolic and nutritive substances including oxygen, carbohydrate, fats, and protein as well as to transport cells helpful in protection and repair. The elasticity and pliability of the skin requires moisturization and a controlled distribution of water in the dermis. The vasculature must assist in thermoregulation by appropriate diversion of blood supply and the support of perspiration. It must remove waste products and foreign material and encourage monitoring by the immune system. These functions are maintained as a result of a finely tuned system of blood vessel and lymphatic control maintaining flow and the integrity of the vessel wall while at the same time allowing selective passage of blood contents into the tissues.

The skin is at the interface between the organism and a potentially threatening environment. These threats are both physical and chemical. Threats are very few in utero but postpartum they are numerous, varied, and potentially overwhelming. The neonatal period is a transition period in which the threats are buffered by a protective armory ranging from the vernix caseosa to the embrace of the mother. It is during this period that

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the cutaneous vasculature learns to adapt to cooling, to greater mechanical stresses, and to the invasion of foreign agents.

Adaptation at birth requires that transepidermal loss of water be controlled and that the barrier function of the epidermis be strengthened. Water that is distributed to maintain pliability is located under the epidermis (58) and must not be too easily expelled from the tissue by greater mechanical distortion experienced from birth. This together with the adaptation to cooling requires a change in the structure of the vasculature in the upper dermis and an enhanced role for adipose tissue.

The removal by improved drainage and lymphatic function of excessive macromolecules such as protein and lipid in the tissue fluid is also important for the maintenance of oncotic and hydrostatic pressure. Tissue planes and low-resistance pathways for lymph flows are less well defined in the fetus than in the older child or adult. Mature elastic fibers, for example, which may be one low-resistance pathway, appear postnatally (59).

These changes are taking place at the same time as growth of the whole organism and the consequent lengthening of the distance between core and the periphery, between heart and skin. Strengthening of soft tissues, like the skin, must also occur to resist external forces never encountered in utero. This requires a strong skeletal system and will entail some concomitant loss of the "flexibility" of the tissues of the fetus. The switch from scarless to scarred wound healing reflects these changes.

VII. ADIPOSE TISSUE

Adipose tissue in lower mammals is a store of fat for energy provision. At birth in humans, it is as yet not fully formed to fulfill acquired functions of pressure dispersion and thermoregulation (20), but during the first few months postpartum, body fat increases from about 16% at term to 25% at 6 months (60). Much of this is sub-cutaneous and induced by cooling from the moment of birth. It brings to the skin a dense sympathetic innervation.

It is not certain from which cells adipocytes develop. Adipose tissue differentiates around a rich bed of capillaries, and capillary endothelium itself may give rise to adipocytes. The development of vasculature in adipose tissue has been well studied because of the interest in brown versus white fat (20). Mature fat cells have a relatively enormous size compared with adjacent capillaries, and thus, in histological sections, adipose tissue seems to be sparsely supplied by blood vessels. However, investigations of blood flow suggest that adipose tissue is, in fact, very well supplied. By contrast, studies by Ryan suggest that the interstitium of fat lobules in adipose tissue is very

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poorly supplied (if at all) by lymphatics (61). In the embryo, at the junction of the dermis with the subcutaneous tissue, there is a rich vascular membrane that ultimately separates into the deep beds of adipose tissue. In addition, there is a predictable relationship between fat cell size and capillary density. As blood flow to adipose tissue increases, fat cells become smaller. Smahel (62) suggested that one of the purposes of a highly developed capillary bed in the embryo is to slow down the circulation and permit the deposition of fat. Conversely, it has been demonstrated that hyperemia of adipose tissue promotes depletion of the fat cells. Available evidence suggests that for adipose tissue to develop there must be a sluggish blood supply. Nevertheless, blood flow to adipocytes must be maintained even when vasoconstriction of the arterial system has been induced, for instance, in cold exposure. The thermoregulation of the skin requires that, on cold days, fat must be burned and its blood supply maintained, but heat loss by vasodilatation must be avoided.

It is difficult to know where the idea originated that there was a relationship between the lymphatics and fat deposition, but adipose tissue is derived from the same reticuloendothelial system that generates the thymus, bone marrow, and lymph glands. Clark and Clark (63), studying the development of adipose tissue in the rabbit ear chamber, concluded that fat was likely to develop in a chamber devoid of lymphatics, and the few fibroblast-like cells observed for many days, which eventually developed into adipocytes, occurred in chambers in which lymphatics were either absent or virtually nonfunctioning and unable to clear fat. Most tissue fat that accumulates in the form of lipoproteins, cholesterol, and other fatty material is derived from the bloodstream. Unless this fat is removed from the tissues by the lymphatics, it will accumulate in macrophages or adipocytes.

VIII. THE LYMPHATICS

The lymphatic system does not attract much interest when it is functioning normally, in spite of the fact it is essential for removing unwanted material such as proteins, lipids, and cells, from the tissues and it is the conduit of the immunological system (64,65). The initial lymphatics lie mostly at the junction of the upper dermis and the middermis surrounded by elastin, which extends tangentially to the epidermal basement lamina and is horizontally disposed deep to the plexus (Fig. 7). It is the deep dermis and subcutaneous tissues that are poorly protected against edema and show impaired clearance of macromolecules. Hence, they are the main sites for swelling in capillary leak or inflammatory processes. Edema of the newborn skin is common, but maturity develops quickly so that even in conditions like Turner's syn-

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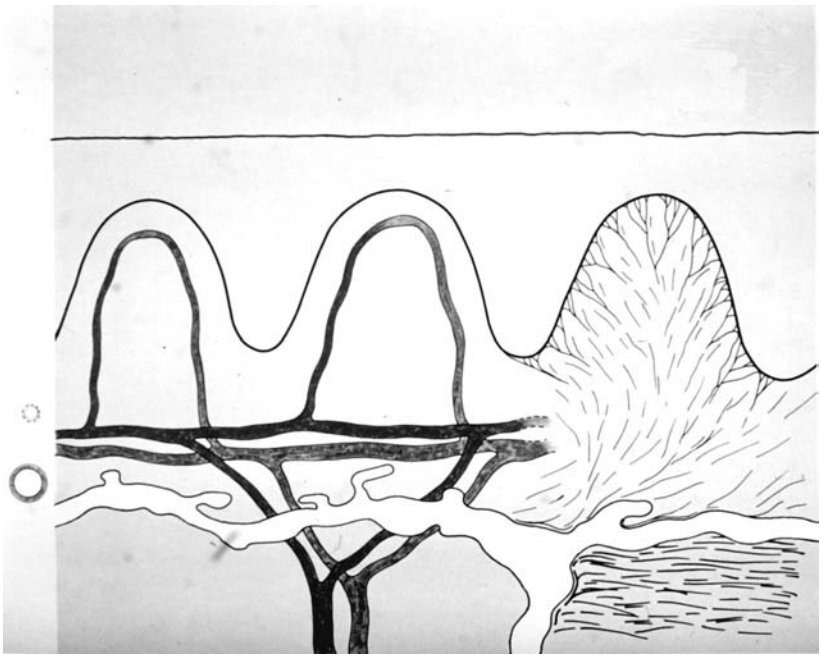

Figure 7 The papillary loops representing blood supply and the deeper lymphatic system are supported by an elastin system, which is perpendicular in the upper dermis and horizontally disposed at the junction between upper and middermis. The rich blood supply of the epidermis is above this level, and the lymphatics lie at the junction.

drome, fluid excess in the tissues with consequent swelling usually lessens within the first week or two of life.

As described by Ryan and De Berker (59), the lymphatic system of the skin has a well-developed associated network of elastin fibers playing a role in assisting responsiveness to tissue movement and possibly acting as a low-resistance pathway for the passage of water and macromolecules through the tissues. It is of interest that, in the adult, such elastin is destroyed by neutrophil elastases and early elastin destruction assists abscess formation. As noted above, the replacement of neutrophils by eosinophils is the typical response of the skin during the early neonatal period and may be advantageous for the development of the lymphatic system as it learns to respond to external environmental threats of infection and antigen exposure. Eosinophils do not destroy elastin.

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In order to mature properly, the lymph node requires cell traffic from the skin as it is exposed to a foreign environment after birth. It is possible that the lymphatics of the skin function poorly (and are relatively unneeded) until systemic blood pressure rises and/or the epidermis is perturbed by an angiogenic and/or toxic environment. The lymphatics of the skin are organized into a superficial and a deep dermal plexus. The mesh of lymphatics in the upper dermis is never as dense as that of the blood capillary network, and lymphatics maintain an even wider network in the deep dermis. It is likely that removal of macromolecules such as protein and lipid is more effective in the upper dermis than in the deep dermis. Consequent accumulation of lipid in the deep dermis, where it is taken up by primitive mesenchymal cells, may be an explanation for the development of adipose tissue in the capillary bed of the deep dermis. This occurs especially around the hair follicles and sweat glands and in the vascular plexus of the deep dermis. It is prominent around lymph nodes.

There have been few studies of the early development of the lymphatic system in humans. The mammalian studies by Clark and Clark (66) prove that lymphatic capillaries grow only out of existing lymph vessels and not from veins. Tonar et al. (67) found that the lymphatic system developed from slits or spaces in the mesenchyme in the juguloaxillary region during the fifth to sixth week of gestation and followed the iliac veins in the pelvis. In lymphatic disorders, such as hygroma, the central spaces fail to link up with peripheral lymphatic spaces. Healthy tissues develop lymphatics in the periphery during the third month of life, giving rise to a network of lymphatic vessels and nodes. Between the tenth and fourteenth weeks of gestation, the development of the lymphatics rapidly progresses in both the upper and lower limbs, and during the fourth month, both the superficial and the deep systems of the lymph vessels are formed. Probably the lymphatic system, like the blood vessel system, requires cytokines for its genesis as well as for its maturation. There are specific VEGF receptors for the lymphatic system (42).

It is not known how well lymphatics work in utero. The function of removing macromolecules and the control thereby of blood and tissue oncotic pressure is probably relatively unimportant until birth, at which time rising blood pressure causes macromolecules to be flushed out of the vessels into the tissues. The important rapid flow of antigens from the surface of the skin to the lymph node may require a rise of blood pressure to flush the system and to initiate flow.

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IX. CUTANEOUS RESPONSES

The reticulate pattern of the skin of a child that resolves when the child is well heated is commonly noted at room temperature during infancy. This phenomenon is probably due to reductions in blood flow secondary to vasoconstriction and increased blood viscosity. The greater cooling of blood in the upper dermis (coupled with the slow flow) gives rise to a flush-red reticulate pattern. Conversely, the paler center supplied by the arteriole may occasionally be almost white, probably due to vasoconstriction; however, pallor resulting from a greater presence of tissue fluid cannot be wholly excluded. The common telangiectasia of the nape of the neck, known as the "stork mark," presents in a high proportion of children but may be undetectable during the first week of life.

The permeability and capacity of newborn blood vessels to wheal have been the subject of a number of studies. The capacity of skin to demonstrate whealing depends on (a) vascular permeability, (b) clearance mechanisms for edema, and (c) the nature of ground substance with respect to retention of water. Sulzberger and Baer (68) inoculated the skin of infants with a 1:1000 dilution of histamine and were able to consistently produce whealing. Matheson and colleagues (69), using more dilute concentrations of histamine, found newborn skin less likely to wheal but capable of erythema. The vasoconstrictor response of the skin has been less well studied. Hinrichs and coworkers (70) tested 100 newborn infants with methacholine and found that 16% gave a definite delayed blanch reaction, 21% a questionable reaction, and 63% no reaction. Suter and Majno (71) examined lipid transport across vascular endothelium and found histamine had little effect in the newborn "because the gaps in endothelium were already as large as possible."

Young (72) studied clearance of a technetium-labeled colloid innoculated intradermally in the neonatal pig and noted a fast and a slow component to the clearance of the radioisotope. She believed that the fast phase represented movement of technetium from the upper dermis and that the slow phase corresponded to removal of the technetium from the deeper dermis. She found that there was an increase in the "fast" half-clearance time between 3 and 6 months of age but no further changes beyond that time. The slow component showed a doubling in the half-clearance time between 3 and 18 months of age. She believed that this was due to a gradual decrease in the relative vascularity of the deep dermis as the tissues widened with maturity.

Yippo (73) studied the fragility of the newborn capillaries using a vacuum or suction-cup method. They demonstrated that a vacuum of -1 mm could rupture the capillaries in the majority of premature infants

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weighing less than 1 kg; however, those of infants weighing more than 3 kg could withstand pressures of up to −500 mm.

X. WOUND HEALING

Even the slightest scratch in the adult will trigger an instant reflex that transiently enhances blood supply for repair some 200-fold. When a persistent wound requires a prolonged enhancement of blood supply, a new organ of repair is formed known as granulation tissue. For normal functioning of repaired skin, granulation tissue must eventually be removed. In the case of neonatal skin, its vascularity at birth is almost equivalent to poorly innervated granulation tissue. In order to develop mature skin function, such vascularity has to be reduced at birth and replaced by a system that has reflex hyperemic nervous responses that are instantly responsive.

It is particularly in the field of wound healing that attention has been drawn to the initial fibrin scaffold, the absence of an inflammatory response and scarring in fetal wounds (74). This changes to an inflammatory response and fibrotic scars by the time of the neonatal period. The author has himself examined the changing control factors in the fibroblast's response to mechanical forces as it ages from the fetus to the neo-natal and finally to the adult (75). It is likely that the neonatal period is a brief transformation characteristic of all cells as they meet the altered environment at birth that demands a qualitative and quantitative change in the response to new physical and chemical stimuli.

There are several interesting models of transition from fetus to neonate. Many studies of scarless healing have been performed on marsupials in which the young are born early but spend their fetal to neonatal period in a pouch (76). The hair cycle also offers a miniature model of rebirth. The mechanical forces of the early budding process and the influence of the arrector pili muscle on the hair bulge are examples of the interactive forces that play a part in rapid remolding. Variations in the composition of the ground substance and the fact that mast cells and histamine are associated with anagen while adrenaline is associated with telogen should not be forgotten in an era in which only cytokines are studied (77).

Many studies (78,79) suggest that transforming growth factor beta (TGF-β) is responsible for scar formation as well as for granulation tissue. It needs regulatory proteins (1GFBP-3E and LTB-1 protein) to bind to its receptor. Such proteins are in lower concentration in the tissues of the foetus. Angiotensin II (80) is another angiogenic factor that acts as a cofactor like PDGF in the production of granulation tissue. Angiotensin II is a vasoconstrictor that maintains oxygenation of essential central organs by

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shutting down the peripheral vascular bed. Adult receptors eventually replace fetal receptors, the latter of which are activated by stem cells (81).

Proteases are necessary for unlinking cellular attachments and for the activation of cytokines such as TGFβ and VEGF. Such activation is controlled in part by protease inhibitors. The fetal fibroblast cannot sustain plasminogen activator inhibitor production, whereas adult cells have difficulty in switching it off (75). The stimulus to production of inhibitors is stick and grip (82). For flexibility and remolding there is a need for inhibition of proteases in the short term. Maturity and skeletal strength require that grip and stick be prolonged. Indeed some authors use the level of protease production as an indication of the amount of mechanical stimulus experienced (83). It might be relevant that weightlessness in utero due to the effect of floating in amniotic fluid is a feature that protects the tissues from the stresses of gravity. This becomes a significant distorting force after birth.

In its chemical complexity, as well its mechanical behavior, it is important to recognize that the expansion of ground substance as a result of taking up fluid may influence intercellular relationships. Physical distortion may even occur to the extent that intracellular signals transmitted by attachment by fibrils from receptors to nuclei are altered. Proteases have to be controlled to prevent the dislodgement of attachments. In the adult, such controls are stable and allow the development of a strong skeletal system. In the neonate, more flexibility for the purposes of remolding are still necessary, and the fact that the fibroblast cannot sustain its production of inhibitors of proteases was thought by Ryan to be central to the differences between life in utero and postnatal existence. In the adult, increasing distances are controlled by lengthening of collagen fibers, e.g., the replacement of collagen III by collagen I. Both the contractility of cells in the dermis, such as myofibroblasts and arrector pili muscle, as well as the swelling of ground substance can transduce biochemical signals on the cell membrane.

XI. SUMMARY

From the moment of birth, the skin of the newborn is exposed to abrupt changes in temperature, mechanical stresses, bacteria, and foreign proteins from the environment. At the same time, birth results in a cutaneous vasculature, which must adapt to higher pressures, greater pulsatility, increased oxygenation, and faster flow. The macro experience of the organism must be translated into subcellular adaptations so that mechanical transduction of biochemical signals are enhanced and new enzymes manufactured. Many of the tissue components of the skin change qualitatively and quantitatively during the perinatal period. Preterm birth poses profound additional pro-

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blems for the organism. The skin of the newborn gradually toughens with age and becomes more protective, and its repair processes become more inclined to scar. While becoming skeletally stronger, it loses some of the flexibility required for growth and repair. Never again is adult skin able to replicate the features of the newborn. Understanding of the processes of normal adaptation of newborn skin and the role and response of the vasculature, lymphatics, and adjacent tissues in wounding will undoubtedly yield new insights into therapies and interventions for both affected newborns and adults.

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8
Prematurity

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I. INTRODUCTION

It is important to recognize that if an infant is born early, organ immaturity is not confined to the lungs, brain, and gut. All the organ systems of the body, including the skin, are immature and may give rise to illness and difficulty in management, particularly if the infant is extremely preterm. The skin of an infant of 25 weeks gestation is structurally and functionally different from the skin of an infant born at term. Problems arise as a result of this immaturity that need to be understood and managed. These problems are predictable and range from difficulties in fluid and electrolyte management to temperature regulation and infection control.

In the extremely low birth weight (ELBW) preterm infant, i.e., those infants weighing less than 1000 g at birth, it is particularly the outermost barrier layer of the epidermis, the stratum corneum, which is most immediately important for survival (1,2). Without the prevention of water and evaporative heat loss provided by the stratum corneum, life in a terrestrial environment is impossible (3). Immaturity of the dermis and the cutaneous appendages pose problems of lesser magnitude for the EBLW infant. This chapter focuses, therefore, primarily on issues relating to the development of the epidermal barrier. This focus is framed within the context of the complex environment of the newborn intensive care unit and the idea that the developing skin surface serves to interface the premature infant with that envir-

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onment (4). Advantages accruing to evidence-based standardization of skin care practices and opportunities for future work are highlighted.

II. DEVELOPMENT OF THE EPIDERMAL BARRIER

Embryologically, the epidermis is an ectodermal derivative like the brain. Structurally, the development of a large, versatile brain and a unique, relatively hairless skin surface are two of the primary characteristics distinguishing humans from other primates (5,6). Physiologically, the close interconnectivity of brain and skin is manifest in the ongoing circular organization of the feedback mechanisms governing visual and tactile development. This approach stresses the importance of co-evolution of both central nervous system structures and an adaptive and dynamic environmental interface (5). According to this concept, the epidermis functions as both a cellular and molecular interface and a psychological and perceptual interface with immediate structural and functional connectivity to neuroperception. The complexity of this interconnectivity includes the fact that the skin serves to interface both the patient to the environment and the patient to the caregiver. Attention to the development of the epidermal barrier in ELBW infants, therefore, is anticipated to have consequences for both the design of the neonatal intensive care unit (NICU) (temperature, humidity, lighting, choice of heating device, etc.) as well as function of caregivers (handwashing, touch, monitoring, adhesives, blood drawing, etc.).

Biochemically, there are intriguing similarities between the epidermis and the brain that have yet to be elucidated. Ceramides, for example, are a major lipid component of both the brain and the lipid lamellae of the stratum corneum (7). Whether this unusual lipid pattern has functional significance or not is unclear. As an incipient environmental interface, the structure of the epidermis more closely resembles other epithelial structures, such as the developing lung. Table 1 lists a number of similarities between the epidermis and the lung, beginning with their mutual function as interfaces between the internal milieu and the environment. Both the epidermis and the lung are active in lipid synthesis with such metabolic activities residing in the epidermal keratinocyte of the stratum granulosum and the pulmonary Type II alveolar cell, respectively. These cells undergo an orderly program of terminal differentiation to form corneocytes and Type I alveolar cells, respectively. These highly differentiated cells form the primary structural interface between the body and the gaseous environment.

Both the epidermis and the lung package barrier lipids within lamellar bodies, which contain a number of enzymatically active proteins as well as barrier lipids (8,9). The epidermal phospholipids are removed during pro-

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Table 1 Similarities Between the Epidermis and the Lung

	Epidermis	Lung
Function	Interface between the internal milieu and environment, enhancing innate immunity	Interface between the internal milieu and environment, enhancing innate immunity
Lipid-synthesizing cell	Keratinocyte	Type II alveolar cell
Primary cellular constituent at air/gas interface	Corneocyte	Type I alveolar cell
Primary mode of packaging lipids	Lamellar body	Lamellar body
Lamellar body contents (proteins)	Acid phosphatases, glycosidases, proteases, lipases	Acid phosphatases, proteases, glycosidases, surfactant apoproteins
Lamellar body contents (lipids)	40% phospholipids, 20% glycosphingolipids, 20% free sterols, 20% other neutral lipids	85% phospholipids, 10% free sterols, 5% other neutral lipids
Hormonal responsivity	Epidermal growth factor, glucocorticoids (rodents)	Epidermal growth factor, glucocorticoids
Time of terminal differentiation in utero	Last trimester	Last trimester

cessing such that the stratum corneum is nearly devoid of phospholipids, whereas in the lung, phospholipids constitute the primary lipid component of pulmonary surfactant. Both lipid and DNA synthesis are affected by exogenous hormones during the last trimester of pregnancy. Epidermal growth factor is a prototypic molecule with effects on differentiation and growth of both epidermis and lung. Maternal glucocorticoids accelerate maturation of epidermis and lung in rodent species (10,11). It is unclear at present, however, whether prenatal glucocorticoids accelerate epidermal maturation in humans to the same extent as lung development. There is some evidence that they do not (12).

Barrier maturation in utero proceeds initially in the vicinity of the pilosebaceous apparatus with secretion of sebaceous lipids and possibly fetal corneocytes to form a protective mantle of vernix caseosa (13,14). Development of the interfollicular epidermis temporally follows cornification of the hair follicle. The preterm infant less than 25 weeks gestation has very poor development of the interfollicular stratum corneum (Fig. 1).

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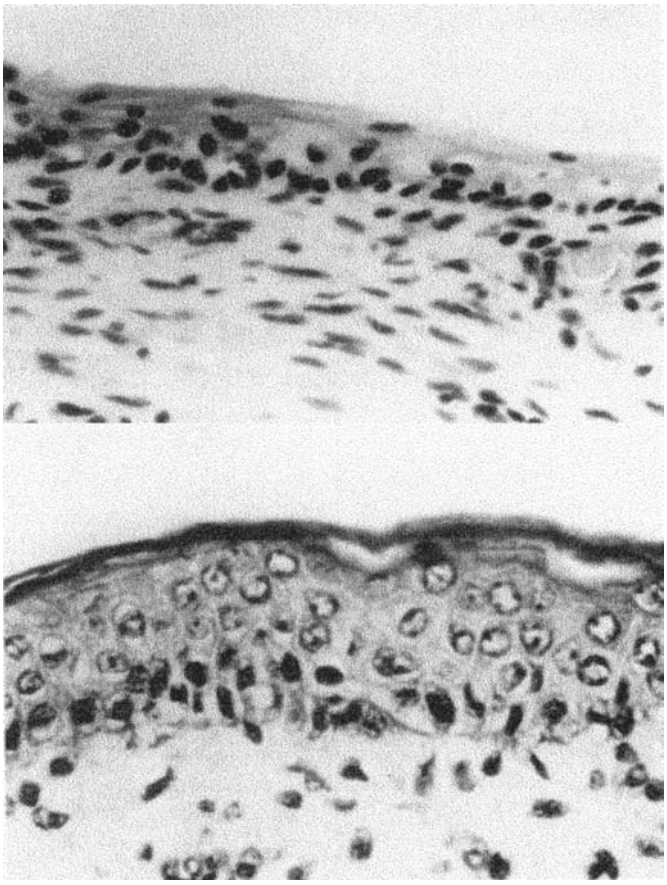

Figure 1 Effect of exposure to ambient environment on epidermal barrier maturation in the very low birthweight preterm infant. This figure shows the epidermis of a 26-week infant on day 1 (top panel) and a 26-week infant on postnatal day 16. Birth of the very low birthweight preterm infant results in marked acceleration of epidermal barrier maturation and formation of a stratum corneum.

Exposure to air following birth, in conjunction with other unknown stimulatory factors, results in marked acceleration of barrier maturation (8,15, 16). The development of therapeutic strategies for facilitating epidermal barrier maturation is an active area of neonatal investigation. At the cellular level, formation of the epidermal barrier (stratum corneum) is generally likened to a brick wall in which corneocytes constitute the ''bricks'' and barrier lipids such as cholesterol and ceramide constitute the ''mortar''

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(Fig. 2) (3). Current thinking envisions lamellar body synthesis and exocytosis in both epidermis and lung to occur with extrusion of barrier lipids into the extracellular space and spontaneous self-assembly at the air interface. New models of barrier lipid assembly based on minimal energy considerations and cubic morphologies have recently been proposed which deserve further investigation (17,18).

Increasing evidence supports the dynamic interaction prior to birth between developing epithelial surfaces such as the lung, skin, gut, and kidney (13). Increasing amounts of pulmonary surfactant within the amniotic fluid, for example, are associated with detachment of vernix from the skin surface and increased turbidity of the amniotic fluid (19). This finding is associated with improved survival and decreased incidence of respiratory distress syndrome in premature infants. Whether vernix has a role in ''waterproofing'' the fetus prior to birth remains to be seen. Recent evidence, however, clearly supports the hydrophobic nature of vernix (20). The presence of anti-infective molecules such as surfactant protein D in both pulmonary surfactant and vernix provides evidence for a possible protective role against prenatal chorioamnionitis and/or facilitation of the birth process with transition to a nonsterile environment (21).

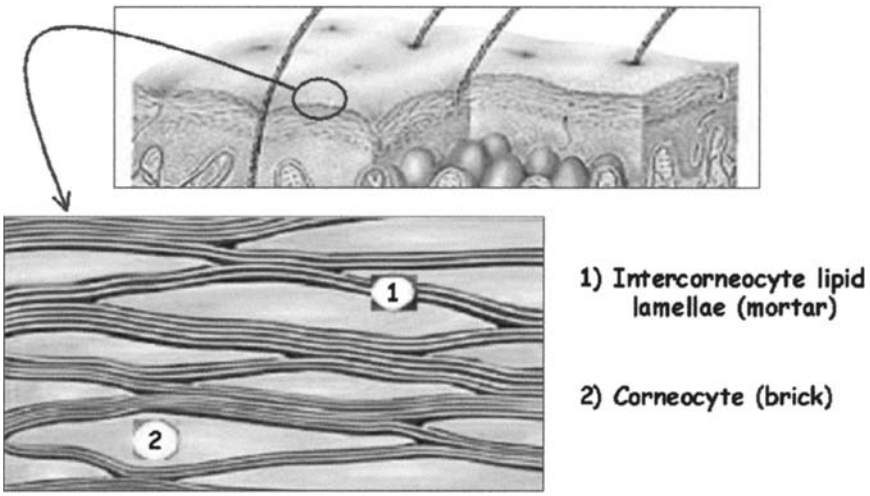

Figure 2 The ''brick and mortar'' model of the human stratum corneum. The organization of the stratum corneum is traditionally considered as a bipartite ''brick-and-mortar'' model in which the bricks are composed of terminally differentiated corneocytes embedded in a complex multilamellar lipid mortar consisting of ceramides, cholesterol, and other lipid moieties. Unlike the lung, the barrier lipids of the skin contain little to no phospholipid.

III. TRANSITION AT BIRTH

Birth marks a sudden change in the physiological requirements of all organ systems, including the skin. Transition to a cold, microbe-rich, gaseous environment places severe demands upon epithelial surfaces in contact with that environment. In the term infant, a number of physiological mechanisms are brought into play in an orchestrated manner to allow smooth, seamless adaptation with the external world. Figures 3 and 4 depict schematically some of these functional adaptations:

1. The skin surface of the term infant is covered with a rich, putatively protective mantle of sebaceous secretions. Preterm infants lack hyperplastic sebaceous glands and, hypothetically, are deficient in potential protective effects associated with sebum such as antioxidants and/or anti-infective molecules (13).
2. Barrier lipid synthesis and programmed cell death occurring at the upper nucleated layer of the epidermis are mechanisms devel-

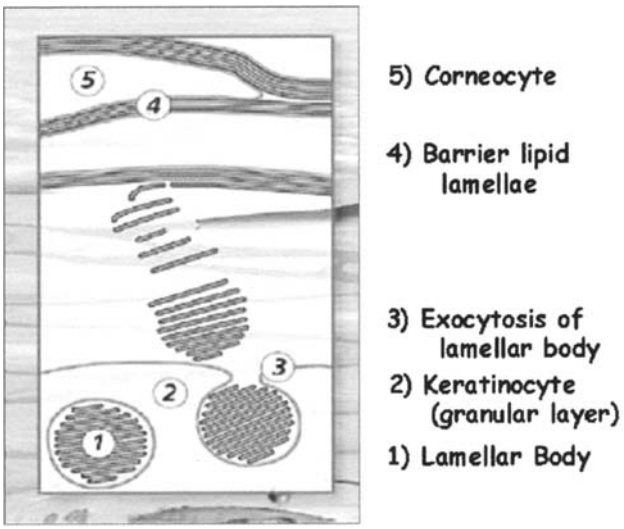

Figure 3 Assembly of epidermal barrier lipids following lamellar body exocytosis. In a process similar to surfactant synthesis and excretion, barrier lipids in the epidermis are packaged in lamellar bodies (1) within the outermost nucleated layer of the epidermis [stratum granulosum (2)]. Lamellar bodies undergo exocytosis (3) and spontaneous self-assembly into highly organized lipid lamellae (4) within the interstices of the intercorneocyte space of the stratum corneum. Individual corneocytes (5) are surrounded by the lamellar lipid matrix.

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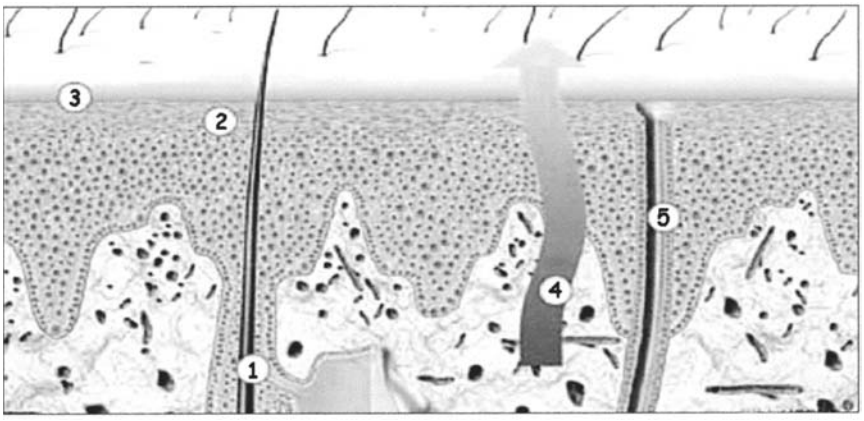

Figure 4 Physiological mechanisms contributing to formation of the epidermal barrier. The epidermal barrier is a combination of multiple physiological mechanisms which are generally deficient in the very low birthweight preterm infant. These mechanisms include (1) sebum secretion, (2) cornification and epidermal barrier lipid synthesis, (3) desquamation and acid mantle formation, (4) control of water transpiration, and (5) sweating. The seamless coordination of all these functions constitutes the epidermal barrier of the skin.

oped during the last trimesters of pregnancy, which are responsible for formation of the terminally differentiated epidermal barrier (stratum corneum) (8).

3. Desquamation of the skin surface and formation of the acid mantle following birth are both important processes for self-cleaning of the organism/environmental interface and adaptation to a new world with surface colonization by ''friendly'' bacterial species.
4. Increasing evidence indicates that the transpiration of water itself may be regulatory for DNA and lipid synthesis resulting in epidermal barrier formation. The level of environmental humidity, for example, markedly influences the rate of epidermal barrier formation in cultured skin substitutes as well as in wounded animal skin (22–24). These results indicate the complex interplay between the environment and the organism in formation of the epidermal barrier.
5. Finally, eccrine sweating is an important mode of protection against overheating in humans. This mechanism is active in the term infant, although to a lesser degree than in older children, but is inadequate in preterm infants (25). Additional effects of

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eccrine sweating include hydration of the stratum corneum and a possible role in infection control.

All these mechanisms of barrier development are inadequate or impaired in the ELBW preterm infant.

In addition to the mechanisms listed above, the epidermis also exhibits specific cell-mediated host defense. Langerhans cells, for example, are the only cell type in the epidermis at birth that is not a permanent resident. Langerhans cells are dendritic, bone marrow–derived cells which migrate into the epidermis and function in the role of antigen presentation (Fig. 5). These cells are strategically positioned in the midepidermis, where they "float," holding a position against a steady stream of differentiating keratinocytes moving from the basement membrane to the environment. The strategic location of these antigen-presenting cells provides a peripheral outpost for the immune system whereby "breaks" in the epidermal barrier can be translated into antibody production following migration of the Langerhans cells to the adjacent regional lymph nodes. It is unknown to

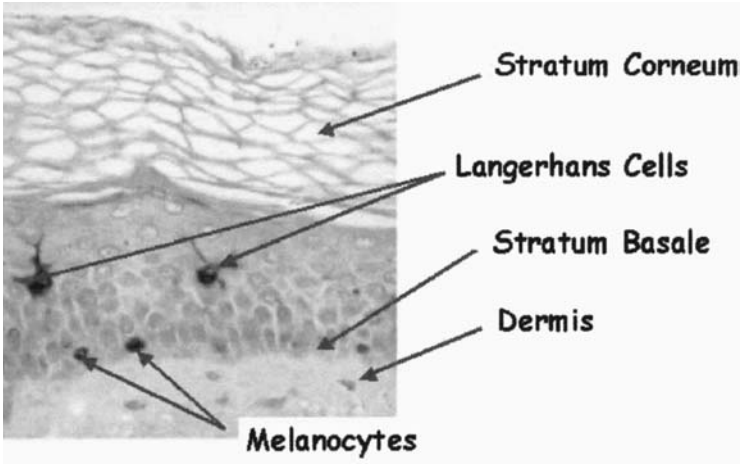

Figure 5 Immunolocalization of Langerhans cells in human epidermis by S100 staining. Dendritic Langerhans cells can be immunolocalized in adult epidermis and are strategically located approximately four to five cells up from the epidermal-dermal junction. As shown, the dendrites of Langerhans cells typically extend upwards towards the stratum corneum. Melanocytes also stain with S100 antibody and are shown in their typical location near the stratum germinativum. The density and location of Langerhans cells in premature infant skin have yet to be determined. Recent work in term infant skin has demonstrated Langerhans cells at significantly lower levels in the epidermis compared to adult skin.

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what extent Langerhans cells are structurally and functionally equivalent in epidermis of preterm infants compared to the skin of term infants, older children, and adults.

Focus on the multiple aspects of skin function at the time of birth highlights the role of the skin as a primary care interface (4,26). Table 2 lists a number of aspects of the skin in this capacity. Clearly, these aspects have broad implications for the delivery of primary care beyond the scope of the neonatal intensive care unit. Nevertheless, skin care begins at birth and is a particularly critical component of care for the ELBW preterm infant. Such an infant interacts with an extraordinarily complex environment constituted by a plethora of heating devices, monitors, adhesives, topical soaps and surfactants, in addition to interaction with parents, nurses, and other caregivers. An appreciation of the clinical consequences of skin immaturity requires recognition of the structured environment in which preterm infants are currently housed.

IV. CLINICAL CONSEQUENCES OF IMMATURE SKIN

The best known consequence of an immature epidermal barrier is increased transepidermal water loss (27). Fig. 6 shows the well-known exponential curve of transepidermal water loss as a function of gestational age. ELBW preterm infants have TEWL in excess of 60 $g/m^2/h$. Such infants are at risk for dehydration, hypernatremia, and temperature instability. Each milliliter of water evaporated from the skin surface carries with it 580 calories, which must be recouped from metabolic sources or supplied by exogenous heating systems such as radiant warmers. The impossibility of

Table 2 Aspects of Skin as a Primary Care Interface

Interface for bedding, clothing, and the environment
Support for tapes and other adhesives
Surface of interaction with soaps, surfactants, disinfectants, and bacteria
Site of most laboratory blood drawing
Platform for percutaneous catheters
Barrier for transdermal drug delivery
Site of action of topical anesthetics and analgesics
Surface of care for wound practices, ostomies, and pressure sores
Boundary for noninvasive monitoring and skin-based sensing techniques
Medium of interaction in massage therapy, acupuncture, and healing touch
Basis for initial clinical evaluation of patient well-being (appearance)

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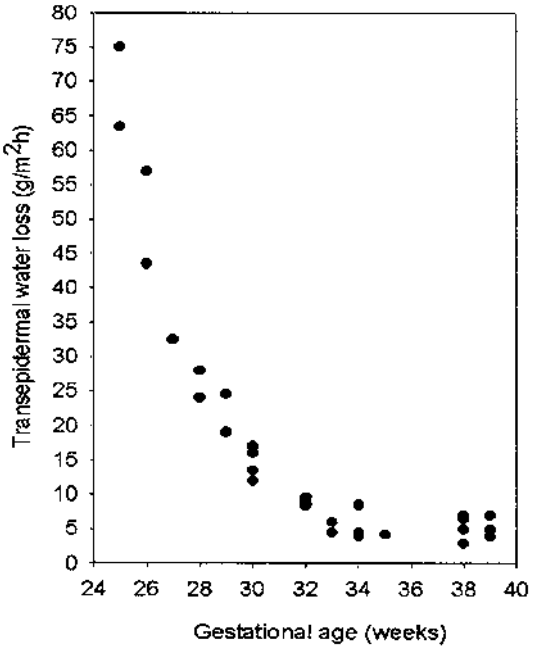

Figure 6 Transepidermal water loss as a function of gestational age. These data demonstrate the well-known inverse relationship between transepidermal water loss (TEWL) and gestational age. Very low birth weight preterm infants have extraordinarily high TEWL in the range of 50–70 $g/m^2/h$. Of note, term infants have low TEWL in the range of 5 $g/m^2/h$, which is comparable to or lower than adult values.

survival with such extremely high water loss requires immediate corrective measures on the part of infant caregivers.

Following preterm delivery, the normal slow intrauterine rate of epidermal barrier maturation is foregone. Birth of the ELBW infant triggers immediate lipid and DNA synthesis with subsequent cornification of the nucleated keratinocytes of the epidermis. The rapid transition from a sticky, wet, translucent skin surface of the ELBW to the dry, opaque stratum corneum of the older preterm infant is familiar to infant caregivers. Such rapid stratum corneum formation often results in excessive desquamation and scaling noted several weeks following preterm birth. Measurements of TEWL and surface electrical capacitance have been used to track the rate of barrier formation following preterm birth (16,27–30). As shown in Fig. 7, TEWL in immature 24 to 25-week gestation infants decreases during the

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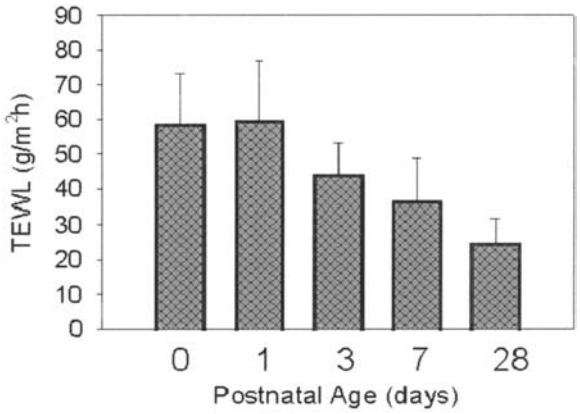

Figure 7 Transepidermal water loss as a function of postnatal age in preterm infants. At birth, very low birthweight premature infants typically have extraordinarily high water losses. After birth, the skin surface rapidly undergoes visible cornification over the first few postnatal days. Subsequently, the skin surface of the VLBW infant will exhibit desquamation with development of a dry scaly skin. This desquamation follows the initial hyperproliferative response. Comparison of these values with Figure 6 indicates that transepidermal water loss in VLBW infants remains elevated above values of term newborns. This elevation persists up to the 4th postnatal week indicating ongoing barrier compromise. (Data from Ref. 28.)

first few days after birth, but still remains markedly elevated on the 28th postnatal day compared to expected levels of 5–10 $g/m^2/h$ in term infants (28). The etiology of the poor stratum corneum barrier in these infants is unclear but may relate to the extreme rapidity of barrier formation in this vulnerable population. Adult skin conditions characterized by rapid epidermal turnover, such as psoriasis, have similar high water loss rates, although histologically the epidermis of a 25-week infant on the 28th postnatal day is similar to that of a term infant, not to the grossly thickened epidermis observed in psoriasis.

The immature epidermal barrier of the ELBW infant is permeable to other substances in addition to water. Respiratory gases, for example, move across the epidermal barrier under normal conditions. The ability to augment respiratory gas exchange across the stratum corneum by surface heating forms the basis of transcutaneous gas measurement in the neonatal intensive care unit. The immature barrier of the preterm infant allows efflux of carbon dioxide (31) and the influx of oxygen (32) in a similar manner to water vapor (Fig. 8). These findings support the contention that increasing ambient oxygen in a convective incubator during the first few postnatal days may lead to an appreciable augmentation of systemic oxygen. The rapid

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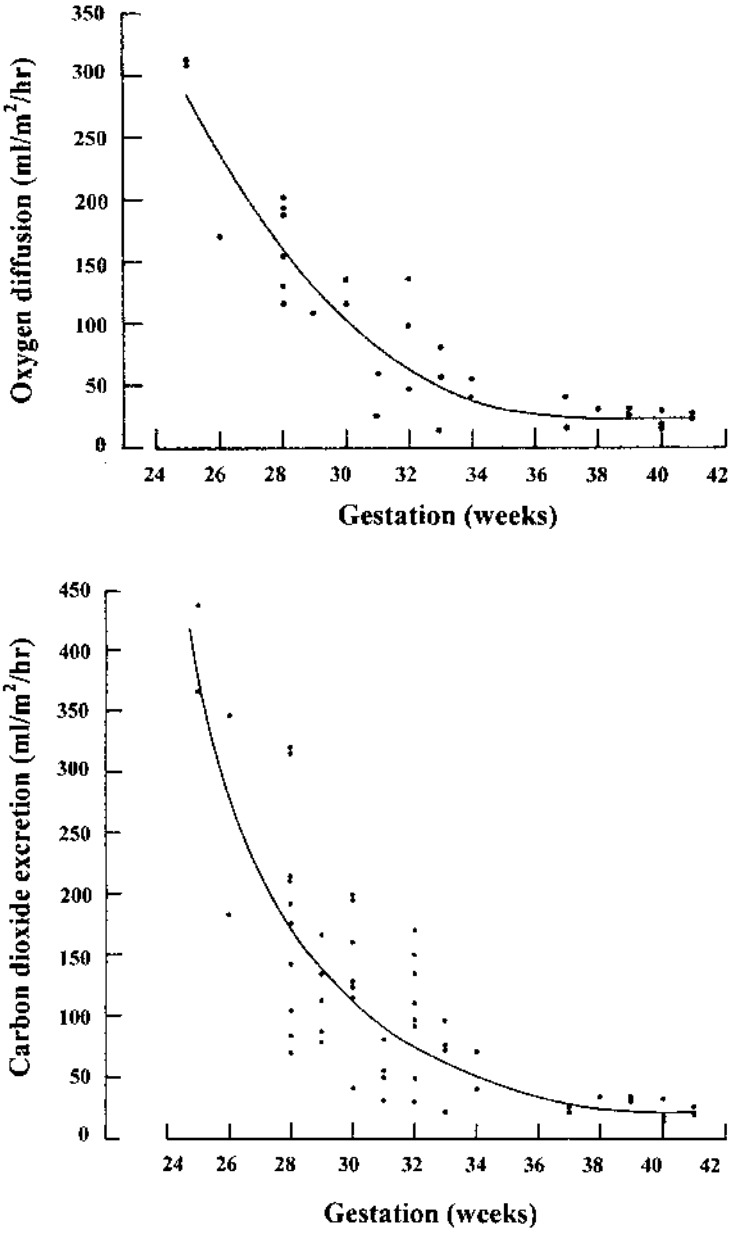

Figure 8 Effect of gestational age on transcutaneous passage of respiratory gases. (A) Diminution of percutaneous oxygen absorption as a function of gestational age. (B) The effect of gestational age on percutaneous carbon dioxide excretion.

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development of the stratum corneum, however, precludes the long-term utility of this novel therapeutic strategy.

Figure 9 shows the blanching response following topical application of phenylephrine in a graded cohort of infants of varying gestational ages. The increased permeability of the barrier to topical phenylephrine is indicated by an increased blanching response. There is a dramatic decline in the drug response as a direct function of both gestational age at birth and postnatal age. The risk of accidental percutaneous and poisoning in the newborn (Table 3), for example, is obviously greater at lower gestational and postnatal ages. These dynamic changes pose difficulties for the design of protocols and devices for the transdermal delivery of pharmacologically active substances. The transdermal delivery of caffeine, for example, would encounter markedly different epidermal barriers in a 27-week premature infant on the 5th postnatal day compared to a 32-week premature infant on the 15th postnatal day.

Not surprisingly, given the incompetence of the epidermal barrier, the incidence of sepsis is markedly greater in preterm infants compared to term infants. In developing countries, preterm infants have a prevalence of sepsis

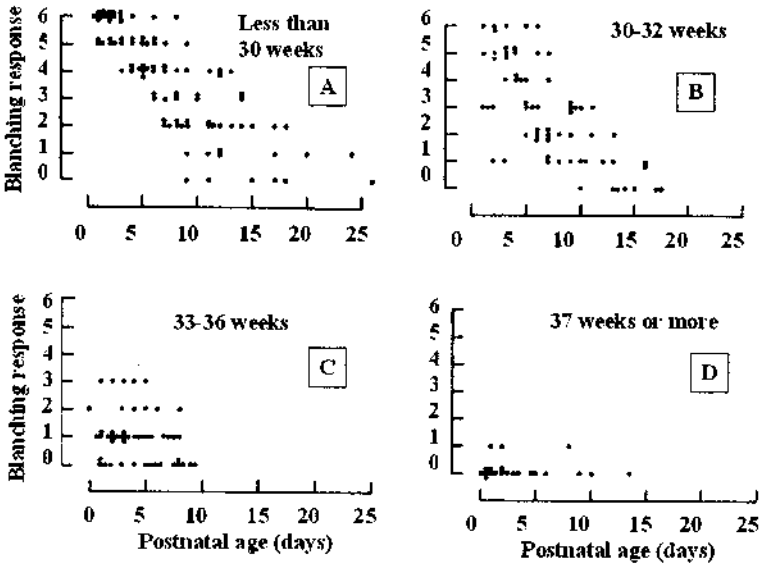

Figure 9 Blanching of the skin following topical application of phenylephrine in infants of varying gestational and postnatal ages. The blanching response is greatest in the most premature infants and decreases as a function of postnatal age.

Table 3 Effects of Accidental Percutaneous Absorption in the Newborn[a]

Chemical or drug	Pathological effect
Topical antiseptics	
Chlorhexidine	None recorded
Hexachlorophene	Vacuolar myelinopathy
Iodine	Hypothyroidism, goiter
Neomycin	Nerve deafness
Alcohols	Hemorrhagic necrosis of the dermis
Pentachlorophenol	Metabolic acidosis; hepatomegaly
Aniline dyes	Methemoglobinemia
Dermatological preparations	
Steroids	Growth retardation; Cushing's features
Boric acid	Gastrointestinal, neurological
Lindane	Neurological
Adrenaline	Pallor, tachycardia
Urea	Raised blood urea
Estrogens	Feminization

[a]See also Ref. 67.

estimated at 30–60% with a mortality of 40–70% (33,34). Approximately one fourth of all infants weighing less than 1500 g at birth in the United States have at least one episode of sepsis after the 3rd week of life (35). This incidence is increased in the more susceptible population of ELBW infants. These facts indicate a need for better scientific understanding of the epidermal barrier in utero and ex utero, particularly in the vulnerable ELBW population. Moreover, the most common organism resulting in systemic infection in ELBW infants is *Staphylococcus epidermidis* (35), suggesting a combination of contributory factors ranging from a poor epidermal barrier to relative immunocompromise.

Physical injury leading to epidermal tears and fissuring is a common concomitant of newborn intensive care (36,37). ELBW infants are particularly prone to damage from monitors, tapes, and physical abrasion. Very immature infants may develop atrophic scars (anetoderma), most likely the result of injury from monitors and adhesives (38–40). Techniques to minimize injury to the skin form an important component of newborn care delivery (41). Attention to such techniques has revealed surprising findings such as the relatively thick heel pad of the preterm infant as demonstrated by postnatal ultrasound (42). Studies such as this have led to reduction of physical injury and pain in the NICU.

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Over the years, a number of therapeutic strategies have been developed for facilitating management of epidermal barrier development (Table 4). In other organ systems, prenatal administration of glucocorticoids to the mother has been a mainstay of treatment. This is particularly evident in steroid-induced maturation of the fetal lung and the dramatic effects on perinatal outcome (43). In rodent models, prenatal steroids administered to the pregnant dam markedly accelerate development of the epidermal permeability barrier as well as maturation of the periderm, a hydrophobic surface film that limits evaporative heat loss (10,11,44). Such dramatic effects are less evident in preterm human infants, with conflicting results in the literature. Omar et al. noted lower estimated insensible water loss associated with a decreased incidence of hypernatremia and an earlier diuresis and natriuresis in ELBW infants exposed to prenatal glucocorticoids (45). In contrast, Jain et al. measured TEWL from abdominal skin by evaporimetry in infants born before 34 weeks gestation (12). In this study, no influence of antenatal steroids or gender could be demonstrated. The authors concluded that epidermal maturation in the preterm infants is not influenced by prenatal maternal glucocorticoids, suggesting a mechanism of maturation differing from that of the rodent. This conundrum deserves further investigation.

Table 4 Therapeutic Strategies for Facilitating Epidermal Barrier Development in the Extremely Low Birthweight Preterm Infant

Prenatal hormone exposure
Do maternal glucocorticoids accelerate epidermal maturation?
Environmental manipulation
Increased ambient humidity decreases transepidermal water loss
Convective incubation allows air and skin servocontrol
Infrared warmers provide direct heating and ease of accessibility
Avoidance of exposure to cold objects, e.g., windows
Semipermeable membranes
Use in delivery room markedly decreases TEWL
Degree of occlusion may facilitate epidermal wound healing
May be combined with other heating modes to create local "environment"
Theoretically can be used for emollient transfer as in diaper products
Emollients—Barrier lipid–replacement strategies
Nonphysiological (petrolatum-based)
Physiological (native lipids)

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It is noteworthy in this regard that the fetal adrenal glands are markedly enlarged in the term newborn infant with combined adrenal gland weights at term equivalent to adult human subjects. Following birth, the adrenal cortex undergoes rapid involution. Prenatally, the primary component of fetal adrenal steroid synthesis are androgenic steroids. The possibility that such steroid synthesis is responsible for the third trimester hyperplasia of the sebaceous gland and subsequent vernix secretion is an intriguing possibility. The overwhelming effect of maternal glucocorticoids to mature pulmonary function has possibly overshadowed the study of lesser or potentially antagonistic effects on other organ systems.

The less obvious effect of high TEWL associated with an incompetent epidermal barrier can be seen in ELBW preterm infants housed in convective incubators. Such heating modes rely upon provision of an insulating layer of heated and humidified air to minimize TEWL with associated evaporative cooling. In ELBW infants it is common to note a higher air temperature than skin temperature during the first few postnatal days (46). The development of an epidermal barrier is associated with a decrease in the time to reach skin–air temperature equilibration, as shown in Fig. 10. Of

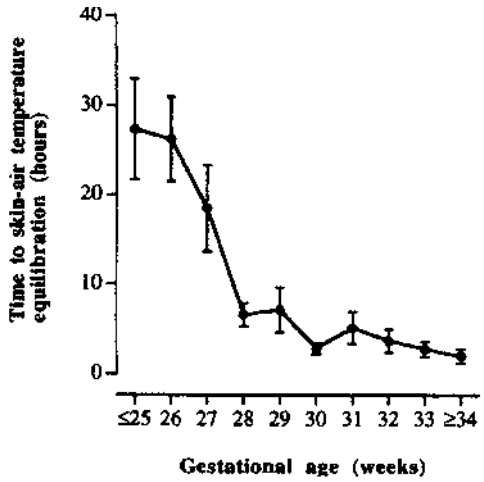

Figure 10 Effect of gestational age on the time required to reach environment–skin temperature equilibration in a cohort of low birthweight infants. The cohort consisted of 120 AGA infants < 34 weeks gestation and < 1500 g birthweight. The time to reach equilibrium under the conditions of convective incubation decreased precipitously between 26 and 28 weeks. There was a significant exponential correlation between gestational age and the time required to reach environment-skin temperature equilibration.

interest, this study failed to demonstrate any effect of exogenous glucocorticoids to mature epidermal barrier function as evidenced by this quantitative measurement.

Other therapeutic strategies for facilitating epidermal barrier development and protection of the preterm infant include adjustment of environmental humidity (15). As shown in Fig. 11, increasing ambient humidity results in decreasing TEWL as a function of gestational age. High relative humidities create a microenvironment in which TEWL is lessened. Such humid conditions can only be provided in convective incubators or within microenvironments created under semipermeable membranes placed beneath radiant warming devices. High humidities may result in "rainout" and obscure the infant with the potential for increased infections in the warm, wet environment. Recent studies have demonstrated that the increase in TEWL associated with infrared warmers is due entirely to the low-humidity state of the overlying air rather than to any direct drying effect of the infrared radiation (47).

Interposition of a semipermeable membrane between the skin and a dry environment is an effective means of decreasing TEWL. Vohra et al. recently studied the use of polyethylene films on premature infants in the delivery room (48). These authors demonstrated better temperature stability and a significant decrease in mortality in EBLW infants treated with vapor-reducing semipermeable membranes at the time of birth. This study focused on delivery room management and supported the concept of a "golden

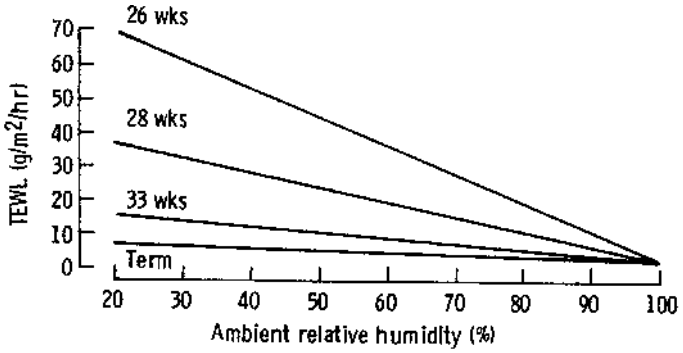

Figure 11 Inverse effect of ambient humidity on transepidermal water loss (TEWL) as a function of gestational age. TEWL decreases as relative humidity increases. This effect is most marked in the very low birthweight preterm infant during the first few days of life when the epidermal barrier is the most immature. At 100% relative humidity, there is no TEWL regardless of gestational age. (Adapted from Ref. 27.)

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hour'' following birth in which regulation of the skin-environment gradient may be important in reducing morbidity and mortality. The classic study of Adamsons et al. in term infants clearly demonstrated the importance of the skin-environment gradient in regulating systemic oxygen consumption as opposed to core body temperature (49). These findings support a direct regulatory role of the skin surface at birth in the control of evaporative heat loss, surface temperature, and the metabolic response to cold. Factors affecting surface water evaporation such as the presence or absence of vernix may have subtle regulatory roles that are overshadowed by our abilities to manipulate environmental temperatures (13). Such properties may be important in developing countries for determining standards for delivery room management.

In addition to humidity and the application of semipermeable membranes, the administration of petrolatum-based ointments to the skin surface has been used to improve barrier function in preterm infants. The latter modality has received attention recently following reports that Aquaphor® administration improved skin condition and reduced nosocomial infections in a small cohort of preterm infants (50). A more recent study performed on more than 1200 low birthweight preterm infants demonstrated an increase in late-onset nosocomial infections mainly secondary to coagulase-negative staphylococci without demonstrating any beneficial effect (51). These findings indicate the need for more attention to therapeutic strategies for facilitating epidermal barrier development.

An important concept emerging from this work is the idea that therapeutic strategies are best constructed as combinations of methods. Maternal hormone therapy (52), for example, coupled with delivery room management (48) and a seamless transition to a controlled NICU environment with judicious use of physiological emollients (50), may provide the best means of coupling the preterm infant to therapeutic environment. On the other hand, the efficacy and/or safety of both prenatal steroid therapy (12) and topical emollients (51) have been questioned for the very low birthweight preterm infant. The development of ''intelligent fabrics'' with heat and motion activated transfer of topical emollients and wound-healing ointments to the skin surface has been developed for treatment of diaper rash (53,54). The concept that clothing and/or bedding material, in addition to wound dressings, may contain physiological emollients and growth factors for facilitation of epidermal barrier development is attractive. Recent studies of epidermal wound healing in adult humans indicates that regulation of surface water vapor flux in the range of 20–60 $g/m^2/h$ may be particularly beneficial in accelerating epidermal barrier repair (55). These therapies draw attention to the role of the skin in environmental coupling and the close interconnection between the human skin surface and the caregiver.

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V. NEW CONCEPTS: THE SKIN AS A NEURODEVELOPMENTAL INTERFACE

The epidermis is an ectodermal derivative linked embryologically to the brain and postnatally to tactile and visual perception (5). The epidermis, therefore, is simultaneously a cellular and molecular interface and a psychological and perceptual interface. Even the stratum corneum is amenable to diametrical perspectives. On the one hand, the stratum corneum is a dead, effete, composite of anucleated cell bodies destined for desquamation to the environment. On the other hand, the stratum corneum has all the hallmarks of a "smart material" in an engineering sense. It is highly organized and strategically positioned such that changes in its physical properties couple the organism in a dynamic manner to a changing environment (3).

The development of the stratum corneum is closely linked to the progressive electrical isolation and autonomy of the fetus in utero. Wakai et al. demonstrated that prior to formation of vernix and the associated stratum corneum, the fetal electrocardiogram is easily detected from the mother's abdomen (56). Following development of the high impedance barrier provided by the vernix and stratum corneum, the fetal ECG electrically disappears. In evoked potential studies, the electrical resistance of the stratum corneum is the primary barrier to obtaining high-quality tracings of voltage amplitudes (5). Noninvasive monitoring, therefore, particularly in visual, tactile, and auditory evoked potential testing, requires increased attention to the epidermal barrier. Pivotal to this concept is the recognition that nerve endings never directly touch the environment. The elaboration of feedback control between the central nervous system and environment, however, requires not only sophisticated central mechanisms (synapse formation, neuronal plasticity) but also an adaptive dynamic interface. Environmental interfacing is not directly a function of nerve endings, but rather of peripheral structures such as the epidermis/stratum corneum which form the actual interface with the environment (5). Because it is right before our eyes, we tend to trivialize or underestimate the importance of this interface. For example, in textbooks dealing with human thermal physiology, the fact that the presence of a stratum corneum is the *sine qua non* of successful temperature regulation is treated as a "given" and is rarely discussed in detail. In preterm infants, however, it is obvious that the stratum corneum develops *pari passu* with mechanisms for controlling body temperature and metabolic heat production.

As in the example above, the study of preterm infants may be particularly illustrative of the interconnection of the skin/environment interface and central homeostatic mechanisms. The complex field of touch, pain, and the withdrawal of body surfaces from noxious environmental stimuli is a

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classic example of adaptive environmental interfacing. Figure 12 shows touch thresholds obtained by use of von Frey hairs (57). As shown, the threshold for response is progressively increased with advancing gestational age, consistent with increased flexor withdrawal in the very preterm infant. This finding is usually attributed to increasing inhibition of central nervous system structures. Similar results have been obtained using light touch in the form of brushing in newborn rats (58,59). This work indicates that light touch is an important determinant of metabolic state and growth potential in central organs such as heart, brain, and liver (58). Light touch also results in increased levels of serum lactate levels in the immediate newborn period in rodents (59). Lactate is an important metabolic substrate for the developing brain in this species. This response is extinguished with advancing gestational age in the same manner as the cutaneous flexor response. These data indicate changes in organism/environment interfacing in terms of dynamic coupling and metabolic state response.

VI. FUTURE DIRECTIONS

Further work is required to understand the remarkable ability of the human fetus to develop a superb epidermal barrier under conditions of total aqueous occlusion. The finding that epidermal barrier development is patterned

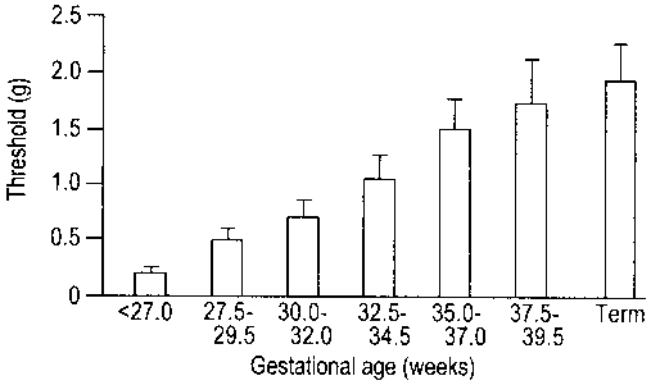

Figure 12 Threshold stimulus for eliciting flexor withdrawal in newborns of varying gestational age. Flexor withdrawal is elicited by stimulation of the foot with nylon filaments (von Frey hairs) of graded thickness. Preterm infants exhibit progressively lower thresholds for withdrawal than term infants. (Data from Ref. 57.)

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and connected to development of the pilosebaceous apparatus is intriguing and supports a potential role for sebaceous secretions and barrier development in utero (14). Current evidence supports a dynamic interaction between developing epithelia such as the skin, lung, and gut prior to birth. This interaction involves the uniquely human skin barrier cream, the vernix caseosa. The prominence of sebaceous glands in the term infant and their relative absence in the preterm has not received adequate attention. Whether new technologies can be developed wherein lipid replacement films analogous to pulmonary surfactant can be used on the skin of the ELBW infant deserves further consideration (60).

The skin of the preterm infant contrasts with the superb epidermal barrier of the term infant. Earlier studies have clearly indicated that the epidermal barrier of the term infant rivals that of the adult and, in some cases, exhibits a better barrier to TEWL or percutaneous drug transport (61). A better understanding of the mechanisms by which this barrier forms in utero may allow translation of these processes to skin and wound care postnatally. In particular, it is unclear whether glucocorticoids enhance barrier maturation in humans in a manner similar to rodents.

Neonatology is well poised to make a significant impact on skin science in general. Many aspects of primary care delivery directly involve the skin (Table 2). Broadening our view of the skin to encompass disease-independent functions such as noninvasive monitoring, adhesion of tapes, and choice of surfactants for skin cleansing and infection control emphasizes the importance of the skin in care delivery (26). The skin surface is highly complex. Mechanisms of bacterial colonization, acid mantle formation, and surface electrical properties of the skin are clinically important but, as yet, poorly understood. Given the complexity of the skin surface and its central position in primary care delivery, it is important to guard against its trivialization (26).

Focus on the role of the skin as a primary care interface and attention to the scientific evidence underlying skin care practices in the nursery creates distinct opportunities for neonatology in general. Recognition of the skin as a primary care interface for patient care and parental satisfaction creates an opportunity for true collaborative practice between nursing and medicine (62). Coupling between the infant and the critical care environment figures prominently in the scientific basis of new fields such as ''developmental care'' (63). The dual nature of the skin as a boundary organ interfacing cellular and molecular domains with psychological and perceptual domains is consistent with this approach (5). Finally, a better understanding of stratum corneum development and the role of the epidermal barrier in infection control have implications for improving infant health care in developing countries (34,64). These approaches require persistent recognition of the

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complexity of the developing skin surface and the need for objective, evidence-based guidelines for infant skin care practices in neonatology (36,37,41,65,66).

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9
Electrical Properties of Newborn Skin

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I. INTRODUCTION

In general, babies' skin is considered cherubic and flawless, appearing so smooth and soft that it is hard to resist the temptation to touch it. However, it is not in such a state from the beginning. At birth, babies are suddenly exposed to a dry environment after residing in utero continuously immersed in amniotic fluid. Thus, birth marks the onset of an acute physiological adaptation process during which the skin transitions to a totally new environment during the neonatal period. This transitional adaptation is evident in changes in the skin surface, particularly the stratum corneum (SC).

The human body is covered by the SC, which consists of about 15 tightly stacked layers of flattened dead bodies of lipid-laden keratinocytes, called corneocytes, forming "a brick-and-mortar" organization with the corneocytes serving as bricks and the intercellular lipids as the mortar (1). Although the SC is a thin biological membrane about 15 μm thick, it plays a crucial role in maintaining our life in the atmosphere, because beneath it fully hydrated living tissues function to sustain our existence. The SC is such an effective barrier that it allows water loss only in minimal amounts, around 5 $g/m^2 \cdot h$, enabling our survival even in a dry environment. This transepidermal water loss (TEWL) can be utilized as a parameter of the in vivo barrier function of the SC, but only when measurements are performed at a room temperature of less than 22°C to eliminate completely the influence of sweating (2). We can compare the skin in such a state to a bag of

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polyethylene film containing water that is placed in a dry environment. The SC protects our body not only from desiccation but also from external invasion of various kinds of injurious agents.

Remarkably, normal SC can remain soft and flexible in the usual ambient conditions, allowing free body movement without producing any cracking or fissuring on the skin surface (3). The application of oily substances may induce smoothness on the surface of dry and firm skin, but it cannot make it soft and flexible (4). It is water that provides the stacked layers of corneocytes with suppleness and smoothness. Although there is a constant water supply from the underlying living tissue in vivo, the superficial portion of the SC may become dry and brittle in dry ambient conditions such as the heated indoor condition of winter. If the water-holding capacity of the SC is functionally deficient, dry skin (xerosis) develops even in normal individuals. In addition to the well-known physiological xerotic conditions that are noted in normal infants, middle-aged females, and elderlies, neonates also show such xerotic changes characterized by fine desquamation on the trunk and extremities (5).

II. IMPEDANCE MEASUREMENTS

In contrast to the ease of measuring the water content of the SC in vitro, it was not until 1980 that we could adequately assess the hydration state of the skin surface in vivo quickly and accurately in a noninvasive manner. Such an assessment is based on the measurement of the skin impedance using dry surface electrodes (6). The impedance (Z), the total electrical opposition to the flow of an alternating current, depends on two components, the resistance (R) and capacitance (C), and their relationship may be formulated as follow:

$$Z = [R^2 + (1/2\pi.fC)^2]^{1/2}$$

where f stands for the frequency of an applied alternating current. In the past, because of the technical simplicity, many researchers could study the impedance of human skin, which was modeled electrically as a resistor in parallel with a capacitor (7). However, it had been difficult to interpret the meaning of the results so obtained in terms of skin physiology, because the impedance changes in a complex fashion with the season, time, circumstances, and the electrode paste used (8). Dry SC is a dielectric medium, a medium of weak electrical conduction. However, when it is moistened there occurs a dramatic change in its electrical properties. Because water is strongly dipolar, H_2O molecules combine easily with the structural components of the SC, rendering it more sensitive to an electrical field (7).

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Starting from the practical need for in vivo methods of making relative measurements of the hydration state of the skin surface, we found that we could evaluate it quickly and quantitatively in a noninvasive way by measuring the skin impedance using dry electrodes (6). Our evaluations were based mostly on our own data obtained with a high-frequency instrument (now available from the IBS Company, Hamamatsu, Japan, under the name of Skicon®), which operates at 3.5 MHz, serving as an automatic device for measuring the skin conductance ($= 1/R$) and capacitance separately. Likewise, Leveque and de Rigal (9) also reported good sensitivity to the water content of the skin surface with a similar instrument developed by them. These obtained results are minimally influenced by the electrical properties of applied water, e.g., between buffered saline with high conductance and distilled water with very low conductance, but only by the amount of water absorbed into the SC (6).

The skin measurements should be done in an environment in which the temperature is lower than 22°C to prevent the influence of sweating. In general, the skin conductance and capacitance measured with this instrument showed similar behavior ($r = 0.952$; $p < 0.01$). However, we chose to use conductance rather than capacitance because the values of capacitance on the palmoplantar skin surface were disproportionately low compared with conductance, in addition to the fact that capacitance is less sensitive as a parameter for the water content in the SC (6). In a simulation model of in vivo SC that has a water gradient that progressively decreases from the deepest layer to the superficial layer, high-frequency conductance values correlated well with the actual water content in the uppermost portion of the SC as well as with the water content of the whole SC (10). Subsequently, a similar instrument has become commercially available from Nova DPM 9003 (Nova Technology Corporation, Gloucester, MA).

Another instrument, Corneometer (Courage-Khazaka Electronic GmbH, Cologne, Germany) is based on the measurement of electrical capacitance. With SC samples Werner (11) demonstrated that the capacitance method detects changes in hydration. Blichmann and Serup (12) found that the capacitance and conductance methods showed parallel changes with skin hydration, although the former shows higher sensitivity to decreased hydration as noted in dry skin, whereas the latter is more sensitive for the measurement of increased hydration such as noted after the application of moisturizers. This is because the conductance method measures very superficially in contrast to the capacitance method, which depicts changes in hydration down to a depth of about 0.1 mm. In fact, based on measurements in a simulation model of in vivo SC, we found that the conductance device showed a much closer correlation with the hydration state of the surface SC ($r = 0.99$) than did the capacitance device ($r = 0.79$), suggesting

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that the conductance method can accurately assess the hydration dynamics of the superficial portions of the SC, particularly that due to the accumulation of easily releasable secondarily bound water and free water (13).

III. CLINICAL FEATURES OF NEONATAL SKIN SURFACE

At first we carried out clinical assessments of the skin condition in 103 healthy newborns within 6 days after birth during the spring (14). We chose nine sites: the forehead, cheek, dorsa of the hand and foot, flexor aspect of the forearm, chest, antecubital fossa, axilla, and inguinal fold. Only 10 babies were free from scaling, but the remaining 93 (90%) of the 103 newborns showed at least one scaly area. It was remarkable that none had scales on the forehead and cheek, both of which are seborrheic areas. In other sites there were fine white scales attached to normally appearing skin that could be graded as 0, none; 1, mild; 2, moderate; and 3, severe. Most had some scaling tendency on the flexor aspect of joints such as the antecubital fossa, axilla, and inguinal fold. The dorsum of the foot was significantly more scaly than the dorsum of the hand in 2- to 6-day-old infants ($p = 0.01$ with two-sample Wilcoxon test). Although the total score of scale severity of the newborns did not change significantly in the first 6 days (Kruskal-Wallis test), there was a gradual increase in those showing grade 0 at the antecubital fossa, axilla, and inguinal fold in the first 6 days.

IV. HIGH-FREQUENCY MEASUREMENTS OF THE SKIN SURFACE

Mize et al. (15) found by measuring low-frequency impedance that there was a statistically significant inverse relationship between the skin impedance and age during the first year of life. They reported that the highest impedance values were in newborn infants and that a drop in skin impedance during the first few postnatal months could be attributed to an increase in skin hydration as a result of the greater function of eccrine sweat glands. Our high-frequency measurements performed on the dorsum of the hand and foot in the warm environment of a nursery (room temperature 26°C, relative humidity 52%) where sweating could occur showed that the conductance values of neonates were significantly lower than those of 10 healthy adults (mean age 32 years, range 22–47 years); the conductance value of adult volunteers was over 10 times higher than that of newborns (14). There were no significant differences in conductance in the skin among 0-, 1-, 2-, 3-, 4-, and 5-day-old newborns. Those values obtained from the dorsum of the hand were almost the same as those on the dorsum of the

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foot. Similarly, conductance values measured on the flexor surface of the forearm of 5-day-old infants in winter were also much lower than those of 1-, 2-, and 6-month-old infants and adults. Thus, there seems to take place a great change in the skin of newborns between the fifth day and the first month. Yosipovitch et al. (16) also found significantly lower hydration levels at various anatomical sites of neonates 10 and 24 hours after birth by using a capacitance method. Our recent study conducted in a climate chamber with a room temperature of 21°C and relative humidity of 50% where no sweating could take place disclosed that the skin of 6 neonates began to show conductance values close to those of their mothers within 14 days on the flexor aspect of the forearm and within one month on the cheek (Fig. 1).

The functional deficiency of the SC of the newborns that show low conductance levels can be analyzed by performing the water sorption-desorption test (17). This procedure consists of high-frequency measurements performed before and after application of a droplet of water for 10 seconds on the skin followed by repeated measurements every 30 seconds thereafter. We can evaluate the hygroscopicity and water-binding capacity of the SC from the initial increase in conductance after water absorption and from the subsequent water desorption curve, respectively. The obtained test results on newborns' skin showed that all the conductance values measured before and after absorption of water were invariably lower than those for their mothers' skin, indicating decreased hygroscopicity, i.e., hydrophobicity, as well as reduced water-holding capacity of the SC (14).

By measuring the capacitance of newborn rats whose external body surface is also highly hydrophobic, Wickett et al. (18) demonstrated beautifully that a single tape-stripping of the peridermal layer remaining on the surface resulted in exposure of the underlying SC and abolished the low hygroscopicity of the newborns' skin as well as the low water-holding capacity of the neonatal skin surface. They further demonstrated the potential role of lipids acting as a water barrier by removing them with acetone treatment. Postnatally, this hydrophobic effect of the rat neonate's skin is gradually lost over the first 3 days of life (18). However, in humans, the periderm is sloughed off from the fetal skin surface at the time of SC formation in utero at approximately 160 days of gestation. In humans, Okah et al (19) found by using capacitance measurements that isopropanol treatment of neonates' skin increased the hygroscopicity as well as the water-holding capacity of the SC. These findings suggested that the fetal SC is hydrophobic in order to prevent excessive water loading from the amniotic fluid. After the abrupt change of the environment at birth, this hydrophobic SC is thought to induce xerotic changes in the skin surface of neonates due to exposure to the dry atmosphere. As mentioned above, it takes more than 5 days in human newborns for the superficial portion of the SC that played

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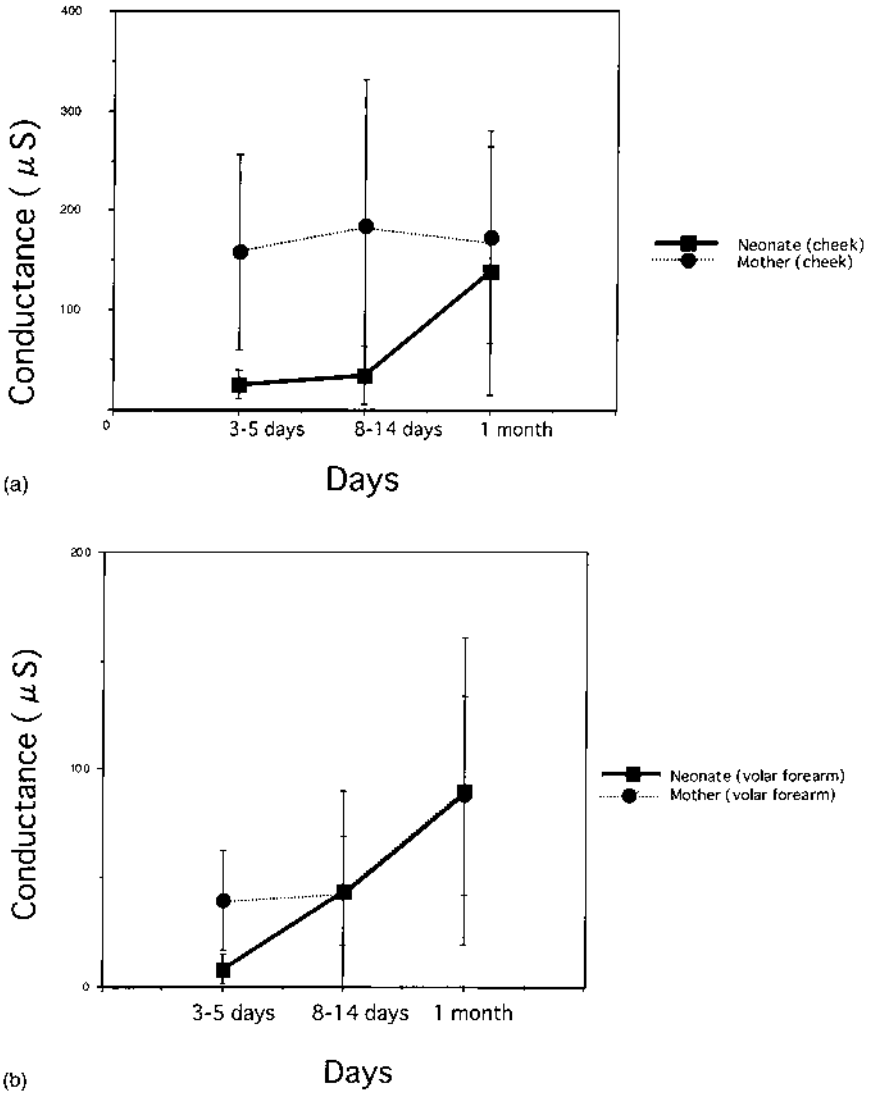

Figure 1 High-frequency conductance measured on the cheek (a) and flexor aspect of the forearm (b) of 6 newborn infants (■) and their mothers (●). The data are shown as the mean of 6 subjects and the standard deviation.

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an important role in utero in keeping water out to be replaced by ordinary SC.

V. CORRELATION OF THE ELECTRICAL PROPERTIES WITH OTHER BIOPHYSICAL PARAMETERS OF THE SKIN

When evaluated with the conductance measurements, the SC of newborn babies showed greatly decreased hygroscopicity and water-holding capacity similar to scaly inflammatory dermatoses such as psoriasis and atopic dermatitis (20–22). In these dermatoses, there is an inverse relationship between TEWL and the water content in the SC (22). However, the SC of newborn baby's skin is not deficient in the water barrier function (14,23). Our recent TEWL data obtained on the cheek and volar forearm also showed TEWL values of the 6 neonates being rather lower or comparable to those of their mothers in the first 2 weeks after birth (Fig. 2). Thus, the skin of the newborn seems to resemble more that of senile xerosis in which the SC barrier function remains intact (24). The epidermis of senile xerosis is rather atrophic, being associated with a slower turnover of the SC (24). In contrast, there is epidermal hyperproliferation together with rapid turnover of the incompletely differentiated corneocytes in the scaly inflammatory dermatoses (20,21). In fact, in their study of SC formation in perinatal rat, Hoath et al. (25) found that SC formation rather slows postnatally.

The skin surface pH values showed a decrease during the first postnatal month (Fig. 3). This change was much less prominent on the cheek, where we did not find any scaly changes because of the presence of sebum. Sebum is efficient for preventing dry skin in adults (26,27). In fact, the sebum levels measured on the cheek of the 6 neonates with the Sebumeter SM 810 (Courage-Khazaka Electronic GmbH, Cologne, Germany) were relatively high (Fig. 4), reflecting the influence of the maternal hormonal environment (28,29). A significant decrease occurs after 6 months. Thus, this high excretion of sebum is thought to be responsible for the well-hydrated state of the faces of neonates.

When we estimate the water barrier function of the SC, it is necessary to perform measurements of TEWL at a room temperature of 22°C or less to eliminate the influence of sweating. However, in our previous study (14) we placed all study subjects in the warm environment of a nursery (room temperature 26°C), finding a great difference in conductance values between the newborns and their mothers. Thus, in this case we cannot rule out the influence of sweating activity, because the lower sweating activity in newborn infants is more clearly shown as low water evaporation (30). It has been demonstrated that minimal effective thermal stimulus to newborns

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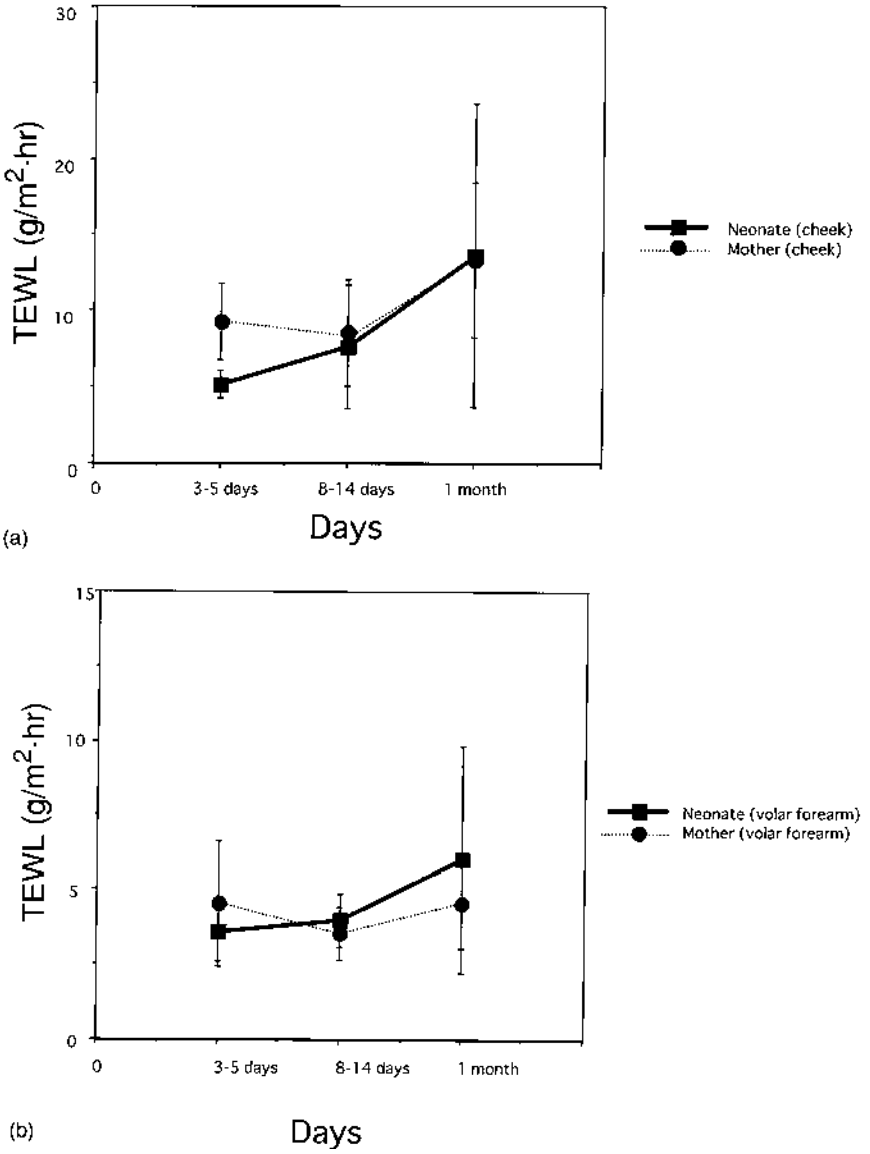

Figure 2 Transepidermal water loss measured on the cheek (a) and flexor aspect of the forearm (b) of 6 newborn infants (■) and their mothers (●). The data are shown as the mean of 6 subjects and the standard deviation.

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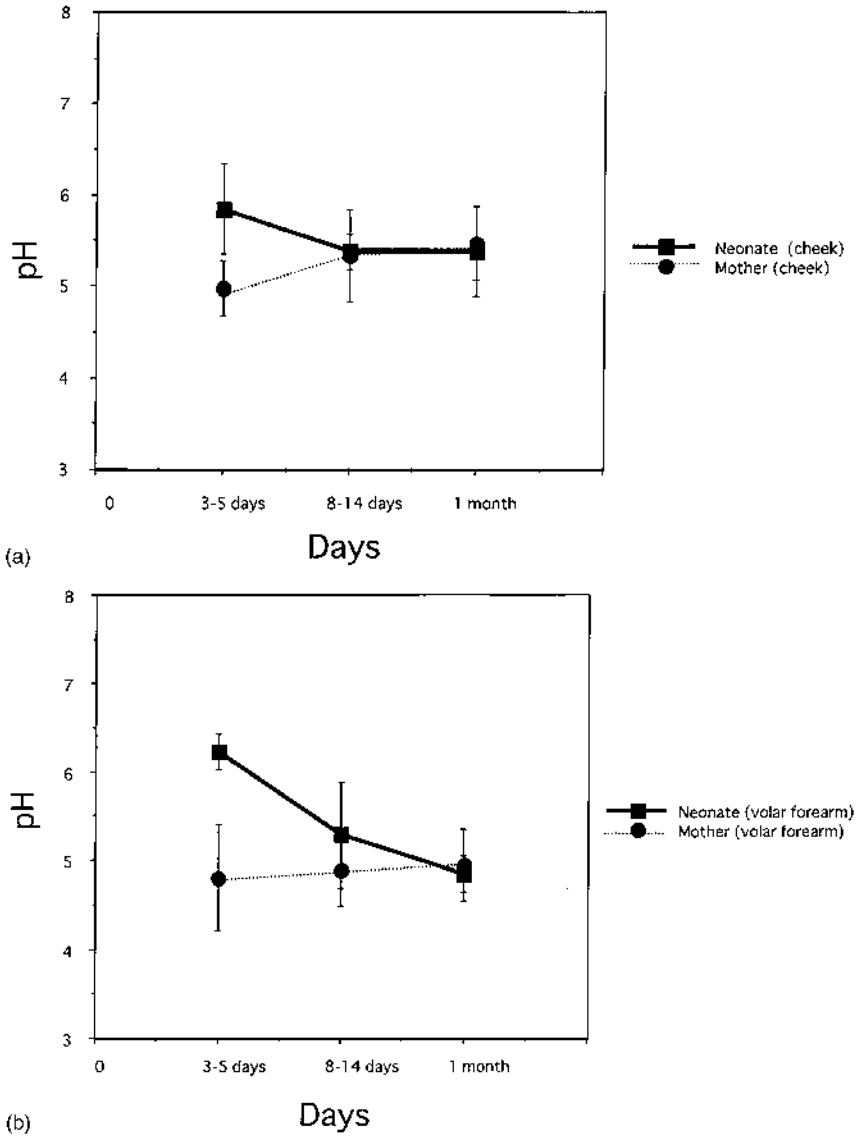

Figure 3 Skin surface pH measured on the cheek (a) and flexor aspect of the forearm (b) of 6 newborn infants (■) and their mothers (●). The data are shown as the mean of 6 subjects and the standard deviation.

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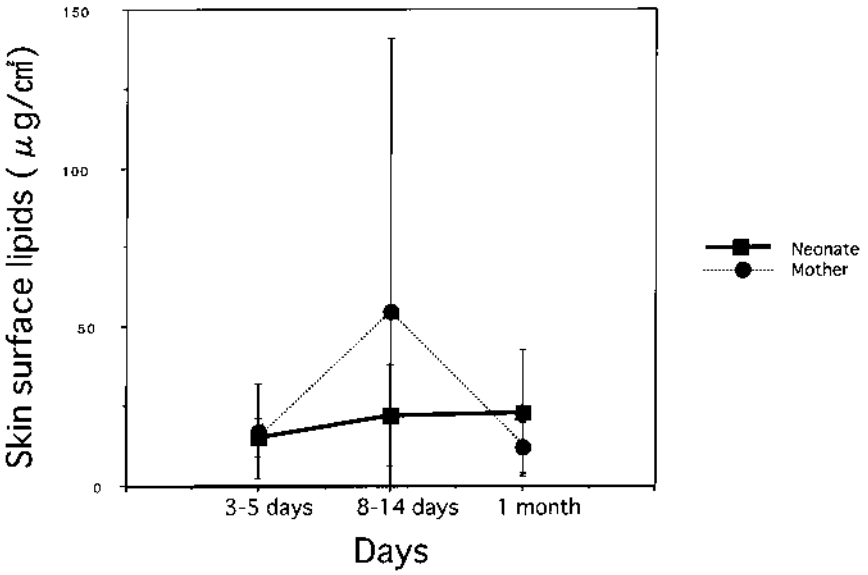

Figure 4 Skin surface lipids measured on the cheek of 6 newborn infants (■) and their mothers (●). The data are shown as the mean of 6 subjects and the standard deviation.

exceeds the thresholds defined for adults (31). In our data, the decreased conductance in newborns seems to reach adult levels within 2 weeks after birth. In fact, because 1- to 6-month-old infants do not remain calm and motionless, they sweat even more than adults and show higher conductance values.

VI. CONCLUDING REMARKS

Our clinical observations disclosed that most newborn infants showed scaling on at least some parts of the body except for the face, which is a seborrheic area. Measurements of high-frequency conductance, which assesses the skin surface hydration, demonstrated that the skin of newborns showed surprisingly lower values than their mothers without any significant change in the water barrier function. The water sorption-desorption test of the neonatal skin showed that the SC is defective in both hygroscopicity and water-holding capacity. All these defects become normal within 2 weeks after birth with the desquamation of the hydrophobic superficial portion

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of the SC that is formed during the fetal stage to prevent excessive water loading from the surrounding amniotic fluid.

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10
The Biology of Vernix

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I. INTRODUCTION

Few events in mammalian life are as physiologically abrupt as birth. Within seconds the newborn organism must move from a warm, sterile, aqueous environment to a cold, microbe-laden world and an obligate air-breathing state. Successful transition to postnatal life requires prenatal maturation of incipient environmental interfaces such as the lung, skin, gut, and kidney. Functional and structural maturation of these interfaces occurs primarily in the third trimester of pregnancy. Recent evidence indicates these epithelial systems interact within the intrauterine milieu prior to birth, as manifested by changes in amniotic fluid composition and associated structural and biochemical maturation of the epithelial surfaces themselves. The prenatal formation of the epidermal barrier is illustrative of this highly coordinated maturational process leading to the ultimate coupling of the newborn with the postnatal environment.

This chapter focuses on the developing epidermal barrier and summarizes the existing data on the uniquely human skin cream called "vernix caseosa." 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 chapter is subdivided into discussion of vernix composition, morphology, physical characterization, and biological properties. What emerges from the available data is a coherent and intriguing glimpse of a new area of perinatal biology.

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II. VERNIX COMPOSITION

Early studies on vernix composition focused primarily on characterizing the lipid contents of vernix following extraction with organic solvents (1–4). The total amount of lipid present in vernix as quantified by Folch extraction is approximately 10% (w/w) (unpublished data). A breakdown of this complex mixture reveals the presence of wax and sterol esters, ceramides, squalene, cholesterol, triglycerides, and phospholipids. Furthermore, vernix also contains a substantial cellular component (5). 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 (6,7). Animals such as sheep produce lanolin, which also contains wax and sterol esters (8). 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 (9–11). A hypothetical working mechanism for the participation of vernix in epidermal barrier maturation is shown in Chapter 4. This model is consistent with a mechanism whereby vernix lipids limit water transport across the developing epidermis, thereby facilitating the cornification process. This process has similarities to maturation of cultured skin following lifting to an air/liquid interface (12).

A recent extensive analysis of the lipid constituents within vernix was published by Sumida et al. (13). 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, while triglycerides, wax and sterol esters, squalene, and phospholipids are components of sebum (Table 1). 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 (13).

The protein constituents of vernix are not as well characterized as the lipid constituents. The abundance of fetal corneocytes present in vernix has not yet been systematically compared to adult corneocytes for the presence of distinguishing molecular constituents such as involucrin, filaggrin, and

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Table 1 Comparison of Lipid Components of Vernix Caseosa, Stratum Corneum, and Skin Surface (Sebaceous) Lipids (Percent of Total)

Lipid fraction	VC lipids	SC lipids	Skin surface lipids
Cholesterol esters	30.6	—	3.0
Ceramides	17.9	40.0	—
Triglycerides	15.1	—	41.8
Cholesterol	7.5	25.0	—
Free fatty acids	6.5	25.0	18.4
Phospholipids	6.1	—	1.5
Wax esters	6.0	—	20.3
Squalene	4.0	—	12.2
Wax diesters	3.7	—	—
Cerebrosides	2.4	—	—
cholesterol sulfate	0.3	10.0	—
Alkanes	—	—	2.8

Source: Data from Ref. 13.

keratin subtypes. Analysis of free amino acids following chloroform-methanol extraction of lipids and acid hydrolysis of the precipitated residue revealed an abundance of asparagine and glutamine (14) (Table 2). The latter is particularly significant insofar as vernix is known to detach from the fetal skin surface prior to birth and is subsequently swallowed by the fetus. Glutamine is currently under investigation as a trophic factor for the developing fetal gut (15).

The authors performed a series of experiments to characterize the water content of vernix. Dry weight-to-wet weight ratios of vernix caseosa and standard topical creams used in the newborn nursery were compared (16). It was found that approximately 82% (w/w) of vernix is volatile. Analysis by Karl-Fischer titration revealed that the volatility is due exclusively to its high water content. Upon in vitro exposure to a dry ambient environment, a situation analogous to the transition that vernix encounters at birth, vernix slowly releases its water content (Fig. 1). Placement of completely desiccated vernix in normal saline or deionized water results in slow rehydration over a period of days, with greater rehydration rates following exposure to deionized water (Fig. 2).

Table 2 Amino Acid Composition of Vernix Caseosa[a]

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

[a] Native vernix was collected at birth from 20 newborns. Following lipid extraction, the specimens were acid hydrolyzed and analyzed by thin layer chromatography to determine the amino acid composition. The abundance of asparagine and glutamine residues is likely derived from the numerous fetal corneocytes found in vernix.

Source: Data adapted from Ref. 14.

III. MORPHOLOGY OF VERNIX

Phase-contrast and standard light microscopy reveal that vernix caseosa is a highly cellular material (Fig. 3). Agorastos et al. provided the first systematic characterization of the cells within vernix (5). 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 fetal cells can be distinguished from corneoctyes found in mature stratum corneum by the lack of desmosomal attachments (16). Ultrastructural analysis of vernix corneocytes by transmission electron microscopy shows a sparse network of keratin filaments with little evidence of tonofilament orientation (Fig. 4). The vernix corneocytes are approximately 1–2 μm thick and are surrounded by a layer of amorphous lipids lacking the typical lamellar architecture present in stratum corneum. This structural arrangement of corneocytes and lipids imparts a different architecture to vernix compared to the stratum corneum, which constitutes the human permeability barrier during postnatal life. The lack of a lamellar lipid matrix surrounding the fetal corneocytes as well as the infrequent appearance of intercorneocyte

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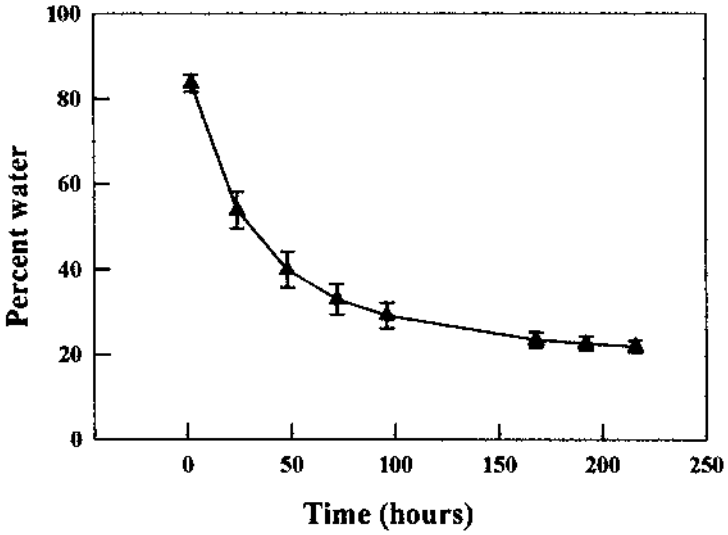

Figure 1 Dehydration kinetics of vernix caseosa. Freshly harvested vernix caseosa was collected from 10 newborn infants. A small aliquot (~ 50 mg) from each infant was placed under vacuum at room temperature for a period of 9 days. Weights were recorded daily. After the specimens attained constant weight, the percentage of water in the vernix was back-calculated for each weighing. Determination of the initial water content (82%) was later confirmed by Karl-Fischer titration analysis. Two 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.)

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.

Cryoscanning electron microscopy coupled with x-ray beam analysis was used to localize the high water content within freshly collected vernix caseosa (16). Figure 5a depicts a cryofractured corneocyte. In Figure 5b, the same corneocyte is examined by elemental analysis 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.

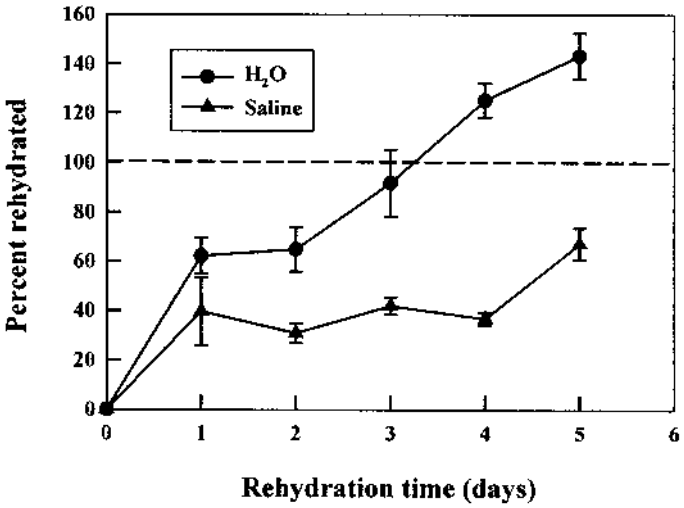

Figure 2 Rehydration kinetics of vernix caseosa. Following complete desiccation, vernix was rehydrated over a period of 5 days in either normal saline or deionized water. After 3 days, vernix immersed in deionized water was rehydrated to $92 \pm 14\%$ of its original weight, while vernix immersed in normal saline rehydrated to only $42 \pm 4\%$ over the same period. Over the 5-day test period, vernix continued to rehydrate to $143 \pm 9\%$ and $67 \pm 6\%$ of its original weight in deionized water and normal saline, respectively. These data illustrate the capacity of vernix to imbibe moisture over a protracted period of time. Data reported as mean $\pm$ SD. (From Ref. 16.)

IV. PHYSICAL PROPERTIES

Understanding of the biological functions of vernix caseosa requires characterization not only of the composition and morphology of vernix, but also of 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 fetus with the amniotic fluid. During the third trimester of pregnancy there is a progressive increase in the turbidity of the amniotic fluid surrounding the fetus (17). This turbidity has been assayed as an index of fetal 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 (18). These authors suggested that amniotic fluid turbidity resulted from increasing amounts of vernix within the amniotic fluid,

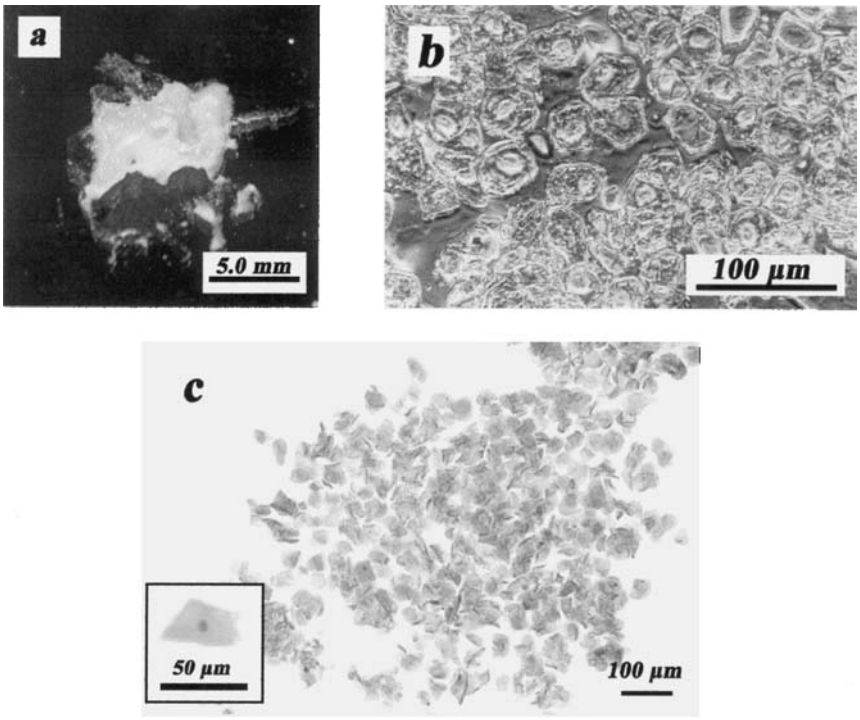

Figure 3 Fetal corneocytes in vernix caseosa. Macroscopically, vernix is a thick, viscous, white paste (a). The phase contrast image of native vernix (b) reveals a dense packing of fetal corneocytes surrounded by a lipid matrix. Many nuclear ghosts are evident. Extraction of vernix in chloroform-methanol followed by staining of the aqueous precipitate with hematoxylin and eosin shows many flat polygonal cells (c). A subset of the cells isolated from vernix by this method retains nuclear remnants. (From Ref. 16.)

although the mechanism underlying this progressive increase was unclear. In order 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 (19). Following incubation, the overlying solutions were spectrophotometrically analyzed. The exposure of vernix to phospholipids 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

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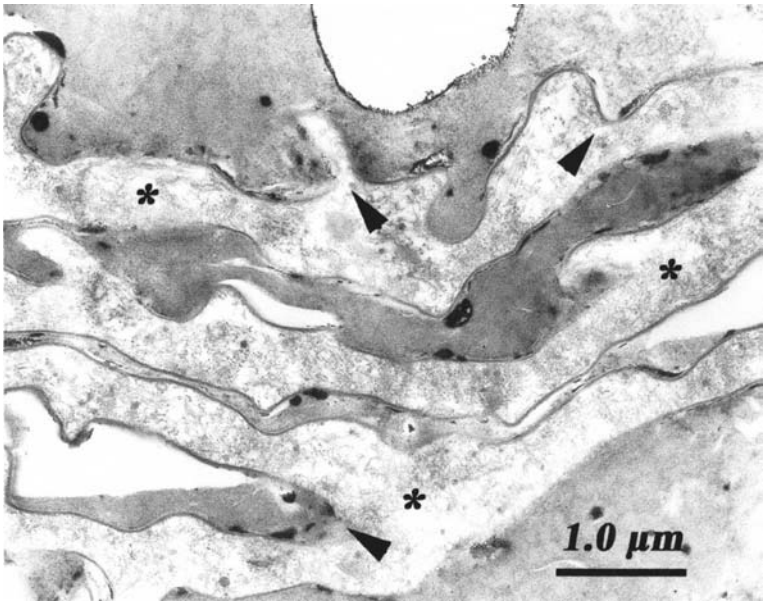

Figure 4 Transmission electron microscopy of vernix caseosa. This photomicrograph shows three cross-sectioned corneocytes depicted by asterisks within fresh vernix obtained at the time of delivery from the skin surface of a normal term infant. Note the sparse network of tonofilaments. The arrowheads show apparent sites of bifurcation and curvature of the cornified cell envelope indicative of malleability. No intercorneocyte desmosomal attachments are observed. The lipid matrix surrounding the corneocytes is generally nonlamellar. (From Ref. 16.)

(37°C) compared to 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 behavior of vernix caseosa. Tests performed in a controlled stress rheometer indicate that vernix rheological behavior is characterized by plastic flow with a decrease in viscosity occurring with increasing shear stress (shear thinning) and the presence of a yield value. The calculated yield value for vernix was 6.7×10^3 dyne/cm^2 at 25°C (Wael Youssef, Ph.D. thesis, University of Cincinnati, 2002). Vernix plastic rheological behavior shows clear temperature dependence over the range of 25–40°C with viscosity decreasing with increasing temperature. The addition of pulmonary surfactant to vernix markedly changes the rheological behavior of vernix with a precipitous drop in viscosity.

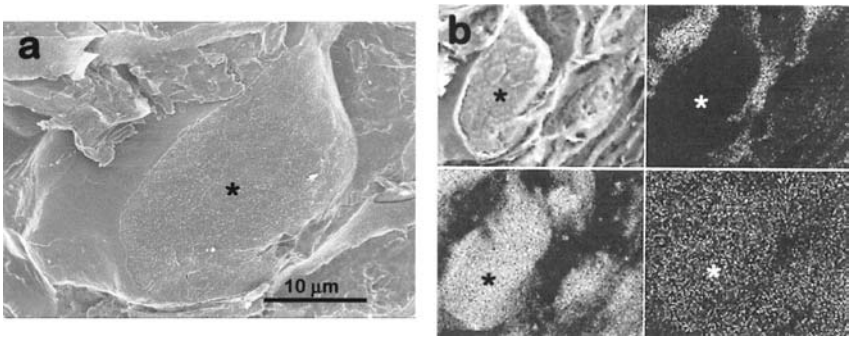

Figure 5 Cryoscanning electron microscopy and elemental analysis of vernix caseosa. These two photomicrographs depict vernix that was examined by cryoscanning electron microscopy and x-ray beam analysis. (a) The single lipid-covered corneocyte is partially fractured and reveals intermediate filaments within the cell. (b) This same corneocyte is imaged throughout the four panels with its position denoted by asterisks. X-ray maps for 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 sulfur and is used as a background element to check for x-ray absorption artifacts due to surface topography effects. (From Ref. 16.)

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 (82%). 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 (16). 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 (20). The critical surface tension of vernix was approximately 39 dyne/cm, indicating a relatively low energy surface comparable to that of petrolatum (35.8 dyne/cm). 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. 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 (nonpolar) and nondispersive (polar) components using Owens/Wendt analyses indicates that vernix is essentially nonpolar despite its very high water con-

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tent. 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 (21). The authors in that study found that the major part of the surface free energy of forehead skin was nonpolar, 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 fetus. 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 absence of a lipid lamellar architecture within vernix, however, suggests that water transport through this material may be markedly increased compared to native stratum corneum. Riesenfeld et al. studied the influence of vernix caseosa on water transport through semipermeable membranes under controlled environmental conditions (22). These authors reported that the evaporation rate over semipermeable membranes covered with vernix caseosa was approximately 50 $g/m^2/h$ at a relative humidity of 50%. This value is comparable to the evaporation rate of a premature infant at 27 weeks gestation on the first day after birth. In the in vitro experiment described, the evaporation rate fell exponentially over the first 3 hours to 10 $g/m^2/h$. In contrast, vernix spread on a semipermeable membrane maintained over a water reservoir exhibits a much slower decrease in evaporation rate over the 3-hour period, with values reaching 30 $g/m^2/h$. Transepidermal water loss in the term newborn infant is approximately 5 $g/m^2/h$ (23). These data indicate that vernix is much more vapor permeable than native stratum corneum, presumably secondary to the nonlamellar structure of the lipid matrix and the high water content of the embedded corneocytes.

Using a similar in vitro system, water vapor transport was studied through vernix films of controlled thickness as a function of temperature (Wael Youssef, Ph.D. thesis, University of Cincinnati, 2002). Vernix was contrasted with other topical emollients typically used on human skin. Figure 6 shows the results of an experiment in which films measuring 2 mg/cm^2 were layered over Gore-Tex® films and mounted tightly on water-filled plastic weighing boats. As shown, there is a marked temperature effect with increased water vapor transport occurring at higher environmental temperatures. Films of vernix decrease water vapor transport compared to Gore-Tex® alone in a similar manner to moisturizing creams such as Curel® that contain high levels of glycerin as a humectant. In contrast, petrolatum-based films such as Aquaphor® produce a nearly impermeable barrier to water vapor transport with values approximately 15× lower than films of vernix of comparable thickness. These findings 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

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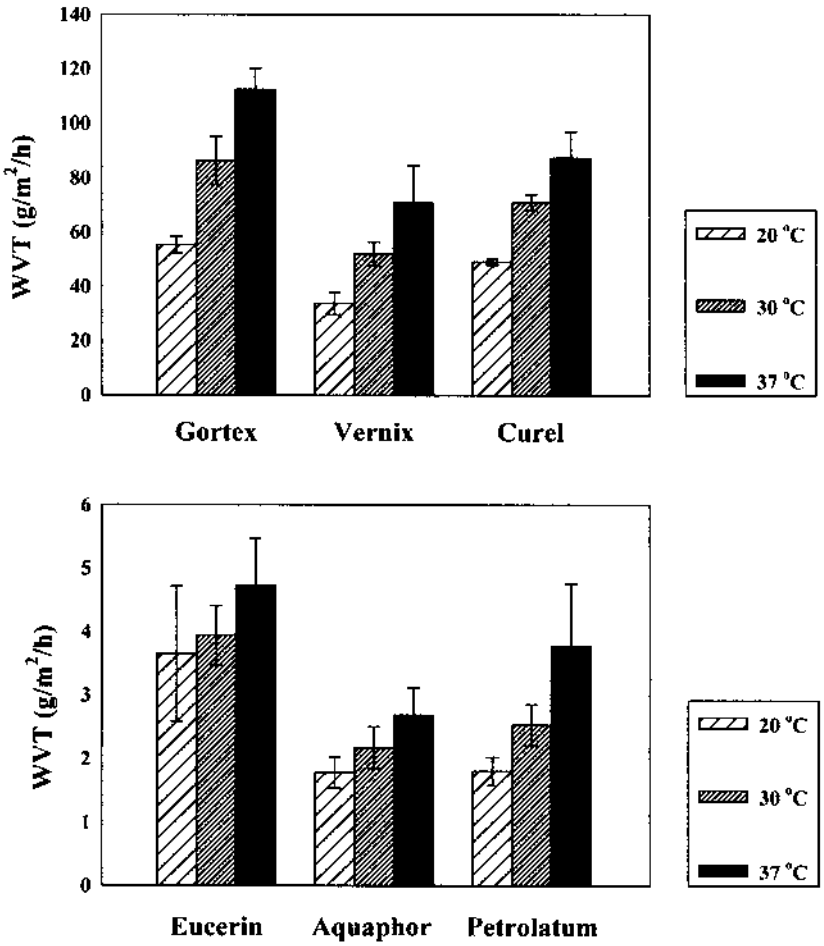

Figure 6 Effect of temperature on water vapor transport through Gore-Tex® membranes covered with vernix and other barrier creams. Gore-Tex® membranes were mounted over water-filled plastic boats. Vernix and other common barrier creams were applied with an Accugate® film applicator to a thickness of 2 mg/cm^2 and the boats placed in a controlled thermal environment. Water vapor transport rates were determined gravimetrically by weighing the boats at consecutive 6-hour intervals at ambient temperatures of 20°C, 30°C, and 37°C. All films produced significant reductions in water vapor transport compared to uncoated Gore-Tex®. Vernix, and Curel® produced similar reductions but were not as occlusive as the Eucerin®, Aquaphor®, and petrolatum. Results are reported as means ± SD for 6 separate samples. (From Wael Youssef, Ph.D. thesis, University of Cincinnati, 2002.)

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vapor permeability in the range of 20–60 $g/m^2/h$ are preferable to either no occlusion or full occlusion (24). These findings may be relevant to proposed uses of vernix as a prototypical wound-healing ointment.

V. BIOLOGICAL PROPERTIES

Understanding the biological functions of vernix caseosa must take into account the multifunctional needs of the organism both 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 (12,25). A similar mechanism may be responsible for initiating the rapid postnatal cornification observed in extremely low birthweight premature infants (26). Whether or not this mechanism can be ascribed to the intrauterine environment is unclear. 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 fetal skin leading to formation of a high impedance skin surface (27). Electrical isolation of the fetus in utero is presumably an important aspect of developing fetal 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 (28–30). Whether a similar influence of water flux on epidermal biology occurs in the fetus or newborn term or preterm 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 (unpublished data). These preliminary experiments, however, cannot dissociate direct effects of vernix on the transepidermal water gradient versus the possibility of a putative growth factor effect. Epidermal growth factor, for example, is present in high concentrations in urine and amniotic fluid (31). Its presence in vernix, therefore, would not be unexpected with possible secondary effects on adjacent cellular

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epithelia. The complex nature of vernix composition makes the ascription of biological effects to single causes more 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 (32). Surfactant protein D is a molecule present in tracheal fluid with responsibility for maintaining airway sterility (33). 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.

In addition to entry into an environment teeming with microorganisms, birth also marks a time of high oxidative stress. Recently, Thiele et al. reported that human skin exhibits antioxidant properties with high levels of alpha-tocopherol in human stratum corneum and sebum (34,35). Alpha-tocopherol has also been reported to be present in vernix (36). In contrast to term infants, very low birthweight preterm infants do not exhibit sebaceous gland hyperplasia and have 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. The increasing concentrations of pulmonary surfactant in amniotic fluid suggest a possible initiating mechanism for the induction of amniotic fluid turbidity in vivo (19). This mechanism is highly attractive as a means of ''cross talk'' between different epithelial surfaces during the third trimester of pregnancy (37). As illustrated in Fig. 7, vernix production results in excess vernix on the skin surface towards term. The developing lung secretes increasing concentrations of pulmonary surfactant into the amniotic fluid with possible effects to emulsify and detach superficial vernix. The fetus subsequently swallows the detached vernix 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 (38). 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 evapora-

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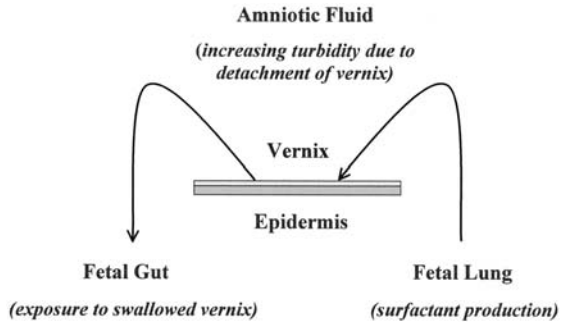

Figure 7 Proposed mechanism for surfactant-mediated vernix detachment. During the last trimester of gestation, the fetal lung produces and secretes increasing amounts of pulmonary surfactant into the amniotic fluid. Concomitantly, vernix on the skin surface builds up and detaches into the surrounding milieu, resulting in increasing amniotic fluid turbidity. Recent data indicate a role for pulmonary surfactant to emulsify vernix and aide in the detachment mechanism (19). Vernix within the amniotic fluid is subsequently swallowed by the fetus with potential effects on the fetal foregut and/or systemic absorption of vernix components. (Adapted from Ref. 37.)

tive heat loss compared to the surface of newborns in which vernix is left in situ (22).

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 (7). 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) (39). Scott et al. determined that NMF production is optimal at relative humidities over 90% (40). 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 (41). 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. Further studies need to be conducted to evaluate this possibility.

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Review of the older literature contains sporadic reports of vernix as a potential wound-healing ointment (42). Other reports describe mechanical barrier properties of vernix with respect to bacterial invasion (43). Direct anti-infective action or possible roles of vernix Ito impede or guide the bacterial colonization at birth are equivocal (14,44). Vernix on the fetal 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 birthweight infants. Recent studies evaluating the use of Aquaphor as a topical emollient have shown increased rates of nosocomial infection secondary to *Staphylococcus epidermidis* in extremely low birthweight infants (45). The application of physiological barrier creams based on vernix caseosa as a prototype have yet to be explored.

Finally, preliminary data indicate that vernix may function as an endogenous skin cleanser (46). In experiments performed using human skin soiled with fine carbon particles, vernix had comparable efficacy to standard commercial skin cleansers. 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.

VI. SUMMARY

The field of vernix biology and its investigation is literally in its infancy. The available data support a role for vernix in epidermal barrier maturation in utero with particular emphasis on the importance of the pilosebaceous unit in the initiation of barrier formation. The source of both the lipids and the corneocytes in vernix is potentially multifactorial, with the interfollicular stratum corneum and the pilosebaceous apparatus having contributory roles. At birth, the exigencies of terrestrial adaptation pose multiple challenges for the organism. Such challenges include regulation of body temperature, facilitation of bacterial colonization, moisturization of the skin surface, wound healing, prevention of fissuring, minimization of oxidative damage, and cleansing. The available data support the hypothesis that

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vernix has evolved as a multifunctional barrier film representing the ''best compromise'' solution to facilitate epidermal barrier adaptation at birth.

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Bathing the Term Newborn: Personal Cleanser Considerations

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The importance of infant cleansing to maintain good hygiene and skin health is well recognized; however, the pediatric and dermatological communities have not reached a consensus on what constitutes appropriate cleansing practice. For example, sponge bathing or wiping gently with a soft, moistened washcloth is often recommended for the term newborn until his or her umbilical cord drops off (1,2). Others suggest that immersion bathing can begin immediately after birth (3–5). Henningsson et al. (4) and Hylén et al. (5) compared cleansing by immersion bathing to wiping with a moistened washcloth following the first few days after birth. Both authors reported no impact of cleansing method on the rate of clinical infection or bacterial colonization but noted that infants cleansed by immersion bathing had less heat loss and cried less, i.e., immersion bathing increased the infants' comfort. Immersion bathing might also provide other benefits, e.g., better cleansing efficiency and increased stratum corneum hydration (6,7). Viewpoints also differ on the appropriate frequency for infant bathing. Recommendations for infrequent bathing remain common (7,8). However, several studies have shown that infants' skin will tolerate, and might benefit from, daily bathing (9–11). Finally, there is the question of whether to cleanse with water only or to use a personal cleansing product and, if the latter, which product to use.

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I. PHYSIOLOGICAL CONSIDERATIONS

The skin of term infants undergoes a number of changes and adaptations in the period following birth. For example, there are shifts in the skin surface pH and surface lipid composition as well as changes in the skin's electrical impedance (hydration) properties (12–16). The state of the stratum corneum barrier is of primary interest, as this barrier represents the primary impediment to the penetration of noxious species in both adults and children. Cetta et al. (17) reported that infants are exposed to a surprisingly large number of chemical species via the application of over-the-counter products; the average newborn is exposed to 8 different skin care products and 48 different environmental chemicals, hence the importance of a competent stratum corneum barrier. Infants' skin is generally thought to be more susceptible to irritant penetration and damage than is adult skin, and there is some evidence suggesting that infants have a lowered irritation threshold compared to adults (18). The belief that infants' skin is more delicate might also stem from a number of cases of infantile poisoning that followed topical application of various materials, though it seems that factors other than an incompetent stratum corneum barrier, such as a larger body surface area-to-body mass ratio, might account for these events (19).

A healthy term newborn's stratum corneum is structurally and functionally similar to that of an adult. Histologically, epidermal thickness and the number of cell layers in each epidermal compartment are comparable in adult and newborn skin, as are the cellular structure, number of cell layers, and thickness of the adult and newborn stratum corneum (20). Fairley and Rasmussen (21) also found no histological difference in stratum corneum thickness between infants (mean age 17.1 days), young children (mean age 3.9 years), and adults (age range 17–46 years).

Transepidermal water loss (TEWL) measurements provide a noninvasive assessment of stratum corneum barrier function, a higher TEWL value corresponding to a less efficient barrier. Elevated TEWL is also predictive of higher irritant susceptibility (22,23). Yosipovitch et al. (15) measured TEWL on 44 healthy newborn infants 5–10 hours after birth. Measurements were taken on five body sites and on the corresponding body sites of 20 adult volunteers (mean age 24 years). Mean TEWL values for the newborns were lower than the adult values at all body sites except the volar forearm. Cunico et al. (24) and Wilson and Maibach (25) also reported a lower TEWL in newborns than in adults. These same authors also reported no significant difference in the carbon dioxide emission rates measured in newborns and adults, providing further evidence of comparable stratum corneum barrier function (24,26). Giusti et al. (27) reported no difference in TEWL measured on the forearms and buttocks of a group of somewhat

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older infants, aged 8–24 months (mean age 14.3 months), and a group of women aged 25–35 years. Overall, these studies indicate comparable barrier function in infants and adults. However, this is not always observed in skin penetration studies. McCormack et al. (28) compared in vitro penetration of various compounds through full-term infant skin and adult skin and reported slightly higher penetration for ethanol in infant skin, but lower penetration for benzyl alcohol, decanol, and cetyl alcohol. Several fatty acid species were also examined, and, as with the alcohols, mixed results were obtained. Penetration of caprylic, lauric, and oleic acids was higher in infant skin than in adult skin, but penetration of stearic acid was slightly lower, though only very small amounts of these fatty acids penetrated either skin type. The authors proposed that the higher level of stratum corneum lipids found in infant skin might account for the greater penetration of lipophilic materials. While these in vitro penetration results indicate that infant and adult skin exhibit differential permeability to some compounds, the absence of a consistent trend showing that infant skin is more permeable than adult skin supports the presence of a competent stratum corneum barrier in the healthy, term newborn.

II. STUDIES EXAMINING THE POTENTIAL FOR CLEANSERS TO IMPACT INFANTS' SKIN

A number of reports in the literature describe beneficial effects when personal cleansing bars, even soap-based bars, are used as therapeutic adjuncts (29,30). However, personal cleansing products are more frequently implicated for their potential to negatively impact skin, either directly or by acting as triggering factors in disease conditions such as atopic dermatitis or rosacea (31–34).

Personal cleansing bars' potential to impact infants' skin has been a subject of study for many years. In 1937 Goldman (35), citing the vast amount of misinformation on the topic at the time, described work in which he examined the skin effects of seven commercial toilet soaps on a total of 127 children, including 82 newborns. These children had normal skin and no personal or family history of hay fever or asthma. Exposure involved patching 5 or 10% soap solutions on the flexor surfaces of the arm or forearm for 24 to 48 hours or swabbing the skin with 10% soap solutions for up to 8 days. The latter procedure involved wiping the medial aspect of the arm with a large cotton applicator dipped into a soap solution and allowing the arm to air-dry. Goldman reported no gross changes resulting from the soap swabbings. Other than a severe reaction to one product containing cresol, which was apparently not unexpected given other infor-

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mation available at the time, there was a low incidence of patch reactions even among the newborns. In fact, the author noted that newborns reacted much less frequently than older infants and children. Despite the low incidence of reactions, Goldman recognized a potential for individuals with a contact dermatitis to react more readily to a cleansing product. However, he attributed this to other materials that might be present in the bar rather than to soap itself.

In 1956 Swanson (36) reported results from a study in which a synthetic detergent bar was used by a group of 200 patients, aged between 1 and 79 years, who were selected on the basis of having soap-intolerant dermatoses. Thirty-one of these patients were aged from 1 to 4 years. The patients were divided into three treatment groups based on the expected tolerance of their dermatoses to soap: intolerant (group I), sometimes intolerant (group II), and usually tolerant and often benefiting (group III). Details of product usage are not given, but patients presumably used the bar daily for a period of several months. Tolerance to the synthetic detergent bar was rated ''good'' in 86% of the patients overall and in 87% of the patients aged 1– 4 years. In terms of treatment groups, tolerance to the synthetic detergent bar was rated 'good' in 83% of group I patients, 85% of group II patients, and 97.5% of group III patients. No age effect was found for tolerance. Swanson observed that the test bar was better tolerated by the patients in the study than common toilet soap and inferred that the difference was due to a pH difference between the lather from the synthetic detergent bar (pH 7) and common toilet soap (pH 10).

Peck et al. (37) conducted a cooperative study involving several hospital sites among a total of 568 children ranging from premature to 10 years of age to assess tolerance to bathing with a synthetic detergent bar. Most of these patients were observed under hospital conditions for a minimum of 4 weeks. Bathing frequency is not specified, but presumably the patients were bathed daily. Of the 141 patients enrolled who had soap intolerance, only 2 did not tolerate the synthetic detergent cleansing bar. All of the remaining patients who had normal skin or some form of dermatitis tolerated the product. The authors emphasize the lower pH of the synthetic detergent bar compared to regular soap in their discussion of possible reasons for the observed high tolerance to the synthetic detergent bar.

Peck et al. (9) conducted a follow-up crossover study to compare the skin effects from washing with a synthetic detergent bar or a soap bar. Three hundred children aged 10 weeks to 4 years (mean age 25 months) were enrolled; 211 children completed the study. Children with dermatitic skin were not specifically enrolled, but product assignments were balanced for incoming skin condition. Usage consisted of cleansing daily with one pro-

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duct for 4 weeks, switching to the other product for 4 weeks, then returning to the original product for 4 weeks. Dermatological evaluations were conducted weekly. Skin condition was classified as uninvolved, slight, moderate, or severe on the basis of evidence of dryness, scaling, erythema, or pustules. Eighty-four percent of the children had some skin condition during the course of the study. The authors did not attribute this to either cleansing product as they noted that over a 3-month period most children's skin will show some deviation from the normal for a variety of reasons. The results, expressed in terms of the frequency per 1000 incidences, showed that the soap bar yielded a lower number of uninvolved or mild observations than synthetic detergent bar (817 vs. 904), but a higher number of moderate (176 vs. 93) and severe (14 vs. 2) observations. The authors proposed removal of superficial skin lipids and liberation of free fatty acids from soap adsorbed on skin as a probable mechanism for the higher incidence of moderate and severe reactions observed for the soap bar, although a role for cleanser pH was also inferred.

In 1967 Ellickson and Jungermann (38) reported results from an 8-week double-blind crossover study that compared the skin effects of two soap bars in 101 children up to age 14 months (mean age 5 months, median age 4 months). The study was conducted in a hospital setting with nurses washing or bathing each child with one of the two test soap bars during each of the two 4-week phases of the study. Washing frequency is not specified, but was presumably conducted daily. The children's skin and the hands of nurses who conducted the bathing were evaluated weekly during treatment and 2 weeks after the end of the 8-week treatment period. Thirty-eight children completed the full course of treatment; the remaining children were discharged from the hospital at some point during their participation. The mean time completed in the study was 5.3 weeks (median 6.0 weeks). The authors reported no evidence of primary irritation, contact dermatitis, or allergic response in any of the children during the test period or at the final evaluation. These conditions were also not observed on the hands of any of the wash nurses, who presumably had a much higher exposure to the test soaps than the subjects.

Kadar and Osbourn (10) compared the skin effects of two soap bars, one of which contained the antibacterial ingredient triclocarban. One hundred and five children ranging in age from 3 months to 3 years were recruited. The 8-week study employed a crossover design. Children were bathed daily with one of the test bars for 4 weeks and then switched to the other test product for the next 4 weeks. The children's skin condition was evaluated weekly. The authors found no difference in the mildness of the two soap bars used in the study and reported no evidence of irritation that could be attributed to either soap.

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Ertel et al. (11) examined tolerance to daily bathing in two ad lib usage studies. One of these studies was conducted among a group of normal newborns aged up to 8 weeks ($n = 53$, mean age at enrollment 34 days). The other study was conducted among a group of young children aged 6 to 36 months ($n = 54$; mean age at enrollment 19 months) with mild to moderate atopic dermatitis. Children using high-potency topical corticosteroids or systemic therapies other than antihistamines for their atopy were excluded from the latter study. Diaper wipes, a nonmedicated moisturizing cream, and terry washcloths were provided in both studies. Parents bathed their child daily with an unscented synthetic detergent bar for a period of 3 weeks. Erythema, induration, and dryness on children's arms, trunk, and legs were scored weekly by the dermatologist investigator, as was the percent area affected on each body site. Tolerance was judged after 3 weeks. Moisturizer use was tracked by diary and by weighing the moisturizer containers at baseline and at study end. Daily bathing with the synthetic detergent bar was well tolerated by the 106 children who completed these studies. One child with atopy was withdrawn for reasons unrelated to the study. Mean total body scores for the scored endpoints decreased over the course of treatment, as did the mean total percent area affected. There was no significant relationship between the change in any of the clinical endpoints and the amount of moisturizer applied to a child.

These examples, which encompass a range of subject populations and cleansing bar compositions, suggest that, as is true in adult populations, most infants and young children will not experience untoward effects from normal use of most personal cleansing products. However, this does not mean that some children will not have issues with certain types of cleansers or that personal cleansing products do not have a potential to negatively impact skin. In adults, controlled arm wash methods based on consumer washing habits and practices are frequently used to dimension the potential for a personal cleansing product to negatively impact skin (39,40). Bathing infants or young children often involves sitting the child in a bathinette or tub of water that quickly becomes a warm surfactant solution in contact with the skin if a cleansing product is used. In this situation a different controlled exposure model, a mini-immersion model, might be a more appropriate predictor of cleansers' potential to negatively impact skin (41). This method involves exposing the volar forearm to warm 5% or 10% aqueous solutions prepared from bar or liquid cleansers. These concentrations encompass reported in-use concentrations of personal cleansing products (42). Healthy adult females are used as subjects. All procedures are conducted in a controlled environment room, and subjects remain in this room throughout the study. Three application sites are marked on each forearm surface corresponding to cutouts in a foam block. Chambers to

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hold the test solutions are fitted into the cutouts and the blocks are positioned on subjects' forearms and held in place with Velcro™ straps. A measured volume of warm test solution is transferred to the appropriate chamber through a hole in its top, which is then stoppered with a foam plug. After 15 minutes the test solutions are emptied from the chambers, the foam blocks are removed from the arms, and the application sites are rinsed with tap water and patted dry. This procedure is repeated four times at 1-hour intervals. Skin effects are evaluated by measuring skin capacitance at baseline and 40 minutes after each exposure. Our experience is that the mini-immersion exposure level and duration are not sufficient to produce visible changes in skin or alter barrier function (TEWL), but differences in treatments' potential to dry skin are apparent after a single 15-minute exposure (Fig. 1). Experience also shows that the trends shown by mini-immersion often predict clinically observable changes in wash tests. Wilhelm et al. have also reported that skin capacitance changes following patch exposure correlate with in vivo irritation (43). Thus, the mini-immersion method demonstrates the potential effect different personal cleansing products might have on infants' skin during bathing. A number of factors, such as the presence of

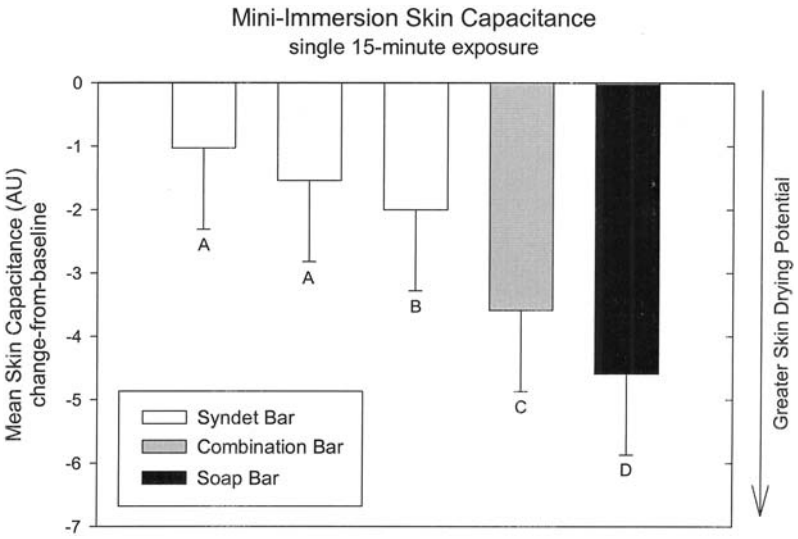

Figure 1 Skin capacitance change after a single, 15-minute exposure to 10% solutions of cleansing bars based on different surfactant systems. A larger capacitance decrease corresponds to a greater skin-drying potential. Bar length represents the mean value, error bars represent the standard deviation. Letters below the bars indicate whether results are significantly different at $p \leq 0.05$.

predisposing skin disease, will determine if and to what extent these changes will manifest clinically.

III. PERSONAL CLEANSER INTERACTIONS WITH SKIN

Personal cleansing products are often referred to simply as "soap," a practice that is undoubtedly a holdover from the days when true soap-based bars were the only cleansers available. This descriptor belies the composition and complexity of most cleansing products currently in the market. For example, other surfactants have replaced soap and products frequently employ mixtures of surfactants, namely, a primary surfactant that determines a product's primary characteristics and one or more secondary surfactants to modify some performance attribute or improve skin compatibility. The range of personal cleanser product forms available to consumers has also been extended to provide improved performance and enhanced skin benefits. However, personal cleansing bars remain the predominant product form in the market. Because of their historical and continued significance, and because their effects on adult and infant skin has been the subject of considerable scientific study, much of what follows focuses on cleansing bars. However, these same principles also apply to newer product forms such as liquid hand soaps, body washes, and disposable cleansing cloths.

IV. SURFACTANT COMPOSITION

Soap is the prototypical surfactant and is chemically the alkali salt of a fatty acid. The regulatory definition of soap is actually quite narrow, and only a few true soaps remain in the United States market (44,45). The fatty acid raw material used in soap manufacture is often a mixture derived from tallow and coconut oils, although starting materials derived from sources such as palm oil, palm kernel oil, and palm stearin have become more common in recent years (46,47). These raw materials contain fatty acids of various chain lengths, usually C_8–C_{22}, with both saturated and unsaturated species present. Thus, saponification yields a material containing a variety of soap species.

The composition of the finished soap determines its biological impact. Dahlgren et al. (48) used a series of soap bars prepared with different relative amounts of tallowate and cocoate soaps to demonstrate that the level of dryness and erythema following controlled washing is dependent on the ratio of these soap species. Studies conducted with pure fatty acids demonstrate a more fundamental relationship. For example, Kellum (49) reported

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that the most prominent irritation reactions developed when saturated fatty acids with even chain lengths from C_8 to C_{12} are patched on skin. Stillman et al. (50) reported similar results for a series of saturated fatty acids that included species with odd-numbered chain lengths. Dugard and Scheuplein (51), working with C_8–C_{16} homologues of the sodium salts of primary aliphatic acids, sodium *n*-alkyl sulfates and *n*-alkylamine hydrochlorides, showed maximal increases in epidermal membrane permeability upon exposure to the C_{12} and C_{14} members in each series. These studies show that soap composition, in particular the fatty acid chain length distribution of the soap species, is an important determinant of soaps' skin compatibility. Using tailored mixtures of fatty acid starting material in which longer chain length species predominate is one approach that has been used to effectively improve the skin compatibility of soap bars (52–54).

The majority of personal cleansing products currently in the market are based wholly or in large part on synthetic detergents or syndets, i.e., nonsoap surfactants. Synthetic detergents were developed during World War II in response to a shortage of the fatty acid raw materials needed to make soap and the need for cleansers that performed well under a wide variety of usage conditions (46,55). Examples of synthetic detergents that are commonly found in personal cleansing products include cocoyl isethionate, alkyl sulfates, and betaines. Synthetic detergents vary not only in terms of their charge (soap is an anionic surfactant) and performance characteristics but also in terms of their effect on skin. Cocoyl isethionate, which is found in many beauty bars, is an anionic surfactant. It has excellent skin compatibility and good lime soap, i.e., calcium soap, dispersant properties. Alkyl sulfates are also anionic surfactants and have a marked potential to irritate skin. For this reason alkyl sulfates are more often used in liquid cleanser formulas than in bars. The high irritant potential of alkyl sulfates makes them popular as model irritants, for example, sodium lauryl sulfate. Alkyl sulfates have good foam-forming properties and produce creamy lather but do not perform well in hard water. Betaines are amphoteric; their charge depends on the pH of their environment. They generally have good skin compatibility and will reduce the irritancy of anionic surfactants when combined with them. Betaines have good lather characteristics and can be used to increase the viscosity of liquid formulas. Several instances of contact allergy have been reported to cocamidopropyl betaine, one of the most commonly used betaines in cleansers and other personal care items (56). This issue might be product specific, as there is some evidence that the response is due to impurities rather than to the surfactant itself (57,58).

Synthetic detergents' skin compatibility exhibits a structural dependence similar to that discussed previously for soap. For example, Kligman

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and Wooding (59) reported a minimum ID_{50}, the concentration needed to produce a discernible irritant reaction in 50% of the population in 24 hours, for the C_{12} homologue in a series of alkyl sulfates applied under patch. An identical pattern was found for the IT_{50}, the estimated number of days of continuous exposure to produce a threshold reaction in 50% of the population. Robbins and Fernee (60) reported a maximum in the swelling behavior of epidermal membrane, a parameter reported to parallel anionic surfactants' ability to elicit erythema in vivo, for the C_{12} homologue in a series of alkyl sulfates. Rhein et al. (61) examined a variety of surfactants and showed that these materials produced a range of epidermal membrane swelling responses. Again, a maximal swelling response was obtained for the C_{12} or C_{14} homologues of alpha olefin sulfonates, paraffin sulfonates, linear alkyl benzene suflonates, and alkyl sulfates. These authors also demonstrated that interactions between surfactant species could reduce the membrane swelling response. As mentioned previously, secondary surfactants are sometimes used in personal cleansing products to improve skin compatibility. They also noted a surfactant counterion effect, reporting that the magnesium and triethanolamine (TEA) salts of surfactant molecules induced significantly less membrane swelling than their sodium counterparts. Imokawa and Takeuchi (62) assessed the skin-roughening potential of soaps with carbon chain lengths ranging from C_8 to C_{14} and of various synthetic surfactants. Their work again showed a maximum response for C_{12} soap and the C_{12} homologues of a number of the other surfactants examined. Wilhelm et al. (43) conducted in vivo work showing maximal increases in skin surface water loss, transepidermal water loss, and skin color reflectance for the C_{12} homologue in a series of alkyl sulfates. And as noted previously, Dugard and Schueplein (51) found that the C_{12} and C_{14} homologues of *n*-alkyl sulfates or *n*-alkylamine hydrochlorides produced maximal increases in epidermal permeability.

The above examples illustrate a common feature that reduces surfactant skin compatibility, namely, a chain length of approximately C_{12}. This suggests that one way to improve skin compatibility of synthetic detergent–based products is to reduce the amount of short-chained surfactant species they contain, analogous to the soap bar example given earlier. Skin compatibility might also be improved by modifying the surfactant molecule. For example, Rhein et al. (61) observed a reduction in stratum corneum swelling produced by C_{12}–C_{14} alkyl ethoxy sulfates when the degree of ethoxylation was increased. Finally, skin compatibility can be improved by combining different surfactants, e.g., adding a betaine surfactant to a cleanser that contains a primary anionic surfactant.

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V. DELIPIDIZATION

Epidermal lipids play a key role in maintaining the skin's barrier integrity and health (63–66). Populations that exhibit heightened sensitivity and reactivity to cleansing products, such as individuals with atopic dermatitis, tend to exhibit aberrant epidermal lipid composition and structure (67,68). Di Nardo et al. (69) reported an inverse relationship between surfactant reactivity and levels of certain stratum corneum ceramides in normals. Findings such as these, coupled with surfactants' natural ability to emulsify oils and lipids, have prompted hypotheses that surfactants' negative impact on skin results from delipidization or selective removal of lipid components from the stratum corneum.

Morganti (70) reported that washing the skin with water decreased surface lipids by about 24%, while washing with soap reduced surface lipids by about 35%. Surprisingly, washing with a synthetic detergent bar reduced surface lipids by an even greater amount, about 53%. Lipid removal was linked to a decreased ability of the skin to retain natural moisturizing factors and, ultimately, to dry skin. Imokawa et al. (71) reported that the stratum corneum lipid lamellar structure of forearm skin was disrupted following a 30-minute exposure to 5% aqueous sodium dodecyl sulfate. Lipid analysis after the exposure showed a selective loss of various components including cholesterol, cholesterol ester, free fatty acids and sphingolipid. The authors noted that surfactant exposure produced an enduring chapped, scaly appearance and reduced the hydration level. Recovery studies conducted by applying isolated lipid fractions to surfactant treated skin suggested a role for sphingolipids in helping to restore the skin's ability to retain water. Gfatter et al. (72) examined the effect of washing on skin surface fat content in a group of infants aged 2 weeks to 16 months (mean age 3.2 months). Treatment consisted of a 1-minute wash performed on each child's chest and buttock with tap water (control), a synthetic detergent liquid, a synthetic detergent bar, or a soap bar. Skin surface fat content and several other parameters were measured 10 minutes after washing. All of the washes removed a significant amount of skin surface fat. Not unexpectedly the least removal was observed for the control group ($-0.93\ \mu g/cm^2$), the greatest removal for the soap bar group ($-4.81\ \mu g/cm^2$). The authors concluded that removal of surface lipid might reduce stratum corneum hydration and lead to dryness and scaling.

Rawlings et al. (73) examined lipid structure and composition in the normal skin of adult females and in xerotic skin induced by exaggerated soap washing. Xerotic skin samples were obtained by tape stripping the backs of subjects' hands following one week of three times daily washing with soap; normal skin samples were obtained similarly from a control

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group of subjects. The authors noted an apparent perturbation of desmosomal degradation, with intact desmosomes persisting to higher stratum corneum levels in soap-treated skin. The lipid bilayer structure in the outer stratum corneum was degraded in both skin types, but the normal and soap-treated structures had a different appearance. The authors found a decreased stratum corneum ceramide content in soap-treated skin, with a progressive, deeper loss accompanying more severe dry skin grades. However, the relative levels of the various ceramide species were not different in the two skin types. The authors concluded that alterations in stratum cornuem lipid composition and organization, along with reduced desmosomal degradation, are responsible for the scaling that can accompany soap washing.

Fulmer and Kramer (74) also compared lipid content in normal and surfactant-induced dry skin in a paired, dry leg study. Subjects were randomly assigned to wash one leg three times daily with a 4% sodium dodecyl sulfate solution for a period of 2 weeks with the other leg remaining untreated as a control. Clinical evaluations were made on each leg, and 8 mm shave biopsies were taken for lipid analysis. In contrast to the results reported by Rawlings, these authors found no alteration in the total amount of lipid per gram of stratum corneum protein as a result of surfactant washing. In particular, the total ceramide level was not changed. However, ceramide, cholesterol, and free fatty acid profiles were altered in the surfactant-treated skin compared to control. The authors concluded that surfactant washing alters the quality, but not the quantity, of stratum corneum lipids, suggesting that surfactants' role in the dry skin process is related to perturbation of the stratum corneum formation process, not lipid extraction.

Dugard and Scheuplein (51), in their in vitro work with human epidermal membranes, found that the surfactant-induced membrane permeability changes were reversible, suggesting that extraction of lipids or other epidermal components by the surfactants is not the sole mechanism responsible for the increased membrane permeability. Rhein et al. (61) also reported that the swelling response of stratum corneum exposed to surfactant solutions was reversible, again suggesting a limited role for lipid extraction in surfactant interactions with skin. Froebe et al. (75) examined in vitro stratum corneum lipid removal by sodium lauryl sulfate and linear alkyl benzene sulfonate. Both materials were found to remove detectable levels of lipid only above their critical micelle concentration, demonstrating that lipid removal is a micellar phenomenon. The primary lipid species involved were cholesterol and free fatty acids; little or no ceramide was extracted. Even at the highest surfactant concentrations used (2%), the amount of lipid material removed from the skin represented less than 7% of the total stratum

corneum lipid, indicating that delipidization, or at least the removal of sizable amounts of stratum corneum lipid, is not a primary mechanism for surfactant irritation.

VI. SURFACTANT BINDING TO STRATUM CORNEUM

These latter studies suggest that a mechanism other than surfactant interaction with or removal of stratum corneum lipids governs cleansers' skin effects. Imokawa et al. (62,76) used a dye-displacement technique to examine surfactant adsorption on skin and reported that the skin-roughening effects of surfactants are related to their ability to adsorb onto skin. These authors proposed that surfactant remaining adsorbed to skin after rinsing denatures keratin, leading to skin roughness. Kawai and Imokawa (77) later extended this proposal to include the induction of skin tightness. In this case lipid removal from skin was related to the sensation of tightness. However, delipidization of the skin with ether did not result in marked tightness, and surfactants' ability to remove lipids did not always parallel their potential to induce tightness. The authors reported a strong correlation between surfactant adsorption and tightness and showed that removal of skin surface lipids enhanced tightness from subsequent surfactant exposure. Based on these results, the authors proposed a model in which extraction of stratum corneum lipids by surfactant is a necessary, but not sufficient, step in the induction of skin tightness. Ananthapadmanabhan et al. (78) examined the binding of a range of surfactants to isolated guinea pig and human stratum corneum and reported that the extent of surfactant binding correlated well with the irritation potential predicted by in vitro and in vivo methods. Rhein et al. (61) noted a time-dependent effect on stratum corneum swelling for sodium lauryl sulfate, which was ascribed to the interaction of surfactant with keratin and disruption of the keratin's secondary and tertiary structure. The reversibility of surfactant effects on swelling was taken as further evidence of this interpretation. Mukherjee et al. (79) examined the interaction of pure anionic surfactants and cleansing bars based on anionic surfactants with isolated stratum corneum in vitro by measuring displacement of 1-anilinonaphthalene-8-sulfonic acid (ANS), a fluorescent probe known to bind to stratum corneum proteins. Their results showed agreement between surfactants' ability to displace ANS from stratum corneum samples and their potential to irritate skin as predicted by in vitro and in vivo methods, suggesting that surfactants' propensity for binding to proteins in the stratum corneum determines their in-use skin compatibility. One caution, however, is that predictions of cleansers' in-use skin compatibility

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are often method dependent and overly exaggerated exposure protocols do not always predict consumer experience with personal care products (42,80).

Hard water can impact dermatological conditions. For example, it is identified as a potential risk factor for atopic eczema development in children (81). The role that water hardness might play in mediating surfactant-skin interactions is often overlooked. Water hardness is determined by dissolved mineral content, primarily dissolved calcium and magnesium species. It is responsible for the formation of soap scum, insoluble calcium or magnesium soap species, on surfaces. Water hardness affects the skin compatibility of personal cleansing products, and the skin compatibility of soap-based cleansers is more affected by water hardness than that of products based on synthetic detergents (82). Warren et al. (83) examined the relationship between water hardness and the skin compatibility of personal cleansing products at a more fundamental level. These authors reported results from controlled arm wash experiments conducted with three cleansing bars: a synthetic detergent bar, a TEA soap bar, and a sodium soap bar. The treatment process was divided into two phases, a wash phase and a rinse phase, which were conducted with various combinations of deionized water (0 grains of calcium per gallon) and water treated to contain 11 grains of calcium per gallon. There are two notable results from this work (Figs. 2 and 3). First, while the dryness and erythema differences between the bars appear as expected when calcium is present in the water, these differences become negligible in deionized water ($p \geq 0.48$ for inter-product dryness and erythema comparisons). Second, the rinse step appears particularly important in determining the cleansers' impact on skin, an outcome attributed to the presence of excess calcium ion during rinsing. Calcium soap deposition on skin measured by FTIR after washing with different wash water/rinse water hardness combinations showed similar trends. Although the specific interaction between the calcium soap and skin was not examined, this work demonstrates that deposition of calcium soap species on skin is an important determinant of soap's skin compatibility.

VII. CLEANSER PH EFFECTS

Soap is neutral unless it is formulated with excess alkali or free fatty acid (84). Solubilized soap will react with water to form free fatty acid and strong base, e.g., sodium soap will react with water to produce small quantities of free fatty acid and sodium hydroxide. As a result, soap-based cleansing bars often exhibit a higher lather pH than products based on synthetic detergents, a point that is frequently used to advocate bars based on synthetic detergents over those based on soap even though some evidence suggests

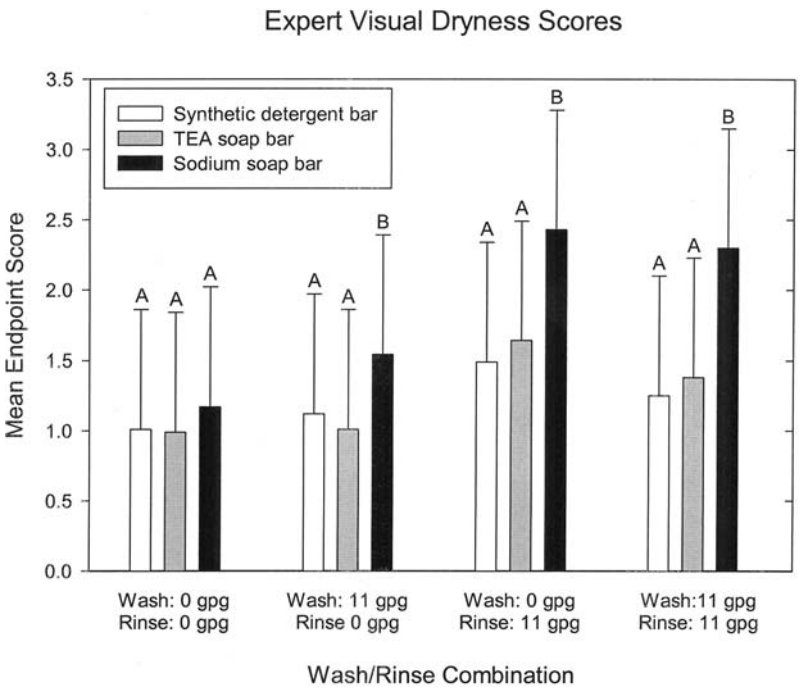

Figure 2 Endpoint dryness scores from a study examining the impact of water hardness on different cleansing bar compositions ($n = 25$). Bar length represents the mean value, error bars represent the standard deviation. Letters above the bars indicate whether results are significantly different at $p \leq 0.05$. (From Ref. 83.)

that synthetic surfactants might penetrate the skin more deeply and be more efficient at removing pH-stabilizing substances from the skin's surface than soap (85). For example, the authors of several of the previously cited infant bathing studies infer that cleanser pH is a key determinant of skin compatibility (9,36,37). Specifically, the enhanced tolerance to a synthetic detergent bar is attributed to the fact that it yields lather with a more neutral pH than soap-based bars.

At a fundamental level, Ananthapadmanabhan et al. (78) reported a pH dependence for sodium lauroyl isethionate adsorption to skin, showing a minimum from pH 7 to pH 9, suggesting that pH might play a role in determining surfactant-skin interactions. However, van Scott and Lyon (86) examined the potential for tap water with pH adjusted from 4.5 to 10.5 or 1% solutions of various soap and detergent products to denature keratin. Water had no effect on the denaturation of defatted keratin or keratin plus 1% sebum over the pH range studied. Similarly, there was no

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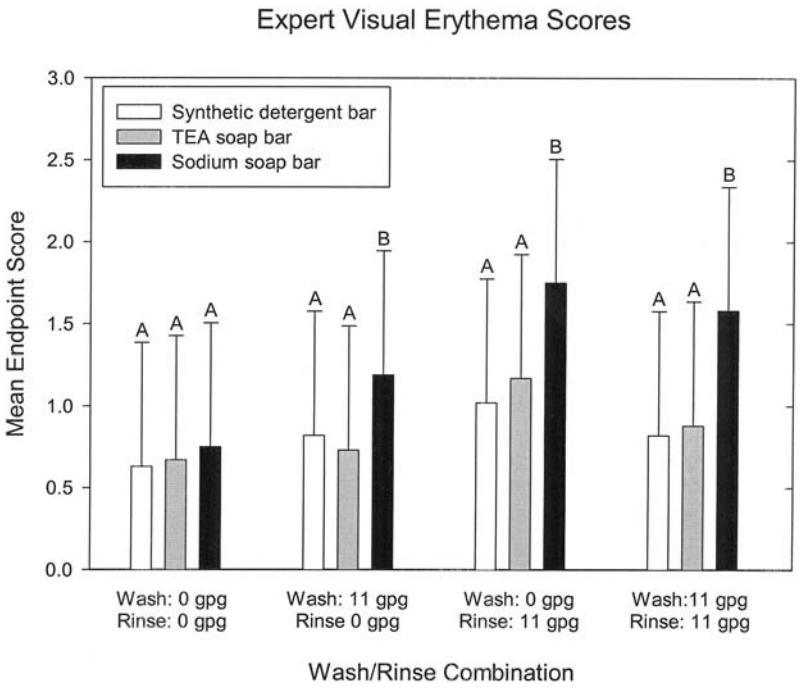

Figure 3 Endpoint erythema scores from a study examining the impact of water hardness on different cleansing bar compositions ($n = 25$). Bar length represents the mean value, error bars represent the standard deviation. Letters above the bars indicate whether results are significantly different at $p \leq 0.05$. (From Ref. 83.)

significant relationship between product pH, which ranged from 6.7 to 10.1, and denaturation of any of the keratins studied. Robbins and Fernee (60) reported no significant in vitro swelling change when stratum corneum was exposed to water with pH values adjusted to between 3 and 9. They also examined the effect of pH on stratum corneum swelling response using three different surfactants: sodium lauryl sulfate (SLS), dodecyl benzene sulfonate (LAS), or dodecyl trimethyl ammonium bromide (DTAB). The first two surfactants are anionic; the latter surfactant is cationic. Lowering the pH from 9 to 3 reduced swelling response. However, the swelling response was unchanged or increased when the pH was lowered from 9 to 6, a range that is relevant to many bar cleansers. Similarly, Dugard and Schueplein (51) observed that buffer in the pH range 3.0–9.5 produced no increase in stratum corneum permeability in the absence of surfactant. These authors also found no change in the rate of permeability increase as a function of pH for the three surfactants studied: sodium dodecanoate (pH range 7.5–9.5),

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sodium dodecyl sulfate (pH range 5.0–9.0), and sodium dodecylamine hydrochloride (pH range 3.0–7.5). These latter studies suggest that pH over the range encompassed by many personal cleansing products has limited impact on surfactants' compatibility with skin.

Similar behavior is observed for fully formulated cleansing products. Bettley and Donoghue (87) reported patch test results comparing a toilet soap and a triethanolamine (TEA) soap. The TEA soap was less irritating than the toilet soap, although the solutions prepared from each product had a similar pH. As mentioned previously, Rhein et al. (61) also reported a reduced potential for skin irritation from TEA salts of surfactants. Frosch (42) reported the relative skin irritation potential determined using a soap chamber test for 23 cleansing bars marketed in the United States and Germany. These products covered a pH range from 5.4 to 10.7 and represented a range of surfactant compositions. There is a good negative correlation between the reported erythema scores and cleanser pH values, i.e., for the 23 products tested the trend is for the erythema score to decrease as cleanser pH increases. The correlation between the reported scaling scores and cleanser pH values is not significant, but the trend is again negative. Van der Valk et al. (88) conducted a similar experiment and assessed the skin compatibility of 13 marketed personal cleansers. The impact of 2% aqueous solutions of the products applied to subjects' volar forearms on stratum corneum barrier function was assessed by evaporimetry. All of the cleansers significantly increased TEWL compared to control, but the results showed no correlation between cleanser pH and irritation, as indicated by TEWL. Van Ketel et al. (89) examined the irritation potential of several liquid hand cleansers by applying 8% aqueous solutions of each product under patch. The results again showed no correlation between a product's pH and its potential to irritate skin. While these patch studies were all conducted among adult populations, it seems reasonable to assume, given the highly exaggerated exposure conditions used, that they also predict the likelihood that pH alone is a determinant of a personal cleanser's potential to irritate healthy infants' skin in normal use.

Taken together, these in vivo and in vitro results indicate that the skin irritation potential of a personal cleansing product is driven by differences in the chemical and physical properties of its surfactants, not by its pH. However, personal cleansing products might induce other changes in skin condition that could be important in infants. In adults the skin's surface is normally slightly acidic, giving rise to the concept of the so-called acid mantle. Healthy adult skin exhibits a very good ability to recover from pH changes even when challenged with alkaline solutions having pH $>$ 13 (90). At birth, term infants' skin has a higher surface pH than adult skin, but this pH differential disappears over a period of days or several

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weeks (7,14,16), although some have reported a significantly higher skin surface pH in infants as old as 8–24 months (27). Literature indicates that personal cleansing products can transiently affect the skin surface pH in both adults and infants. As mentioned previously, Gfatter et al. (72) examined the effect of washing infants' skin with synthetic detergent–and soap-based cleansing products. Washes were conducted with water (pH 7.9–8.2), a synthetic detergent bar (pH 5.5), a liquid synthetic detergent cleanser (pH 5.5), or a soap bar (pH 9.5). Skin surface pH measurements were made 10 minutes after washing. All washes increased the skin surface pH, with the control producing the smallest increase (+0.20 units). Both synthetic detergent cleansers increased the skin surface pH by +0.29 units, significantly greater than the control. The soap produced the greatest skin surface pH increase, +0.45 units. This increase was significantly greater than that produced by the control or the synthetic detergent cleansers.

Changes in the skin surface pH resulting from washing with personal cleansing products can persist for longer periods. Bechor et al. (91) examined the time course of skin surface pH following washing. Adult volunteers washed their faces for 30 seconds with one of 41 cleansing products covering the surfactant composition range from soap to synthetic. The skin surface pH was measured at defined times for up to 200 minutes after washing. The results from this study, summarized in Table 1, show that cleanser-induced elevation of skin surface pH persisted for up to 94 minutes, though on average recovery occurred more quickly, in 36 minutes. Not surprisingly, there is a good positive correlation between the mean skin pH after washing and the mean recovery time.

Thune et al., (92) Korting et al. (93,94), and Barel et al. (95) also reported that personal cleansing products can elevate skin surface pH skin in adults for several hours after washing. It appears, however, that surface

Table 1 Summary of Changes in Skin Surface pH and Skin Surface pH Recovery Time for 41 Cleansing Products After 30-Second Wash

	Skin surface pH	Skin surface pH recovery time (min)
Mean (std dev)	0.6 (0.3)	36 (22)
Median	0.6	36
Minimum value	0.0	0
Maximum value	1.3	94

Source: Ref. 91.

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pH differential between skin washed with a more alkaline cleanser and skin washed with a more neutral cleanser is variable and can be relatively small. For example, in the study conducted by Barel et al., (95) in which measurements were made at least 3 hours after washing, the mean skin surface pH measured after 10 weeks of normal product use among groups using a soap or synthetic detergent bar for washing differed by ≤ 0.4 pH units at all body sites. The range of skin surface pH values measured at baseline and endpoint in this study was from pH 5.3 to 6.0. Choo-Ik (96) reported that the skin surface pH increased, on average, by 1.1 units after washing with water alone, by 0.9 units after washing with synthetic detergent–based cleanser, and by 1.2 units after washing with soap. Korting et al. (93,94) reported a surface pH differential between adult skin washed with a pH 5.5 cleanser and a pH 8.5 cleanser of about 1 unit at some evaluations over the course of their 8-week crossover studies. These authors again found no effect of cleanser preparation pH on skin roughness or TEWL (93). They did, however, note population shifts in some resident bacterial flora species on skin with an elevated surface pH (94). The long-term implication of such shifts is unclear. Cleanser pH effects have been inferred for infantile conditions such as diaper rash (9). Carlucci et al. (97) reported results from a study conducted among infants aged 4–30 months that showed that use of a detergent-based cleansing wipe did not significantly impact skin surface pH in the diaper area. The product also had no significant impact on irritation, microbial flora, or TEWL. However, these authors did not specify the product's surfactant composition or its pH. Kadar and Osbourn (10) reported that several cases of diaper rash that developed during a study examining the effect of daily bathing with soap bars resolved without discontinuance or change of cleanser. Thus, it seems that an assessment of whether the transient changes in skin surface pH that have been reported following personal cleansing use represent a real risk factor in diaper dermatitis requires further investigation.

VIII. OTHER INGREDIENT CONSIDERATIONS

Personal cleansing products can contain a variety of other ingredients that might present issues for infants. Siegfried (98) recommends that cleansing products containing triclosan, propylene glycol, benzethonium chloride, or glycerin should be used with caution on newborn skin. Fragrances are widely used in personal cleansing products and frequently implicated as a cause of contact dermatitis and as a potential triggering factor in adults with predisposing skin conditions such as atopic dermatitis. There is evidence that infants are susceptible to developing irritant reactions to concentrations

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of standard patch test tray chemicals that would be nonirritating in adults (18,99). Thus, fragrance cannot be excluded as potential source of skin irritation in infants. Fragrance might also put infants at an increased risk to develop future sensitization to topical agents (7,17). Since manufacturers rarely identify specific fragrances or fragrance components, identifying an offending agent is difficult. Using a cleanser that is labeled as ''unscented'' or ''fragrance-free'' does not guarantee that fragrance will not be an issue. Fragrance-free, for example, implies that a product has no perceptible odor, but these products can contain a low level of fragrance, smaller than the amount needed to impart a noticeable scent, to mask the offensive odor from raw materials (100). A complicating factor is that some fragrance-free products contain ingredients such as preservatives or natural oils that provide scent as a secondary function, but that can also be a covert source of dermatitis (101,102).

IX. NEW POSSIBILITIES FOR INFANT CLEANSING

Much of the previous discussion has focused on cleansing bars, but a number of new personal cleanser product forms have been introduced in recent years that remove many of the formulation constraints imposed by having to make a solid bar. As a result, there are now personal cleansing products available that go beyond simply minimizing the potential for skin drying and irritation that has for years been a focus with bars and liquid cleansers. For example, a body wash is now available that contains a relatively high level of petrolatum. This product deposits a clinically relevant amount of petrolatum onto the skin during use. Dermatologists recognize petrolatum as the gold standard moisturizer (103,104), and petrolatum or petrolatum-based products are often recommended as a treatment for infantile conditions such as diaper rash (105,106). Petrolatum is often viewed as functioning purely as an occlusive or protectant, but evidence suggests that petrolatum can permeate the stratum corneum and improve barrier function (107). The beneficial effects of petrolatum do not require application of 100% petrolatum to skin. For example, Odio et al. (108,109) reported that a diaper with a petrolatum-impregnated topsheet improved infants' skin condition and diaper dermatitis. Clinical studies show that the petrolatum-depositing body wash product described above is mild to skin and can improve dry skin condition under both controlled use (Fig. 4) and home-use conditions (110–112). Additionally, Warner and Boissy (113) reported improvement in Landmann unit structure in adult skin following use of this body wash product, which is consistent with observations made by Ghadially for topically applied petrolatum. Thus, a petrolatum-delivering

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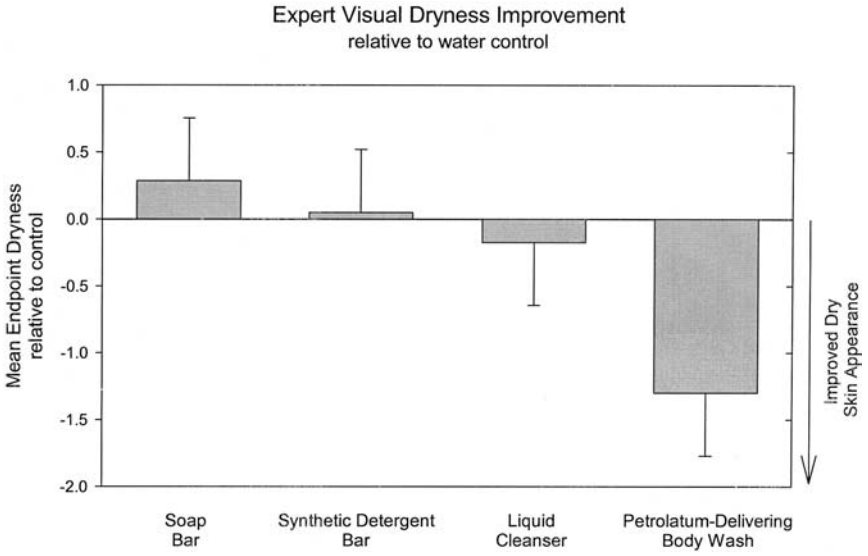

Figure 4 Endpoint dry skin improvement after 5 days of controlled leg washing with a range of personal cleaning products. Only the petrolatum-delivering body wash provides a significant ($p < 0.001$) dry skin improvement compared to the water control. Bar length represents the mean value, error bars represent the standard deviation.

body wash such as this might represent a good alternative to traditional personal cleansing products for infant bathing to not only minimize the potential for irritation but also help to improve skin condition.

X. CONCLUSION

Opinions vary on what constitutes appropriate cleansing practice for infants. Available information suggests that daily bathing with a suitable cleansing product will not harm a healthy infant's skin and might benefit their skin condition. Normal infants' skin will tolerate a range of cleansing products. However, soap-based cleansers generally have a greater likelihood of drying and irritating skin than cleansing products based on synthetic surfactants, particularly when used under hard water conditions. Even products based on synthetic detergents, however, can differ substantially in their potential to irritate skin due to the particular surfactants chosen and their concentrations in the formulation. Data from normal and controlled-use tests can provide some guidance in choosing products that have the least

potential for irritating the skin of infants. While surfactants are the primary determinant of cleansing products' skin compatibility, pediatricians, dermatologists, and parents must be aware of other ingredients in cleansing products that have a potential to cause issues when used on infants.

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12

Aesthetics of Newborn Skin: Biophysical Aspects

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The skin of most newborns, infants, and young children is soft, velvety, and smooth. Its color is quite uniform, and its texture has a special feel of its own. As such, infant skin is commonly perceived as ideal or perfect, and this organ is a source of admiration and wonder to the adult. The pleasing aspect of infant skin is largely due to the physical properties of the skin itself and to the sensorial perceptions these properties generate in the observer. In addition to functioning as the outer supporting sheath of the body, the skin also serves as a sensory organ closely linked to motor response. As such, the skin of the newborn is an important adjunct in the initial attachment phase between parents and infant. The attraction of infant skin for the caregiver presumably provides a strong survival advantage for the newborn infant during adaptation to postnatal life. Maternal-infant bonding, for example, implies close physical contact between the baby and the mother while simultaneously shielding the baby from the environment and providing a source of nutrition for the infant.

The complexity of such interactions and their importance for survival cannot be overemphasized. Presumably, the role of the skin as both a perceived surface and an organ of perception (with the associated release of appropriate neuromediators) depends on the biophysical characteristics of human skin in general. In this context, baby skin, with its preeminent role as an aesthetic ideal, is a natural focus for quantitative characterization in dermocosmetology. Biophysical measurements of newborn skin, therefore, may provide an excellent means for linking the biochemistry and molecular

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biology of the skin with the complex psychophysics of human perception. This approach recognizes the role of the skin as a neurodevelopmental interface with close embryological connections to the brain (1).

Most skin functions are common to all individuals, but the ability of the skin to perform those functions is decidedly influenced by age, environment, and exposure to various drugs, clothing, bathing practices, and cosmetics. These conditions are minimized by study of newborn skin. Considering the importance of the human skin, it is surprising how little research has been devoted to healthy newborns. Indeed, the scientific literature abounds with information on skin diseases in infancy and childhood, but references to the characteristics of healthy neonatal skin are few. With the advent of consumer-centered concepts of health care, however, the specific aspects of normal healthy skin and dermocosmetology should not be neglected (2). Skin care, in general, has an inordinate impact on consumer satisfaction. It is likely, therefore, that health care institutions will increasingly discover that focus on "skin care" will improve all aspects of care delivery.

It is the intent of this chapter to review some of the available information on the physical, physiological, and cosmetic aspects of healthy skin in the newborn infant. Describing the biophysical characteristics of newborn skin calls for noninvasive techniques assessing specific functional properties of the tissues. For years, while numerous biophysical methods have been proposed, only those providing precise, reproducible and easily interpretable parameters in terms of skin structure and physiology have been retained.

I. THE SKIN SURFACE

During late gestation and soon after birth, the skin interface with the environment undergoes profound ontogenic changes and acquires different functional characteristics. Postnatal maturation and adaptation of the skin surface are impressive, particularly in preterm infants. The skin of premature infants represents more than 10% of the body weight, compared with 3% in adults (3). Such a difference is impressive. Preterm infants lack a competent epidermal barrier and present a glistening, moist, sticky skin surface, which contrasts greatly with a term infant and adult. Because much of skin development takes place relatively early in gestation, the skin of a young baby born at term and that of an adult have a few qualitative differences but many similarities. Indeed, many more significant structural and functional differences exist between premature and full-term infants than between term infants and adults.

At the moment of birth, skin surface characteristics depend largely on the structure of the stratum corneum and on the amount and composition of

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vernix caseosa covering it. The development of vernix in the last trimester of gestation (4) and the formation of the stratum corneum as the result of terminal differentiation of epidermal keratinocytes lead to the formation of a relatively water-impermeable barrier (5). This composite structure hinders the egress of water through the skin, thus reducing skin surface moisture, a characteristic identified by skin surface electrical measurements (5–10). The epidermal barrier properties progressively change over the first month of life, showing an increase in surface hydration, a decrease in transepidermal water movement under occlusion, and a decrease in surface water desorption rate (9). These functional aspects are not perceived directly by the naked eye. However, the transparency and gloss of the stratum corneum may be modified and thus influence the visual perception of baby skin by the observer. Understanding these global characteristics and changes requires focus on the role of sebum and sweat excretion at the skin surface and the functional properties of the stratum corneum itself.

A. Sebum and Vernix Caseosa

Skin surface lipids consist of a mixture of compounds of sebaceous and epidermal (stratum corneum) origin. The sebaceous glands are distributed over most of the body surface. They empty their sebum through a small duct into the neck of hair follicles. Sebum production is, to some extent, a function of sebaceous gland size, and it is under the control of androgens, other hormones, and probably some neuropeptides. The sebaceous glands are already active by weeks 13–15 of gestation. Sebaceous glands produce, in part, the greasy vernix caseosa with which the full-term infant is covered at birth. By contrast, very low birthweight preterm infants lack coating with this surface film. It has been shown experimentally that the application of vernix caseosa to freshly bathed human skin results in a unique profile of temporal change in baseline surface hydration, moisture accumulation, and water-holding capacity (10).

The sebaceous glands are large and well developed in the full-term newborn as a result of the effect of maternal androgens and possibly fetal steroid secretion. Shortly after birth, the glands begin a period of quiescence, which lasts until puberty. Hence, young children produce little or no sebum. These age-related changes result in differences in surface film composition.

Depending on the body site, the lipid mixture at the skin surface differs largely in composition. The face, thorax, and shoulders are rich in sebaceous lipids, while epidermal lipids are predominant in the extremities. Sampling methods should be adapted to the quantities of lipids present on the skin surface (11). On the face of a newborn, these quantities are of the order of

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tens or hundreds of micrograms, whereas only a few micrograms per square centimeter are present on the limbs. One of the most appropriate devices for such assessments is a video camera equipped with an internal ultraviolet light–emitting unit (Visioscan®, C+K Electronic, Cologne, Germany). Interposing a lipid-sensitive film between the camera and the skin allows recording of the follicular sebum excretion pattern after only a few seconds of contact. The number of lipid dots and their surface area can be measured using image analysis. The results provided by this method have been correlated with those of other ancillary techniques (12).

Observations made at different stages of life (13) indicate that a low amount of sebum on the skin surface has little or no direct consequence from a cosmetic point of view. Hence, in contrast with their prominent physiological roles, vernix caseosa and sebum are not considered to be of prime cosmetic importance in the newborn. The decision to remove and discard vernix at the time of birth, however, may be influenced by cosmetic considerations.

B. Sweat Production

Similar to sebaceous glands, the density of eccrine glands is greater in the neonate than at any other time of life, because of the smaller skin surface area relative to body size. In the full-term newborn and the adult, sweat glands are similar in size, structural maturity, and position within the dermis. Those of the premature baby, however, resemble more those of the fetus than the adult. The coiled secretory part is not completely differentiated, although the ducts are fully formed and patent.

The cosmetic importance of sweat production is almost nonexistent in contrast with its thermoregulatory role. The eccrine sweat glands respond differently in the young infant and child than in the adult. Secretory activity begins shortly after the 29th week of gestation. However, for the very first years of life, the number of active glands is small and they function irregularly. Palmar sweating is detectable in most infants by the third day of life, but emotionally induced eccrine sweating is almost absent.

C. Stratum Corneum

From the moment of birth, or shortly thereafter in preterm babies, the stratum corneum is remarkably capable of fulfilling most of its critical functions. Aspects of the superficial stratum corneum can be documented by examining cyanoacrylate skin surface strippings (14,15). This structure, in the adult appears as individual corneocytes that tend to separate from one another. In the full-term newborn, it appears as larger units of adherent

sheets of multiple layers. In the premature infant, only isolated clusters of corneocytes are loose at the skin surface. In some newborns corneocyte clumpiness can be recognized as discrete xerosis commonly called ''dry skin'' by laypersons. Such a skin condition is easily documented using a video camera under ultraviolet light illumination (16).

Compared with older infants and adults, and particularly with preterm infants, the full-term newborn has a low rate of transepidermal water loss (TEWL) (6,17–20). The mechanisms underlying the low TEWL in the neonates are unclear but may relate, in part, to a well-developed stratum corneum and to contributions from surface lipids of vernix caseosa, which partially impede transepidermal movement of water (10). In premature infants, however, a marked impairment in barrier function with a greater TEWL is demonstrable (10,17–19,21) consistent with poor stratum corneum formation and a diminished coating of vernix caseosa. The presence of a poor epidermal barrier places the very premature infant at a greater risk of heat loss because of water evaporation (18,19,21,22).

Excessive hydration is equally disruptive to the skin barrier. The mechanism of action by which water damages the stratum corneum is incompletely understood, and more is involved than simply the establishment of aqueous channels for water-soluble agents. The ability of the infant to develop a competent stratum corneum barrier under conditions of total aqueous immersion is remarkable in this regard.

Whereas excess water can be harmful, the proper degree of skin hydration is critical for maintaining optimal appearance of the skin surface. If the water content of the stratum corneum, normally 10–20% by weight, drops below 10%, the layer becomes cracked and brittle, and various xenobiotics can penetrate more readily. In the case of irritation, the resulting inflammation can further impair keratinocyte maturation. Ambient temperature and humidity affect the barrier effectiveness. Hence, there are seasonal conditions that affect the skin surface. A rise in temperature increases water diffusion through the epidermis. In addition, it can cause excessive sweating in the diaper area and flexures. This situation is worse when the environmental humidity is high. By contrast, there may be exceptional skin dryness during winter time associated with low environmental humidity. The decrease in humidity in centrally heated homes is also a key element favoring stratum corneum dehydration. Also implicated is damage to the corneocyte membranes following frequent, vigorous scrubbing of the skin or excessive exposure to surfactants. When the cell membranes are damaged severely enough, hygroscopic natural moisturizing factor (NMF) molecules, which normally bind intracellular water, are lost, and irreversible alteration in corneocyte function may ensue.

Excessive skin surface hydration is associated with pronounced permeability (23,24) and impaired elasticity of the stratum corneum (25). In addi-

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tion to cosmetic implications, the former characteristic places the premature newborn infant at risk for absorbing various xenobiotics. This situation last only a few days because a remarkably rapid development in the epidermal barrier takes place during the first week of life in the premature infants born at less than 30 weeks of gestational age (7).

Friction is another problem to which babies are particularly susceptible. This action may occur between clothing and skin, between diaper and skin, or in intertriginous areas where two facing areas of skin rub together, such as the axilla or buttocks. Hydration of the stratum corneum significantly increases its coefficient of friction. Removing one of the opposing surfaces, by decreasing skin hydration or by applying lubricants, can reduce friction. Products such as creams, emollients, baby lotions, and oils are used for both term and premature newborns. The use of lubricants is based on the subjective feeling of dryness, as well as traditions of skin care that may include elaborate oiling practices. There are concerns, however, that lubricants can alter skin pH and microorganism colonization patterns, so lubricants should only be used sparingly and only when excessive dryness can lead to fissures.

Racial differences have been shown in the epidermal barrier function of the adult population (26,27). Similarly, by term black infants appear to have a more mature barrier function than Caucasian infants as assessed by the moisture accumulation test (7). Race-dependent differences in epidermal barrier maturation have been offered to explain better survival in black infants than in white infants in the extremely premature population (28). These important physiological aspects do not appear to have cosmetic implications.

Another developmental variation deals with the skin surface pH. A value in skin surface pH lower than 5 is normally found in both adults and children. In a large number of term newborns the pH is much higher reaching 6.3 immediately after birth, with a decline to less than 5 within less than a week (7). In low birthweight infants, the mean pH falls from 6.7 at birth to 5 by the eighth day of life. An acid skin surface is credited with bacteriocidal effects against some pathogens and serves in the defense against infection. Occlusion of the diapered area raises the skin pH, which gradually shifts from the acidic range to neutral.

II. SKIN MICRORELIEF

The microtopography of the skin affects such factors as the softness of the skin and its velvety aspect. Most of the microrelief characteristics depend on the organization of collagen bundles in the upper dermis. A simple magnifying lens reveals the complexity of human skin topography and the extensive

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variations from one anatomical site to another. Microscopic examination better reveals the details. The different types of skin relief are generally classified according to the depth of primary and secondary lines (discrete and shallow, respectively), tertiary lines (edges of corneocytes), and quaternary lines (trabecular network on the corneocyte itself) (Fig. 1). The mean depth of these lines does not change from birth until early adult life. However, their orientation and depth differ according to body site and change with age and the influence of environmental factors (29).

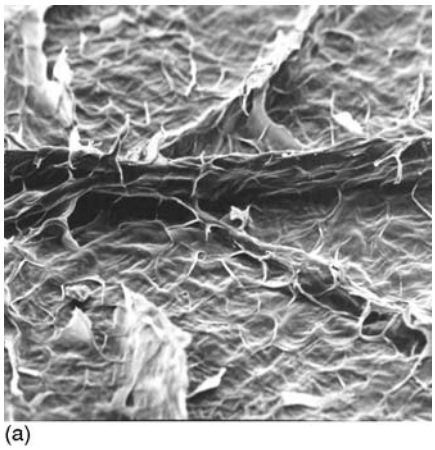

Figure 1 Cyanoacrylate skin surface biopsy showing (a) primary, secondary, and tertiary lines (×300), (b) quaternary lines (×2500).

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Slight abnormalities in the skin's normal smoothness and softness may lead to parents seeking corrective measures. The accessibility of the skin and its easy observability make this organ amenable to routine care and early intervention. Conditions leading to increased friction, roughness, and irritation can be corrected early in order to maintain in optimum surface condition and decrease the risk of overt skin disorders.

III. SKIN COLOR

Skin color depends largely on the superficial blood supply and on the epidermal melanin content. In addition, many newborn infants suffer from transient jaundice. The bilirubin content in the skin is responsible for the typical yellowish hue. When conditions are ideal, newborn Causasian skin, for example, exhibits a uniform peach color. This skin hue depends largely on the underlying vascular network, which consists of a web of superposed plexuses. The blood supply underlying the epidermis in the newborn depends on the rich capillary network of the dermis. This network gradually matures to produce the characteristic structure of capillary loops and a subpapillary plexus. A mature upper dermis has many capillary loops, and the tissue fluid transuding from them tends to obscure the subpapillary plexus, which is mainly venular and bluish. At certain sites where the capillary loops are well developed in newborns, there is a heightened pink color. This is particularly so over the cheeks, which are often rosy in color. Direct arteriovenous anastomoses are abundant and are responsible for thermoregulatory shunting. The skin appendages are encased in special vascular networks.

Melanin production in the newborn is low, even though the mean density in melanocytes is similar to that of an adult. Melanosomes formed in the melanocytes move via dendritic processes into basal and suprabasal keratinocytes. The melanin content is dispersed as fine intracellular granules. In contrast with adult skin, such a distribution is very uniform in young children.

Skin color can be measured using devices that break down color into its three principal components corresponding to luminance (L*) and chromaticity (a*, b*). The response of the system is similar to that of the human eye. Luminance represents the shade of grey (between white and black), while the chromaticity parameters a* and b* measure the color of the object on the red/green and yellow/blue axes, respectively (30). Compared to the skin of their parents, newborn skin often exhibits higher L* and a* values. The b* value is also higher during the icteric phase and falls afterward below the comparable adult value.

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Another convenient way to assess skin color relies on narrow band spectroscopy, which derives the so-called melanin (M) and erythemal (E) indexes (30). Newborn skin is often characterized by increased E values and lower M values compared to the parents.

IV. SKIN MECHANICAL PROPERTIES

Several structures condition the overall mechanical properties of skin, which are important from a cosmetic point of view. The stratum corneum is responsible for most of the tensile strength of the epidermis. This property is important for maintaining epidermal integrity. It is, however, too weak to ensure the whole mechanical function of the skin. The elasticity and suppleness of the stratum corneum are of cosmetic importance. These physical properties are largely governed by the capacity of the stratum corneum to absorb and retain water.

At the dermo-epidermal junction, the premature infant has fewer hemidesmosomes per equivalent linear distance, and the dermis below is more edematous and has a looser organization of collagen fibers. Thus, with a less secure epidermal attachment, the premature infant has a greater propensity to blister formation in the first weeks of life.

The major tensile properties of skin depend on the structure of the dermis and hypodermis (31). The dermis forms the preponderant mass of the skin connective tissue. Its thickness varies substantially among different regions of the body. The dermis contains fibrous elements, amorphous ground substance, free cells, nerves, and blood and lymphatic vessels. Between 10 and 30% of the wet weight of the dermis is fibrous protein, mostly collagen fibers and bundles admixed with fewer elastic fibers. The collagen fibers form an interlacing network with elastin fibers woven in a meshwork pattern. Thus, the collagen gives the skin high resistance to mechanical stress, whereas elastin fibers restore the skin to its original condition once the deforming stresses are removed. Surrounding the fibers is an amorphous and viscous mucopolysaccharide gel.

The subcutaneous tissue, lying beneath the dermis, is composed of areolar and fatty connective tissue. Its thickness depends on the individual and body region and insulates the underlying structures from heat, cold, and mechanical pressure. Strands of collagen extend into this layer from the dermis.

The organization, structure, and composition of the dermis of the term newborn exhibits features that are intermediate between the adult and the premature infant. The collagen bundle density and thickness are considerably smaller in the infant than the adult, and the total thickness of the

dermis is less. Most of the elastic fibers are formed after birth and may not become fully adult-like in structure until 3 years of age. Thus it would be expected that infant skin might have less resiliency than in later years. The proportion of the hygroscopic amorphous material interposed between the fibrous structures is higher in the perinatal period than later in life. Hence, when the body is adequately hydrated, skin is turgescent. This feature is of utmost importance in the appreciation of the tactile perception of skin softness in infants.

Several physical systems can be used to measure the viscoelastic properties of the human skin in vivo. Measurements are usually performed parallel or normal to the skin surface (31). The curves show an initial skin deformation corresponding to immediate extensibility, followed by a slow increase corresponding to a creeping phenomenon, which represents the viscous and plastic characteristics of the skin. The most strongly affected mechanical parameter affected by age is the elastic return of the skin, which decreases linearly when adulthood is reached. Another measurement method relies on the assessment of the speed of shear wave propagation (32,33), which progressively increases from birth to puberty (Fig. 2).

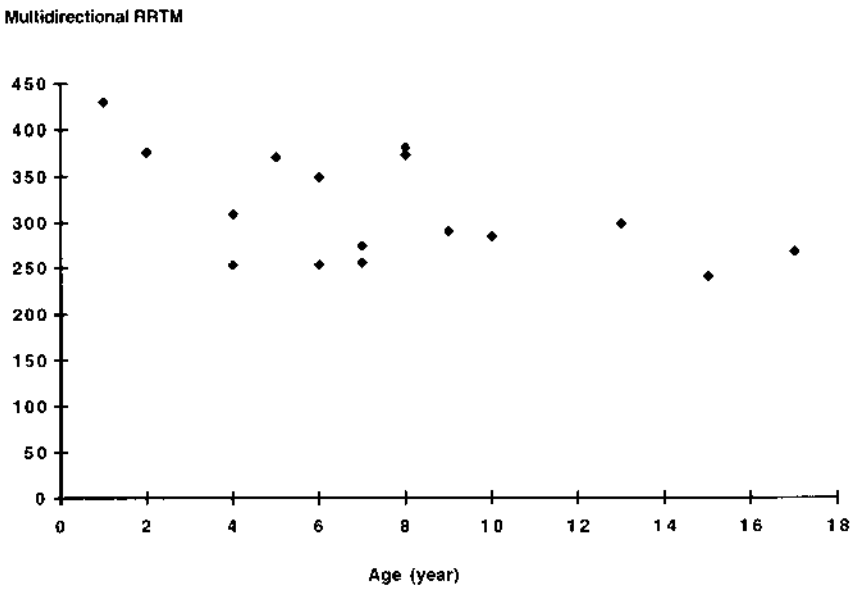

Figure 2 Progressive decrease with age of the resonance running time measurement (RRTM, arbitrary units). Multidirectional measures are taken along 4 axis at 45° intervals on the forearm.

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V. CONCLUSION

Structurally and biochemically, differences between the skin of an adult and that of a newborn may appear minor. As a whole, however, these differences have important effects on skin perception and contribute to the impression that the skin of the newborn infant is perfect or ideal. The quantitative and qualitative description of such skin states is central to the field of dermocosmetology. Bioinstrumental methods for assessment of hydration, color, gloss, friction, mechanical strength, and tissue plasticity offer excellent tools for measuring newborn skin and comparing such measurements to adult states and disease conditions. Overall, the strategy of quantitatively describing the properties of newborn and infant skin provides a set of standard parameters defining normal skin that is free of the effects of age, environment, trauma, and disease. The ''aesthetic'' aspects of baby and infant skin, studied in conjunction the caregiver, moreover, may have application to complex emerging fields such as neuroperception and psychophysics.

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13
Transepidermal Water Loss

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I. INTRODUCTION

It has long been known that humans lose water and weight insensibly. As early as 1614, Sanctorius (1) observed an insensible reduction in body weight over a period of several hours, during which the subject was placed on a sensitive balance. With the introduction of increasingly sophisticated balances, insensible weight loss (IL) has been one of the principles for determination of insensible water loss (IWL). In 1917 Söderström and Du Bois (2), using a calorimeter, found that healthy men lost about 25% of their produced heat through evaporation of water from the skin and respiratory passages. Later, Levine and coworkers found that compared with adults, infants lost slightly more of their produced heat by evaporation under basal conditions (3,4). These authors conducted most of their studies by accurately weighing the lightly clothed infant, but they also measured IWL by means of a ventilated respiration chamber. These studies indicated that the insensible weight and water loss was considerably greater when the infant was outside than when it was inside the respiration chamber. The only plausible explanations for this difference were a slight difference in ambient humidity and a difference in the activity of the infants, who tended to be awake for longer periods when not restricted in the respiration chamber. In later studies with use of a ventilated chamber, Hey and Katz (5) determined both the total IWL and the respiratory water loss (RWL) in infants born at term or near term, and the results indicated greater losses at a low ambient humidity than at a higher one. These authors also calculated the water loss from the skin by subtracting RWL from IWL.

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II. BODY COMPOSITION

In early gestation, the body water of the fetus accounts for more than 90% of the fetal body weight. The total body water and extracellular water decrease during gestation and the intracellular water increases towards term. A full-term newborn infant has a water content of 77% of the body weight. In preterm infants the total body water at birth is higher than in full-term infants, namely 86% at 24 weeks, 84% at 28 weeks, and 82% at 32 weeks of gestation. (6).

After birth, decreases take place in total body water, extracellular water, and body weight. These events are considered to be a ''physiological weight reduction,'' the magnitude and duration of which are influenced by the supply of water, electrolytes, and nutrients. In very preterm infants who had survived the first 2–3 postnatal days, disturbances of water balance were often observed in the 1970s. The findings by Fanaroff et al. (7) suggested that IWL determined through weighing indicated larger losses than had previously been reported in appropriately sized 2- to 10-day-old infants weighing less than 1.250 kg and with gestational ages of less than 32 weeks (7). It seemed probable that the water loss through their thin skin might be very high. The study by Fanaroff et al. (7) further emphasized the need for new methods to measure water transport through the skin separately. Previously applied gravimetric methods and the use of ventilated chambers enclosing the whole body did not allow separate determinations of water loss through the skin and from the respiratory tract. A new technology for addressing this and similar problems seemed to be needed, i.e., a device for measurement of the evaporation rate (ER) from the skin surface.

III. INSENSIBLE WATER LOSS–TRANSEPIDERMAL WATER LOSS

Insensible water loss is the sum of two components: insensible loss of water from the skin and water loss from the respiratory passages (1). Within the frame of projects at the University of Linköping and Uppsala University, Sweden, methods have been developed and applied for direct determination of transepidermal water loss (TEWL) (8–11) and for determination of respiratory water loss (12,13).

IV. MEASUREMENT OF WATER LOSS FROM THE SKIN

A. Unventilated Chambers

Local water loss from the skin can be determined by means of an unventilated chamber placed on the skin surface and with a bag of hygroscopic salt placed within the chamber (14–16). Before each measurement, the salt has to be desiccated by heating it to about 200°C and then placing it in an airtight bottle. The bag is then weighed immediately before and immediately after a period of measurement of 15–30 minutes, and the increase in mass then constitutes a measure of the loss of water from the skin surface. The two disadvantages of this method are that the salt initially absorbs the vapor already present in the measurement chamber and the salt may be saturated with water before the end of the measurement period. In addition, the hygroscopic properties of the salt may alter the microclimate above the skin surface (9).

B. Ventilated Chambers

With this method evaporative water loss from the skin is estimated by passing a continuous flow of gas with a known water content and at a known velocity through a measurement chamber placed on the skin surface. The humidity of the gas and the velocity of its flow are measured both before entry of the gas and when it leaves the chamber (17). The outflow and inflow of gas have to be monitored to detect any leakage of gas. Too low flow velocities cause condensation in the tubing and too high flow produces convective currents that will influence the microclimate over the skin surface and hence the evaporation rate. A number of principles have been applied as a basis for measuring water vapor (9). The most serious drawback of the ventilated chamber technique is that the gas flow may influence the vapor pressure gradient close to the skin surface and water loss through the skin (18).

C. Electrolytic Water Analyzer

The Mecco electrolytic water analyzer (Mecco) has been used to determine water loss from the skin in adults and in full-term and preterm infants (19). Wilson and Maibach (20) found a higher water loss from the skin of preterm than of full-term infants. This method has been used in a small number of studies in infants.

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D. The Gradient Method—the Evaporimeter

In order to measure water moving through the skin and evaporating from its surface, a number of criteria need to be fulfilled. Most important is to ascertain that there is free evaporation from the skin surface. Further, the method should allow direct measurement of the rate of evaporation from the skin surface, quick measurements, repeated measurements at short intervals, measurements of relatively rapid changes and measurements without causing discomfort to the infant (9,11). A method that is based on measurement of the vapor pressure gradient in the boundary layer close to the skin surface would be ideal. The underlying theory is that in the absence of forced convection there will be a linear relationship between the vapor pressure and the distance from the evaporative surface (21,22). The thickness of this boundary zone is about 10 mm. If the vapor pressure distribution in the boundary layer is known, the amount of water evaporated per unit time and area (i.e., the evaporation rate, ER, can be calculated:

$$\frac{1\,dm}{A\,dt} = -D' \frac{\delta p}{\delta x}$$

where $1\,dm/A\,dt$ is the evaporation rate ($g/m^2/h$), D' is a constant ($0.670\ 10^{-3}$ g/m/h Pa), and $\delta p/\delta x$ is the vapor pressure gradient in the layer of air immediately adjacent to the skin (Pa/m). To examine the validity of the theory, the vapor pressure profile above a salt solution was determined. The relationship between vapor pressure and distance from the surface is shown in Figure 1 (9).

Under steady-state conditions, the vapor pressure gradient and the evaporation rate are constant and proportional to the difference between the vapor pressure at two separate points located on a line perpendicular to the evaporative surface. The actual vapor pressure at each point is calculated as the product of the relative humidity (RH) and the saturated vapor pressure. Since the latter is a function of the temperature, the vapor pressure at the two levels above the skin surface can be determined by measuring the relative humidity and temperature at two distances (A and B) from the skin surface, as shown in Figure 2. For measurements of relative humidity, capacitive sensors are used. These are only sensitive to changes in relative humidity (23,24) and have a size of 4×6 mm. For determination of temperature, fast thermistors are used at each point of measurement. An open cylindrical Teflon capsule protects the sensors from mechanical damage and maintains a stable zone of diffusion in the measurement area (9). To avoid influencing the vapor gradient, the height of the capsule has to be limited (9,25).

The vapor pressures and the differences between them are processed electronically, and the relative humidity, the vapor pressure at each of the

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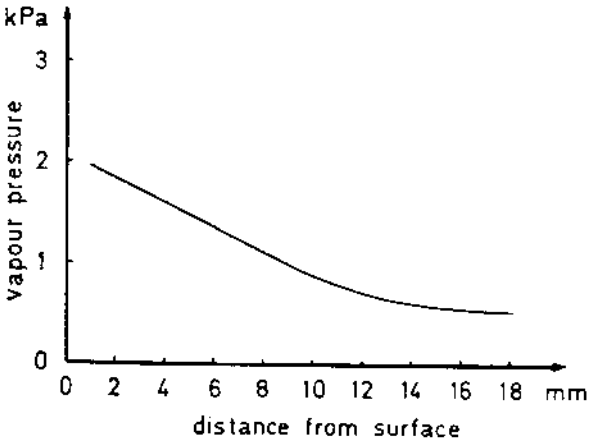

Figure 1 Example of vapor pressure distribution above the surface of a salt solution. (From Ref 9.)

measurement sites, and the evaporation rate are displayed by the instrument. The system is calibrated by placing the probe above saturated salt solutions (8,9,26). To calibrate the thermistors, the probe is placed in an environment with a known ambient temperature.

During measurements, the probe is held lightly against the skin surface. There should be no leakage of air between the lower rim of the Teflon cylinder and the skin, as air currents could disturb the vapor pressure gradient. During measurements, water accumulates on the skin underneath the

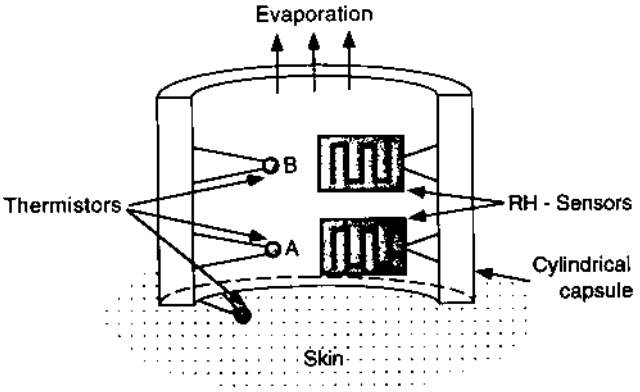

Figure 2 Sensors for relative humidity (RH) and termistors positioned in the Evaporimeter probe. (From Ref. 11.)

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Teflon. The accumulated water therefore will be recorded as higher ER values if the probe is moved than if the probe is kept at the same surface (9,11,27,28). At very high ambient humidity, condensation of water at the sensors and the Teflon cylinder may influence the ER measurements, making it difficult to obtain a steady-state reading (8,11).

E. Environmental Conditions

All measurements of evaporation rates using the evaporimeter should be made at air flow velocities of less than 15 cm/s (9,11) and with well-defined conditions concerning ambient humidity, water vapor pressure, and body and skin temperatures. In our studies at Uppsala University, the body temperature has always been maintained at 36.0–37.0°C except when the influence of body temperature on ER and TEWL has been analyzed, and the infants have always been studied when calm during the measurements except when the influence of activity has been investigated. In all of our studies of ER and TEWL in infants nursed in incubators, the ambient relative humidity (RH_{amb}) has been kept at 50% (27–43) except when the influence of ambient humidity has been examined (11,27,30,35,37,57). Hands placed in the incubator during the measurement procedure should be inserted in a way that will not allow any leakage of air through the openings of the incubator (11,29,35,36).

V. EVAPORATION RATE FROM THE SKIN OF FULL-TERM NEWBORN INFANTS AND ESTIMATION OF TRANSEPIDERMAL WATER LOSS

When of the rate of evaporation was measured from different skin surfaces in full-term infants, great differences were found, for instance, between the forehead and the palm of the hand, both of which showed high evaporation rates, and the chest and abdomen, both of which had much lower values (see Fig. 3), i.e., evaporation rates were higher from the skin of some peripheral parts of the body and lower from the trunk.

The transepidermal water loss could be determined by estimating different body surface areas (14) and inserting the measured ER in the following equation (11):

$$TEWL = \frac{\sum_{i=1}^{18} ER_i \, Area_i}{BSA}$$

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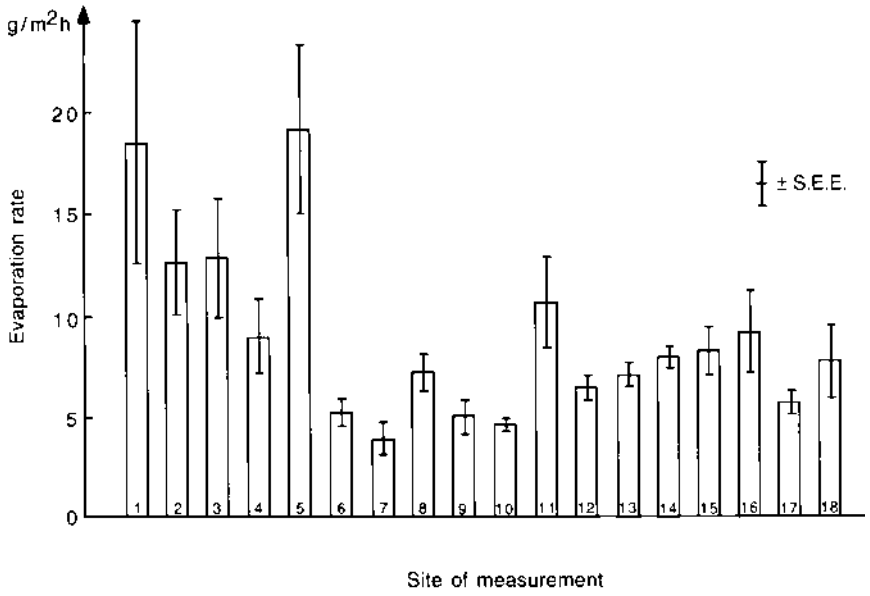

Figure 3 Evaporation rate from different skin sites in full-term newborn infants. (From Ref. 11.)

The different body surface areas were determined according to the linear formula of Du Bois (44), and the total body surface area (BSA, m^2) was calculated from the Du Bois length-height formula (44) in which the proportionality constant was changed, as proposed by van Graan (45), to $BSA = 0.2157\,W^{0.425}\,H^{0.725}$, where W is the weight (kg) and H the height (m) of the infant. Calculations of the arithmetic mean of ER from different combinations of a few measurement sites showed that measurements from the chest, an interscapular skin area, and a buttock gave the highest correlation coefficient of 0.945 (11). TEWL could thus be estimated with reasonable accuracy using the following equation (11):

$$TEWL = 0.92\,\overline{ER}_{abc} + 1.37$$

where $\overline{ER}_{abc}$ is the arithmetic mean of ER measured on the skin surface at the three above-mentioned sites. Since the evaporation rate differs less between different sites of the trunk in very preterm infants, the equation is probably also valid for these infants (27,30,46), which is further supported by the results of Rutter and Hull (46). The fact that the transepidermal water loss, being the mean value for the whole body surface, could be

determined on the basis of only a few measurements, opened the possibility of using the gradient method for estimations not only of local rates of evaporation, but also of the total loss of water from the skin surface (11).

VI. TEWL IN FULL-TERM NEWBORN INFANTS

In full-term infants less than 30 hours old, at rest and with a body temperature of 36.2–36.9°C, data from measurements at the chest, an intrascapular skin area, and the buttock gave a mean TEWL value of 8.1 $g/m^2/h$, obtained at an environmental humidity of 50% (11). This was somewhat higher than the value of 6.8 $g/m^2/h$ obtained by Hey and Katz (5) at an ambient humidity of 60%, using a technique in which the baby was placed in a ventilated chamber.

VII. TRANSEPIDERMAL WATER LOSS IN RELATION TO GESTATIONAL AGE

Preterm infants born at a gestational age of less than 35 weeks were never examined in studies using body-enclosing chambers for measurements, such as those used by Hey and Katz (5) and Zweymüller and Preining (47). When insensible water loss was measured it was discovered that some very preterm infants lost weight dramatically (7). With the introduction of the gradient method (8–11) it became possible to study the evaporation rate and transepidermal water loss in very preterm infants also. The equation for determination of TEWL that was based on measurements in full-term infants was also used in studies of preterm infants (30).

In studies of 32 infants with gestational ages of 25–39 weeks in whom estimations of TEWL ($g/m^2/h$) were made, this water loss showed an inverse relationship to gestational age (30) (Fig. 4), which can be described by the following equation obtained by regression analysis:

$$\text{TEWL} = 4.17 + 64.76e^{-\frac{(\text{G.A.}-24.99)}{2.73}}$$

where G.A. is gestational age (weeks). The residual standard deviation of the equation is 2.72 ($n = 32$; 28 degrees of freedom).

TEWL is considerably higher in infants with a gestational age of less than 30 completed weeks than in those born close to or at term. During the first day of life, TEWL is 15 times higher in infants born at 25 weeks of gestation than in full-term infants (27,30) in an environment with 50%

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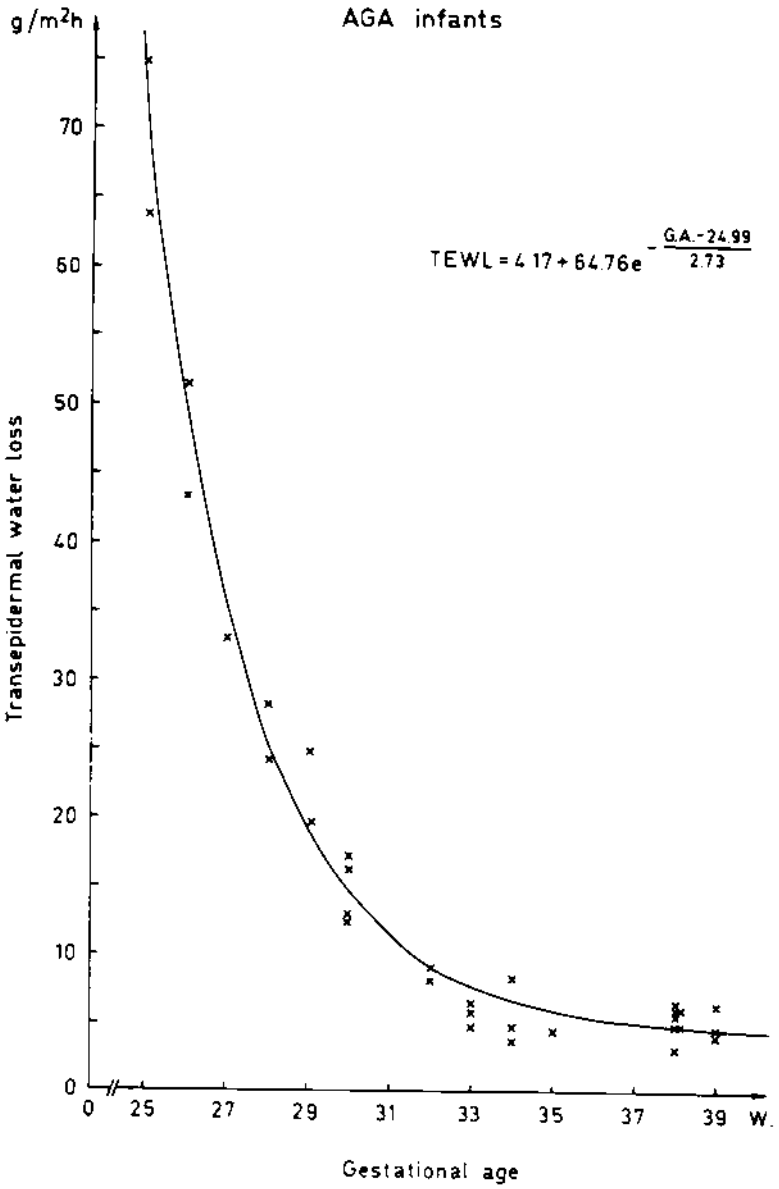

Figure 4 Transepidermal water loss (TEWL) in relation to gestational age (GA). AGA = appropriate for gestational age; W = completed weeks of gestation. (From Ref. 30.)

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ambient humidity. These observations of high evaporation rates in preterm infants compared to full-term were verified by Rutter and Hull (46).

VIII. TEWL AT DIFFERENT POSTNATAL AGES IN AGA AND SGA INFANTS

The relation between TEWL and gestational age at different postnatal ages during the first 4 weeks after birth can be described with exponential equations (36) (see Fig. 5). TEWL remains highest in the infants born most preterm, but the difference in TEWL between the most preterm infants and the full-term infants gradually diminishes with age (Fig. 6). At a postnatal age of 4 weeks, TEWL is still twice as high in the former as in the latter (Table 1) (35,36). Also in SGA infants there is an exponential relationship between TEWL and gestational age (Fig. 7). On the first day after birth

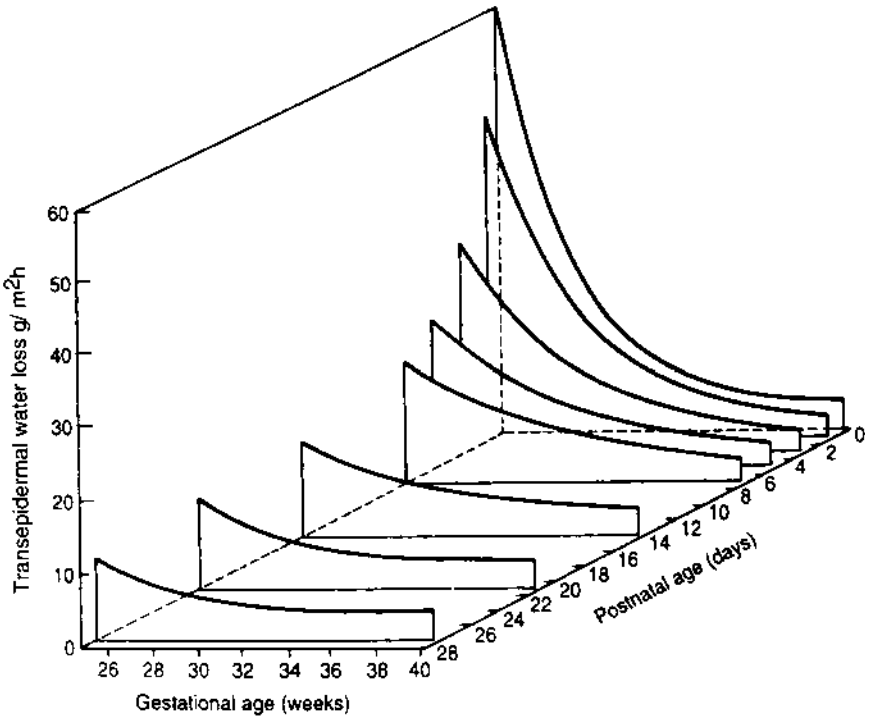

Figure 5 Regression of transepidermal water loss ($g/m^2/h$) on gestational at birth at different postnatal ages in appropriate for gestational age infants. (From Ref. 36.)

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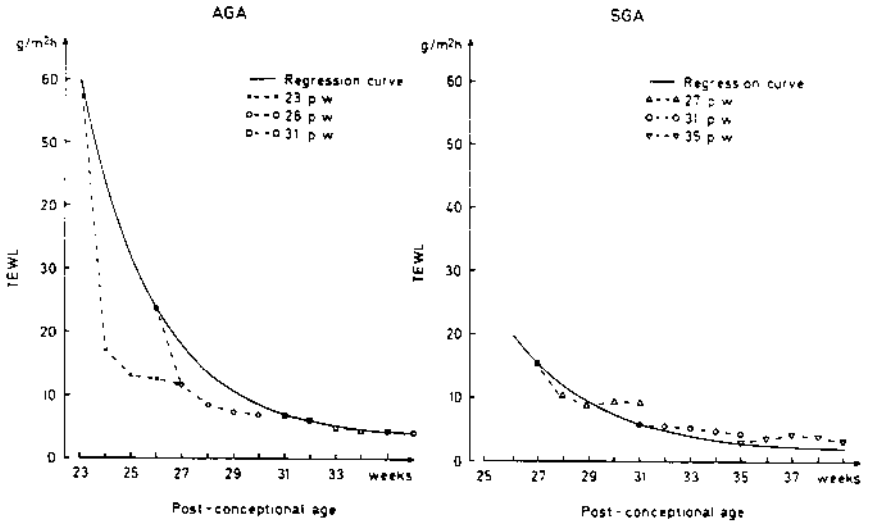

Figure 6 Transepidermal water loss (TEWL) in three groups of appropriate for gestational age (AGA) infants (left) and in three groups of small for gestational age (SGA) infants (right) during the first 4 weeks postnatally (dashed lines) compared with TEWL on the first day after birth (regression curves). p.w. postconceptional age at birth (weeks). (From Ref. 36.)

SGA infants born at different gestational ages have a lower TEWL than AGA infants born at a corresponding gestational age. Postnatally, in full-term SGA infants TEWL tends to increase somewhat (32), but in full-term AGA infants (36) TEWL remains at almost the same level during the first 2 weeks after birth. The reason for the lower TEWL in full-term SGA than in full-term AGA infants is still not fully understood, but might be related to the water content of the skin.

In extremely *preterm infants born at 24 and 25 weeks of gestation* (48), measurements of ER and calculations of TEWL at 50% ambient humidity ($TEWL_{50}$) revealed that $TEWL_{50}$ remained very high both on the first day and on the second day after birth (59.3 ± 17.6 g/m^2/h), and then gradually became lower, reaching 36.1 ± 12.6 g/m^2/h at 7 days and 24.2 ± 7.7 g/m^2/h at 28 days (Fig. 7). Thus, in these infants transepidermal water loss did not change from the first to the second day of life. In addition, TEWL decreased with postnatal age at a slower rate in these infants than had been observed previously (33,34) in infants born at 25–27 weeks of gestation. At a postnatal age of 28 days TEWL was twice as high as that reported for infants born at 25–27 weeks and four times as high as that found in infants born at term (28,35,36).

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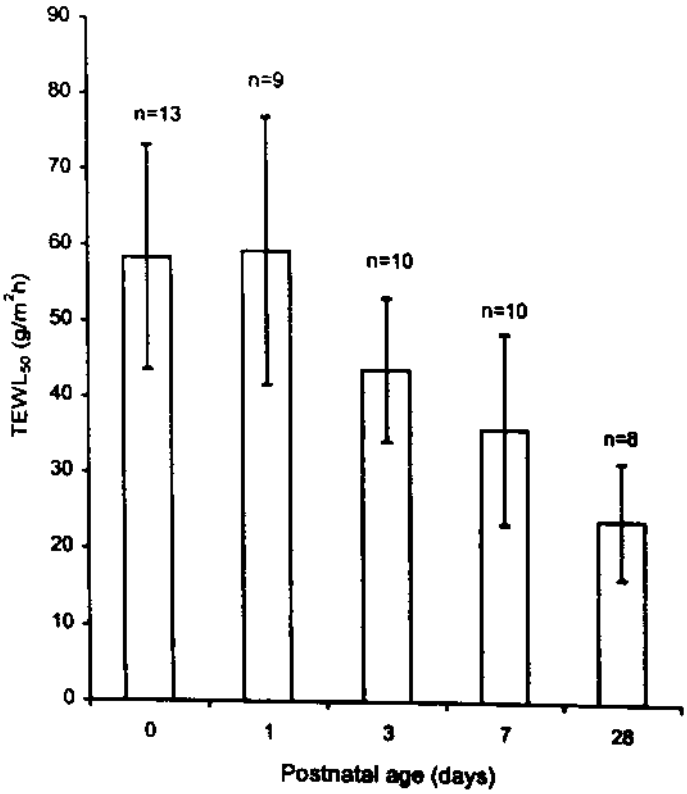

Figure 7 Transepidermal water loss, corrrected to 50% relative humidity ($TEWL_{50}$), in relation to postnatal age in 13 infants born at 24–25 completed weeks of gestation. (From Ref. 48.)

The barrier properties of the skin depend to a great extent on the thickness and integrity of the stratum corneum (49,50). A correlation between the structure and lipid content of the stratum corneum and TEWL has also been demonstrated (51,52). This epidermal structure is well developed in term infants, but in those born at 24–25 weeks of gestation the epidermis is only two to three cell layers thick and the stratum corneum is poorly developed (49). The high permeability of the skin of very preterm infants may at least partly be due to the poor development of the stratum corneum (49) and to the high hydration of the skin (53). Measurements of surface electrical capacitance (SEC) in infants born at a gestation of less than 30 weeks indicated a higher surface hydration in very preterm infants than in more mature preterm infants (54).

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Postnatally, the barrier function of the skin in AGA infants born at 25–27 weeks of gestation rapidly matures (34,35). In those born even more preterm, the process of maturation of epidermal structure may need more time. Exposure to air may be one of the factors influencing this maturation (55). In fetal rat skin explants, antenatal corticosteroid administration has been found to influence the maturation of the stratum corneum (56). No clear evidence has been presented, indicating a possible effect of antenatal steroids on skin maturation in infants.

IX. EFFECTS OF DIFFERENT AMBIENT HUMIDITIES ON THE EVAPORATION RATE

In studies of the evaporation rate from an intrascapular skin area in full-term AGA infants on their first day after birth at normal body temperature and at different ambient humidities a linear relationship was found between this rate and RH_{amb}, with a much higher ER at a low (20%) than at a high (60%) humidity. The correlation coefficient between ER and RH_{amb} was -0.986 (11) and between ER and ambient water vapor pressure (PH_2O_{amb}) it was -0.971. The same linear relationship was also found in full-term SGA infants (32) (Fig. 8).

Preterm infants also showed a linear relationship between ER and RH_{amb}, and between ER and PH_2O_{amb} (30,57) (Fig. 9), but the ER values were much higher in these infants (27,30). The relationship between ER and ambient humidity persists at higher postnatal ages of at least up to 4 weeks (35).

Regression analyses of evaporation rate on ambient relative humidity in AGA infants born at different gestational ages and studied on the first day after birth were performed in order to estimate ER at 100% RH_{amb} by extrapolation of the regression line for infants born at different gestational ages from 25 weeks up to term. The ER values obtained at 100% RH did not differ significantly from zero evaporation. This might indicate that the change in water loss from the skin in relation to gestational age at birth is due solely to changes in the permeability of the skin (40) (Fig. 10). Other mechanisms are now being studied (see below).

X. TRANSEPIDERMAL WATER LOSS IN RELATION TO ACTIVITY

Studies in infants in ventilated chambers showed an increase in IWL in relation to activity (5). With the use of the gradient method (8,9), a distinction could be made between the effect of physical activity and the effect of an

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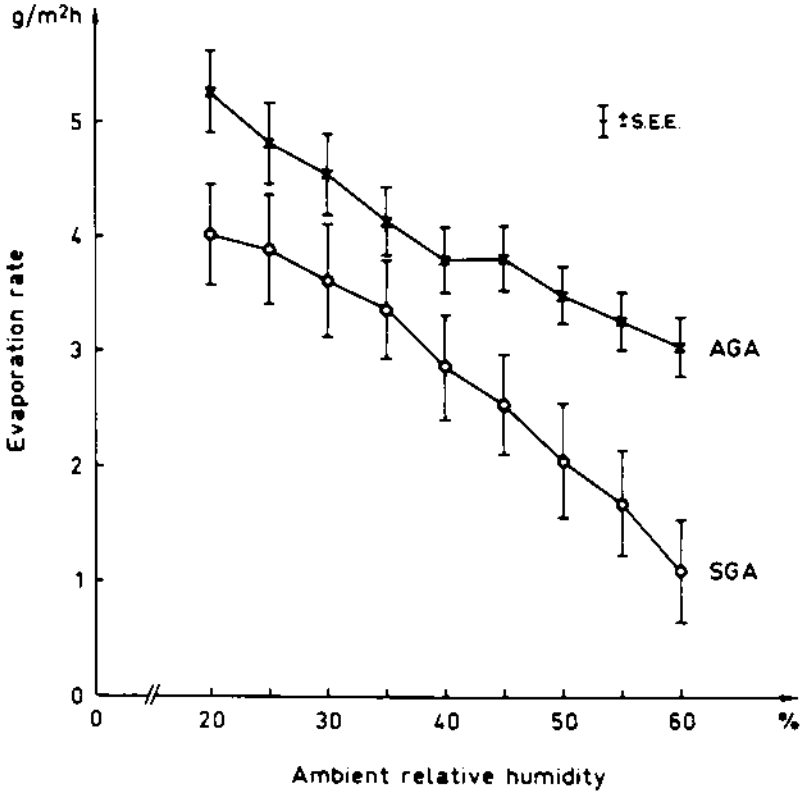

Figure 8 The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) in full-term small for gestational age infants (SGA) and full-term appropriate for gestational age infants (AGA). SEE = standard error of the estimate. (From Ref. 32.)

increase in body temperature on IWL. In full-term infants studied at a postnatal age of 1–5 hours, measurements before, during, and after a 10-minute period of physical activity and crying revealed that during a period of sleep without crying for at least 20 minutes, TEWL was 5.3 ± 1.1 g/m^2/h (SD) (29). During activity, TEWL increased to 6.4 ± 1.8 g/m^2/h (SD) (28), i.e., by 20 ± 14% (SD). The mean TEWL after a period of rest following activity was 3.9 ± 0.6 g/m^2/h (SD). The mean TEWL during activity was 37 ± 23% (SD) higher than the mean value for the periods of rest in each infant. During activity the mean skin temperature (T_{skin} abc) was 0.2°C higher than during the periods of rest ($p < 0.05$), and the mean body (rectal) temperature (T_{body}) was 0.1°C higher both during and after activity than

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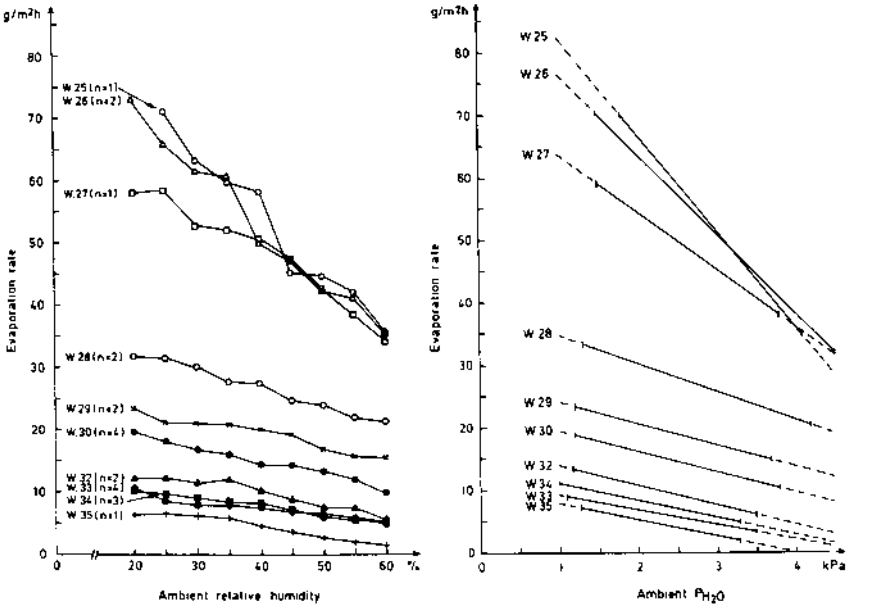

Figure 9 The evaporation rate from the skin at different ambient humidities and at different ambient PH_2O in AGA infants of different gestational age. (Left panel from Ref. 30; right panel from Ref. 57.)

before activity (29) ($p < 0.01$). In this study all infants showed a visible increase in skin perfusion during activity, but no visible sweating was observed. The small increase in skin temperature in this study can only have contributed to a minor part of the increase in TEWL during activity by increasing the diffusion rate of water through the skin. The increase in TEWL might therefore be partially mediated through the sweat glands as a consequence of an increased skin temperature and possibly activation of central control mechanisms (29).

It is clear from the above-mentioned study (29) that the increase in TEWL during activity (37%) is much smaller than the increase in IWL, by a factor of at least 1.7, observed during activity in the studies by Zweimüller and Preining (47), in which respiratory water loss was also included. Direct comparisons with earlier studies are not possible, as neither the duration nor the degree of activity (5) nor the environmental humidity (47,58) have been specified exactly in previous reports.

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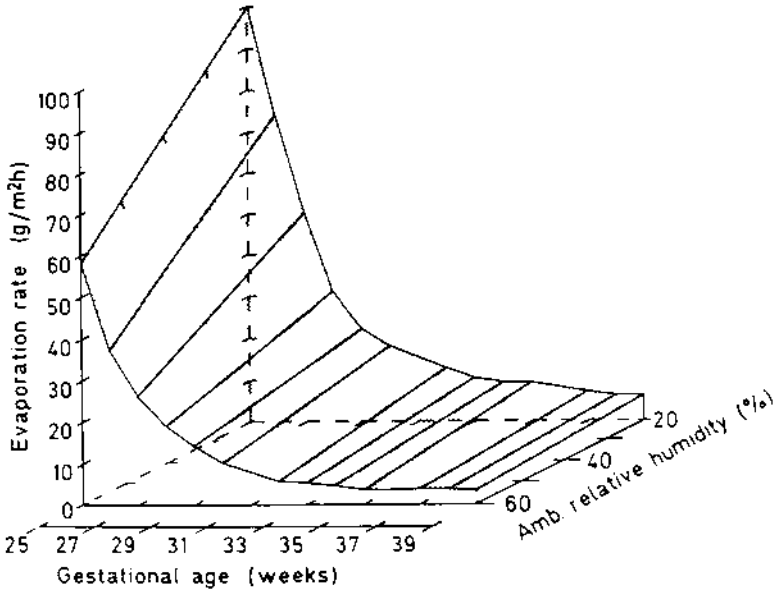

Figure 10 Regression lines of evaporation rate on ambient relative humidity at different gestational ages in AGA infants on their first day after birth. (From Ref. 40.)

XI. TRANSEPIDERMAL WATER LOSS IN RELATION TO BODY TEMPERATURE

In studies on IWL (including both transepidermal and respiratory water loss) in relation to body temperature, Hey and Katz (58) found a marked increase in water loss when the body temperature was above 37.2°C. In a later study using an evaporimeter, full-term infants placed in an incubator within 30 minutes after birth were studied while nursed in an environment with a temperature slightly above the thermal neutral range and with an ambient humidity of 50% (28). Repeated measurements showed that the transepidermal water loss changed very little, whereas T_{body} increased to 37.1°C. When T_{body} rose from 37.1 to 37.2°C, the mean TEWL increased by nearly 80%, and scattering of the individual TEWL values occurred. In a few infants, the changes in TEWL were very small (29) (Fig. 11). At a body temperature of 37.2°C visible sweating occurred, and in most infants this was followed by an increase in activity, flushing, and an increase in the respiratory rate. Thus full-term infants have a capacity for physiological sweating and can increase their loss of heat through evaporation. At a

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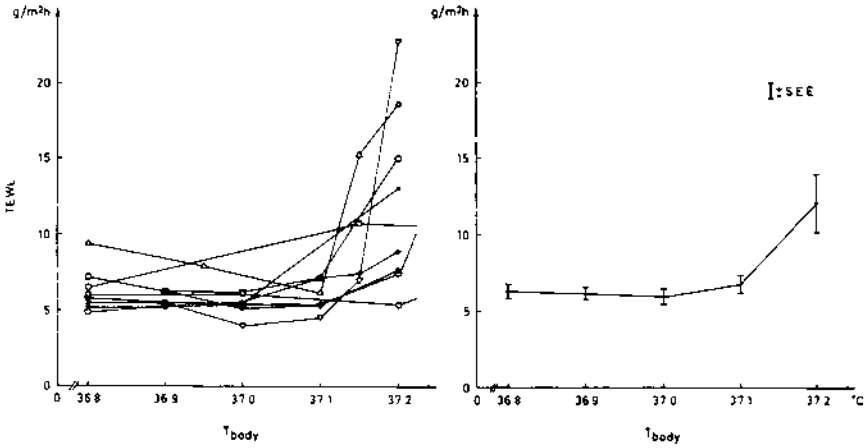

Figure 11 TEWL in relation to body temperature in nine newborn infants. Individual values on the left, mean values on the right. SEE = standard error of estimate. (From Ref. 29.)

body temperature of $\geq$37.2°C, the effects of body temperature and activity on TEWL cannot be kept separate.

In full-term infants nursed in a warm environment (41), the mean skin blood flow (Q_s) as measured with a laser Doppler technique (59,60) started to increase, simultaneously with an increase in the mean skin and body temperature, without any effect on the evaporation rate. Again in this study, many infants, but not all, started to sweat at a body temperature above 37.1°C (41). This raised a question concerning the role of central cold receptors and their interaction with peripheral heat receptors in newborn infants (61–63). Two further series of measurements were therefore made in full-term newborn infants placed in a warm environment within two hours after birth, for almost two hours (mean T_{amb} 36.6 $\pm$ 0.1°C (SD). In the first series, among the 17 infants studied, all born by caesarean section, eight started to sweat and nine did not. In the infants who started to sweat, ER increased from 7.0 $\pm$ 0.7 (standard error of the estimate; SEE) to 19.4 $\pm$ 4.8 g/m^2/h during the 2 hours in the warm environment ($p < 0.05$) and Q_s started to increase before ER increased. These infants responded to feeding with cold glucose with an immediate decrease in skin blood flow and inhibition of sweating. In infants who did not start to sweat, ER did not change at all and Q_s increased moderately from the initial value and was not altered by feeding with cold glucose (42).

In a further study on full-term infants exposed to an air temperature of 36.6°C (64), i.e., above the thermal neutral range, the body and skin tem-

peratures increased, and at the same time the difference between the oesophageal and leg skin temperatures decreased (Fig. 12A) ($p < 0.001$). Visible sweating occurred in 9 out of 14 infants, and in these infants the evaporation rate increased from 5.6 ± 2.8 to 15.7 ± 10.6 g/m^2/h ($p < 0.005$). When these infants were fed cold water, ER decreased and within 10 minutes it had returned to a value that was not different from the presweating value. Q_s increased by 42% ($p < 0.01$) up to the time of sweating and decreased by 22% ($p < 0.001$) after the infant had been fed with cold water. In infants who did not begin to sweat, no increase in the evaporation rate was observed (Fig. 12B) and the changes in skin blood flow were not statistically significant. Thus, there seem to be individual differences in the regulation of

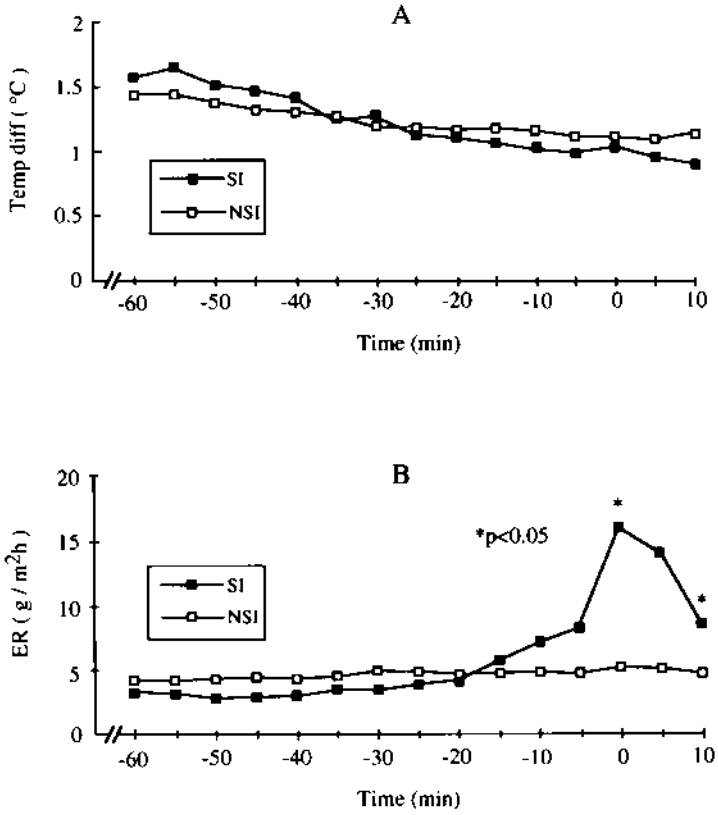

Figure 12 Mean difference between esophageal and leg skin temperature (ΔT) before and after feeding of cold water (at 0 min) in infants who started to sweat (SI) and those who did not (NSI). (From Ref. 64.)

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body temperature in newborn infants postnatally. As stated by Benzinger (65), the human thermostat is situated in the hypothalamus and works with a temperature set-point that controls sweating. There may be a delayed postnatal change in the central temperature set-point in some infants.

In full-term newborn infants nursed in a warm environment, it has also been observed that the breathing pattern becomes more irregular and the rate of breathing gradually higher with increasing body temperature (66).

XII. EFFECT OF ALBUMIN INFUSION ON TRANSEPIDERMAL WATER LOSS

Theoretically the plasma albumin concentration could influence the water transport through the skin in preterm newborn infants. In the fetus, this concentration is low and increases with gestational age (67). Albumin infusion increases the blood volume in preterm infants (68), but albumin escapes from the circulation much faster in preterm infants than in more mature individuals (69). An infusion of 0.5 g of human albumin per kilogram body weight was given through an umbilical artery catheter in 6 hours on the first day after birth in 17 preterm infants born at 25–30 weeks of gestation. This caused a significant rise in the plasma albumin concentration and simultaneously a significant decrease in TEWL from 26.6 to 23.5 $g/m^2/h$. This effect, however was, transient. The plasma albumin concentration remained constant during the last hours of infusion but returned to the preinfusion value within 2 hours after the end of the infusion. At the same time, TEWL returned to the preinfusion value. It is difficult to determine from this study whether the transient effect on TEWL is due solely to the change in albumin concentration or to a change in skin blood flow or to other unknown factors.

XIII. WATER LOSS FROM THE SKIN IN INFANTS EXPOSED TO PHOTOTHERAPY LIGHT AND RADIANT HEATERS

In the beginning of the 1970s, when measurements of insensible water loss were made with gravimetric methods, Oh and Karecki (70) found that newborn infants nursed in incubators and exposed to phototherapy light showed an increase in insensible water loss. Similar observations were made by other investigators (71,72). A few years later it was noted that the insensible water loss was very high in preterm infants nursed under a radiant heater (73). The question was raised whether this could be an effect of

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convective air currents under the radiant heater (74,75). It was also observed that a heat shield placed over the infants, probably providing a higher humidity and a lower air flow rate close to the infant, lowered the insensible water loss (75). In addition it was suggested that the increase in insensible water loss may have been due to a change in the resistance of the skin to water vapor diffusion as a direct effect of the radiant heat (76).

A. Phototherapy

Using the gradient method (8–11) in preterm and term infants studied naked in an incubator with RH_{amb} of 50% and with a controlled environment with respect to temperature and air velocity, no change in ER from the skin ($g/m^2/h$) was noted during periods of 30–120 minutes of phototherapy (77). Similarly, no changes in respiratory water loss were observed (78). In these studies (77,78), however, the ambient temperature had to be reduced somewhat during the course of the treatment to avoid heat stress and an increased body temperature. It was therefore concluded that in thermally stable infants there is no increase in insensible water loss from the skin or respiratory tract during phototherapy.

B. Radiant Heaters

In studies in preterm and term infants nursed either in an incubator or under a radiant heater, mean values for ER from the skin could be determined under these two conditions and related to the ambient vapor pressure (79). In term infants nursed in an incubator the mean ER from the skin was 3.2 ± 0.7 $g/m^2/h$ (SD), and when they were nursed under a radiant heater, this value was $4.3 \pm 0.6\,g/m^2/h$ (SD) ($p < 0.001$). The mean PH_2O_{amb} in the incubator air was 2.3 kPa and in the room air under the radiant heater 0.7 kPa. In moderately preterm and in very preterm infants there was again a difference in ER between infants nursed in incubators at 50% RH_{amb} and those nursed under radiant heaters, i.e., in a much drier environment. When radiant heater data were compared with those from infants nursed in an incubator at an ambient vapor pressure that was closer to that prevailing under the radiant heater, the difference in evaporation rate was much smaller. Thus, ER from the skin of newborn infants in thermal balance was found to depend on the PH_2O_{amb}, irrespective of whether the infant was nursed in an incubator or under a radiant heater. The higher ER under a radiant heater is due to a lower ambient humidity and not to any direct effect of nonionizing radiation on the infant's skin. Results of comparisons of ER values calculated from measurements in an incubator and corrected for the difference in PH_2O_{amb} and measured values for ER during care

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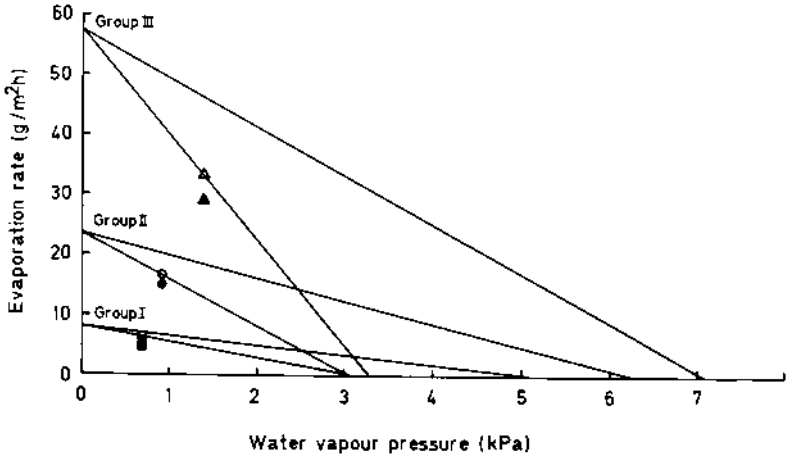

Figure 13 Calculated (open symbols) and measured (filled symbols) values for ER ($g/m^2/h$) during care under a radiant heater, together with the corresponding regression lines for ER on PH_2O at the air temperatures in the incubator and under the radiant heater, in three infants, one from each of the study groups I–III. (From Ref. 79.)

under a radiant heater are presented in Figure 13 (79). These data indicate that measured values for ER during care under a radiant heater are lower than can be expected from ER values corrected for ambient PH_2O, obtained in an incubator in representative infants born at term (group I), moderately preterm (group II), and very preterm (group III) (79).

XIV. THE INFLUENCE OF VERNIX CASEOSA ON WATER TRANSPORT THROUGH THE SKIN OF FULL-TERM INFANTS

An in vitro study of ER from a semipermeable membrane covered with vernix caseosa showed that ER fell from about $50\,g/m^2/h$ initially to about $10\,g/m^2/h$ after 3 hours. Thus, the vernix caseosa contains water that evaporates postnatally. When the same study was repeated with water placed under the semipermeable membrane covered with vernix caseosa, ER gradually fell to about 30 $g/m^2/h$ (39). Further, in full-term newborn infants ER decreased more quickly postnatally from a washed than from an unwashed skin area. This indicates that water in the vernix caseosa contributes to the ER from the skin of infants early after birth (39). Later studies with analyses of the composition of vernix have resulted in the

proposal that vernix, with its water content, may serve as a protection for the fetal skin during a critical period of epidermal barrier development (80).

XV. WATER LOSS FROM THE SKIN AND FLUID BALANCE

Many factors, such as body temperature, activity, ambient temperature and humidity, gestational age at birth, and postnatal age, all influence the loss of water through the skin. The most important of these factors is the relation to gestational age, with up to 15 times more water loss per unit surface area in the most preterm infants than in full-term infants during the first day after birth. This loss is also greatly dependent on the ambient relative humidity or vapor pressure.

TEWL is expressed in $g/m^2/h$, but a clinically more useful expression, insensible water loss from the skin (IWL_s) (g/kg/24 h) has to be used in clinical neonatology. IWL_s can be calculated from data for TEWL ($g/m^2/h$) body surface area (m^2) and body weight (kg) (36). IWL_s values for AGA infants nursed at an ambient humidity of 50% are presented in Table 1, where it is seen that IWL_s in infants born at 25–27 weeks of gestation decreased from 129 to 24 g/kg/24 h during the first 4 weeks after birth. In infants who were a little more mature, but still preterm, there was again a clear decrease in IWL_s during that period, whereas in term infants IWL_s remained almost the same during that time period (36). Data for SGA infants have also been presented (36).

The results concerning IWL_s indicate that an ambient humidity of 50% the water loss through the skin of very preterm infants may exceed the fluid volume excreted as urine during the first days after birth (81), the insensible water loss through the urine in these infants being about 40 mL/

Table 1 Mean Insensible Water Loss from Skin in 68 Newborn AGA Infants at Ambient Humidity of 50%

Gestational age (weeks)	Infants no.	Mean birthweight (kg)	Post natal age (days)					
			< 1	3	7	14	21	28
25–27	9	860	129	71	43	32	28	24
28–30	13	1340	42	32	24	18	15	15
31–36	22	2110	12	12	12	9	8	7
37–41	24	3600	7	6	6	6	6	7

Source: Ref. 36.

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kg during the first day after birth and up to 130 mL/kg on the third day after birth. Infants with a very low birthweight have a low loss of water in the stools.

The factor with the most dramatic influence on IWL_s is the ambient humidity. Infants nursed in a very dry environment have a very high insensible water loss during the first 24 hours after birth. At an RH_{amb} of 20% a very preterm infant born at 25–27 weeks of gestation will lose 20% of its body weight through IWL_s during the first 24 hours after birth. Infants of the same maturity nursed in a more humid environment of 80% will lose only 5% of their body weight through IWL_s (Table 2) (36,57,82,83).

When infants are nursed in a warm and humid environment and in good heat balance, the supply of fluid during the first days after birth can be kept fairly low, with an increase from 65 to 90 mL/kg over the first 3 days and up to 150 m/kg by the seventh day (83). When infants are nursed in RH_{amb} of 85–90%, signs of hyperosmolality or hypernatremia are uncommon.

The observation during recent years of even higher TEWL in infants born at gestational ages $\leq$24 weeks (48) indicates that in this group the skin is even more permeable to water over a longer time period. In these infants increased serum sodium values and increased serum osmolality have been observed on the second and third days after birth despite the very high ambient humidity (83). These findings imply that other factors may be involved in the transepidermal transport of water (83). If these mechanisms are located in the skin, active transport or facilitated transport of water might be alternatives. Preliminary studies have indicated expression of selective water channels in the skin of preterm and term rat pups (84), but the action of these water channels under different conditions has not yet been evaluated. No data for human infants are available at present.

Table 2 Insensible Water Loss from Skin in Infants Born at 25–27 Weeks Gestation at Different Postnatal Ages and Nursed at Ambient Humidity of 20, 50, or 80%

Ambient humidity (%)	Postnatal age (days)				
	< 1	2	3	5	7
20	205	171	105	75	63
50	130	108	65	46	40
80	53	43	26	19	15

Source: Ref. 36.

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XVI. INSENSIBLE WATER LOSS THROUGH THE SKIN AND WATER LOSS FROM THE RESPIRATORY TRACT

Respiratory water loss (mg/kg min) in infants, both full-term and preterm, depends in the first place on the temperature and humidity of the inspired air and on the respiratory rate (13) and may also be influenced by the tidal volume, dead space ventilation, and the ability to dehumidify and cool the expiratory air (13). RWL increases when dry and cool gas is inspired (13), when activity is increased (85), and with an increasing rate of breathing (86,87). The increase in IWL observed in some studies (68) may at least partly be due to the observed increased rate of breathing.

XVII. TRANSEPIDERMAL WATER LOSS AND HEAT BALANCE

Heat exchange between an infant's skin and the environment occurs through evaporation, radiation, convection and conduction (34,37,88). The heat loss by evaporation from the skin per unit area and unit time ($W/m^2/h$) is a function of the latent heat of evaporation of water (2.4×10^3 J/g) and TEWL. The loss of heat through radiation (W/m^2) depends on the temperature of the skin and the temperature of the surface facing the infant, and the heat exchange through convection (W/m^2) is dependent on the skin temperature, the temperature of the ambient air, and the air flow velocity. The heat exchange through conduction to the bedding is usually very low in newborn infants and is often omitted from the calculations.

Very preterm infants with a high ER from the skin surface also have a high evaporative loss of heat (88) (Fig. 14). These infants are all kept at a normal body temperature. As observed in Figure 14, heat exchange through radiation is fairly low in very preterm infants on account of the high ambient temperature in which these infants are nursed, which influences the temperature on the incubator walls. There is a gain of heat through convection in very preterm infants. Evaporative losses from the skin in such infants can be kept lower by nursing the infant in a high ambient humidity, thereby decreasing the evaporative heat exchange (34,88). In a dry environment (RH 20%), the evaporative heat exchange remains the most important mode of heat exchange in very preterm infants during the first week of postnatal life, whereas in a more humid environment of 60%, this form of heat exchange will be the dominating mode only during the first 5 days after birth (89).

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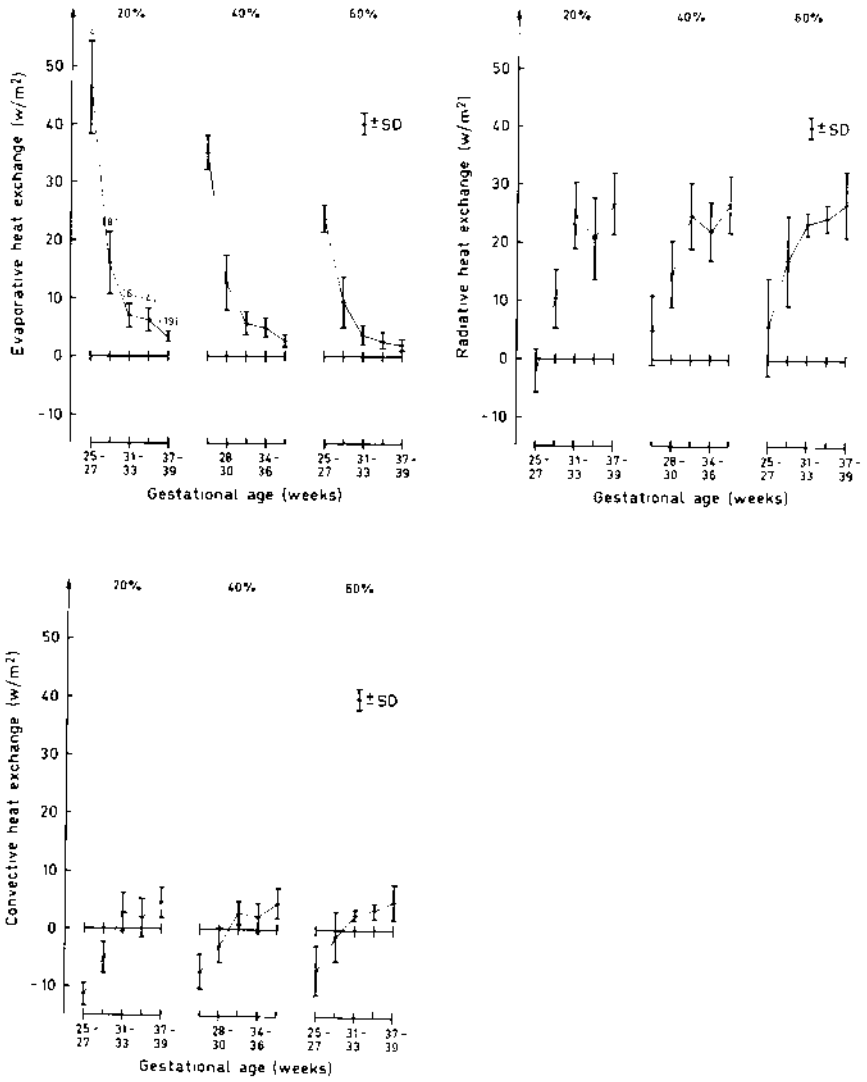

Figure 14 Heat exchange through evaporation, radiation, and convection in relation to gestational age at relative ambient humidities of 20, 40, and 60%. SD = standard deviation. (From Ref. 34.)

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XVIII. CONCLUSION

The transepidermal water loss in newborn infants depends mainly on the gestational age at birth, which determines the permeability of the skin to water, but also on other factors, such as postnatal age, state of activity, and body temperature, and whether the infant is appropriate or small for gestational age. The environmental conditions constitute other factors influencing transepidermal water loss. The most important of these are the environmental humidity and temperature. Transepidermal water loss is the principal factor in the loss of body water early after birth and has a great impact on fluid and heat balance postnatally.

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14
Percutaneous Penetration

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The skin provides the barrier between the body and the environment. The skin of the premature neonate comprises approximately 13% of the body weight, compared to only 3% of an adult (1). The surface area–to–body weight ratio of the neonate is four times that of an adult (2). Factors influencing cutaneous absorption include the physicochemical characteristics of drugs or chemicals, vehicles, and dosing conditions as well as the biological characteristic of skin (3). Biological factors such as skin thickness (4), anatomical site (5), age (6), blood flow rate (7), and skin conditioning (8) can influence permeation. The epidermal barrier of the newborn, particularly of the preterm infant is immature leading to problems of ineffective thermoregulation (9), percutaneous absorption of toxins (10), tissue injury (11), fluid imbalance (12), infection (13), and delayed healing (14).

In practice, the transdermal delivery of drugs in neonatal care is not widespread. In theory, this method should reduce the negative consequences of traditional methods of drug delivery (intravenous and oral). The introduction of an intravenous cannula is painful and stressful and may damage the insertion site. An inappropriate infusion rate can result in under- or overdosing. Small variations in infusion rate can have dramatic consequences, particularly in the newborn (15). Oral drug delivery is problematic due to unpredictable absorption from the developing gastrointestinal tract of the premature neonate (16). Oral delivery of high osmolarity drugs may also irritate the neonate's immature gastrointestinal tract. Transdermal drug delivery can, thus, provide a potentially noninvasive, continuous, and controlled delivery method for the neonate (17).

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Transdermal delivery of drugs in neonates, however, has limitations. The stratum corneum (SC) forms the rate-limiting barrier to most topically contacting substances in adult and full-term neonates. In premature neonates, the SC is of variable thickness and undergoes rapid maturational changes following birth. The thinner SC, in particular, cannot ensure effective protection from negative environmental influences including toxic exposures.

Stratum corneum lipids play a major role in barrier function. The unique mixture of lipids in adult human stratum corneum, consisting primarily of ceramides, cholesterol, and fatty acids, prevents desiccation and serves as a barrier to diffusion of substances across the skin. Knowledge of the lipid composition of the stratum corneum of the neonate is, therefore, critical to understanding barrier function development, as variation in lipid concentration may influence permeation rates across different anatomical regions.

The term infant has a well-developed epidermis, multiple cell layers thick, with a well-defined keratinized stratum corneum. In utero, the epidermis matures over a 10-week period from 23 to 33 weeks gestation (18). Infants born prematurely within this gestational period have an underdeveloped barrier. During the early part of this period, the epidermis is only a few cells thick, the stratum corneum is barely detectable, and there is little keratinization. Structural immaturity of the preterm infant's epidermis is mirrored by functional immaturity of the epidermal barrier (19).

The term infant's epidermis has barrier properties similar to those of a child or adult (12,20,21). The structure of the epidermis of the preterm infant develops rapidly after birth, so that by 2–3 weeks of age it resembles the epidermis of a term infant in structure (18) and function (21). Keratinized stratum corneum develops over several weeks. Newborn infant skin is easily traumatized by adhesive tape removal, which may lead to increased water loss and drug absorption (21).

I. ANIMAL MODELS

Most investigations have focused on the neonatal rodent as an experimental animal model, primarily because the epidermis and stratum corneum of these animals is well developed with similarities to human skin. Furthermore, their skin is hairless, obviating the need for potentially traumatic shaving. Because the animals are newborn, cutaneous exposure to environmental pollutants is minimized (22). Besides rodents, the newborn rhesus monkey and pig skin have also been investigated.

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A. In Vitro Measurements

Insofar as the SC constitutes the epidermal barrier to percutaneous absorption, the barrier is dead and, therefore, can be studied in vitro using a skin cell (23). With this method, skin removed at operation or autopsy is treated to isolate the stratum corneum and clamped between two enclosed reservoirs. Tissue culture models can also be used with this system consisting of neonatal keratinocytes and fibroblasts grown on a nylon mesh. In the dermal model proposed by Slivka et al.., for example, fibroblasts are seeded onto nylon mesh and grown for 4 weeks until a physiological dermal-like matrix is formed. This matrix consists of collagens I and III, fibronectin, and glycosaminoglycans (24). Keratinocytes can be overlaid on this matrix.

Diffusion parameters of hairless mouse stratum corneum/epidermis (SCE) resemble neonatal SCE. In contrast, adult SCE exhibits different diffusion characteristics from neonatal or hairless mouse SCE. The viability of hairless mouse SCE, as measured by glucose conversion to lactate, is comparable to human neonatal SCE. This study suggests that, if species correction factors can be developed, hairless mouse SCE may serve as a useful model for human neonatal SCE in percutaneous absorption studies (27,28).

The fetal rat also provides a convenient model for the in vivo and in vitro study of mammalian barrier ontogenesis. The barrier in this animal model forms during late gestation; i.e., on day 20, such that by day 21 of gestation, all pups demonstrate a competent barrier to excess water loss (term = 22 d) (25,26). The similarity between fetal skin and fetal lung development has allowed studies of comparative hormonal control.

In general, barrier development depends on hormonal regulation. Ballard (29) demonstrated that glucocorticoids accelerate fetal lung surfactant maturation, and subsequently, several hormones (including thyroid hormone, estrogens, androgens, prolactin) and growth factors (such as epidermal growth factor-α (TGF-α)), as well cytokines (such as interferon-α), have been shown to regulate lung development (30).

Using an in vitro fetal rat skin explant model, thyroid hormone was demonstrated to accelerate epidermal maturation, with a competent barrier forming after 2 days in culture, accompanied by morphological and ultrastructural features of barrier maturity (25,31). In contrast, other growth factors and vitamins, several of which have been shown to accelerate lung maturation, did not accelerate barrier formation in vitro. Agents tested and found to have no effect in this model system include (31):

1. Peptide growth factors and cytokines, i.e., IGF-1, EGF,KGF,TGF-α,TGF-β1, IL-1α, and TNF-α
2. Retinoids, all-*trans*-retinoic acid and 9-*cis*-retinoic acid
3. 1, 25-Dihydroxyvitamin D_3.

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The absence of an effect on barrier maturation following addition of a growth factor, hormone, or vitamin to the incubation medium, however, does not exclude a potential role for that substance in barrier ontogenesis, because these tissues may have been exposed previously to sufficient levels of this factor in utero to permit barrier maturation to proceed in vitro. Moreover, some factors could be generated locally during organ culture in quantities sufficient to maximize their effect on barrier maturation (25).

The role of the sex hormones estrogen and testosterone in fetal rat barrier maturation has been investigated in submerged organ culture. In this model system, estrogen accelerates barrier formation, increases stratum corneum thickness, leads to deposition of lipids in a membrane pattern in the stratum corneum, and augments formation of mature lamellar unit structures (32). In contrast, testosterone delays barrier maturation both in utero and in vitro, delaying the reorganization of initially secreted lamellar material into lamellar unit structures (32).

These studies indicate the complexity of organization of the SC, which provides both a permeability and a mechanical barrier between the outside environment and the internal milieu of the organism. The SC is composed of corneocytes, which provide strength and rigidity, and an extracellular lipid-enriched matrix, providing a barrier to water transit (33–34).

Corneocytes are postapoptotic, anucleated cells, consisting of proteins, such as involucin and loricrin, cross-linked by transglutaminase and cytoplasmic keratin filaments aggregated into macrofibrils by filaggrin (35).

Many of the important barrier properties of the epidermis to percutaneous transport are due to the extracellular lipids in the SC which are enriched in ceramides, cholesterol, and fatty acids. These barrier lipids are organized as multiple lamellar membranes (33) and are delivered to the extracellular space as a mixture of polar precursors by the exocytosis of lamellar body contents. Lamellar bodies contain abundant quantities of glucosylceramides, whose catabolism to ceramides, by the enzyme β-glucocerebrosidase, is essential for normal permeability barrier function (36,37). Permeability barrier function in the epidermis is closely linked to epidermal lipid metabolism. Disruption of the permeability barrier stimulates epidermal lipid synthesis (38,39). Increased cholesterol synthesis in the epidermis, for example, follows an increase in the activity of HMG CoA reductase (40,41), whereas increased sphingolipid synthesis follows an increase in serine palmitoyl transferase (SPT) (42).

Skin culture from fetal rats can be used for determining SC enzyme activity (43) and the role of air exposure (xeric stress) in inducing accelerated SC and barrier formation. Air exposure leads to an accelerated appearance and an overall increase in the activity of key enzymes required for the formation of glycosylceramides and cholesterol sulfate. Glycosyl-

ceramides are required for the formation and secretion of normal lamellar bodies (44) and cholesterol sulfate for normal SC cohesion (45). Peak activity of these enzymes facilitates the accelerated formation of a functional SC.

Air exposure also increases the activity of β-glucocerebrosidase and steroid sulfatase, enzymes that function primarily in the SC rather than the Malpighian epidermis (46,47). β-Glucocerebrosidase catalyzes the cohesion of glucosylceramides to ceramides crucial for the formation of mature extracellular lamellar membranes and a competent permeability barrier (48). Steroid sulfatase in the SC catalyzes the conversion of cholesterol sulfate to cholesterol, an enzymatic step essential for both normal desquamation and barrier function (45,49).

Thus, air exposure of fetal skin explants stimulates the activity of epidermal GC synthase, CSTase, β-glucocerebrosidase, and steroid sulfatase. These lipid enzymes are crucial for the formation and function of the SC. Air exposure also stimulates the expression of structural proteins, such as involucrin, profilaggrin/filaggrin, and loricrin, which are required for formation of cornified envelopes and normal corneocyte function (43).

Knowledge of the biochemical and structural requirements for epidermal barrier development and the factors affecting such development can be used to study the percutaneous absorption and metabolism of drugs that may behave in a different manner in neonatal skin as compared to the skin of older animals and adults. Dinitrochlorobenzene (DNCB) application, for example, has been studied in the skin of newborn and 26-day-old rats utilizing the flow-through diffusion cell. In this system, DNCB penetrated neonatal rat skin much less than adult rat skin. Because it contained higher levels of GSN than 26-day-old rat skin, a far greater proportion of the DNCB was metabolized (50).

Drug metabolism may be affected by different cell populations in the epidermis. Coomes et al. (51) demonstrated that two populations of epidermal cells (sebaceous and basal) can metabolize benzo(a)pyrene and 7-ethoxycoumarin. In particular, sebaceous cells were highly active in metabolizing these xenobiotics. The high level of xenobiotic-metabolizing enzyme activities may relate to the presence of steroid hormone receptors under hormonal control. Pohl and Fouts (52) also showed that Zymbal's gland, a specialized sebaceous gland, contains inducible cytochrome P-450–dependent xenobiotic-metabolizing enzymes. Bickers et al. (53) demonstrated that the catalytic activities of cytochrome P-450–dependent monooxygenases are highest in the epidermis of neonatal Sprague-Dawley rats.

Das et al. (54) reemphasized that the epidermis is the major component of the skin of neonatal mice in which cytochrome P-450-dependent metabolism of benzo(a)pyrene and benzo(a)pyrene-7,8-diol occurs. This

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skin compartment is also a major site for carcinogen-DNA adduct formation and enzyme-mediated mutagenicity.

B. In Vivo Measurements

For the most part, the skin of rats and mice has been used for the study of the metabolism of different drugs. A single topical application of benzo(a)-pyrene-4-5-oxide (BP-4-5-oxide) to neonatal rats, for example, resulted in the formation of benzo(a)pyrene-4-5-dihydrodiol (BP-4-5-dihydrodiol) and water-soluble metabolites (55). The metabolism of BP-4-5-oxide to BP-4-5-dihydrodiol by rat skin is time-dependent (55); the formation of BP-4-5-dihydrodiol in the skin was maximum at 2 hours after topical application of BP-4-5-oxide to the rat.

Topical application of the polychlorinated biphenyl mixture Aroclor 1254 to neonatal rats results in a near doubling of hepatic epoxide hydrolase (EH) activity without any detectable effect on skin enzymes (22). Cutaneous application of Aroclor had earlier been shown to result in the induction of epoxide-degrading enzymes in liver (EH and glutathione-S-transferases), which are also induced by topical application of Aroclor 1254. NADPH–cytochrome *c* reductase was not induced in skin or liver following topical Aroclor treatment.

Asokan et al. (56) studied the effect in neonatal rats of a single topical application of several nitroarenes (1-nitropyrene, nitropyrenes mixture, nitrobenzo(ghi)perylene mixture, 3-nitrofluoranthene, nitrofluoranthene mixture, and nitropyrene mixture) and their corresponding parent arenes on hepatic and cutaneous drug and carcinogen metabolism. Topical application of each nitroarene (10 mg/kg) resulted in significant induction of aryl hydrocarbon hydroxylase, 7-ethoxyresorufin-O-deethylase, and 7-ethoxycoumarin-O-deethylase activities in both skin (1.5- to 14.6-fold) and liver (1.3- to 41.9-fold). Their studies suggest that nitroarenes are inducers of hepatic and cutaneous monooxygenases in neonatal rats after topical administration.

The newborn rhesus monkey provides an evolutionarily close model for the study of percutaneous transport across neonatal human skin. Wester et al. (57) compared the absorption of testosterone in the full-term newborn to the adult. Absolute flux was similar. Values for the newborn are shown in Table 1. Of interest, when a topical dose of 40 $\mu g/cm^2$ was applied to the ventral forearm and the area was occluded with plastic and adhesive tape for 24 hours, percutaneous absorption was 14.7%, a value twice that obtained from nonoccluded absorption (compare to Table 1).

This study showed that a high percentage of a steroid could be absorbed through the skin of an infant. The study also suggested an inter-

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Table 1 Percutaneous Absorption of Testosterone in Newborn Rhesus Monkey

	% dose absorbed[a]	
Time (h)	4 μg/cm^2	40 μg/cm^2
0–24	6.3 ± 0.9	1.3 ± 0.4
24–48	8.0 ± 0.7	2.1 ± 0.8
48–72	4.3 ± 0.2	1.6 ± 0.6
72–96	2.5 ± 1.1	1.0 ± 0.1
96–120	1.4 ± 0.4	0.7 ± 0.2
Total	22.5 ± 2.2	6.8 ± 2.1

[a]Mean values and standard derivations of three animals. Topical values were corrected for incomplete urinary excretion with the formula:

$$\% \text{ absorbed} = \frac{\% \text{ urinary radioactivity topical}}{\% \text{ urinary radioactivity iv}} \times 100$$

esting relationship between the skin surface area of a newborn and the system availability of the applied compound. Once the compound and/or metabolites are absorbed, they are available systemically. In the newborn, the ratio of surface area to body weight is three to four times that of the adult. Therefore, given an equal application area of skin for the newborn and adult, the systemic absorption seen in the newborn can be much higher when based on kilograms of body weight (Table 2).

Therefore, by topically applying the same strength compound to both the adult and the newborn, the systemic availability in the newborn is 2.7 times that of the adult. With a different ratio of skin surface to body weight, the therapeutic ratio probably is lower in the newborn than in the adult when the compound is topically applied. In designing a transdermal drug-delivery system for newborns, this increased systemic availability must be interrelated with any differences in systemic metabolism between the newborn and the adult.

II. HUMANS

The stratum corneum of a premature neonate is much thinner than that of adults or full-term infants (18,58). Full-term infants (40 weeks gestational age) have a competent barrier, which is comparable with that of adults. In contrast, very low birthweight premature infants have far less developed dermis/epidermis at birth and consequently are ill equipped to cope with

Table 2 Systemic Availability in Newborn and Adult Following Topical Application[a]

	Adult	Infant
Surface area	17,000 cm^2	2200 cm^2
Topical dose	100 mg	13 mg
Patient weight	70 kg	3.4 kg (neonate)
Systemic dose	$\frac{100\,mg \times 0.2\,abs}{70\,kg} = 0.28\,mg/kg$	$\frac{3\,mg \times 0.2\,abs}{3.4\,kg} = 0.76\,mg/kg$

[a]Systemic dose = mg/kg; compound = 20% absorbed.

ex utero conditions (59). A number of different in vitro and in vivo systems have been developed for assessing epidermal barrier function during development.

A. In Vitro Measurement

The epidermal barrier to percutaneous absorption can be studied in vitro using a skin cell (23). Using this method, the drug is introduced into the reservoir on the outer surface, and its appearance on the dermal side is measured (19). This method has been used for study of percutaneous absorption of alcohols, using abdominal skin obtained at autopsy (60). McCormack et al. (60) found that, although skin sections from adults and term infants were relatively resistant to alcohol penetration, the skin of preterm infants was more permeable by a factor of 3–50 times, depending upon the alcohol studied. The same method was used for studied salicylate absorption in term and preterm infants (61). Percutaneous absorption varied greatly with maturity and was 100–1000 times higher in infants born at 26 weeks gestation than in an infant at term.

B. In Vivo Measurement

In general, drugs can penetrate more readily through the skin of preterm neonates than that of adults. Transdermal drug delivery, therefore, may offer advantages compared with the traditional intravenous or oral routes of drug administration used in neonatal intensive care. The transdermal route of drug delivery is potentially a convenient, painless, and noninvasive method of drug therapy without the difficulties, discomfort, and scope for error of intravenous or oral administration (62).

Topical application of 4% amethocaine gel (Ametop), for example, has been used for local anesthesia in neonates (63). Amethocaine gel was

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applied to the dorsum of one foot and the foot was occluded for one hour. Local anesthesia was then assessed by eliciting the cutaneous withdrawal reflex in response to stimulation with a series of graded nylon filaments. Over half (54.8%) of the infants treated with amethocaine showed evidence of local anesthetic action. Thus, topical amethocaine gel has a local anesthetic action on neonatal skin, which merits further investigation (63). Focus on the use of penetration enhancers or adjuvant methods for improving transdermal delivery may greatly augment efficacy.

Amato et al.. (64) demonstrated that the topical application of caffeine was an effective treatment for neonatal apnea in a study of 57 low birthweight infants (< 1500 g birthweight). One focus of this study was the determination of the effect of anatomical and functional immaturity of the epidermal barrier on the transdermal absorption of drugs and chemical agents. These investigators studied the efficacy of percutaneous application of caffeine using high-pressure liquid chromatography for evaluation of plasma levels. Doses of caffeine were graded for babies less than 1000 g (15 mg) versus babies greater than 1000 g (20 mg). Caffeine was applied transcutaneously in the form of a gel to the abdominal skin in a standard dose equal to 0.06 g of gel equivalent to 10 mg of caffeine citrate. The gestational age of the babies was 29.4 ± 1.7 weeks, mean birthweight 1025 ± 240 g. The mean postnatal age at the beginning of treatment was 25.5 ± 18 hours. Of the treated babies, 73% had serum levels in the therapeutic range 48 hours after the first 5 doses of caffeine application. After 10 doses, 97% of patients had serum levels in the therapeutic range. The authors concluded that percutaneous caffeine application may be a useful approach for treatment of apnea in premature infants.

Nachman and Esterley (65) noted that the α-adrenergic agonist phenylephrine caused blanching of the skin in premature infants. Phenylephrine drops are commonly used to dilate the pupil for funduscopy, and it was noted that if excess drops ran down the baby's cheek, they left a trail of blanched skin. They applied a drop of 10% phenylephrine to the skin of infants of differing maturity and age and observed a blanching response. Infants below 34 weeks gestation showed a marked response, although by 3 weeks of age, the responsivity had disappeared. Full-term infants showed no response. Harpin and Rutter (21) quantified this technique further by using solutions of different strength (1% and 10%). Percutaneous absorption was most marked in infants below 30 weeks gestation in the first few days of life. By 2 weeks of age, the blanching response was weak in the cohort of preterm infants, suggesting a rapid maturation of the epidermal barrier. Stripping of the stratum corneum with adhesive enhanced absorption (19).

Successful transdermal therapy in the neonate has been achieved with theophylline and caffeine, with therapeutic blood drug concentrations being

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achieved for up to 7 days after topical application (62). Several other drugs in common use in neonatal intensive care therapy have potential for transdermal delivery. There is a need, however, for further development of neonatal transdermal delivery systems that provide controlled rates of drug delivery. Challenges posed for such systems include (a) rapid alteration in the epidermal barrier following transition to a dry environment after birth (particularly in very low birthweight preterm infants), (b) high surface-to-volume ratios for neonates, and (c) possible differences in systemic metabolism between newborns and adults, making extrapolation of effective dosing more difficult. The advantages of transdermal drug delivery, however, make this mode of therapy an attractive alternative to conventional oral and intravenous routes.

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15
Adhesion and Newborn Skin

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Adhesives are applied and removed many times a day in the typical neonatal intensive care unit (NICU). Premature and full-term infants who require medical interventions and constant observation are regularly exposed to adhesive placement for a wide variety of indications. Adhesives are used to secure critical life support equipment such as endotracheal tubes, intravenous and arterial catheters, and chest tubes, as well as monitoring devices such as electrocardiogram electrodes, pulse oximeter probes, and temperature sensors.

The need for secure attachment of life support and monitoring devices creates a dilemma for the protection of an underdeveloped epidermis. The constant attachment and reattachment of adhesives, which can result in skin trauma and barrier disruption, challenges the delicate newborn skin surface, particularly in very low birthweight preterm infants. A nursing research utilization project involving 2820 premature and term newborns in 50 nurseries in the United States found that adhesives were the primary cause of skin breakdown among NICU patients, occurring on the extremities most often, followed by the trunk and face (1).

Injury from adhesives is not only an unsightly source of discomfort for newborns and caregivers in the NICU, it can also lead to other morbidity, especially in the very low birthweight infant. Significant disruption of the epidermis from adhesives interrupts the already deficient barrier function of the premature infant's skin (2–4). An increase in transepidermal water loss

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(TEWL) and evaporative heat loss follows, necessitating more frequent monitoring of fluid status, increased fluid intake, and stringent temperature measures to compensate for heat losses. The compromised skin barrier places the infant at higher risk for toxicity from topically applied substances such as disinfectants (5,6) and provides a significant portal of entry for pathogens, especially with microorganisms that are already colonized on the skin surface such as coagulase-negative staphylococcus (7,8) and fungi (9). In addition, the wounded epidermis requires additional calories and nutrients for tissue repair processes, which are often difficult to provide, due to the necessary fluid restrictions and limitations in nutrient delivery for these tiny patients.

Thus, the problems posed by the use of adhesives in the NICU require careful consideration and knowledge. In this chapter, the basic principles of adhesion are explained, including the fundamentals and terminology of skin adhesion, commercially available materials used in skin adhesives, and the testing of skin adhesives by industry. Following this, adhesive use in the NICU and a review of existing research are explored. Finally, some of the future goals for product use and development are identified.

I. PRINCIPLES OF SKIN ADHESION

The adhesion of a material to skin occurs when the adhesive material comes into intimate contact with skin and the intermolecular forces across this interface are maximized (10). These intermolecular forces are a sum of many types of forces, including dipole-dipole interactions, dispersion forces, hydrogen bonds, covalent bonds, and diffusion. Dispersion forces and dipole-dipole forces (collectively called van der Waals forces) are very small, but they occur everywhere and can lead to large adhesive forces.

Virtually all of the adhesives that are applied to skin are pressure-sensitive adhesives (PSAs). They are termed ''pressure sensitive'' because they will flow when a slight external pressure is applied to the adhesive device. Initially, the PSA will contact only the outermost points on the surface of the skin. With time the adhesive will flow laterally and downward into the folds, creases, and pores of the skin. This process of flowing and spreading of the adhesive on the surface of the skin is termed ''wetting'' or ''wet-out,'' and a bond will form between the adhesive and the skin. As this wetting process continues, the adhesive bond will continue to build strength. This process can be accelerated by the addition of a tackifier into the adhesive. Tackification results from the addition of certain low molecular weight materials into the adhesive polymer matrix. While this process provides a very perceptible tack to an adhesive product, it also helps to increase the

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cohesive strength of the adhesive when it is under stress. Tackifiers are usually derived from natural products, such as rosin or citrus peels, or from petroleum streams (11).

A good PSA requires several attributes. The adhesive should be viscoelastic in order to achieve intimate molecular contact. This means that it should be able to flow into the smallest valleys of a surface but not flow beyond its intended application area. It should be aggressively and permanently tacky and should adhere with simple finger or hand pressure. It should require no activation by water, solvent, or heat. It should provide a strong holding force and have sufficient cohesiveness and elasticity that it can be removed from surfaces without leaving a residue (12). These attributes arise primarily from the molecular architecture of the adhesive polymer. A PSA is compositionally a liquid, but its extremely high molecular weight will provide it with an appropriate viscosity and elasticity.

For a pressure-sensitive adhesive to stick to skin or to any surface, the surface energy of the adhesive should be less than that of the surface to which it is being applied. The surface energy of a material is a function of how tightly bound its molecules are to each other relative to its surroundings. For an adhesive to be willing to "expose itself" to a surface (such as skin), it should have a surface energy less than skin. Surfaces, including skin, have both lipophilic and hydrophilic components. In the case of skin, these components provide a total surface energy of about 40 mJ/m^2. Skin surfaces having a high level of sweat/sebum will have an appreciably higher surface energy (13). The surface energy of the adhesive, therefore, must not be any greater than 40 mJ/m^2 to be able to effectively wet out the skin. Though having an appropriate surface energy is a prerequisite of a pressure-sensitive adhesive to wet out a surface, this feature alone does not guarantee that an adhesive will be strong. Commercial adhesive manufacturers design their skin adhesives to have both polar (hydrophilic) and nonpolar (lipophilic) components. The polar components are primarily there to provide resistance to adhesive shearing (14).

Besides having an appropriate surface energy, an adhesive formulation must have an appropriate viscoelasticity for both fast flow (for quick stick) and good cohesion (for long-term wear), particularly if it is used for fixating a device. An adhesive must be able to perform while being subjected to external intermittent forces. These small forces generally come from either direct skin flexion or external tugs and pulls that transmit through the fixed appliance. When the external forces exceed the capacity of the adhesive device, failure occurs. Thus, a well-designed skin adhesive needs to perform over an extended period. Concurrently, a skin adhesive needs to withstand the insult from sweat, sebum, and salts. And though the skin temperature itself is fairly constant, varying ambient temperatures may require a skin

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adhesive to perform over a modest temperature range. In addition, while a tackifier will help to provide immediate adhesion, the long-term flow of an adhesive must be minimized. This is especially important while the product is stored in a package waiting to be used.

An important property of an adhesive polymer is its glass transition temperature (T_g). The T_g of a polymer is that temperature (range) over which it changes from a stiff (or glassy) material into a rubbery material. The glass transition temperature of a skin adhesive must be below the temperature of the skin/adhesive interface (typically 31–34°C) for the adhesive to be mobile enough to flow. More importantly, the adhesive material should be below a certain level of stiffness (modulus) to become tacky at that temperature range. The value of this so-called Dahlquist criterion for tack is 3×10^5 Pa (15). To reduce the stiffness of an adhesive material to below this criterion and to achieve good long-term performance properties of the skin, adhesive manufacturers have traditionally relied on the use of a tackifying resin. The tackifier and adhesive resin become homogeneous when mixed. This leads to two benefits. First, the modulus of the tackified adhesive is lowered over the temperature range of use (allowing for better tack). Second, the T_g is raised, effectively giving the adhesive better holding power during wear. This is due to the inverse relationship between temperature and rate (Fig. 1). Should the adhesive appliance be suddenly sheared from an external force (such as rubbing against a bed sheet), the higher modulus of the adhesive would cause the appliance to resist shearing or

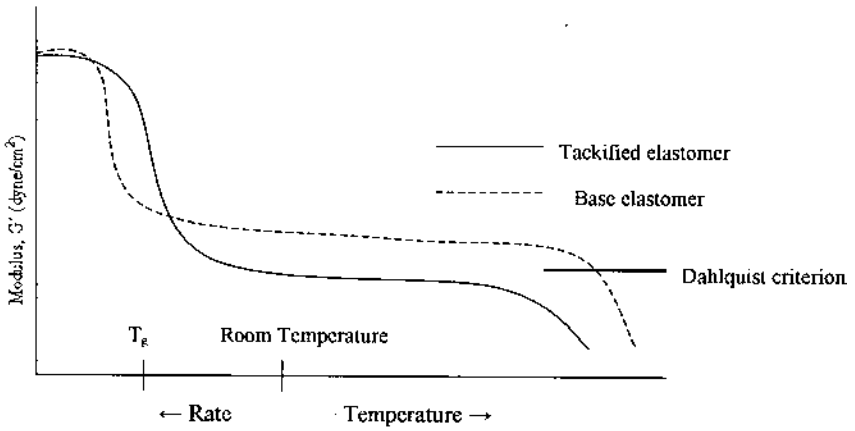

Figure 1 Dynamic mechanical spectrum of a PSA-based elastomer in the tackified and nontackified state. The tackified elastomer has a plateau modulus below that of the Dahlquist criterion. (From Ref. 11.)

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peeling from the skin (at this higher rate). A useful analogy is the behavior of silly putty. It is easily kneaded with one's hands (at a low rate) but will bounce off a wall when thrown (at a high rate).

Adhesive manufacturers have also incorporated reinforcing monomers, such as acrylic acid, to achieve a good balance of properties (16). Cross-linkable monomers (chemical- or photo-induced) as well as low molecular weight ''macromers'' have also been incorporated into the adhesive polymer and used to adjust the short-term and long-term flow of skin adhesives (17).

A. Application and Removal of Adhesive Products

To achieve optimum adhesion with an adhesive appliance, the skin should be cleaned, dried, and clipped of hair if possible; thus, hair found on the heads of neonates can sometimes cause adhesive appliance failure, as it is a physical obstruction between the adhesive and skin. Isopropyl alcohol is often used for preparing the skin of adults, but this would be inappropriate for use on neonate skin due to its drying effect and potential for skin injury (18,19). Very few commercial skin adhesive products have the inherent chemistry or structure to deal with significant amounts of surface moisture, so starting with a dry skin surface will allow an adhesive to perform optimally. Properties of ''restick'' are also not generally built into pressure-sensitive adhesives, as adhesives are designed to have rapid intimate contact with skin. The expected tack of an adhesive device upon reapplication (even within seconds after its initial contact with skin) will not be there, as the adhesive will have already bonded to corneocytes, sebum, salts, and other surface materials. These contaminants will interfere with the ''rewetting'' of the adhesive, although some soft/thick PSAs may ''engulf'' the surface contaminants, and the adhesive surface can renew itself.

Because skin adhesive products are pressure sensitive, the caregiver should ensure that the adhesive appliance is pressed upon with the thumb and fingers immediately after application. This will promote good wetting of the adhesive and help provide good initial product performance. If moisture is initially present on the skin, some hydrophobic adhesive systems will repel the moisture away from its source, while some hydrophilic adhesive systems will absorb moisture and transmit it through the adhesive and backing. Obviously, not all adhesive products will stick to all skin types. The surface of skin is highly variable, and its dynamic biological features will cause some adhesive products to fail prematurely. Studies have shown that adhesion to adult skin, as measured by an adhesive peel force, correlates to several biological parameters. These include sebum, moisture, pH, body location, and gender. The production of sweat underneath an adhesive device, followed by skin drying, can cause even so-called gentle tape products to bond

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very tenaciously to skin. Moreover, while the skin of individuals that produce a relatively high level of sebum may be a slight detriment to the initial adhesion of a device, sebum production over a 2- to 6-hour time period after device application appears to plasticize the adhesive, causing it to spread and ultimately bond more tightly to the skin (20).

Andrews et al. (21–24) and others (25) have published works that examine the failure mechanics of adhesive peel from skin and other test surfaces in an attempt to model the energy transfer processes that occur. The ''strain energy density'' that develops during the peeling process must be successfully transferred from the appliance backing, through the adhesive, to the skin/adhesive interface without causing extraordinary trauma and pain to the skin or leaving a visible residue. The skin is nearly always stretched during the peeling process, and it, too, will build strain energy before the breaking of the adhesive bond (Fig. 2). This results in the inevitable ''skin stripping'' that occurs during adhesive removal, as there will always be corneocytes that have relatively weaker bonds to each other than to the adhesive. Because skin stripping directly relates to the weakened boundary between skin layers, this problem will be compounded if the region becomes overhydrated. Keeping the skin relatively dry prevents the corneocytes from reaching a saturated hydration level and allows them to maintain a higher cohesive strength. This reduces their tendency to be stripped off in large ''cell clusters'' rather than individual cells. Unfortunately, there has been little practical evidence showing that adhesive products can maintain functional performance without accelerating the loss of corneocytes upon appliance removal. Studies have shown that the amount of stripped corneocytes from tape peeling generally correlates to

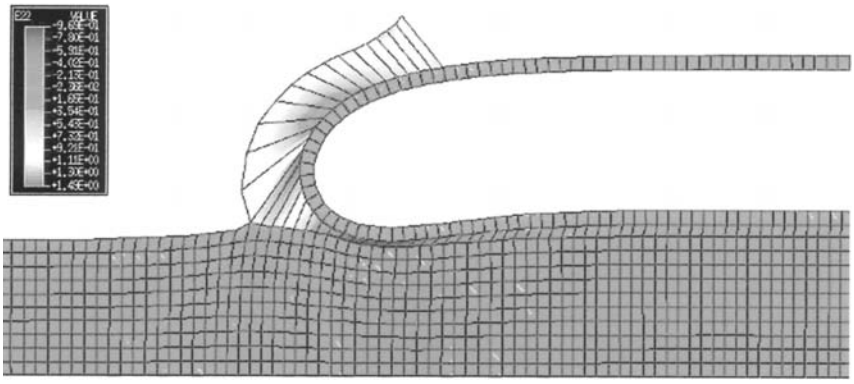

Figure 2 Model of the strain distribution of a peeling PSA from a flexible substrate such as skin. (From Jianguang Du, 3M Company.)

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the peel force of the initial tape pulls. They have also shown that the strength of the stratum corneum can be a limiting factor to obtaining a high peel adhesion value when the skin becomes saturated under an occlusive tape (20,26). Repeated applications of fresh adhesive tape strips to adult noncompromised skin sites will result in successively higher peel adhesion forces. This is due to the removal of loose corneocytes in the outer layers of skin. This trend typically continues for four repeated adhesive applications before leveling off. If tape continues to be applied to that same site, the peel adhesion force will begin to decrease due to a thin layer of interstitial fluid rising to the surface of skin. This will result in poor skin/adhesive contact (20).

A common technique for assessing the holding power of an adhesive tape is the measurement of its peel force as it is removed from a substrate. These measurements have been used from the time of the earliest development of adhesive tapes. Adhesive manufacturers use commercial peel testing equipment such as Instron, MTS, or IMASS testing machines. While these instruments can be adapted to test tapes from skin, it is often convenient to use smaller (and more portable) force transducers that can be brought into close proximity to the skin of test subjects. In the case of skin adhesives, peel force measurements are usually made from the forearms or the backs of healthy adult volunteers. The back is a good surface because it is relatively flat and has a high surface area for testing many specimens. The evaluator of adhesive sample performance must try to minimize the variability in sample application and removal. Adhesion assessment methods must be validated over a large population of subjects, as there can be many sources of variability. While a clinical laboratory and the associated test equipment requires a certain level of investment for a developer of skin adhesives, it should be noted that a suitable synthetic skin test surface for evaluating adhesives does not exist. A number of efforts have attempted to duplicate such a surface for in vitro adhesion testing (27–30), but human skin is still the gold standard, and any serious efforts at improving the properties of skin adhesives require the evaluations to be performed on skin. The dynamic biology of the skin surface cannot be duplicated in vitro, and different classes of adhesives (e.g., hydrophilic vs. hydrophobic) cannot be fairly evaluated and compared when tested on a synthetic surface. And while data obtained from peel tests on skin help to optimize an adhesive formulation, the ultimate clinical screening test is the overall performance of the skin adhesive product during wear. A number of assessments are made during wear testing and product removal, including edge lift, tunneling, roll-up, sebum spotting, adhesive residue, and subject comfort (20).

Peel adhesion studies have looked at the effect of peel angle, peel rate, and tape backing stiffness on the peel force of skin adhesives (31) and those

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effects on skin (32), in an effort to optimize the techniques for tape removal from skin. While the elastic properties of skin will help to dampen the effects of peeling an adhesive too quickly from skin, a higher peel force will accompany a "quick peel." And while conventional thinking states that a higher peel force exhibited by a product will correspond to better adhesive wear time, a product that exhibits a higher peel force may also predispose it to producing a higher degree of skin trauma. A number of techniques are used to minimize the adhesive peel force upon appliance removal (33). One technique relies on slowly "pulling" adhesive appliances at a very low angle to the skin while holding the surrounding skin in place. This technique is dependent on the appliance having a stretchy, compliant backing such as would be found on IV dressings like Tegaderm™ and OpSite™. This technique takes advantage of the fact that, while these products develop a high strain energy density while being pulled, the actual peel force on the skin is relatively low. This concept has been exploited in a variety of non–skin-adhesive applications and has now appeared in products such as the new Ease-Off™ first aid dressing.

Besides the concept of using a stretchable backing at a low-angle pull, other adhesive concepts have been commercialized and promoted to reduce irritation and pain during adhesive removal. One concept is the use of a perforated soft silicone adhesive. The adhesive is purported to have very intimate contact with the skin, thus distributing the peel force over a large area. This allows the appliance to be removed before the pain threshold is reached. Skin stripping is also greatly reduced (34). The perforations allow moisture to diffuse away from an exuding wound. Another long-held concept is the use of an adhesive product that is designed to keep the covered skin dry during wear. These products incorporate the use of a discontinuous adhesive and an open-structured backing, typically made from a woven or nonwoven material. Examples include the so-called paper tapes, such as Micropore™ (35), as well as the Comfort Strip™ first-aid dressing. Conversely, utilizing a tape product that has both a soft (highly plasticized) adhesive and an occlusive backing, but which is intended for only short-term (< 4 h) use, may lead to a weak skin/adhesive interface and thereby reduce skin trauma as it is peeled. Examples of this type of concept are Baby Tape™ and Yu-ki Ban™ (sold primarily in Japan).

B. Categories of Skin Adhesives

While natural products dominate the long history of adhesives used on skin, synthetic polymers, particularly acrylate copolymers, have dominated the modern era of skin adhesives. Synthetic polymers lend themselves to being

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tailored for specific stick-to-skin applications. For example, the copolymer family of 2-ethylhexyl acrylate and acrylic acid is a common adhesive system that provides adhesion to both dry and slightly moist skin. This is also a good system for dissolving pharmaceuticals used in transdermal drug-delivery systems (36). This class of adhesives can undergo light to moderate crosslinking, providing long-term stability and minimizing cold flow. Crosslinking can occur from ultraviolet (UV), gamma, or electron-beam irradiation of the coated or extruded adhesive. These processes will also help to eliminate much of the residual monomer that may be present from the polymerization step.

Natural rubber adhesive (latex) is still found on athletic tapes and some cloth adhesive tapes, but adhesive manufacturers have generally phased out their U.S. product lines that contained this adhesive. While natural rubber has shown to be an inexpensive and reliable adhesive to skin, it cannot be produced in a 100% pure form and thus has been implicated as a skin sensitizer. Other natural products, however, are still used as tackifiers as previously mentioned.

Another category of adhesives is based on polyisobutylene. Polyisobutylene is primarily used as the matrix for ostomy and hydrocolloid wound dressing products. Typically, high and low molecular weight polyisobutylene elastomers are milled together with tackifiers, crosslinking agents, and water-absorbing components. These so-called hydrocolloid components include pectin, carboxymethylcellulose (CMC), chitosan, and polyacrylic acid. Hydrocolloid ''wafers'' are often used as a barrier (or platform) between the skin and an adhesive in situations requiring multiple applications of an aggressive adhesive. Other synthetic rubbers have been used in adhesive tape products as well. These can be blended with acrylates and are typically hot-melt extruded onto backing materials.

Another category of adhesives that has not seen many skin applications outside of drug delivery is the silicones. The base polymer of a silicone adhesive is polydimethyl siloxane (PDMS). Silicone adhesives are now being used on a variety of wound dressing appliances and some scar-reduction products. Mölnlycke Health Care AB (Sweden) is the primary manufacturer of silicone adhesive product used on skin (34).

While both natural and synthetic adhesive systems are sterilizable, manufacturers need to monitor adhesive properties for changes from the sterilization process. And while medical tapes are typically not sterilized, dressings and other wound contact products are. Sterilization, aging, light, and heat can negatively affect each class of adhesive products. Antioxidants and other stabilizers are usually incorporated into the manufacturing process to counter the effects from these external energy sources and from the high temperatures encountered in hot-melt extrusion.

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II. ADHESIVES AND NEWBORN SKIN

A. Differences in Newborn Skin

Several unique properties of newborn skin influence adhesion and place the newborn at risk for injury from removal of adhesives. By 24 weeks gestation, a thin stratum corneum has developed in the fetus, with a steady increase in epidermal cell layers and epidermal thickness (37). The stratum corneum in infants of 28 weeks gestation consists of only a few cell layers and is markedly thinner than that of term infants and adults. This underdeveloped stratum corneum results in immaturity of barrier function, characterized by increased permeability (2,38) and increased TEWL (4,39). Since some degree of cell removal is inherent in the process of adhesive removal, having fewer layers of stratum corneum makes the premature infant in the first weeks of life at risk for completely stripping all stratum corneum and barrier protection.

By 32–34 weeks gestation, the stratum corneum has developed sufficiently to offer some protection (2,40). Term infants have been shown to have actually lower TEWL levels compared to adults, with the exception of the antecubital region (41). After birth, rapid postnatal maturation occurs, with thickening of the epidermis and development of the stratum corneum. However, in infants of 23–25 weeks gestation, skin barrier function has been shown to reach mature levels much more slowly (42), requiring as long as 8 weeks after birth in a 23-week gestation infant (43).

The undeveloped stratum corneum of the premature infant's skin leads to increased transepidermal water loss and evaporative heat loss (4,44). In addition to the problems of skin disruption with adhesive removal, the moist, glistening skin surface of the extremely premature infant of < 28 weeks gestation makes the adherence of adhesives difficult, since a drier surface is desired for maximal "wetting" of pressure-sensitive adhesives that are currently available in most products.

Another factor complicating the application and removal of adhesive products in premature infants is the use of thermoregulatory devices. Radiant warmers, convectively heated incubators, and high ambient humidity are necessary to maintain a thermal neutral environment. These environmental influences may cause instability of many adhesive products and may result in adhesives inadvertently falling off. Skin injury with adhesive removal may follow enhanced adherence secondary to changes in chemical properties of the adhesive or tackifiers.

Neonatal skin is 40–60% thinner than adult skin, which, along with a body surface/weight ratio nearly five times greater, places the newborn at risk for toxicity from topically applied substances (45). Due to a deficient stratum corneum, the skin of a premature infant is remarkably permeable;

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permeability correlates inversely with gestational age (6). Toxicity due to topically applied substances in newborns has been reported in numerous cases (45,46). Thus, the use of liquid solvents or bonding agents can pose a hazard of toxicity if absorbed through neonatal skin.

Another variation in the premature infant's skin structure and function is the diminished cohesion between the dermis and the epidermis. The junction of the epidermis and the dermis, which is normally connected by numerous fibrils, has fewer and more widely spaced fibrils in the premature infant than in term infants or adults (47). These fibrils become stronger with increasing gestational and postnatal age. The premature infant is at higher risk for blistering and stripping of the epidermis when adhesives are removed. The cohesion between many of the currently used adhesives and the stratum corneum may be stronger than the bond between the dermis and the epidermis (2,3).

The dermis of newborn infants contains both eccrine sweat and sebaceous glands. Although eccrine sweat glands are developed in the fetus by 28 weeks, thermal sweating is not well developed in the newborn (48), so this is not a major concern for adhesive attachment.

The skin of a term newborn is covered with vernix caseosa, a cheesy substance composed of sebum from sebaceous glands, broken-off lanugo, and desquamated cells. Vernix starts to form between 17 and 20 weeks of gestation, becomes a thick covering between 36 and 38 weeks, and by 40 weeks is found primarily in creases. The greasy nature of vernix poses a challenge in terms of adhesion; it may be necessary to remove vernix with cleansers and water or isopropyl alcohol under necessary adhesive appliances such as electrodes or tapes that secure endotracheal tubes or umbilical catheters.

B. Adhesive Products in the NICU

1. Tapes

A wide variety of adhesives and related products are currently in use on both premature and full-term newborns' skin. Cloth and silk tapes are commonly used to attach endotracheal tubes (ETTS) and gastric tubes, and in many nurseries "pink tape" (HyTape^{TM}) is selected due to its aggressive adherence. Stretchy Elastoplast, created for use in orthopedics, is also used for enhanced adhesion in taping ETTs. Plastic perforated tape (Transpore^{TM}) is one of the most popular tapes for securing intravenous catheters and is valued by clinicians for ease of use; it can be torn into small pieces easily and is transparent for visual inspection of intravenous (IV) insertion sites for signs of extravasation. Paper tape has been found to

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cause less irritation and stripping than plastic perforated tape in adults with repeated wound dressing changes (49). Paper tape is not often chosen by neonatal clinicians, however, due to poor adhesion and lack of molding to surfaces. A porous cloth-backed adhesive (Medipore™) has been shown to decrease visible skin injury compared to silk tape in adults (50) and adheres well to clean, dry skin surfaces; this may be useful in infants as well.

2. Natural Adhesives

Products containing natural adhesives include karaya gum (from the Sterculia tree) and pectin, a water-soluble carbohydrate substance (from fruit peels). Karaya rings and pectin products have been used for a number of years to protect the peristomal skin in adult ostomy patients. A number of products containing natural adhesives have also been used in NICUs.

A comparison of regular adhesive electrodes and karaya gum electrodes in premature infants found less skin disruption, as measured by TEWL, from the karaya electrodes, although they did not adhere quite as well as the regular adhesives, especially in high ambient humidity conditions (51). However, some premature infants were later noted to develop skin irritation from the karaya electrodes, and they are no longer commercially available. Pectin barriers (Hollihesive™, Stomahesive™, DuoDerm™, Comfeel™) are used beneath adhesives in premature infants and are reported to leave less visible skin trauma when removed (52–54). Advantages of pectin barrier adhesives include improved molding to skin surfaces and adherence to moist skin. Hydrocolloid adhesives are similar to pectin barriers. Products using hydrocolloid adhesives include endotracheal tube holders, umbilical catheter taping devices, and urine collection bags. Although manufacturers claim that these products cause little or no skin trauma, rigorous studies to substantiate these claims have not yet been performed with premature infants.

3. Hydrogel Adhesives

Hydrogel adhesives are available for electrocardiogram electrodes, temperature probe covers, and as strips or tapes. A number of manufacturers make these products, and clinicians note differences in adherence and skin contact among the different products. An evaluation of two brands of hydrogel electrodes in one NICU found that one brand lasted three times longer than another (55), justifying a significant price difference. Hydrogel adhesives should not be used in situations where adherence is critical, however; even their use to attach temperature probes is a concern because dislodgment or inadequate adherence can cause the probe to sense an inaccurate temperature and could result in either underheating or overheating the infant (3).

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4. Semipermeable Dressings

Semipermeable dressings, also known as transparent adhesive dressings, have a variety of uses including wound coverings, dressings to secure intravenous catheters, and also as adhesives to attach other medical devices. These products are used in the NICU to anchor silicone intravenous catheters, peripheral vascular devices, Broviac and subclavian central venous catheters, nasal cannulas, and nasal or oral gastric tubes. They allow visualization of the insertion of the IV catheters, and are permeable to water vapor, oxygen and carbon dioxide, allowing the skin to ''breathe.'' Because they are lightweight and clear, both comfort and appearance are improved for the infant.

Transparent adhesive dressings such as Tegaderm™, OpSite™, or Bioclusive™ applied to large areas of skin surfaces in very low birthweight infants reduce TEWL and evaporative heat loss (56–58), although significant increases in TEWL were seen when the dressing was removed, likely due to adhesive injury to the epidermis. Mancini et al. (59) studied the effect of a nonadhesive semipermeable dressing on the epidermal barrier of 15 premature infants by measuring TEWL on control and on treated skin. Treated skin showed a significantly decreased TEWL on the treated site; TEWL was measured after temporary dressing removal on days l, 2, 4, and 7. Increased cellular proliferation was documented in human fetal skin grafts in this study. This phenomenon was associated with improved epidermal barrier function. Nonadhesive dressings are not commercially available for this purpose, however, and many NICUs have elected to use other techniques to reduce TEWL in very low birthweight infants rather than induce skin injury when the dressings must be removed.

5. Adhesive Removers

Solvents are sometimes used in hospitalized patients to prevent discomfort and skin disruption and facilitate adhesive removal. They contain hydrocarbon derivatives or petroleum distillates. Toxicity is a major concern with these products, especially in premature infants with their underdeveloped stratum corneum, increased skin permeability, larger surface area-to-body weight ratio, and immature hepatic and renal function.

A case report in a premature infant described serious sequelae from the use of a solvent adhesive remover (60). An 87-day-old, former 26-week premature infant was exposed to a distillate-based adhesive remover (Detachol™) to facilitate removal of electroencephalogram electrodes. After several hours erythema was noted encircling the neck and extending to the shoulder. By the next day, bullae and superficial loss of epithelium

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was noted. On the third day, an acute hemorrhage from the affected area occurred, resulting in a 20 cc blood loss.

Mineral oil or petrolatum ointment may be helpful in removing adhesives but cannot be used if the site must be used again for reapplication of adhesives, such as with the retaping of an endotracheal tube. Water- or saline-soaked cotton balls may loosen adhesives somewhat. Pulling the adhesive parallel across the skin surface instead of straight up, or perpendicular, as described previously, can also facilitate removal and minimize damage from some adhesives. Delaying removal of adhesives for several days may also be helpful, as some adhesives become less aggressive over time, and the skin (particularly of premature infants) is undergoing maturation processes over time and may prove less vulnerable to trauma from removal.

6. Bonding Agents

Skin-bonding agents include Tincture of Benzoin™ and Mastisol®, a resinous liquid that contains gum mastik, styrax liquid, methyl salicylate, and alcohol. These agents are used to increase the adhesive strength of tape and are often used to enhance the adhesion of wound closure tapes applied after surgical procedures. Although Tincture of Benzoin does enhance adhesiveness, Mastisol has been shown to provide more adhesive strength (61,62).

The use of bonding agents for routine adhesive applications in the NICU is not recommended, especially when the removal of adhesives is required in a matter of days. The bond between adhesive and epidermis becomes much stronger than the fragile cohesion of the epidermis to the dermis; when removed, epidermal stripping may result. Tincture of Benzoin™ is not recommended in adults because it is drying to skin, can cause irritation, and can occlude the skin and impair its function (63,64).

7. Barrier Films

Skin-protective sprays and wipes are used in adults to prevent trauma to delicate or friable skin (63,65). Since many contain isopropyl alcohol, however, they cannot be used on skin with compromised barrier function without severe stinging and potential damage occurring. A spray-on, copolymer acrylic dressing (OpSite) was used to prevent damage from adhesive removal in premature infants (66). However, since this product contains alcohol, the same limitations to its use apply.

An alcohol-free skin protectant (Cavilon™ No Sting Barrier Film) is available that is less irritating to skin surfaces in adults than comparable products containing alcohol (67). This product has been approved for infants over 30 days of age to treat mild diaper dermatitis and to prevent skin injury from adhesive removal (68). A study reports less visible skin

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stripping when using this skin protectant to tape intravenous lines in newborns (69); however, it has not yet been approved for use in premature infants or term newborns in the United States. Among the questions about the use of barrier films as skin protectants include residue build-up with repeated use and the effect on adhesion when using these products.

C. Adhesive Damage

Adhesive use is not an innocuous intervention, even in adults. Investigators have found increased bacterial growth beneath occlusive tapes (70,71) and skin impairment ranging from chemical sensitivities to prolonged mechanical force on the skin from tape (72–76).

Adhesive tapes can irritate the skin by occlusion or by altering the skin morphology via epidermal stripping. ''Skin tears'' associated with adhesive removal are described in adults resulting from shearing or friction that separates the epidermis from the dermis (50,77,78). Although allergy to adhesives can occur, it is surprisingly rare due to the change from rubber- and rosin-based adhesives to acrylate-based adhesive tapes (63,72,73,79). Skin irritation from adhesives is sometimes misinterpreted as tape allergy, but differs in its distribution. Skin irritation from adhesives is patchy, mildly uncomfortable, and limited to the epidermis without edema. An allergic response to adhesives is intensely inflamed, edematous, and uncomfortable with itching or pain (63).

1. Types of Adhesive Damage

Damage from adhesives can occur from chemical and physical properties of the materials, improper technique of application, or injudicious removal. Table 1 lists the major types of damage from adhesives including epidermal stripping, tearing, maceration, tension blisters, chemical irritation, sensitization, and folliculitis (80). Two examples of adhesive damage in NICU patients are shown in Figures 3 and 4.

2. Epidermal Injury

Transepidermal water loss is a noninvasive, quantitative measurement of skin barrier function, extending beyond visual inspection that measures changes in the epidermal surface from adhesive removal. Alterations in skin barrier function, evidenced by elevated TEWL measurements, are seen in adults after 10 consecutive removals of adhesive tape (81,82). Harpin and Rutter (2) documented skin damage resulting from removal of adhesive tape and adhesive rings used to secure transcutaneous oxygen electrodes, using two techniques. Thirty minutes after adhesive removal, the skin was assessed and

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Table 1 Potential Types of Adhesive Skin Damage to Preterm Infants

Skin stripping	Due to stratum corneum or malpighian epidermis being stripped away by tape; cumulative damage on repetitive applications or tape removal (nonbleeding injury)
Tearing/ avulsion	Caused by disruption of skin surface (epidermis and dermis) with aggressive adhesives and lack of care in removal; may be exacerbated by presence of hair (bleeding injury)
Maceration	Caused by excessive hydration of the skin; typically this injury would occur following formation of the stratum corneum; typical of prolonged occlusion
Tension blisters	Can occur secondary to tension with stretching and pulling of skin adhesive product
Chemical irritation	Caused by presence of irritants in the adhesive formulation; enhanced by occlusion
Sensitization	Caused by allergens in the adhesive formation; occurs with repeat applications
Folliculitis	Inflammation/infection of hair follicles often associated with occlusion

Source: Modified from Ref. 80.

compared to adjacent undisturbed skin by TEWL measurement (Fig. 5A) and by blanching response to phenylephrine (Fig. 5B). Significant changes in the barrier function of the epidermis were seen using both techniques. Due to the need for regular removal of adhesives and the small surface area for attachment, they estimate that 15% or more of a premature infant's total surface area would be traumatized over a 24-hour period.

Another study quantified the effect of three different adhesive products on skin barrier function in 30 infants between 26 and 40 weeks gestation (birthweight range 690–3000 g) during the first week of life (3). One cm^2 pieces of plastic perforated tape (Transpore™), pectin barrier (Hollihesive™), and hydrogel adhesive (Cleartrace) were placed on undisturbed skin of the back after baseline measurements of TEWL, colorimetry, and visual inspection scores were obtained. Twenty-four hours later each adhesive was removed, and measurements repeated after 30 minutes to allow the linear concentration gradient across the stratum corneum to be reestablished after occlusion (83). Significant differences in TEWL (Table 2) and colorimetry measurements (Table 3) were seen after removal of both the plastic tape and pectin barrier. Colorimetry measurements were also elevated with the hydrogel adhesive, but not as much as with the other two adhesives.

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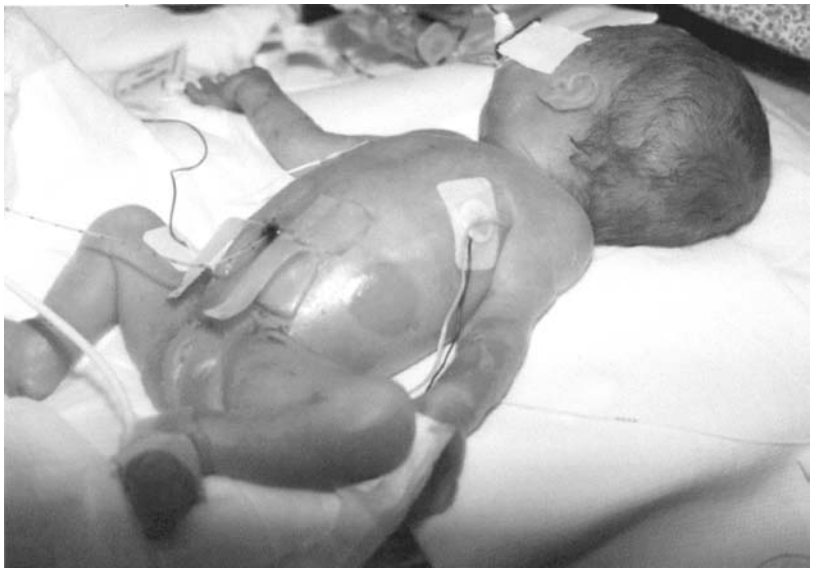

Figure 3 Skin injury due to epidermal stripping in a premature infant.

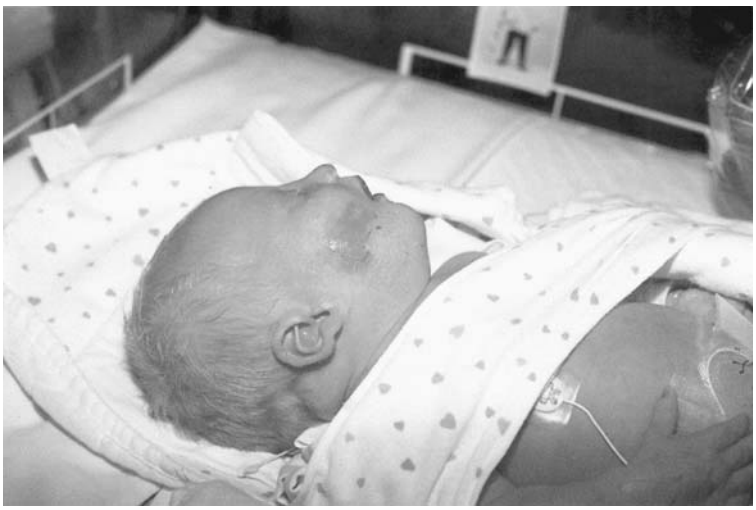

Figure 4 Skin injury due to tape irritation following removal of an endotracheal tube in a term newborn.

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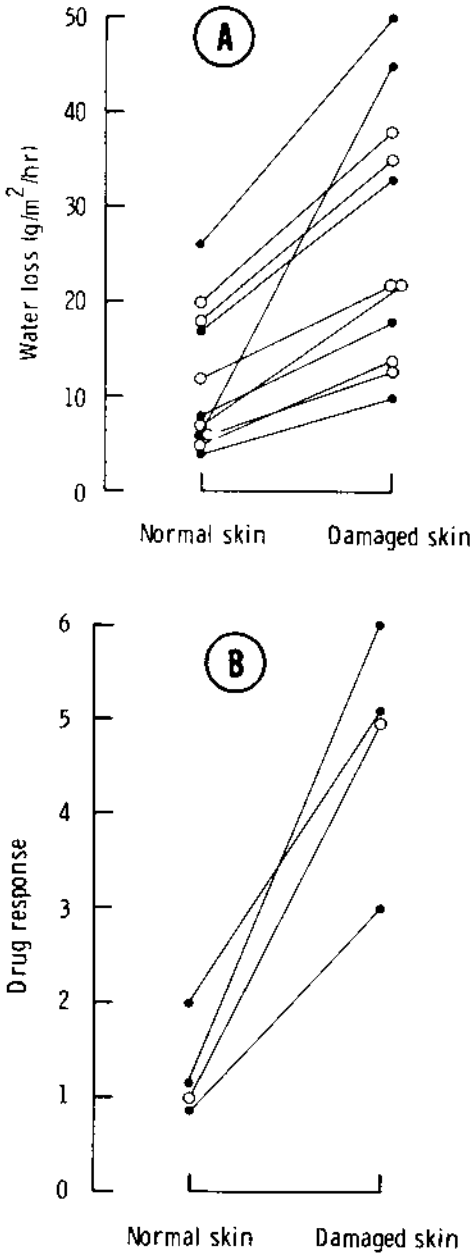

Figure 5 (A) TEWL measurements from skin 30 minutes after removal of adhesive tape in premature infants compared to adjacent undisturbed skin. (B) Blanching response to phenylephrine from skin 30 minutes after removal of adhesive tape in premature infants compared to adjacent undisturbed skin. (Modified from Ref. 2.)

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Table 2 Mean TEWL ± SD (g/m^2h) for Each Adhesive Site

Time	Control ($n = 30$)	Pectin barrier ($n = 30$)	Plastic tape ($n = 30$)	Hydrophilic gel ($n = 23$)
Baseline	21.7 ± 12.48	20.49 ± 11.85	20.86 ± 13.02	21.6 ± 12.72
30 minutes after adhesive removal	16.39 ± 8.18	21.42 ± 9.70[a]	21.6 ± 90.2[a]	15.91 ± 6.90
24 hours after adhesive removal	15.00 ± 6.80	16.81 ± 6.53	16.60 ± 6.94	13.84 ± 5.53

[a] Significantly different versus control site TEWL on the same day of study ($p < 0.01$).
Source: Ref. 3.

When infants were stratified by birthweight (< 1000 g, 1001–1500 g, > 1500 g), baseline TEWL measurements were different due to maturation factors, but the effect of adhesive removal was documented in all three groups (Fig. 6). Also of note, the hydrogel adhesive fell off five subjects with birthweights of < 1000 g before the scheduled removal at 24 hours. These infants had significantly higher TEWL baseline measurements than the other very low birthweight infants studied.

Table 3 Mean Colorimeter a*-axis Values (±SD) for Each Adhesive Site

Time	Control ($n = 30$)	Pectin barrier ($n = 30$)	Plastic tape ($n = 30$)	Hydrophilic gel ($n = 23$)
Baseline	12.49 ± 3.11	12.18 ± 3.37	12.27 ± 3.30	11.88 ± 3.50
30 minutes after adhesive removal	12.65 ± 3.79	14.36 ± 4.52[a]	13.99 ± 4.21[a]	13.17 ± 3.18[a]
24 hours after adhesive removal	12.07 ± 3.03	13.33 ± 3.62[b]	12.36 ± 3.52	12.61 ± 3.48

[a] Significantly different versus control site colorimeter a*-axis value on the same day of study ($p < 0.01$).
[b] Significantly different versus control site colorimeter a*-axis value on the same day of study ($p < 0.05$).
Source: Ref. 3.

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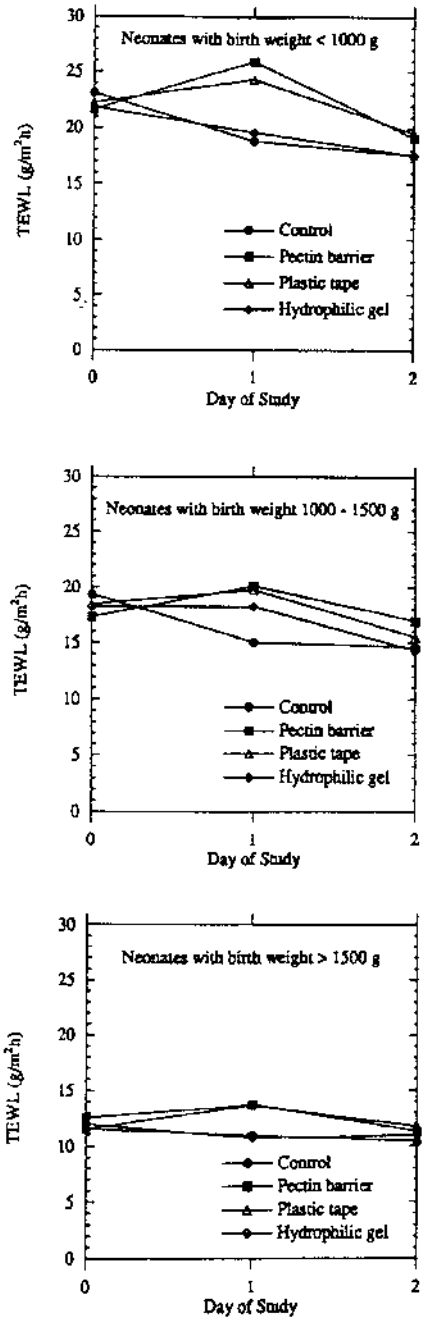

Figure 6 Mean TEWL values for three birthweight groups (<1000 g, $n = 5$; 1000–15000 g, $n = 10$; >1500 g, $n = 8$). (Modified from Ref. 3.)

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An alternative interpretation of this study's findings has been raised, that is, the possibility of occlusion-induced inhibition of epidermal barrier maturation in premature infants as the mechanism for the findings of elevated TEWL and colorimetry readings (84). However, visual inspection scores of the skin indicated evidence of both irritation and skin stripping, so traumatic injury is most likely the cause of the altered skin measurements.

3. Anetoderma of Prematurity

Another potential morbidity that has been reported related to adhesive use in very low birthweight infants is called anetoderma of prematurity. Anetoderma is defined as atrophic patches of skin due to dermal thinning. Nine infants, born at 24–29 weeks of gestation from four different hospitals, were noted to have atrophic skin lesions at 6 weeks to 10 months of age, located on the ventral surface and distributed over both sides of the abdomen, the upper arms, the chest and thigh. Histopathological evidence was confirmed in 4 of 5 patients. A clear association between site of involvement and monitoring leads or temperature probes was identified in a number of the cases. The authors postulate that pressure or a change in flow of ions or water under the electrodes or adhesives in extremely immature skin may have caused an inflammatory reaction and subsequent damage to elastic tissue. They recommend careful consideration given to application of all adhesives and monitoring leads in extremely premature infants in order to prevent permanent skin changes (85).

At the current time, clinicians have an increasing awareness of the problems of adhesives in NICU patients but limited options for reducing adhesive-related injuries while maintaining optimal adherence of life support devices. Guidelines generated by national nursing organizations include recommendations to (a) use adhesives sparingly, (b) use semipermeable dressings for silicone catheters, IV devices, cannulas, and NG tubes, (c) consider using pectin barriers or hydrocolloid adhesive products for protection from tapes and for enhanced adhesion in moist environments, (d) use wraps such as stretchy gauze to anchor extremities instead of tape, (e) use hydrogel electrodes for EKG monitoring, (f) consider use of alcohol-free skin protectants as skin barriers in infants >30 days of age, and (g) avoid use of solvents, bonding agents, and routine use of bandages after lab sampling (86).

III. FUTURE GOALS

The challenges posed by the use of adhesives in both term and premature infants in the NICU are many. Adhesives must firmly secure many life

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support appliances and monitoring devices, with serious consequences occurring with accidental dislodgment. At the same time, skin injury is to be avoided without using agents with potential toxicity. The long-term effects of many aspects of skin care on the future skin development of premature infants remains unknown, with adhesive use high on the list. Research in these areas can assist in answering these and other questions about neonatal skin.

Some of the goals for research and development of improved adhesion systems for the NICU include the following:

- Standardization of techniques to document effects of adhesive products on skin barrier function, including TEWL measurements (87).
- Scientific evaluation of copolymer barrier films for skin protection including effects with repeated use and effect on adhesion.
- Release systems to minimize trauma with removal.
- Potential application of mucoadhesion systems for improved adhesion to skin surfaces with high water loss (88,89).
- Development of silicone-based adhesives with sufficient strength to adhere life support devices.

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16
Environmental Interactions

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I. INTRODUCTION

A. Concepts

The skin forms a continuous, highly organized and responsive interface between the organism and the environment. The skin is a sensory organ that functions in many ways as a "smart material," i.e., a material that shows an oriented response to an external stimulus, e.g., heat, friction, sunlight, water, and chemicals (1). In this view, the skin functions as a sensory signal transducer closely linked to the regulation of central processes. The rate of change of skin temperature, for example, controls the thermoregulatory response to cold (2). Specific receptors in the skin, sensitive to mechanical stimuli, are critical for survival. Researchers are beginning to identify the signaling proteins involved in this transduction (3). Psychological stress due to environmental overcrowding is associated with a delay in skin barrier recovery in mice, an effect attributed to increased production of glucocorticoids (4). Skin-to-skin contact immediately following birth results in increased temperature and blood glucose levels, compared to swaddling next to the mother (5). Tactile stimulation via repeated stroking increases circulating lactate levels by 200% in the neonatal rat model (6). These examples indicate the intimate connection of the skin and the environment. They support an important role of the skin in actively modulating the interaction between the environment and underlying dermal, neural, and neuroendocrine control systems. In this view, the skin constitutes a prototypical smart material interface between the organism and the environment. This chapter explores this concept in the context of the newborn infant.

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B. Approach

Data on skin-environment interactions are presented in the context of the skin as a highly organized and dynamic interface, i.e., the skin adapts and continuously changes its biophysical and physiological properties in response to specific environmental influences. This interaction is discussed separately for full-term and preterm neonates, as the underlying skin condition varies markedly with gestational age. Specific environmental factors include temperature, relative humidity, light, hydration, bathing, surface treatments (cleansers, alcohol), diapers, creams, protective barriers, friction, soils (urine, feces), adhesives, and antenatal steroid exposure. In particular, we examine the endogenous and exogenous water-handling properties of the stratum corneum (SC), since the hydration status of this outermost layer is vitally important for maintaining barrier properties of the skin (7). A variety of optical and biophysical techniques can be used to quantify skin characteristics, including measurement of color, texture, hydration, barrier integrity, barrier function, surface friction, mechanical properties, and blood flow. Importantly, the environmental effects on the skin are frequently combinatorial, e.g., humidity and temperature. The multiplicity of simultaneous environmental effects on skin condition will be addressed.

II. THE BEGINNING: TRANSITION TO A DRY, TERRESTRIAL ENVIRONMENT—EFFECT OF TEMPERATURE AND HUMIDITY

A. Full-Term Neonate

At birth, the human infant must move rapidly from a heated (37°C), water-filled container to an expansive, cold, dry, terrestrial environment. The mechanisms for this adaptation include air breathing, enteral nutrition, maintenance of body temperature, water balance, and waste elimination. The normal full-term infant has a competent epidermal barrier, which provides proper water homeostasis and protects against infection (8,9). The mechanisms whereby a functional stratum corneum is formed under aqueous conditions are unclear insofar as prolonged water exposure causes maceration and barrier compromise (10–13). Nonetheless, transepidermal water loss (TEWL) values are low for full-term newborns at birth, with values comparable to or lower than for adults (9,14).

We hypothesized that infant SC undergoes programmatic changes in hydration and water binding immediately after birth and investigated these parameters in a series of in vivo studies among a cohort of 101 healthy full-term newborns in the context of conventional delivery room practices (15). Typically, following birth, infants are dried with a cotton towel and placed

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under a radiant warmer. Some institutions place the infant in a plastic bag from mid-torso to feet. The effects of both radiant warming and wrapping (i.e., occlusion and high humidity) were determined in a subset of 29 infants by placing them in a bag to mid-torso. SC properties were evaluated for chest (outside bag, under warmer) and an occluded suprapubic region within 4 hours after birth. The mean relative humidity in the nursery was 31%, and the mean radiant warmer temperature was 36.5°C. The baseline hydration (cru) was significantly lower for the non-occluded site (118 ± 4.1 cru) than for the occluded region (137 ± 7.3 cru). The rate of moisture accumulation under the instrument probe (MAT cru/s) was also significantly lower for the nonoccluded region (1.0 ± 0.2 cru/s) than for the occluded site (3.3 ± 0.5 cru/s), as shown in Fig. 1. This result concurs with the results of Hammarlund et al., who investigated water evaporation for healthy infants during the first 30 minutes after birth and found it to be high and the major cause of heat loss (16). The rate depended upon environmental conditions, i.e., incubator vs. delivery room. A linear relationship between the evaporative rate and incubator humidity was documented for 19 healthy newborns, yielding a calculated TEWL of 8.1 $g/m^2/h$ (17).

The effect of anatomical site on SC-water interactions was determined within a subset of 34 infants (15). The infants were dried and placed under a radiant warmer. Moisture accumulation rates were evaluated for the chest

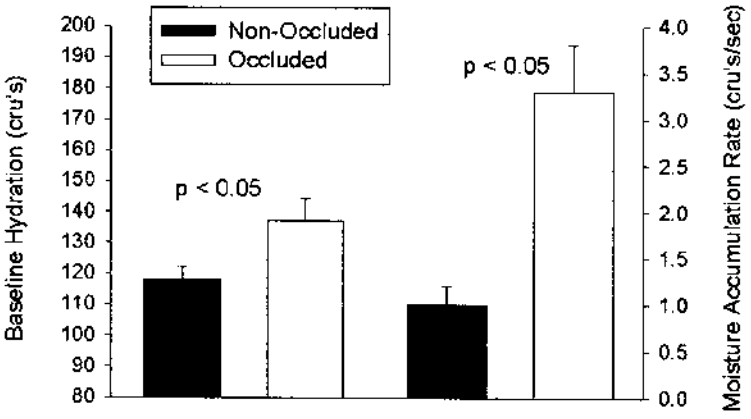

Figure 1 Baseline hydration and rate of moisture accumulation values upon admission to the nursery for term infants. The baseline hydration (cru) was significantly lower for the nonoccluded site (118 ± 4.1) than for the occluded region (137 ± 7.3). The rate of moisture accumulation under the instrument probe (MAT) was also significantly lower for the nonoccluded region (1.0 ± 0.2 cru/s) than for the occluded site (3.3 ± 0.5 cru/s). (Adapted from Ref 15.)

and back twice within 4 hours. The first measurement was made at the time of admission to the newborn nursery, on average 92 minutes after birth (range 36–124 minutes). The second measurement was made about 55 minutes later upon discharge from the nursery to the mother's room. The mean relative humidity was 27%, and the radiant warmer temperature was 36.2°C. The moisture accumulation was significantly higher for the back versus the chest shortly after birth, and values were 2.0 ± 0.5 and 0.9 ± 0.2 cru/s, respectively (Fig. 2). Upon discharge from the nursery, the moisture accumulation rate was lower for both sites, with a significant decrease over the back (Fig. 2). Surface illuminated optical images of the chest and back skin were recorded with a high-resolution digital camera at the time of nursery admission and evaluated for dryness/scaling by the neonatal research staff. The skin on the back was significantly less dry than that for the chest, as shown in Figure 3.

The moisture accumulation rate for the chest was compared statistically for the three nurseries to assess the potential effect of skin care practices on SC hydration (Fig. 4). In this study, infants from Nursery B ($n = 15$) were maintained under the radiant warmer for a significantly longer period of time ($p < 0.01$) than infants from Nurseries A and C, even though the average time from birth was significantly shorter for infants in Nurseries B and C. The moisture accumulation rate for Nursery B infants was signifi-

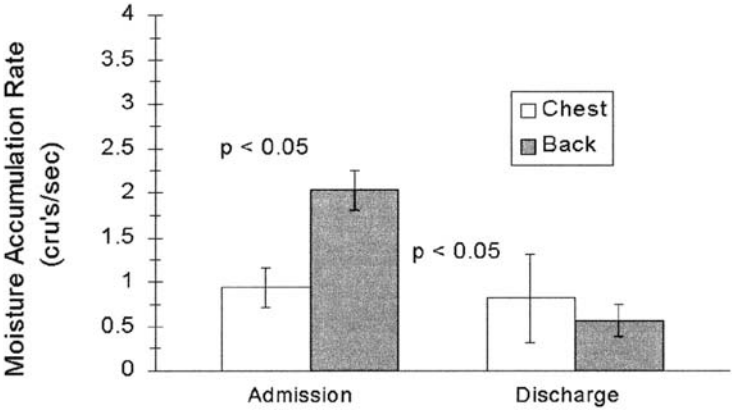

Figure 2 Rates of moisture accumulation for chest and back at time of nursery admission for term infants. The moisture accumulation was significantly higher for the back versus the chest shortly after birth and values were 2.0 ± 0.5 and 0.9 ± 0.2 cru/s, respectively. Upon discharge from the nursery, the moisture accumulation rate was lower for both sites, with a significant decrease over the back. (Adapted from Ref. 15.)

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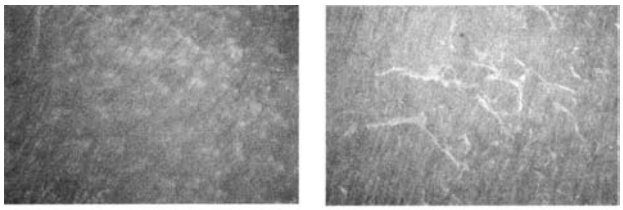

Figure 3 Optical images of back and chest skin. The skin on the back was significantly less dry than that for the chest. (Adapted from Ref. 15.)

cantly lower than for the infants in Nursery A ($p < 0.05$), indicating that the SC water-handling properties may be influenced by the time spent under the radiant warmer.

Regional differences in SC water-handling properties were investigated by comparing the chest and forehead sites of 37 full-term infants in two nurseries within 90 minutes of birth. The moisture accumulation rate was significantly greater for the forehead than for the chest (2.07 ± 0.55 vs.

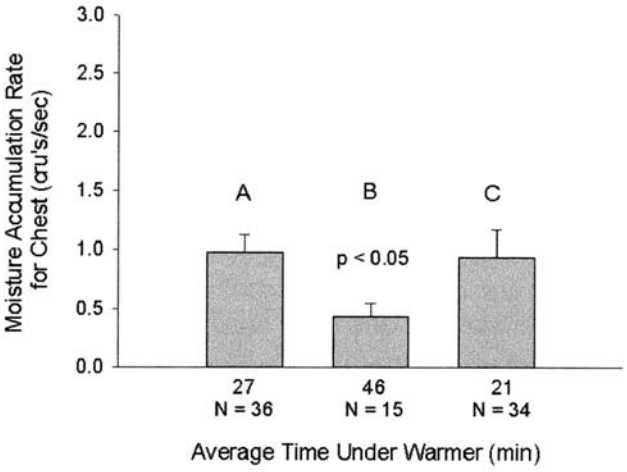

Figure 4 Rate of moisture accumulation for chest skin in infants from three nurseries. Infants from Nursery B ($n = 15$) were maintained under the radiant warmer for a significantly longer period of time ($p < 0.01$) than infants from Nurseries A and C, even though the average time from birth was significantly shorter for infants in Nurseries B and C. The moisture accumulation rate for Nursery B infants was significantly lower than for the infants in Nursery A ($p < 0.05$), indicating that the SC water-handling properties may be influenced by the time spent under the radiant warmer.

0.83 ± 0.14 cru/s, respectively; $p = 0.05$). The surface hydration was directionally higher for the forehead than for the chest (113 ± 11.0 vs. 111 ± 3.5 cru, respectively). The skin temperature of the forehead was significantly lower than for the chest, indicating that increased hydration may have produced evaporative cooling. Regional differences over the skin surface on the first postnatal day have been reported by other investigators (14,18,19).

Investigations of skin surface characteristics at birth have also been studied using the neonatal rat model (20). In this animal, the skin surface of the newborn is very hydrophobic, as evidenced by a rapid rate of water desorption. Removal of the outermost epidermal layer using a single tape strip dramatically decreased the rate of desorption. Further studies indicated that the surface hydrophobicity is secondary to a specialized tissue layer called the periderm, which disaggregates over the days following birth. Skin surface hydrophobicity was also assessed in a cohort of 13 full–term newborns (21). An unperturbed area on the chest was compared with a contralateral control site that had been treated with isopropyl alcohol to remove any hydrophobic components. The sites were exposed to exogenous water for 10 seconds and then blotted dry (22). Capacitance readings indicated a slower desorption rate and a higher peak sorption for the alcohol-treated site, indicative of a less hydrophobic surface. These findings support the notion that the surface of the newborn infant, like the rat, is relatively hydrophobic.

Skin water-handling properties during the first postnatal day were examined for a cohort of 30 full-term neonates and compared to values observed within 90 minutes of birth for a demographically similar parallel group. The moisture accumulation rate decreased significantly during the first day (Fig. 5), indicating that the epidermis undergoes rapid changes as the infant adapts to a dry environment.

Skin adaptation throughout the first month of life was further investigated for 30 full-term newborns in order to describe normative features of SC hydration and barrier properties and compare those features to adult skin under similar environmental conditions (23). The infants' skin exhibited increasing surface hydration and rate of moisture accumulation during the first 2 postnatal weeks, while maternal measurements did not vary significantly over the time period (Fig. 6). The infant skin reached a plateau around day 14, and values were comparable at days 21 and 28.

Most significant was the progressive increase in MAT, the rate of transepidermal water movement, from the initial, low values at birth. The TEWL values for full-term newborns are low (24). Hammarlund et al. reported that TEWL for full-term infants remained unchanged for the first 2 weeks (25). Therefore, TEWL is unlikely to account for the increased

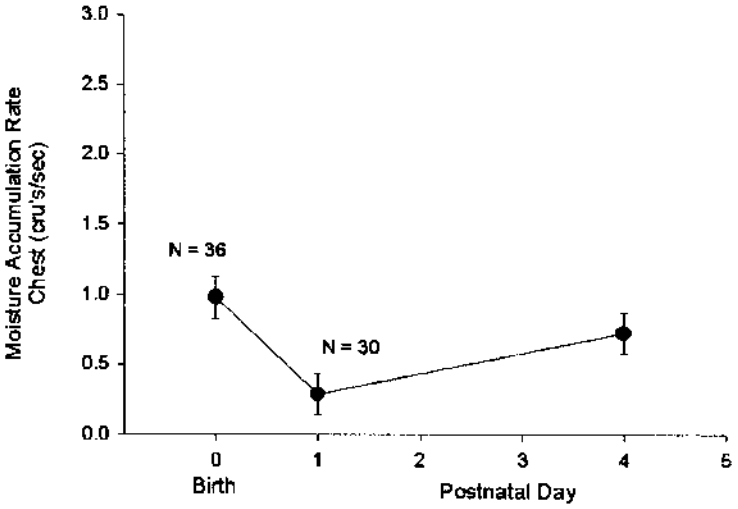

Figure 5 Rate of moisture accumulation during first day of life. The rate of moisture accumulation decreased significantly during the first day in a cohort of 30 full-term infants compared to values observed within 90 minutes of birth for a demographically similar parallel group, indicating that the epidermis undergoes rapid changes as the infant adapts to a dry environment. (Adapted from Refs. 15, 23.)

MAT. In another study, the water-handling properties of the skin were evaluated by occluding the skin for one hour and then evaluating TEWL (18). TEWL was found to be higher than normal, suggesting an increased rate of water movement through the epidermis. Eccrine sweating could be another source of increased MAT. Rutter reported increases in surface water loss in full term infants aged 4 hours to 11 days due to eccrine sweating when incubator temperatures were higher than 34°C (26). In our investigation, infants were in open cribs or at home and the skin sites had equilibrated to environmental conditions for 25 minutes prior to measurements. No sweating was detected, and the low standard error of the mean indicates good experimental control of the measurements.

Water sorption-desorption evaluations also revealed significant changes in the upper SC during the first 14 days followed by a plateau through the next 2 weeks (23,27). The desorption rate decreased for infants during the first 14 days and remained unchanged for mothers (Fig. 7). The relatively rapid desorption rate at birth may indicate a greater capacity to shed exogenous water, thereby minimizing potential evaporative heat loss. The presence of a hydrophobic film on the skin surface, such as vernix caseosa, would result in increased water desorption (28). Alternatively,

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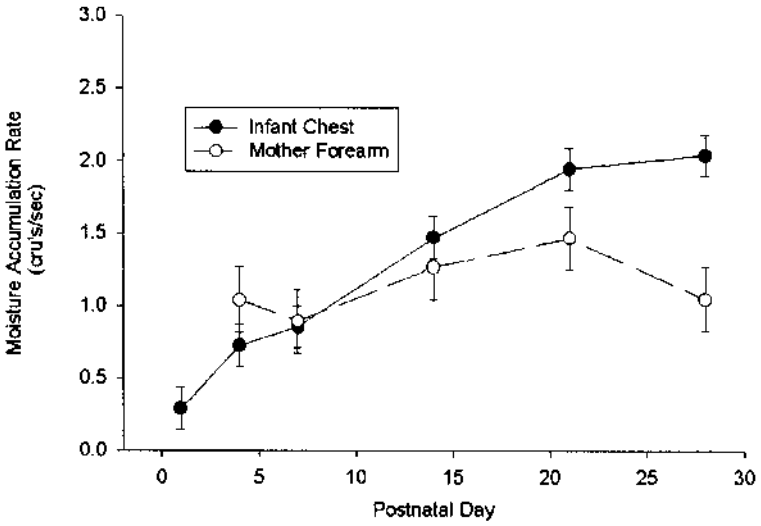

Figure 6 Changes in rate of moisture accumulation rate for infants and their mothers. The infants' skin exhibited increasing surface hydration and rate of moisture accumulation during the first 2 postnatal weeks, while maternal measurements did not vary significantly over the time period. The infant skin reached a plateau around day 14 and values were comparable at days 21 and 28. (Adapted from Ref. 23.)

low water-holding capacity coupled with low hydration is also indicative of dry skin (27). The decrease in desorption rate over time indicates that the skin surface begins to bind exogenous water rather than to shed it. The generation of hygroscopic NMF components in the upper SC would presumably increase water-holding capacity and thereby reduce the desorption rate.

Comparisons of infant vs. adult skin parameters have demonstrated that epidermal barrier properties related to water handling change markedly over the first 4 weeks of postnatal life, indicating a significant period of adaptation (15). Infant and adult comparisons on days 1 and 2 after birth revealed lower TEWL for infants in the forehead, palms, and soles and higher TEWL than adults for forearm sites (14). Comparison of TEWL for day 1 versus day 2 indicated lower values on day 2 in the forearm, palms, and soles. Skin surface hydration was significantly lower in the infants on the forehead, back, and abdomen and higher on the forearms and palms. Skin hydration and TEWL values were correlated in infants (positively) but not in adults. Skin conductance was reported for newborns aged 5 days compared to infants aged 1 month, 2 months, and 6 months and

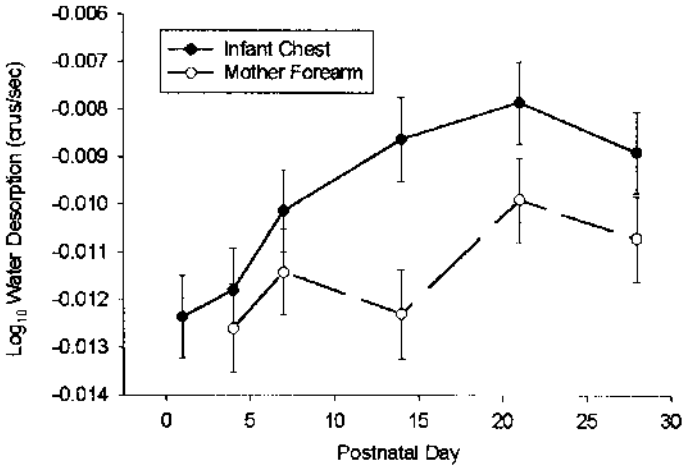

Figure 7 Water desorption rates for infants and their mothers. The desorption rate decreased for infants during the first 14 days and remained unchanged for mothers. The relatively rapid desorption rate at birth may indicate a greater capacity to shed exogenous water, thereby minimizing potential evaporative heat loss. (From Ref. 23.)

to their mothers (27). The skin surface of the newborns was significantly drier than the older infants and the mothers (Fig. 8). Giusti et al. investigated 70 infants, aged 8–24 months, versus a cohort of 30 female adults and found no significant differences between infants and adults in TEWL on either the forearms or the buttocks (29). However, capacitance values were significantly higher for the infants, suggesting higher levels of SC hydration. The authors concluded that the infant skin was immature compared to the adult skin. However, measurement of full-thickness skin from infants, children, and adults showed no significant differences in SC thickness (30). Based on these reports, differences in capacitance values for infants and adults are not attributable to variations in SC barrier thickness or integrity, since TEWL values were not different. Alternatively, the higher capacitance values observed for the infants may indicate that infant skin is actually in better condition, i.e., more well hydrated and plastic, than adult skin.

Environmental humidity as well as liquid water exposure may impact SC structure and function. Sato et al. investigated the effect of a dramatic change in environmental humidity on the SC water-handling properties using the hairless mouse model (31). Young animals were kept at 80% relative humidity for 2 weeks and then transferred to 10% RH. Three days later the SC water content had decreased by 60% (as measured by

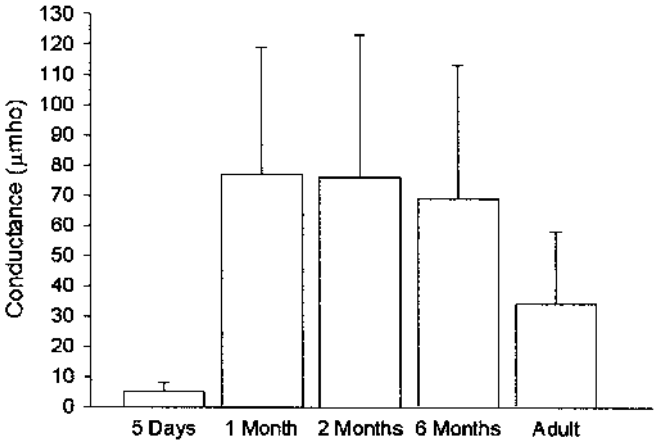

Figure 8 Skin hydration in infants and adults. Skin conductance was reported for newborns aged 5 days compared to infants aged 1 month, 2 months, and 6 months and to their mothers. The skin surface of the newborns was significantly drier than the older infants and the mothers. (From Ref. 27.)

skin conductance), the SC water-holding capacity was significantly lower, and the free amino acid content was significantly reduced. The direct relationship between stratum corneum hydration and amino acid content has been established in adult skin and found to be indirectly correlated with visual skin dryness (32,33). The low amino acid content was attributed to the finding that there were fewer keratohyaline granules in the tissue of animals under high-humidity conditions (34).

The effects of environmental humidity on barrier response were examined with respect to hydration, TEWL, and epidermal cell proliferation (35). Groups of hairless mice were kept at high (90% RH) and low (10% RH) humidity for 5 days following a period of acclimation at 40–50% RH. Epidermal DNA synthesis increased, beginning 12 hours after exposure to low humidity, while the synthesis rate did not change under conditions of high humidity, even though both groups had similar TEWL values. However, stratum corneum hydration was significantly lower for the low-humidity group. The authors concluded that the decrease in SC hydration caused an increase in DNA synthesis (35).

Scott and Harding examined the influence of decreasing humidity at birth on the epidermal protein filaggrin using the newborn rat model (36). Filaggrin proteolysis gives rise to free amino acids, pyrrolidone carboxylic acid, and urocanic acid (collectively, natural moisturizing factor, NMF), which facilitate water binding of the stratum corneum at low environmental

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humidities (7). In the absence of NMF, the stratum corneum is dry, does not desquamate properly, and has poor water-holding capacity. During late gestation, filaggrin is present throughout the stratum corneum in the animal model. Immediately following birth, proteolysis occurred and filaggrin was found in the lower stratum corneum only. In addition, the proteolysis was dependent on the humidity at birth and was observed at ranges of 80–95% RH but did not occur at 100% RH (Fig. 9). The authors hypothesized that the generation of NMF was dependent upon a specific *decrease* in water activity (36). This report provides a possible explanation for the development of a dry, poorly hydrated stratum corneum during the first postnatal day in the full-term neonate reported by our laboratory (15). Specifically, we hypothesize that the current delivery room practice of rapid skin surface drying and exposure of the infant skin to the radiant warmer may bypass the optimum range of 80–95% RH necessary for filaggrin proteolysis and generation of water-binding NMF. A small based ($n = 5$) investigation of the soluble amino acid levels in the outer stratum corneum of full-term infants was recently conducted in our laboratory. Two d'squame samples were collected from each infant, extracted, and analyzed for the total soluble amino acids using high-performance liquid chromatography (HPLC) analysis. The preliminary results indicate that the NMF levels are markedly lower than those of adults (data not shown).

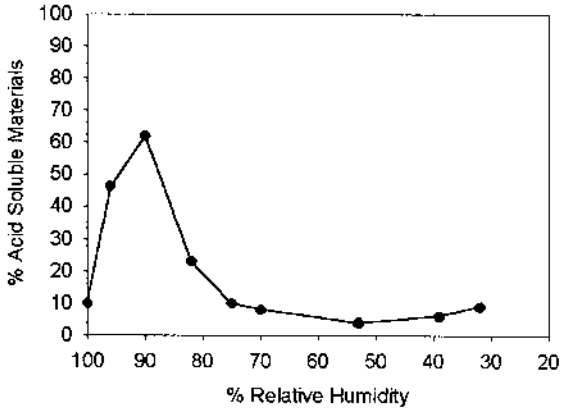

Figure 9 Generation of soluble amino acids from filaggrin. Proteolysis of filaggrin to soluble amino acids was found to depend on the relative humidity with greatest degradation from 80–95% RH. Proteolysis did not occur at 100% RH, leading to the hypothesis that the generation of NMF is dependent upon a specific decrease in water activity (15). (Adapted from Ref. 36.)

B. The Preterm Neonate

1. Environmental Influences on Barrier Development

The preterm neonate has a poorly formed epidermal barrier and experiences high fluid loss, thus presenting a very different clinical picture than the full term infant. TEWL is markedly elevated in infants less than 26 weeks gestational age (GA), as shown in Figure 10 (8,37,38). *The very premature infant has, essentially, a wounded skin surface* with few cornified layers (39,40). As gestational age increases, TEWL decreases until 33–34 weeks, when values are similar to those of full-term infants. Consistent with these findings were those of Okah et al. (41), who reported skin hydration in preterm infants as a function of gestational age (42). Baseline skin surface hydration was significantly related to gestational age and was significantly higher in the infants < 30 weeks GA than in those born after 30 weeks. Additionally, the skin hydration for infants of 32–34 weeks gestation was not significantly different from that of the full-term group.

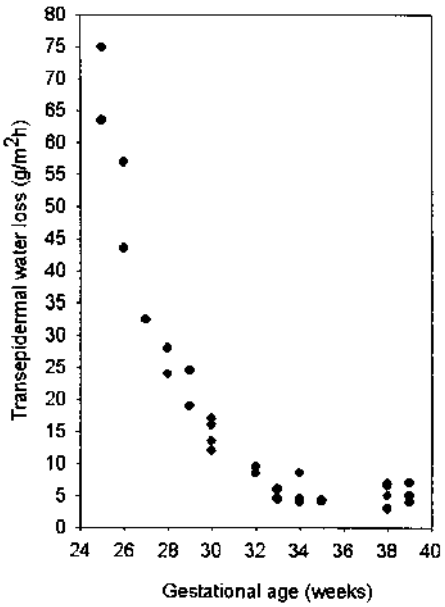

Figure 10 Barrier integrity as a function of gestational age. TEWL is markedly elevated in infants less than 26 weeks gestational age (8,37,38). As gestational age increases, TEWL decreases until 33–34 weeks, when values are similar to those of full-term infants. (Adapted from Ref. 8.)

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Stratum corneum maturity was reflected in investigation of percutaneous drug absorption, which was poorer for older infants than for premature infants (24,43). Once birth occurs, the ambient humidity influences TEWL and direct humidification of the incubator environment has been utilized to minimize fluid loss in the preterm infant (17,25,38,44–46).

Following exposure to a dry environment at birth, TEWL for the preterm infant gradually decreases with increasing postnatal age (25). Small-for-gestational-age preterms have higher values than appropriate for gestational-age infants (37). Transition to a postnatal environment significantly influences epidermal barrier development since the epidermis of a 2-week postnatal preterm infant is histologically similar to that of a full-term infant (39). The morphological changes are directly related to development of epidermal barrier properties.

Okah et al. also examined the ontogeny of epidermal barrier maturation during the first 5 postnatal days as a function of gestational age (41). Changes in skin surface hydration under probe occlusion were evaluated as an indirect measure of transepidermal water movement (42). The rate of transepidermal water movement was significantly higher in infants < 30 weeks GA compared to those > 30 weeks GA. The rate of water movement changed most dramatically in the most premature infants, and by day 5 the rate had decreased substantially compared to postnatal day 1 (Fig. 11). The rapid development of a functional barrier was demonstrated in all very premature infants and did not appear to be influenced by the severity of their illness.

Epidermal barrier development during the first postnatal month was examined for infants born at 24–25 weeks. TEWL decreased over time, indicating barrier maturation, but even after one month TEWL was still significantly higher for this group compared to normal full-term infants (Fig. 12) (47). Therefore, while epidermal maturation occurs, the barrier function is not completely normal. Kalia et al. reported that the development of a fully functional epidermal barrier requires longer than 4 weeks for infants born at 23–25 weeks (48). Significant maturation of the skin required 5–7 weeks in the infants born at 23–24 weeks gestation (49). Within the group of premature infants, those who were small for gestational age (SGA) had a higher TEWL 3 weeks after birth than a group of preterm infants who were appropriate for gestational age (AGA) (37).

Several investigators have utilized semipermeable films and dressings to mitigate the high transepidermal water loss in the preterm neonate by effectively altering the local environmental humidity (50–52). The application of a semipermeable dressing to the leg of 10 infants of less than 32 weeks gestation significantly reduced TEWL relative to the contralateral untreated control and permitted the barrier to mature (50). For a group

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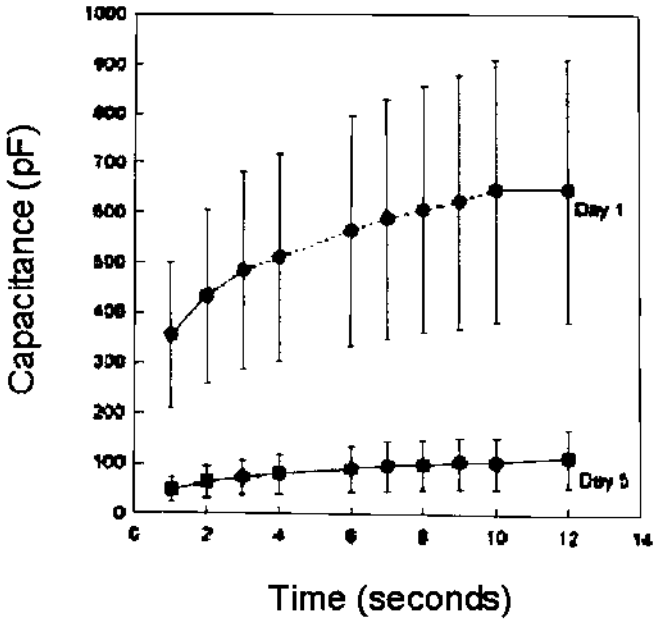

Figure 11 Ontogeny of epidermal barrier maturation. The rate of transepidermal water movement was significantly higher in infants < 30 weeks GA compared to those > 30 weeks GA. The rate of water movement changed most dramatically in the most premature infants, and by day 5 the rate it had decreased substantially compared to postnatal day 1. (Adapted from Ref. 41.)

of 15 preterm infants, the semipermeable dressing significantly reduced the TEWL as measured through the dressing as well as the TEWL of the skin itself following removal of the dressing (51). In a parallel animal study, samples of fetal skin (gestational age 23–26 weeks) were transplanted onto nude mice that were then divided into treatment (semipermeable dressing) and control (no dressing) groups. TEWL was reduced twofold, and keratinocyte proliferation on day 4 was increased in the skin covered by the semipermeable dressing (51). Bustamante and Steslow reported similar results in a study among 13 preterm infants weighing 770–1450 g (53). Eighteen preterm infants (mean 30 weeks gestation) were treated with a polyurethane-semipermeable dressing, again resulting in a twofold reduction in TEWL with the dressing in place (52). Decreases in TEWL were observed throughout the first 4 days.

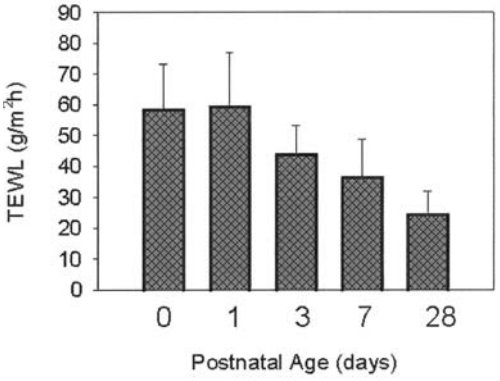

Figure 12 Stratum corneum barrier properties for very preterm infants following birth. TEWL month was examined for infants born at 24–25 weeks and decreased over time, indicating barrier maturation. Even after one month, TEWL was still significantly higher for this group, compared to normal full-term infants. Therefore, while epidermal maturation occurs, the barrier function is not completely normal. (Adapted from Ref. 47.)

2. Literature on Effect of Humidity—Relevance for Preterm Infants

The relevant literature on the effects of environmental humidity on stratum corneum barrier development comes from several sources, in addition to the in vivo studies involving preterm human infants described above. Both in vivo human or animal studies using tape-stripped, i.e., barrier-compromised, stratum corneum as a model for the poorly developed epidermal barrier of the preterm infant and in vivo studies of skin cultures or skin explants can provide insight regarding the effects of humidity in the preterm population. Hanley et al. reported the effects of air exposure using skin explants from fetal rats that were either submerged or placed at the air/medium interface (54). The barrier developed in both systems but was accelerated for the air-exposed samples, as evidenced by histological and biochemical changes. The application of an occlusive film to the explants exposed to air resulted in rates of barrier development similar to those of the submerged samples. In contrast, a semipermeable film accelerated barrier development. Williams and Feingold discussed the implications of this work in the context of applying nonocclusive films to preterm infant skin (55).

Supp et al. reported the effect of humidity on stratum corneum barrier formation using a cultured human skin system (56). Both cultured skin and human cadaveric skin (control) had lower surface hydration at ambient (49–61% RH) versus humid (79–91% RH) conditions. The cultures were followed for 12 weeks after grafting. The graphs grown at ambient humidity

achieved the skin hydration of normal skin more quickly and were more stable than the grafts grown at high humidity.

We investigated the factors that influence skin hydration during barrier repair in two in vivo studies using adult tape-stripped skin as a model for premature, barrier-compromised skin (57). The effects of several semipermeable barrier films relative to complete occlusion and no occlusion were evaluated using measures of TEWL, skin hydration, rate of moisture accumulation, and erythema. Tape-stripped skin treated with semipermeable films underwent more rapid barrier recovery than either nonoccluded skin or skin under complete occlusion. Barrier films that produced intermediate levels of skin hydration during recovery had the highest barrier repair rates, supporting the hypothesis that semipermeable wound dressings augment barrier repair and skin quality. We speculate that the skin condition produced during barrier repair in the presence of the semipermeable membranes is due to the provision of an optimum water vapor gradient for generating appropriate levels of NMF in the recovering SC. We routinely observe infants in the neonatal intensive care unit after they have been exposed to relatively dry conditions for several weeks. Frequently, their skin is dry and undergoing abnormal desquamation, as shown in Figure 13. An important next step in this work involves investigating the effect of an optimum water gradient on the barrier properties and hydration in the premature infant in vivo.

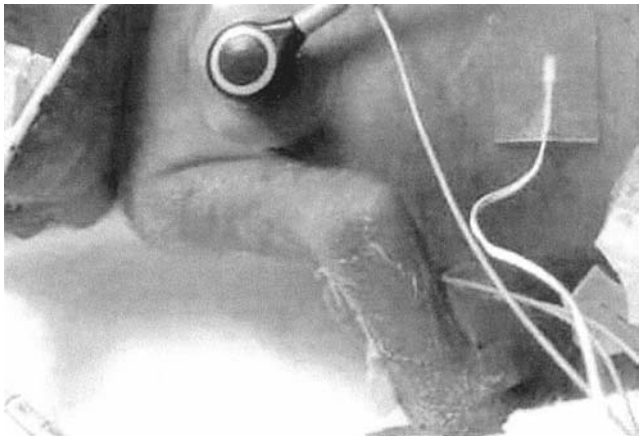

Figure 13 Premature infant skin 3–4 weeks post birth. Premature infants are exposed to relatively dry conditions for several weeks in the neonatal intensive care unit. The skin is dry and undergoes abnormal desquamation in the weeks following birth and barrier development, as shown in Figure 13.

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III. EFFECT OF BATHING

Bathing, a common repeated environmental interaction with the skin, typically occurs soon after birth for the normal neonate. A discussion of the effects of bathing as an environmental perturbation on skin is, therefore, warranted. Bathing practices vary across nurseries regarding frequency, procedure, timing of initial bath, and products used for the bath (58–61). One of the functions of the initial bath is to remove the vernix from the skin surface, although some advocate leaving it in place to take advantage of benefits, including stratum corneum hydration (59,62). One report suggested that vernix insulates the SC and that removal increases the risk of infection (63). However, there have been no published reports of controlled studies comparing the effect of vernix (allowed to remain in place) vs. no vernix on SC hydration, microbial flora, or infection rates for the newborn infant.

Traditionally, bathing involves exposure to water, as well as various types of cleansing agents. Both water and cleansers, singly and in combination, can influence the skin in a dynamic fashion. The effects of water exposure will first be discussed followed by a section on the combination of water with other factors that influence skin condition.

Proper hydration of the SC is essential for effective skin function, e.g., to allow sufficient plasticization and flexibility during movement, to prevent fissuring, and for proper desquamation of the outermost SC layer (64,65). Prolonged exposure of the SC to high levels of water, however, causes maceration, barrier breakdown, and dermatoses, including inflammation, irritation, and urticaria (11,13,66–70). Warner et al., described damage to the SC following prolonged exposure to water (12). Abnormalities included disruption of intercellular lamellar lipid bilayers, degradation of corneodesmosomes, and formation of amorphous regions within the intercellular lipid.

Repetitive exposure of the skin to water during routine bathing and hand washing is a common practice. Imokawa et al. reported the extraction of soluble amino acids following in vivo exposure of human SC to a water soak (71,72). The extracted amino acids were constituents of NMF, the compounds that confer water-holding properties to the SC (73). Ramsing and Agner described the effects of water soaking on irritated human skin in which subjects were exposed to water twice daily for 15 minutes over 2 weeks (74). A significant increase in blood flow was observed, but barrier function (TEWL) and baseline hydration were not significantly impacted. *None of the investigations, however, involved infant skin.*

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A. Full-Term Neonate

1. Effect of Water Soak

Limited in vivo information has been reported about the effects of routine water exposure during bathing on stratum corneum barrier integrity and water-handling properties, i.e., hydration, moisture accumulation rate, and water-holding capacity. We investigated the effects of a 10-minute tub bath on the skin of 52 healthy male and female infants aged 3–6 months (75). Johnson & Johnson Baby Bath® was provided for use during bathing at the parents' discretion. The skin was evaluated before the bath, after removal of clothing and equilibration to room conditions, and again 15 minutes after the bath for both diapered and nondiapered sites, using the same preset timing sequence. Bathing dramatically altered the biophysical properties of the skin. After the 10-minute soak, the skin had significantly decreased erythema and dryness. The skin friction, rate of moisture accumulation, and water-holding capacity were significantly lower after the bath, indicating that bathing markedly alters the SC water-handling properties (Figs. 14, 15). High-resolution optical images were evaluated in pairs several weeks after the enrollment period by mothers (blinded to subject identification). A significant preference for the skin after bathing ($p < 0.01$) was observed.

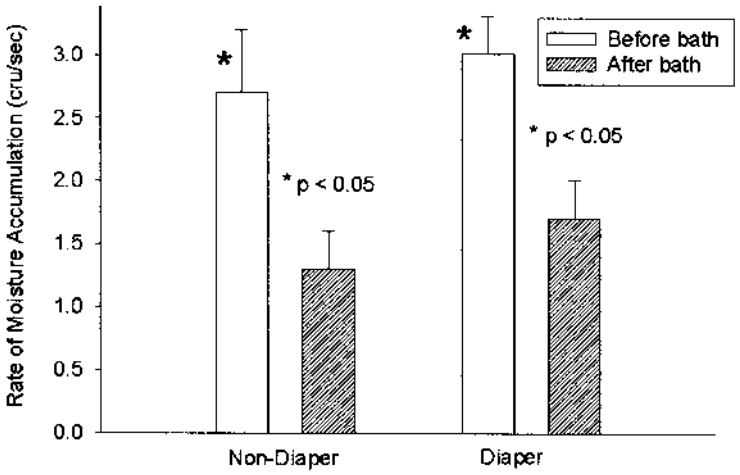

Figure 14 Effect of bathing on SC water-handling properties. The hydration status of the stratum corneum changed significantly 15 minutes after bathing. The rate of moisture accumulation was significantly lower for both nondiapered and diapered skin sites after the bath ($p < 0.05$). (Adapted from Ref. 75.)

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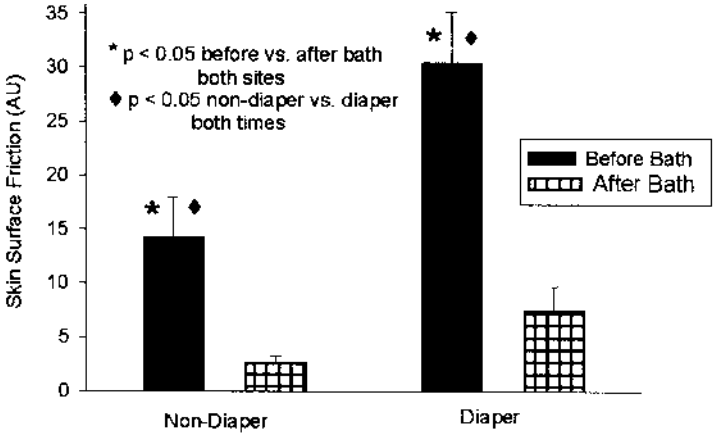

Figure 15 Effect of bathing on SC physical properties. The skin surface friction was dramatically lowered after bathing for both the nondiaper and diaper regions ($p < 0.05$). The skin friction for the nondiaper site was significantly lower than for the diapered site, both before and after the bath ($p < 0.05$). (Adapted from Ref. 75.)

Since NMF consists of water-soluble materials, routine bathing might be expected to alter the NMF content of the stratum corneum. The above results suggest that freshwater bathing removes water-soluble amino acids, i.e., NMF, thereby reducing the amount of secondary bound water in the skin. The reduction in MAT after soaking may be due to a decrease in NMF-dependent bound water that gives rise to the higher capacitance reading prior to the soak. We speculate that, in the presence of a normal SC barrier (i.e., normal TEWL, no damage) and the absence of eccrine sweating, the moisture accumulation test provides a dynamic, functional assessment of loosely bound water associated with the hygroscopic NMF.

2. Effects of Cleansers

Skin cleansing formulations generally contain surfactants that emulsify soils from the skin surface for removal upon rinsing with water. Surfactants may be anionic, cationic, nonionic, or zwitterionic in nature and can be in the bar, liquid, or cream form. Surfactants vary markedly in their effects on the skin and differ significantly in their inherent irritancy or "mildness" to skin (76,77). Surfactants have been shown to vary in their effects on corneocyte swelling, disaggregation, and damage (78). Surfactants, as well as other topical treatments, can vary greatly in their effects on the permeability barrier. For example, Fartasch used electron microscopy to determine the

effects of sodium dodecyl sulfate (SDS) and acetone on human skin in vivo (biopsy specimens) (79). Damage to nucleated epidermal cells and disruption of lipid extrusion were observed for skin treated with 0.5% SDS, even though the upper stratum corneum was intact. In contrast, acetone treatment resulted in disruption of epidermal lipid lamellae and loss of lamellar cohesion throughout the stratum corneum.

The amount of residual surfactant left on the skin surface after rinsing depends upon surfactant properties including the interaction with calcium and magnesium in the rinse water (80). The amount of surfactant used in the procedure and the extent of dilution with rinsing (i.e., volume of rinse water) can impact the residual material remaining on the skin surface after the process is complete (81). Procedures for bathing newborns frequently involve minimal rinsing in order to minimize the cooling effects of full body water exposure. Consequently, the levels of residual surfactant are expected to be high due to the low volume and short duration of rinsing.

Given the irritancy and drying effects of surfactants on skin, the advisability of bathing infants with cleansing products warrants reevaluation. One of the functions of bathing the newborn is the removal of blood and pathogens to prevent transmission to others. Medves and O'Brien investigated the colonization rate for a group of 62 infants bathed using a mild cleanser with the rate for a group of 65 infants bathed with water alone (82). Colonization of the skin increased over time, but there was no difference in type or quantity of microorganisms for the two groups, implying that the cleanser does not impact bacterial colonization (82). Gelmetti recommended the use of mild cleansers with few ingredients to minimize irritant and allergic dermatoses in infants, as well as the use of specialized preparations for specific dermatoses that might come about (83).

Gfatter et al., investigated the effects of cleansing with water alone, a synthetic liquid cleanser, a synthetic bar cleanser, and a fatty acid soap on the skin of infants aged 2 weeks to 16 months (84). Parallel treatment groups of 7–10 infants were washed one time. Measures of skin pH, hydration, and surface lipid content were made before and 10 minutes after washing. All four treatments increased the skin pH versus the starting value, with water alone producing the smallest increase and soap resulting in the largest increase. All three cleansers resulted in a significantly greater increase over water alone, and soap was significantly greater than the synthetic products. The authors indicated that the tap water used in bathing was alkaline (pH 7.8–8.2). However, the extraction of water-soluble amino acids in NMF due to bathing is also expected to give rise to a higher pH. No differences were detected in skin hydration, either as a result of bathing or the cleansing product. This finding is inconsistent with our observation of decreased hydration following bathing, but the time after bathing was shorter (10

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vs. 15 min) and the base sizes were smaller than in our study. The age range of the infants varied from 2 weeks to 16 months, compared to a 3- to 6-month range in our report. Iliev et al. reported the effects of bathing a group of infants over a 2-week period with a whey-based product (85). The authors reported decreases in hydration, pH, and erythema, but the changes were not statistically significant.

The effects of water and surfactants can be further exacerbated by preexisting skin conditions or by other environmental factors. The effects of washing with cleanser on SC and epidermal thickness were evaluated among normal and atopic subjects (86). Soaps decreased the number of cell layers in the atopic group, but not among normal individuals, suggesting an increased susceptibility to cleansers among this population. The combination of washing with surfactants and decreased environmental humidity has been investigated (87,88). The effects of irritant dermatitis due to repeated exposure to water and surfactants were exacerbated at decreased absolute humidity among adult subjects. Animals exposed to low humidity for a short time (2 days) exhibited increased epidermal proliferation following surfactant (sodium dodecyl sulfate) exposure compared to animals housed at normal or high humidity (88). Additionally, animals kept at conditions of high humidity for 2 weeks had greater epidermal proliferation after exposure to surfactant than did animals at low or normal humidities. While similar studies have not been performed in either infants or neonates, the evidence suggests that the effects of water and surfactants may be greater under humidity extremes among this population.

B. The Premature Neonate

The role of bathing and surfactant exposure in the care of the premature infant is the subject of ongoing discussion. The poor epidermal barrier properties and increased susceptibility to damage, coupled with the overall medical instability, are balanced with the need for practices to reduce the risk of infection. Franck et al. (89) investigated the effects of reduced bathing frequency on skin pathogen colonization among a group of premature infants for whom bathing frequency was reduced from once a day to once in 4 days. He found no differences in skin flora on days 2, 3, or 4 after bathing (89). Reduced bathing frequency for premature infants has been recommended as a general standard of care in the neonatal nursery (59). Evaluation of reduced bathing in combination with other evidenced-based guidelines was conducted among 2800 infants (90). Reduction in bathing frequency for the premature infant continues to be recommended. The specific effects of bathing on preterm infant physiology and behavior were investigated in a group of 10 subjects, with responses measured 10 minutes

before, during, and 10 minutes after the bath (91). Significant increases were observed for heart rate, cardiac oxygen demand, and motor behavior, accompanied by a significant decrease in oxygen saturation.

Compared to term infants, preterm infants are more susceptible to transdermal exposures due to their immature epidermal barrier. At one time, cleansing products containing 3% hexachlorophene were used on a regular basis for full body bathing of premature neonates. Subsequent evaluations of the neurotoxic effects indicated that vacuolar encephalopathy was related to hexachlorophene exposure (92). The use of hexachlorophene-containing products was therefore discontinued for this population. Alternatives were proposed through testing on the control of pathogenic bacteria and included Lactacyd and Hibitane (chlorhexidine) (93). Subsequent evaluation of Lactacyd (an alkyl sulfate surfactant), however, showed that it increased TEWL and was inappropriate for premature infants (94). Chlorhexidine was found to be cytotoxic to fibroblasts and keratinocytes in culture and contraindicated for preterm infants (95).

In summary, the use of topical products on premature infant skin, including surfactants, cleansers, antiseptics, etc., should be carefully considered. As described above, the stratum corneum of these infants develops rapidly under the influence of the relatively dry environmental conditions. However, the barrier is not *fully* competent for several weeks after birth. The stratum corneum is more permeable to exogenous materials. In particular, the bathing process involves minimal rinsing of the skin surface, and residual materials undoubtedly remain on the surface. Factors such as SC thickness and integrity, amount of surface residue, inherent irritancy of residual materials, and partition coefficient through the stratum corneum will govern the relative effect on the infant. Most of the surfactants used in commercially available cleansing products interact with and alter the epidermal barrier, as evidenced by numerous studies using in vivo systems (96–103). A consideration of infant skin versus adult skin with respect to the response to topically applied drugs is useful in order to determine the potential effects of topical products in the preterm infant. For example, the infant has a greater surface area-to-body weight ratio and absorbs proportionately greater quantities than adults, tissue distribution depends on age, tissue affinity may vary between infants and adults, and overall effects may be different (104). Future investigations on the effects of surfactants and cleansers using model systems for preterm epidermal barrier structure and function are necessary in order to determine the appropriate products for use in this population.

Disposable cloths containing water, cleansing agents, and other ingredients (e.g., fragrance) are commonly used for cleansing the diaper area skin. In most use situations, the skin is wiped with the cloth prior to diaper

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application but it is not rinsed with water. The scientific information on the effects of diaper wipes and their ingredients is limited. One study among 302 infants compared the effects of four different wipe products on diaper area skin after 10 weeks of use (105). The products varied with respect to wipe substrate and lotion composition, and single variable analysis of specific ingredient effects were not performed. The study did not compare wipe treatment versus any other procedure (e.g., washcloth with water, washcloth with cleansing product, etc.). Therefore, the effects due to product form (i.e., wipe vs. cloth or no cloth) or to method (i.e., wipe ingredients left on the skin surface vs. water rinse) could not be ascertained. There were no significant differences among the four products for erythema, edema, desquamation, or rash frequency. However, skin pH was significantly decreased from 5.6 to 5.0 for the product with pH of 2.8. The implications of a skin pH change with routine use of wipe products have not been described.

Ehretsmann et al. compared a new wipe product to water plus a skin cleanser in a group of 102 infants over a 2-week period. There were no significant differences in severity of erythema and diaper dermatitis among the two test groups (106). The same wipe product was used for 4 weeks on a group of 60 atopic infants. Examination of the treated skin by a dermatologist indicated good tolerance for the product. A controlled study of the effects on the skin condition, i.e., erythema, dryness, rash, barrier properties, of an atopic group compared to normal controls has not been reported.

The results of a 10-week study on four brands of diaper wipes among 317 infants aged 4–12 months concluded that the wipes were safe for daily use (107). The difference among products was their effect on skin pH. The wipes of low pH were associated with low skin pH, but pH changes were not causative for changes in skin condition. The authors did not report the skin grades and did not comment on the skin condition of the infants in the study. An investigation of the effects of diaper wipes on damaged skin was reported, using a skin stripping model on adult skin (107). Adult volar forearms were tape-stripped to TEWL values of $30\,g/m^2/h$ greater than baseline to simulate the TEWL values observed in infants with damaged skin. One of the stripped sites was wiped with a diaper wipe product (Pampers® brand, alcohol-free) four times daily for a total of 135 seconds, and the other served as an untreated control. Skin erythema grades during the 4-day period were reported, as shown in Figure 16. Erythema decreased for both sites from initial mean grades >2, presumably as a result of barrier recovery from tape stripping. The wipe and control sites exhibited similar patterns, although the wipe treatment appeared to have statistically higher erythema on days 3 and 4 (Fig. 16). The authors concluded that the wipes did not interfere with normal healing. However, measurements of barrier recovery, such as TEWL to establish normaliza-

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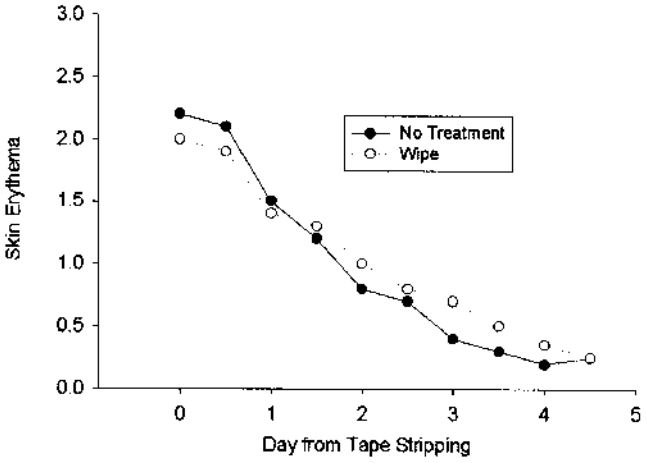

Figure 16 Effect of diaper wipe on tape-stripped adult volar forearm skin. One tape-stripped skin site (adult volar forearm) was wiped with a diaper wipe product (Pampers® brand, alcohol-free) four times daily for a total of 135 s, and the other served as an untreated control. Erythema decreased for both sites from initial mean grades >2, presumably as a result of barrier recovery from tape stripping. The wipe and control sites exhibited similar patterns, although the wipe treatment appeared to have statistically higher erythema on days 3 and 4. (Adapted from Ref. 107.)

tion of the SC barrier, were not reported. The tape-stripped sites recovered without occlusion or exposure to the diaper material, unlike the situation experienced by the infant. The effect of wipes on damaged infant skin has not been reported.

IV. EFFECT OF TOPICAL TREATMENTS

Materials applied to the skin surface represent another set of "environmental" effects, since they may interact with and influence properties such as hydration, barrier function, and elasticity. The "topical treatments" in this section include nonprescription ointments, e.g., petrolatum, Aquaphor®, generic creams, alcohol, and laundry products, e.g., fabric softeners and detergents that transfer to the skin from a substrate. We will also discuss the effects of tactile and photostimulation. The effects of topical treatments on full-term neonatal skin are described in the context of their transfer from skin contact products, e.g., diapers. Therefore, the discussion here focuses specifically on the effects on premature infants.

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A. Ointments

High insensible water loss is a significant problem for the premature neonate and is a function of stratum corneum barrier integrity. Since epidermal cornification occurs during the latter part of gestation, barrier integrity is exponentially related to gestational age (Fig. 10) (8). For example, at 26 weeks gestation, TEWL is about 45 $g/m^2/h$. At week 29, mean TEWL is about 17 $g/m^2/h$. By week 32, TEWL is 8–10 $g/m^2/h$ (8).

In an attempt to reduce water loss, hydrophobic ointments such as petrolatum (mixture of hydrocarbons) and Aquaphor (petrolatum, mineral oil, mineral wax, wool wax alcohol) have been applied to the skin surface of premature infants (108–110). Rutter and Hull reported an immediate, significant decrease in TEWL on each of three infants, aged 26–30 weeks of gestation, following application of paraffin (mixture of hydrocarbons, similar to petrolatum) (108). Initial TEWL values of 18–36 $g/m^2/h$ dropped to 11–14 $g/m^2/h$, corresponding to 40–60% reductions in water loss. TEWL increased over time, but values at 8 hours posttreatment remained lower than the untreated starting values.

The effects of repeated application of Eucerin® cream (petrolatum, mineral oil, mineral wax, wool wax alcohol, water) twice a day for 12 days were reported for a group of older preterm infants (110). Treatment and control (no Eucerin®) groups consisted of 17 infants each with mean gestational ages of 32.3 and 32.5 weeks, respectively. TEWL decreased for both groups from 15 and 14.6 $g/m^2/h$ to 8.6 and 8.2 $g/m^2/h$ at study completion, and between-group comparisons were not significantly different. However, the treatment group had significantly better skin condition than the control group for whom the condition worsened. No differences were observed between groups in quantitative bacterial cultures.

Nopper et al. investigated the effect of topical treatment with Aquaphor versus an untreated control on the barrier function (TEWL), skin condition, and nosocomial infection rate among premature infants of lower gestational age (109). Parallel treatment groups of 30 infants (mean age 29 weeks, range 24–32) were evaluated over 14 days. The short-term effects of Aquaphor, compared to an untreated contralateral control site (same infant), on TEWL were determined using the treatment group. TEWL for the treated site was significantly lower than the control immediately after application. The difference was maintained for 4–6 hours, although TEWL increased over time for the Aquaphor site (Fig. 17). Amounts of 1.5 g of Aquaphor were applied twice a day. To calculate a dose per unit area, the body surface area was approximated using published algorithms and reduced by 20%, since the treatment was not applied to the head and face (111,112). Depending upon the assumptions regarding infant

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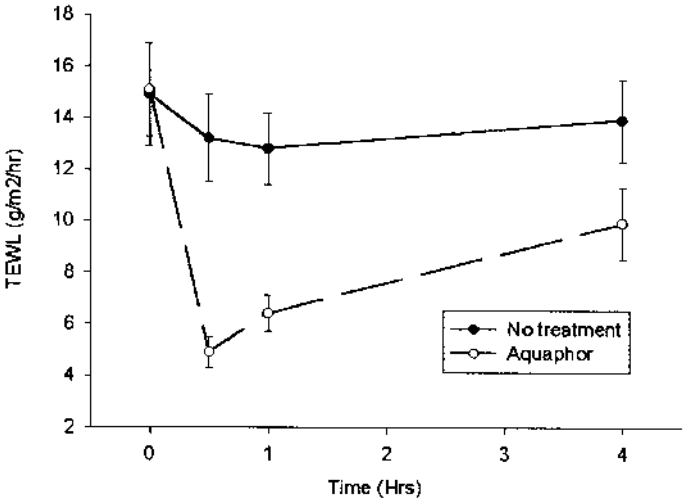

Figure 17 Short-term effects of Aquaphor treatment on SC barrier properties in premature infants. The short-term effects of Aquaphor, compared to an untreated contralateral control site (same infant), on TEWL were determined. TEWL for the treated site was significantly lower than the control immediately after application. The difference was maintained for 4–6 h, although TEWL increased over time for the Aquaphor site. (Adapted from Ref. 109.)

height, the dosage is approximated to be 0.8–1.0 mg/cm^2 for the infants in this study. The effects of petrolatum versus an untreated control on TEWL over a 4-hour period were determined in normal adults ($n = 10$). Thirty minutes following application, TEWL was significantly lower than the control, but the treatments were not different after 4 hours. Similar decreases in TEWL at 15 minutes following application have been observed for Aquaphor and petrolatum at levels of 2.5 mg/cm^2 on normal adult volar forearm skin (113).

The effects of repeated application of Aquaphor on TEWL in the preterm infants during the 14-day postnatal period are shown in Figure 18 (109). TEWL decreased for both treatment sites over the 2-week period with values within the normal range by the end of the period. TEWL was significantly different for the groups on day 14 only. However, because TEWL differed for the groups on day 0, the percent decrease in TEWL was not significantly different between the groups. The decrease in TEWL is consistent with other reports of decreased TEWL over time from birth and with the development of the stratum corneum barrier following exposure to the dry environment after birth (24,39,41,47). *However, lack of differentiation in TEWL over time between treatment and control suggests that*

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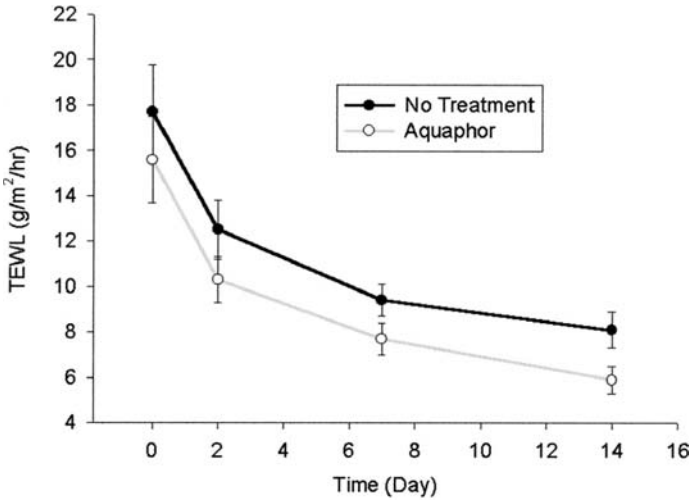

Figure 18 Effects of repeated Aquaphor application on SC barrier properties in premature infants. TEWL decreased for the Aquaphor treated site and for the untreated control site over a 2-week period, with values within the normal range by the end of the period. TEWL was significantly different for the groups on day 14 only. Since TEWL differed for the groups on day 0, the percent decrease in TEWL was not significantly different between the groups. (Adapted from Ref. 109.)

Aquaphor did not increase the rate of SC barrier development for this group of infants (mean EGA 29 weeks).

The skin grades were significantly lower (better condition) for the treatment than the control, which exhibited a significant increase in scaling. The treatment group had significantly less colonization in quantitative skin cultures of the axilla (days 2, 3, 4, 14). The treatment group had 3.3% incidence of positive blood or cerebrospinal fluid compared to 26.7% for the untreated control. Pabst et al. investigated the effects of repeated Aquaphor exposure over 14 days in premature infants of 26–30 weeks gestation and found improved skin scores in the treatment group, worsening skin condition in the untreated control group, and no differences in skin microflora (114). These positive findings for levels of microorganisms and for skin condition suggested that the use of petrolatum-based ointments was appropriate for premature infants (115). An increase in systemic candidiasis was reported among a population of extremely low birthweight infants who had been treated with topical petrolatum (116). The incidence rate decreased to baseline (prior to petrolatum treatment) once applications of petrolatum were discontinued.

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Following an extensive review of the literature regarding skin care practices, including the use of topical treatments, bathing practices, disinfection, and adhesive removal, a skin care guideline for use on neonates was formulated (59). These recommended skin care practices were implemented and evaluated among 51 hospitals and 2820 neonates throughout the United States (90). A total of 11,648 evaluations were made on 2464 sick infants in NICU and SCUs, including premature infants, and 356 healthy infants. The study examined various aspects of skin condition and skin care practice before and after implementation of the guidelines. The use of emollients on premature infants increased, along with a decrease in bathing frequency. Skin condition, measured as dryness, erythema, and skin breakdown, was improved on both groups following the changes in skin care practices. The incidence of nosocomial infection was not reported. The extent of improvement in skin condition could not be established in quantitative terms, e.g., percent reduction in dryness or erythema, since there was no control group for whom skin care practices were not modified.

Given the positive findings for the effects of topical treatments (i.e., emollients, Aquaphor, petrolatum), a large multicenter clinical trial was conducted among parallel groups of premature infants of 501–1000 g from August 1998 through March 2000 (117,118). The primary outcome measures were nosocomial sepsis rates and skin condition. Infants were enrolled after birth and before 48 hours of life, and mean enrollment time was postnatal day 1. The treatment group included 602 infants of mean EGA 26.2 weeks and the control group had 589 infants (EGA 26.2 weeks). Aquaphor ointment (petrolatum, mineral oil, mineral wax, and wool wax alcohol) was administered every 12 hours to the treatment group from enrollment through postnatal day 14. The control group infants received ointment only on the local areas of severe dermatitis or injury for no more than 3 days. A 7 g tube of Aquaphor was provided for every two treatments, with a maximum dose of about 3.5 g. Total body surface areas can be calculated, assuming weights of 500–1000 grams and heights of 0.29–0.36 m (111,112). From the resulting surface areas, dosages were approximated to be 1.0–2.7 mg/cm^2 for 1.5 g of Aquaphor up to 2.3–6.3 mg/cm^2 for 3.5 g of Aquaphor.

In the total population, the infection rate was not significantly different for the treatment versus the control (117,118). However, a significantly higher incidence of nosocomial infection was observed among the treatment infants weighing 501–750 g. This finding was not anticipated, given the favorable results reported in smaller sized studies (109,110). Infants in the large study had a mean EGA of 26 weeks, compared with an average of 29 weeks in the smaller studies. While TEWL was not measured in the large trial, previous investigations found that TEWL for a 26-week-old infant would be about 45 $g/m^2/h$ versus the 15–17 $g/m^2/h$ for the 29-week infant

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(8,109). This represents a substantial difference in TEWL and SC barrier integrity at the beginning of treatment. The skin of the younger, smaller infants corresponded to a more "wounded" state. The differences in mean gestational age would translate to skin surface area. Amounts of Aquaphor per infant also differed, with 1.5 g per dose in the smaller trials up to about 3.5 g per dose for the large study. Therefore, it is reasonable to conclude that the infants in the large study had doses (g/cm^2) up to 2–4 times higher than in the previous reports.

The inherent occlusive properties of the Aquaphor treatments under the clinical conditions may also be relevant to understanding the outcome. The water vapor transport rates of topical treatments, including petrolatum and Aquaphor (glycerin-containing version), have been measured in vivo (119,120). Measured amounts were applied to films of GORE-TEX® (expanded polytetrafluoroethylene backed with a hydrophilic nylon support) sealed to the edges of water-filled reservoirs under specified environmental conditions. Water vapor transport (WVT) rates ($g/m^2/h$) were calculated from gravimetric measurements of the system. Under the experimental conditions, the GORE-TEX control had high WVT rates, from 55 to 70 $g/m^2/hr$. Aquaphor and petrolatum were applied at levels of 0.5–2.0 mg/cm^2 and produced substantially lower WVT rates of 1.5–4.8 $g/m^2/h$, depending upon humidity and temperature. The WVT rates were lower at higher humidity (e.g., 70%). Aquaphor and petrolatum had very similar WVT profiles. By comparison, occlusive plastic films, e.g., Saran Wrap®, had WVT rates of 0.5 $g/m^2/h$. Therefore, for the multicenter trial among ELBW infants, the Aquaphor treatment may have provided an occlusive environment for the skin during at least some of the 12-hour period between applications. If this were the case, the effect of occlusivity on nosocomial infection rates and skin barrier development can be considered. Ghadially et al. investigated the effects of petrolatum (Vaseline® petroleum jelly) on adult volar forearm skin (121). Skin sites were treated with acetone to remove SC lipids and disrupt the barrier, and TEWL values ranged from 16–40 $g/m^2/hr$ prior to application of petrolatum. Petrolatum was found to augment barrier repair in the initial stages, providing a 70% recovery after 3 days. However, this barrier damage model does not remove outer SC layers and decrease the overall thickness, as is the case for ELBW infants' skin, and the findings may not be applicable to the infant.

Total occlusion has been shown to delay barrier recovery by some investigators (122), while others have shown no impairment of barrier formation following occlusion (123,124). We examined the ability of vapor-permeable films to augment wound healing following superficial trauma. The effects of semipermeable membranes on skin barrier repair in an adult model for barrier disruption (i.e., stratum corneum tape-stripping)

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were investigated (57). The barrier recovery process was optimal for conditions of semi-occlusion. The semipermeable films produced a faster rate of barrier recovery than either no occlusion or complete occlusion, as measured by changes in transepidermal water loss over time. Hydration measurements made immediately following removal of the films indicated that the occlusive films resulted in the highest levels of hydration at the skin surface. Evidence supports a role for the water vapor gradient in regulating barrier lipid synthesis as well as DNA synthesis in superficially wounded epidermis (34,122,125). As a consequence, if Aquaphor was sufficiently occlusive in the multicenter trial, barrier development (i.e., barrier repair in the referenced literature) may have been delayed, thereby increasing the risk of infection.

The effects of long-term occlusion on TEWL and microbial skin flora have been reported (126). Healthy, undamaged skin sites on normal adult volar forearms were occluded for five days using plastic film (Saran Wrap). TEWL readings were elevated following occlusion, but the effects of water accumulation under the film could not be separated from the effects on barrier function. The microbial analyses revealed significant increases in total average counts from $1.8 \times 10^2/cm^2$ before occlusion to $4.5 \times 10^6/cm^2$ on day 5. Coagulase-negative staphylococci, a normal well-tolerated flora in healthy adults, were present in the greatest amounts (63%). In the multicenter Aquaphor trial, coagulase-negative staphylococcus was found to be the cause of nosocomial sepsis in over 60% of the cases (118). *Therefore, one possible explanation for the increased incidence of nosocomial infection in the ELBW infants is that treatment with Aquaphor resulted in a sufficiently occlusive skin microenviroment to delay SC barrier development and to facilitate the growth of coagulase-negative staphylococcus, a harmful organism for the vulnerable premature infant.*

B. Phototherapy

Phototherapy is a common treatment in premature infants with jaundice. The impact of phototherapy as an environmental perturbation in newborn infants has been investigated. Kjartansson et al. determined the effect of phototherapy on TEWL in both full-term and preterm infants nursed in incubators at 50% ambient relative humidity (127). TEWL was not significantly altered after 30 minutes of phototherapy in the full-term infants and after 120 minutes in the preterm infants. Wananukul and Praisuwanna studied the effect of phototherapy on TEWL in full-term infants (GA 38–41 weeks) (128). A group of 40 jaundiced infants receiving phototherapy was compared with 40 healthy control infants (no phototherapy). TEWL was measured before, 30 minutes after, and 6 hours after treatment for the

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jaundiced infants and at comparable time points for the control group. TEWL increased significantly in the treatment group but not in the control infants (128). TEWL and skin hydration were measured in seven body sites for 31 premature infants (25–36 weeks GA) prior to phototherapy and during the session (129). TEWL increased on average by 26.4% relative to the pretreatment values and reached levels of 45.9, 36.4, and 29% for the cubital fossa, groin, and back, respectively. Stratum corneum hydration increased significantly in only one site.

In an effort to prevent TEWL increase in premature infants ($\leq$34 weeks GA), 1.5 mL of a clear topical ointment was applied to one half of the infant prior to phototherapy (130). The untreated side served as the control site. TEWL was measured before treatment and at 30 minutes and 4–6 hours after. The topical treatment significantly reduced TEWL (29% at 30 min, 26% at 4-6 hours) relative to the untreated side. Dani et al. compared the effects of conventional phototherapy and fiberoptic phototherapy on TEWL in 20 preterm infants (10 per group) (131). For both modalities, a topical ointment was applied to part of the skin surface, while the contralateral sites were untreated controls. For the conventional group, the two skin sites did not differ significantly in TEWL after treatment. For the fiberoptic treatment, TEWL increased significantly for both sites, but to a lesser extent for the topical ointment site. These findings indicate that phototherapy can have significant negative effects on fluid loss and on the epidermal barrier of the premature infant.

C. Tactile Stimulation

One of the most significant environmental influences in the life of a neonate is the sensory stimulation provided by the mother and other caregivers. Interactions with the infant occur in large part through the skin, in contact through feeding, bathing, and diapering. The infant is held, touched, and stroked for long periods. The effects of tactile stimulation, massage, and holding on the skin properties and barrier function of human infants have not been reported. One indication of the role and importance of the skin comes from studies in the neonatal animal model (6). Tactile stimulation in the form of stroking with a soft brush (to simulate licking/stroking by the mother) was found to increase circulating lactate levels by 207% relative to the untreated control group. Since lactate is an important substrate for neonatal brain development in this species, tactile stimulation may prove to be critically linked to neurological development.

Tactile stimulation, in the form of stroking or massage therapy, has been examined in neonates by several investigators (132–140). In general, the infants were enrolled after medical stability was achieved. A trial among

20 premature infants (31 weeks mean GA, 1280 g mean weight) and 20 control infants showed that infants who received stroking and passive limb stimulation gained more weight (25 g vs. 17 g) and were behaviorally more mature than the control group (141). The treated infants were discharged 6 days earlier than the control group. In an animal study, rat pups were initially separated from their mothers and thereby deprived of tactile stimulation. They had reductions in growth hormone release and protein synthesis (142). Pups separated from mothers had lower synthesis of ornithine decarboxylase, decreased DNA synthesis, and decreased response to growth hormone (139). The decreases were related to the absence of a specific tactile stimulation behavior.

Massage therapy was administered three times a day for 10 days in 15 cocaine-exposed neonates (30 weeks mean GA) (134). The treated infants gained more weight than the parallel control group and demonstrated fewer stress behaviors. In order to identify the hormonal factors responsible for growth, 24-hour urine samples were collected before and after a 10-day treatment with tactile-kinesthetic stimulation (3 times per day) (138). A group of premature infants (30 weeks mean GA) was compared to a control group that did not receive the stimulation (138). Levels of norepinephrine and epinephrine increased significantly in the group receiving stimulation. No significant differences were reported between groups for dopamine, cortisol, and creatinine. Acolet et al. reported a decrease in cortisol for a group of premature infants (29 weeks mean GA) before (45 min) and one hour after a massage (140). However, changes in cortisol for a control group (no massage) were not reported.

Mathai et al. examined the effects of a structured massage in 24 premature infants ranging from 1000 to 2000 g at birth compared to a parallel group that did not receive massage (137). The massage was conducted from day 3 to the term-corrected age, and outcome measures included growth parameters and neuro-behavioral assessments. The massage group exhibited significantly higher weight gain (4.2 g/day) than the control group and had significant improvements in orientation, autonomic stability, range of state, and regulation of state. While heart rate increased during massage, it remained within the physiological range. White-Traut et al. investigated the effect of unimodal and multimodal sensory stimulation in premature infants and concluded that the tactile component alone may cause too much arousal (133). As part of a program to investigate the NICU environment on brain growth and CNS organization, Gorski et al. monitored physiological parameters (e.g., pO_2) as a function of timing of tactile stimulation (143). They concluded that tactile stimulation administered during periods of low pO_2 could predispose to episodes of bradycardia.

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Therefore, timing of the tactile intervention is most important in achieving favorable outcome.

To clarify the impact of neonatal massage on physiological and developmental outcomes and to determine the role of massage for premature infants, Vickers et al. conducted an extensive statistical review of the available information. Specifically, information in the Cochrane Collaboration Field in Complementary Medicine and the Neonatal Collaborative Review Group databases were reviewed and analyzed (135). While increased weight gain and reduced length of stay were attributable to massage therapy, the studies were found to have deficiencies in methodology. The authors concluded that the data were insufficient and did not provide the basis for establishing massage therapy as a treatment modality for premature infants. They recommended additional, well-controlled, and large-base clinical investigations in order to establish the risk/benefit of this modality. *Importantly, none of the published reports have addressed the role of tactile stimulation in the care of the very premature (24–26 weeks GA) or very low birthweight (< 1000 g) infant.*

V. SUMMARY

At birth, the human infant undergoes rapid and significant changes during transition from an aqueous environment in utero to the dry terrestrial surroundings of postnatal life. The skin, particularly the stratum corneum, adapts rapidly to the changing environmental influences. The full-term infant has a superb, fully functional epidermal barrier at birth with respect to protection from water loss and prevention of percutaneous absorption of harmful agents. The stratum corneum, nevertheless, undergoes marked changes in water-handling properties over the first few weeks of life. Thereafter, the skin responds to simple environmental interactions, such as freshwater bathing and changes in relative humidity, to maintain the necessary transepidermal water gradient and hydration for flexibility during movement. The premature infant also experiences major changes at birth. However, the wounded skin state of these infants makes them more vulnerable to their environment. The stratum corneum develops rapidly in response to decreased environmental humidity but does not achieve the normal newborn state, with low TEWL, for more than one month. During this time of epidermal barrier development, the preterm infant is at risk for additional skin damage, due to adhesives and routine handling, and for increased permeability to exogenous agents. The contrast in skin properties and barrier development between the full-term neonate and the preterm infant provides a valuable mechanism for identifying treatment

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modalities that will facilitate optimum barrier development in the preterm. However, common interventions, such as the use of topical ointments, to protect the preterm infant's skin are not always successful. That is, the premature infant does not respond as predicted by models and experience in healthy full-term infants, older children, or adults. This lack of predictive ability is in part due to the relatively limited base of data in this population. Better skin-treatment modalities are needed to assist this group in achieving decreased morbidity. Additional insight regarding the development of the superb epidermal barrier in utero in the full-term is predicated upon a better, mechanistic understanding of the developmental biology and the role of vernix caseosa during the last trimester. This information is necessary in order to formulate optimum conditions for barrier development ex utero.

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