TWO VOLUME SET

Harper's Textbook of Pediatric Dermatology

FOURTH EDITION

EDITORS

Peter Hoeger • Veronica Kinsler • Albert Yan

EDITORIAL ADVISORS

John Harper • Arnold Oranje

ASSOCIATE EDITORS

Christine Bodemer • Margarita Larralde • David Luk

Vibhu Mendiratta • Diana Purvis

WILEY Blackwell

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Contents

VOLUME 1

Section 1 Development, Structure and Physiology of the Skin

Section 2 Skin Disorders of the Neonate and Young Infant

Section 3 Atopic Dermatitis and Related Disorders

Section 4 Other Types of Dermatitis

Section 10 Parasitic Skin Infestations and Sting Reactions

Section 11 Urticaria, Erythemas and Drug Reactions

Section 12 Acne and Acneiform Disorders

Section 13 Nutritional Disorders

Section 14 Blistering Disorders

Section 15 Photodermatoses, Photoprotection and Environmental Skin Disorders

Section 16 Granulomatous Diseases

Section 17 Neutrophilic Dermatoses

Section 18 Lymphocytic Disorders

CHAPTER 1
Embryogenesis of the Skin

Lara Wine Lee[1] *& Karen A. Holbrook*[2]

[1] Departments of Dermatology and Pediatrics, Medical University of South Carolina, Charleston, SC, USA
[2] Formerly Department of Physiology and Cell Biology, Ohio State University, Columbus, OH, USA

Abstract

The skin is a large and complex organ. While skin development begins during early embryogenesis, full development is not complete until well into the postnatal years. Studies of skin development can shed light on a number of basic problems in contemporary biology: epithelial–mesenchymal interactions that establish organs (in skin, these tissue interactions occur in follicle, sweat gland and nail formation); cell–cell interactions through soluble mediators; gene regulation; apoptosis; differentiation (structural, biochemical and functional); and certain longstanding basic phenomena of development such as induction, pattern formation and differentiation. Rigorous understanding of embryogenesis allows definition of critical periods when the skin may be more vulnerable to developmental errors. Further understanding of these critical processes advances the study of developmental disorders of the skin with the promise of improving therapeutic options for these disorders.

Introduction

The skin is an ideal organ in which to study development because it is readily accessible for observation, sampling and evaluation. As an interface, it straddles the internal, systemic world of the individual and the external environment and is modified by both. The skin itself is a complicated and complex organ, with the normal structure and function of each 'part' highly dependent upon what happens in other parts of the skin. In other words, one cannot understand, for example, changes that occur in the epidermis without understanding the nature of the dermis since the dermis has major influences on the activities and functions of the epidermis. This is the case for each region or structure of the skin.

Development offers an opportunity to study skin structure and function under more controlled conditions because the environment of the developing skin is reasonably constant (controlled light, temperature, pressure, etc.). It is therefore possible to investigate how the properties of the different regions and structures of the skin are coordinately established, presumably under the directions of a genetic programme.

Some of the structures of the skin may be fully formed early in the fetal period whereas other structures or regions are not complete until well into the postnatal years. Full establishment of adult functions of the skin always requires an extended period of development beyond the stages *in utero*. Development is the first period in a continuum of events that modifies the skin. It is characterized by morphogenetic processes, activation of new genes and gain of function. In contrast, ageing may involve morpholytic processes in which genes are turned off, resulting in a loss of function. Consideration of this continuum, and the genetic and environmental interactions that come into play throughout life, provides a conceptual framework for discussing the place and role of the events in skin morphogenesis.

Understanding the stages and events of normal human skin development is also important from a biomedical perspective. Skin embryogenesis allows the definition of critical periods when the skin may be more vulnerable to developmental errors. It provides an opportunity to study the evolution of skin function, establishing a background for understanding the natural history of expression of genetic skin disease in its earliest form. Moreover, advances in gene therapy may provide intervention rooted in understanding normal morphological processes.

The unique morphological properties of developing human skin have always intrigued investigators. Specific aspects of the skin that are found only in the fetus, such as the periderm, and specific events that result in the formation of complex structures, such as follicle or sweat gland, were often described for specific ages only (reviewed in [1–4]). Expansion of studies to characterize the complete ontogeny of the tissue, region or structure then began to include data derived from

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biochemical or immunohistocytochemical assays for the expression of specific molecules that were known to correlate with the state of differentiation or with a specific property such as barrier function adhesion. Culturing and grafting human embryonic and fetal skin (reviewed in [5–8]) and skin-derived cells [9,10] and evaluation of skin from fetuses affected with genodermatoses (reviewed in [11–13]), or under conditions of growth retardation, have also provided insight into human skin development. Our understanding of skin development continues to increase as we apply more modern tools of biology to study the skin at all stages of life.

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Time-scale of skin development

There are several schemes for categorizing stages of skin development (Fig. 1.1) [1]. Development is defined by estimated gestational age (EGA) starting at the time of fertilization, which differs from calculations based on the last menstrual period (LMP). Fertilization occurs on average 2 weeks after the LMP. Human development is separated into the embryonic period, before the onset of bone marrow function, which corresponds to fertilization to 2 months EGA, and the fetal period from 2 months EGA until birth. The first trimester includes the entire embryonic period and the first stages of the fetal period. Histogenesis of all skin regions is initiated in the embryo, and differentiation of some of those tissues begins to occur in the first trimester [2]. The boundary between the first and second trimesters, at 3 months of age, is based only on fetal age and not on any specific changes in structure, composition or function of any region of the skin.

The second trimester includes many important events in skin development. Morphogenesis of new structures is initiated and there is terminal differentiation of others. During the third trimester, all parts of the skin are assembled and the functions of each of them are unfolding. The end of this period is not the final state of the skin, as there is significant reorganization of certain units of the skin (e.g. the vasculature), additions to the skin in volume (e.g. the dermal matrix) and functional maturation of many structures of the skin (e.g. nerves, sweat glands and stratum corneum) after birth [3–7].

Other important times that should be recognized in skin development are the ages at which diagnostic procedures are performed for the purpose of evaluating the condition of a fetus at risk for a genetic skin disease (reviewed in [8]). Fetal deoxyribonucleic acid (DNA) can be extracted from chorionic villi sampled around 10 weeks EGA and amniotic fluid cells can be obtained at around 14–16 weeks EGA. Fetal skin can be sampled as early as 16 weeks EGA, however this technique is largely obsolete and has been replaced by more advanced diagnostic and genetic methods. Newer technology allows isolation of fetal DNA from maternal plasma as early as 9 weeks EGA. The cell-free DNA testing is currently not widely used for microdeletion syndromes but offers promising noninvasive first-trimester testing in the future. In addition, preimplantation genetic diagnosis has been used successfully for diagnosis of severe skin disease (reviewed in [8]).

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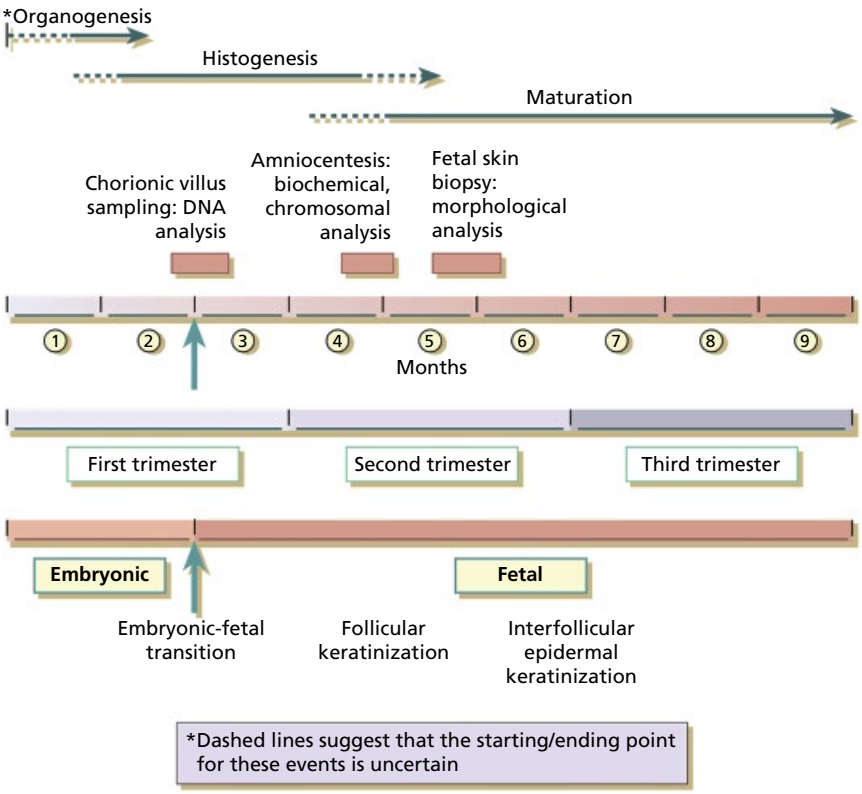
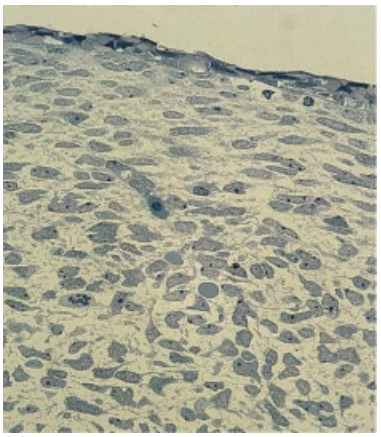


Fig. 1.1 Time-scale diagram identifying specific stages of skin development and identifying the ages at which prenatal diagnosis can be performed using each of the various methods currently employed. Source: Adapted from Polin RA, Fox WW. Fetal and Neonatal Physiology, 2nd edn. Vol. 1. Philadelphia: W.B. Saunders, 1998:730.

Embryonic skin

The primitive ectoderm of the developing blastocyst is established at 1 week EGA, and by 20–50 days EGA the development of major organs and organ systems of the human embryo is initiated. The integumentary system exhibits characteristics of the skin at 30 days EGA. The epidermis, dermoepidermal junction (DEJ) and dermis are well delineated and the tissue is innervated and vascularized (Fig. 1.2). The boundary between the dermis and subcutaneous tissue is not clearly defined in all body sites, but in some regions these two zones are distinct from one another on the basis of a greater density of cells and matrix in the dermis compared with the hypodermis. The skin is closely associated with the underlying developing striated muscle or cartilage on the appendages. There is no morphological evidence that epidermal appendages have begun to form.

In most regions of the embryo, the epidermis is a simple, flat, two-layered epithelium consisting of basal and periderm cells (Figs 1.2 and 1.3). The periderm is a distinct embryonic layer that is eventually shed. Both types of cells are mostly filled with glycogen, a molecule that is characteristic of the cytoplasm of developing and regenerating tissues, where it most likely serves as a source of energy [1] (Fig. 1.3). Microvilli project from the peridermal surface into the amniotic fluid (Figs 1.3b and 1.4). The nucleus is centrally located in periderm and basal cells, and the cytoplasmic organelles are sparse and distributed either around the nucleus or at the periphery of the cell (Fig. 1.3b). Both layers contain distinct keratin intermediate filament proteins (Fig. 1.5)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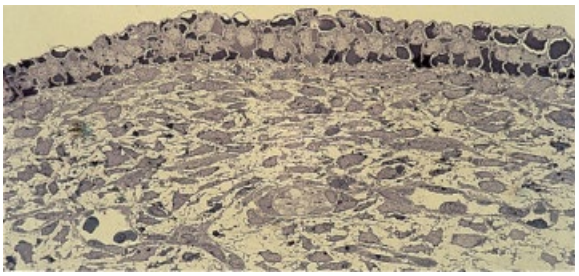
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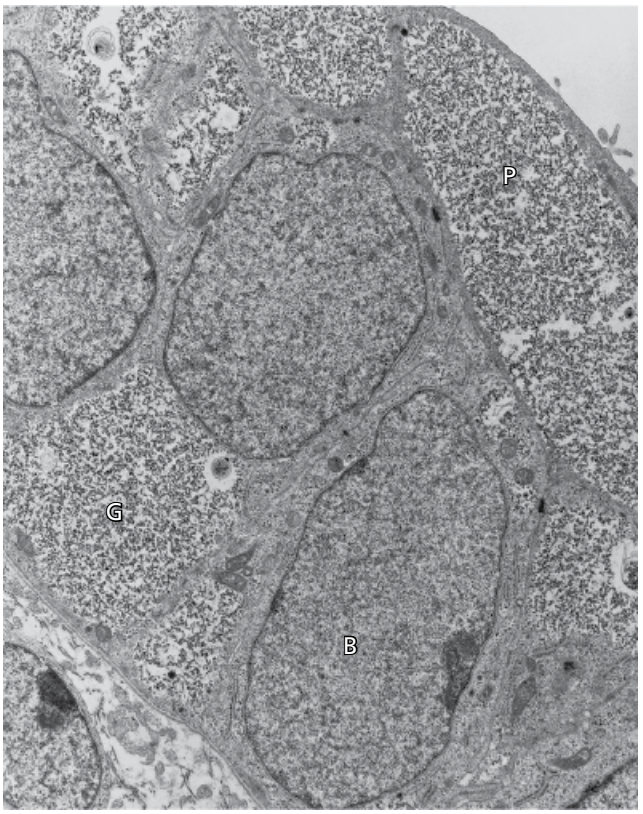
Fig. 1.2 (a) Tissue of the body wall of a 36-day EGA human embryo and (b) the skin from a 45-day EGA human embryo. Note the two-layered epidermis, dermis and subcutaneous tissue and the more linear orientation of dermal cells in contrast to the pleomorphic shapes of the subcutaneous mesenchyme. In (b) note the periderm and basal cells of the epidermis, the closely associated fibroblastic cells in the dermis proximal to the epidermis and a nerve–vascular plane separating the dermis from the subcutaneous tissue (×200).



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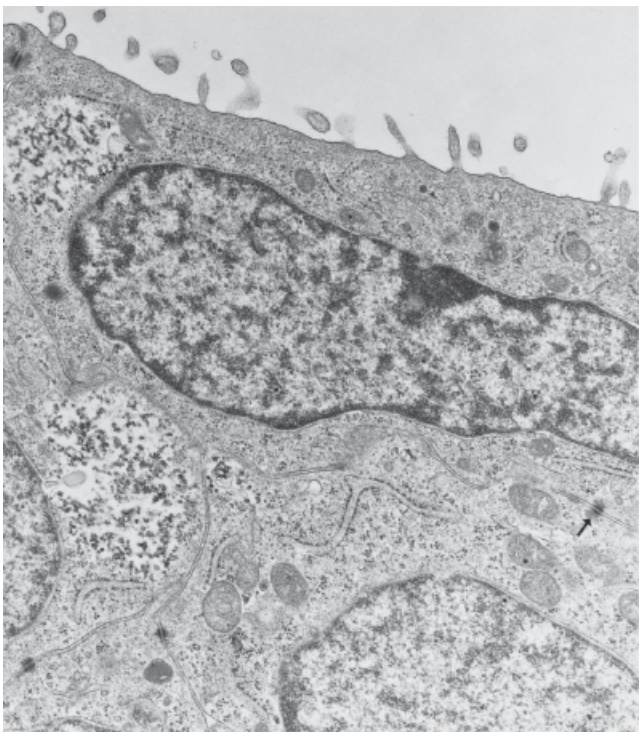


(b)

Fig. 1.3 Transmission electron micrographs of the embryonic epidermis. In (a) note the glycogen (G)-filled basal (B) and periderm (P) layer cells. Desmosomes are evident between basal cells and between basal cells and periderm cells. The DEJ is flat and shows few sites of increased density, suggesting sites of desmosome formation. In (b) one periderm cell and portions of two basal cells are shown. Note the nature and disposition of cytoplasmic organelles within both cell types, the keratin filaments associated with desmosomes (arrow) and the microvilli extending from the periderm surface (a, ×11 525; b, ×25 000).

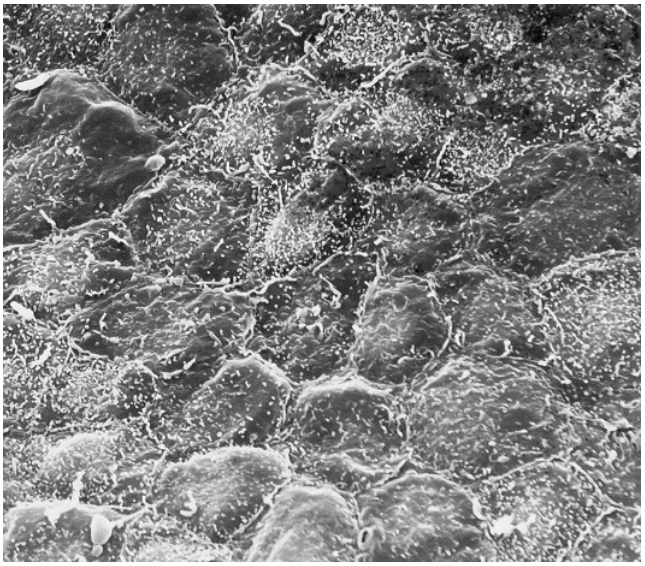
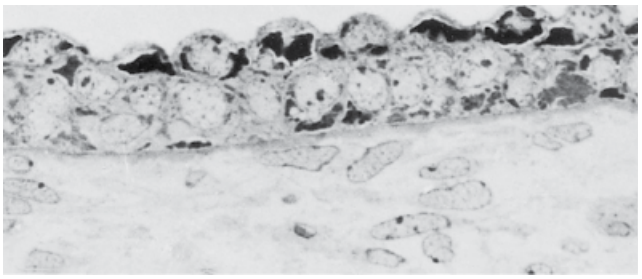


Fig. 1.4 Scanning electron micrograph of the surface of 55-day EGA embryonic skin from the surface of the developing foot. The layer of cells shown is the periderm. Note the microvilli and the variable size and shape of the cells (×1000).

[2,3], and unique cell-surface molecules [4]. The latter markers may reflect the differences in environments surrounding each layer.

The columnar-shaped basal cells of the embryonic epidermis express the keratins, K5 (58 kDa) and K14 (50 kDa), that are characteristic of adult basal layer keratinocytes [2,3] and additional keratin polypeptides, K19 (40 kDa) and K8 (52 kDa), that are specific to embryonic/fetal basal cells and periderm cells [2,3]. At least one keratin polypeptide expressed in periderm cells is different from those in the basal cells, K18 (45 kDa), although it is a marker for Merkel cells [5]. In contrast to the adult tissue, the filaments in fetal embryonic epidermis are dispersed in the cytoplasm or assembled in small, seemingly short, bundles that are associated primarily with desmosomes and hemidesmosomes (see Fig. 1.3b). Periderm cells and basal cells also differ in the expression of many growth factors, growth factor receptors (Fig. 1.6), cell adhesion molecules and other cytoplasmic and cell-surface molecules [6–8].

Two of the immigrant cells that are prominent in adult epidermis, melanocytes (neural crest in origin) and Langerhans cells, are present in the embryonic epidermis among basal cells and associated with the basement membrane. Sheets of embryonic epidermis immunostained with an antibody that recognizes melanocytes specifically (HMB-45, an inducible,



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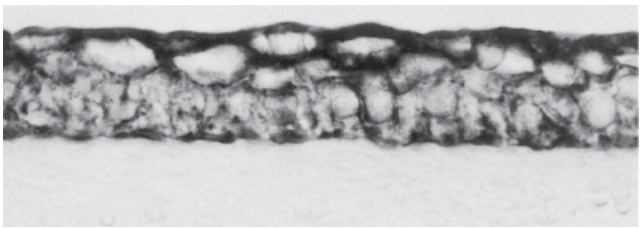

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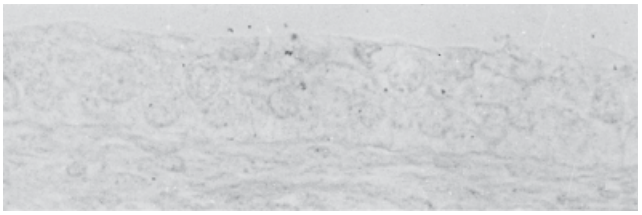

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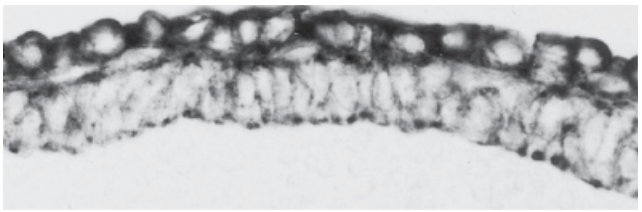


(d)

Fig. 1.5 Immunostained samples of (a) early (~50-day EGA) and (b–d) later (~60-day) human embryonic epidermis showing positive staining of both periderm and basal layers with the AE1 (a) and AE3 (d) monoclonal antibodies that recognize keratins. Both layers are negative when reacted with the AE2 (c) antibody, which recognizes the differentiation-specific keratins (×350).

cytoplasmic antigen common to melanoma and embryonic/fetal melanocytes [9,10]) show a remarkably high density (~1000 cells/mm^2) of these cells organized in a regular pattern of distribution (Fig. 1.7). They are dendritic as early as 50 days EGA in general body skin but there is no evidence of melanosomes in the cytoplasm [11]. Langerhans cells are recognized in embryonic skin as early as 42 days EGA on the basis of a reaction product for membrane-bound Mg^{2+} adenosine triphosphatase (ATPase) and histocompatibility locus antigen (HLA-DR) on the plasma membrane [12–14]. Their truncated or dendritic morphology is also apparent (Fig. 1.8). Interestingly, they are present in skin before the bone marrow begins to function leading to a hypothesis that they are derived from the yolk sac or fetal liver at this age. At 7 weeks EGA, the density of Langerhans cells is about 50 cells/mm^2 [13,14].

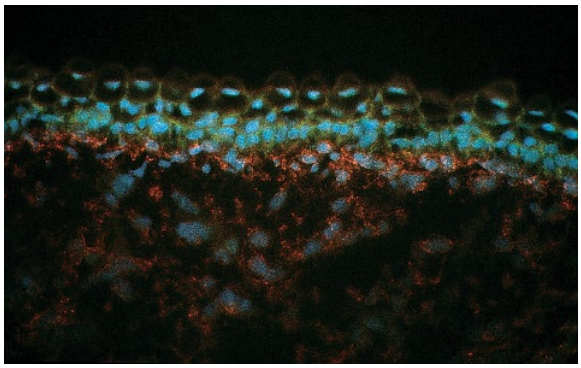
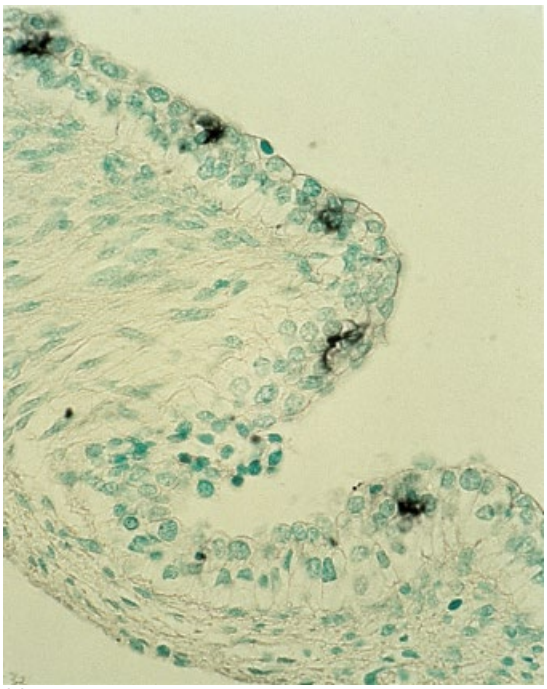


Fig. 1.6 Section of skin from a 78-day EGA human fetus showing differential expression of the A-chain of platelet-derived growth factor (PDGF) in the basal and intermediate cell layers (green) and an absence of staining in peridermal cells. The receptor for PDGFA, PDGFR-α (red), is expressed by cells in the dermis (×350).



(a)

(b)

Fig. 1.7 Embryonic skin from a 54-day EGA human embryo immunostained with the HMB-45 monoclonal antibody, which recognizes an antigen in the melanocyte. (a) Section of skin. Note the abundance and position of these cells within the two-layered epidermis. (b) Epidermal sheet. Note the density, spacing and dendritic morphology of these cells (a, ×350; b, ×25).

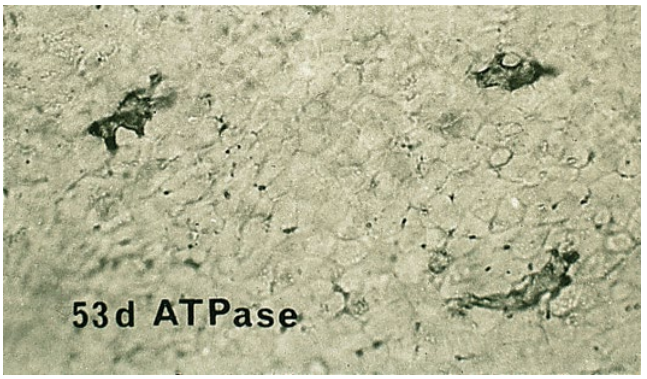


Fig. 1.8 Epidermal sheet from a 53-day EGA human embryo immunostained to recognize HLA-DR antigen in epidermal Langerhans cells (×400). Source: Micrograph courtesy of Dr Carolyn Foster.

The third immigrant cell, the Merkel cell, can be recognized in embryonic palmar skin as early as 55–60 days EGA (see Eccrine sweat gland formation) at a density of ~130 cells/mm^2 [15], using as a marker any one of the set of keratins expressed by Merkel cells (K8, K18, K10 and K20) [5,16–18]. K20 is the only keratin found exclusively in Merkel cells [18]. At this embryonic age, they are distributed randomly and in a suprabasal position. Merkel cells are neuroendocrine cells that were originally thought to function primarily as slow-adapting mechanoreceptors. Studies that have found soluble mediators produced by these cells such as nerve growth factor (NGF) and brain-derived neurotrophic factor [19,20] suggest that it is likely that Merkel cells are targets for ingrowing nerve fibres or other cells such as the smooth muscle cells of the arrector pili muscle [21,22]. Their presence in selected sites of developing epidermal appendages (e.g. sweat glands and hair follicles) has also been suggested to stimulate or to correlate with active proliferation of the tissue. It is generally accepted that Merkel cells are derived from keratinocytes *in situ* [16,18,21,23,24].

A continuous basal lamina (lamina densa) underlies the two-layered epidermis and defines, morphologically, one structural component of the basement membrane zone [25–27]. The basal lamina is patchy, however, in regions of the body where the epidermis may be only a single layer, for example, superior to the spinal cord. The molecules and antigens characteristic of all basal laminae (type IV collagen, laminin, heparan sulphate proteoglycan, nidogen/entactin) are present in the earliest recognized basal lamina of the skin; skin-specific molecules are recognized later during the first trimester in accord with the more prominent development of the attachment structures [28,29]. A thin, mat-like layer of microfilaments lies just inside the basal plasma membrane of the basal cell keratinocytes (Fig. 1.9). It may reinforce this surface of the epidermis and add to the strength of the DEJ at this stage when the structural modifications associated with dermoepidermal adhesion (hemidesmosomes, anchoring filaments, anchoring fibrils) are rudimentary [30]. The same organization of filaments is observed in cultured keratinocytes, which do not typically form hemidesmosomes and anchoring fibrils *in vivo*, and in basal keratinocytes under pathological situations, such as junctional epidermolysis bullosa, in which the epidermis separates from the dermis.

The antigens associated with the attachment structures (laminin 5/epiligrin/kalinin and 19 DEJ-1 for hemidesmosomes and anchoring filaments [31–34]; type VII collagen for anchoring fibrils [35]) are not seen by light microscopic immunostaining methods until early in the fetal period. It is likely, however, that keratinocytes begin to synthesize these proteins in the embryonic period but that the methods used for detection are not sensitive enough to demonstrate their low levels of expression. The dermoepidermal boundary is flat in the embryonic skin (Figs 1.2, 1.3 and 1.9) and thus presents a limited surface area for nutrients to traverse between the dermis and the epidermis. This may be relatively less important in the developing skin than in infant and adult skin because the dermis is thin and the small, dispersed bundles of dermal matrix proteins and the hydrated condition

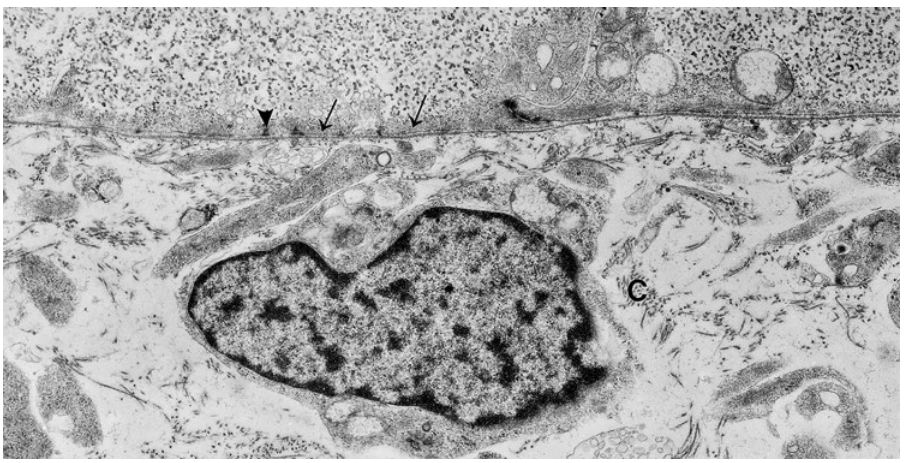
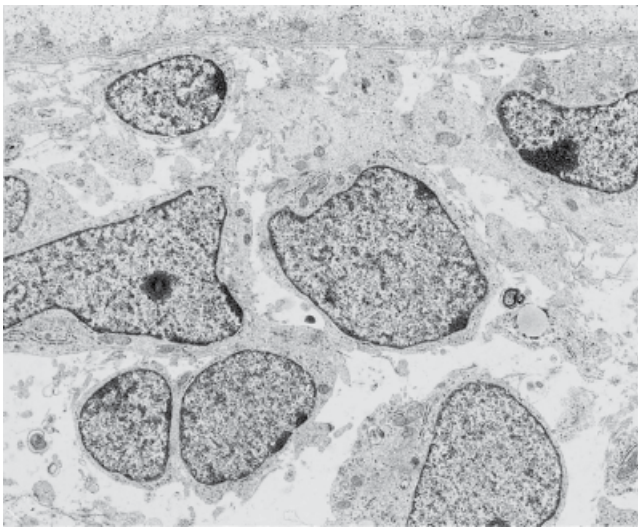
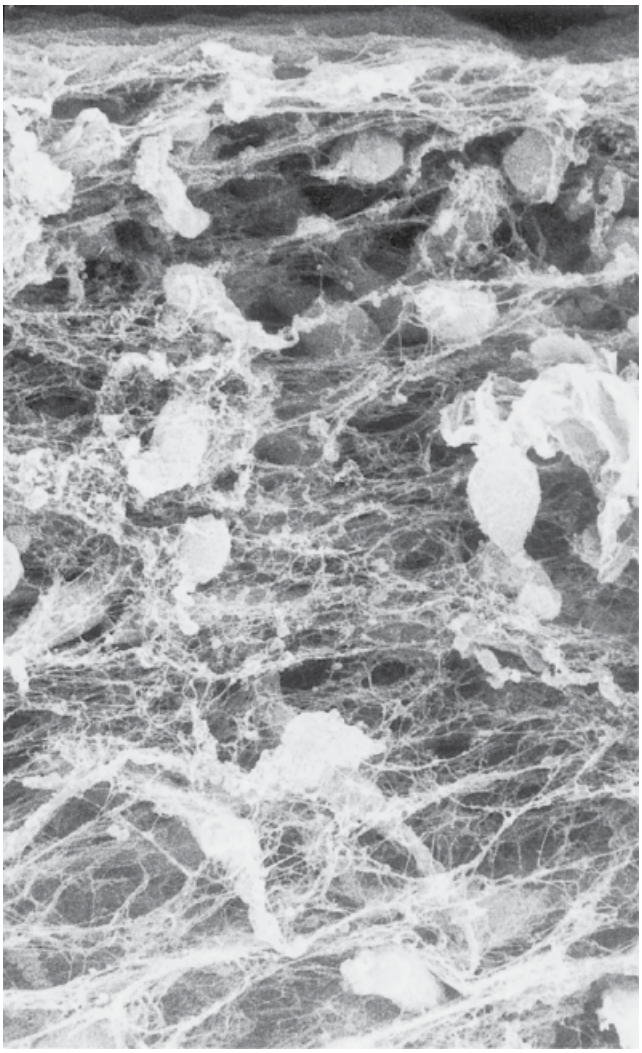


Fig. 1.9 Enlarged view of the DEJ of human embryonic epidermis showing the microfilament network within the basal epidermal cell (arrows), sites where desmosomes are forming (arrowhead) and the lamina densa. Note collagen fibrils (C) surrounding the dermal fibroblastic cells (×11 625).



(a)



(b)

Fig. 1.10 Transmission (a) and scanning (b) electron micrographs of the embryonic dermis at 48 days EGA beginning at the DEJ. The matrix is less evident in the sectioned sample (a) than in the whole-mount specimen (b) (a, ×4500; b, ×1500).

of the interstitial matrix permit more rapid diffusion of substances than the mature skin.

The dermis in the embryo is highly cellular (Figs 1.2 and 1.10), but it also contains the extracellular fibrous matrix proteins, types I, III, V and VI interstitial collagens, characteristic of adult dermis [30,36–43]. Small bundles of collagen accumulate in a thin, dense layer, called the reticular lamina, immediately beneath the dermoepidermal interface (Figs 1.2b, 1.5 and 1.9). They are also dispersed throughout the dermis in varying densities according to the collagen type and age of the embryo. Types I, III and VI collagen are distributed uniformly throughout the dermis. Type V collagen is concentrated primarily along basement membranes (at the DEJ and around blood vessels) and surrounding cells (Fig. 1.11). Fibre bundles within the interstitial spaces are widely dispersed by a hydrated, hyaluronic acid-rich proteoglycan matrix [44,45] (Figs 1.11 and 1.12). The fluidity of the matrix at this stage permits migration of mesenchymal cells to sites of active tissue morphogenesis.

A broader zone of sulphated proteoglycan-rich matrix, called the compact mesenchyme, is delineated beneath the epidermis on the basis of its rich concentration of cells that express growth factor receptors – the platelet-derived growth factor receptor β (PDGFR-β) and PDGFR-α (see Fig. 1.6), nerve growth factor receptor (NGFR) – and cell adhesion molecules (e.g. neural cell adhesion molecule, NCAM) [44,45]. Evidence from the skin of non-human species during development has shown enlargement of the composition of growth factors and receptors and adhesion molecules that are included in this dermal zone (reviewed in [44] and [46–48]). The compact mesenchyme may be involved in the exchange of signals between the epidermis and dermis and may be very important in stimulating the onset of appendage formation. Many of the growth factors that correspond to the receptors on the mesenchymal cells are produced by cells of the developing epidermis (e.g. PDGF-AA, PDGF-BB and NGF) (Fig. 1.6). The compact mesenchyme may also be the earliest evidence of a papillary dermis. In the adult, the modified composition and structure of the papillary dermis probably reflects molecular interactions between the epidermal and dermal cells, similar to the situation of the compact mesenchyme.

Elastic fibres are not formed in the embryonic skin, but fibrillin (the microfibrils of elastic fibres) (Fig. 1.13) and elastin proteins of the elastic fibre can be identified immunohistochemically [30,36–40,42] and microfibrils can be seen by electron microscopy [30].

Fine nerve fibres and capillaries are present within the compact mesenchyme and deeper dermis (Fig. 1.14a), and large nerve trunks and vessels are readily apparent in the subcutaneous region. Reconstructions of vessels from serial sections of developing first-trimester skin have shown that the basic pattern of cutaneous vasculature is established in the first trimester [49]. New vessels presumably both form de novo from dermal mesenchyme and sprout from deeper, established vessels through a process that includes endothelial cell migration, capillary budding and vessel remodelling [50]. Pieces of full-thickness

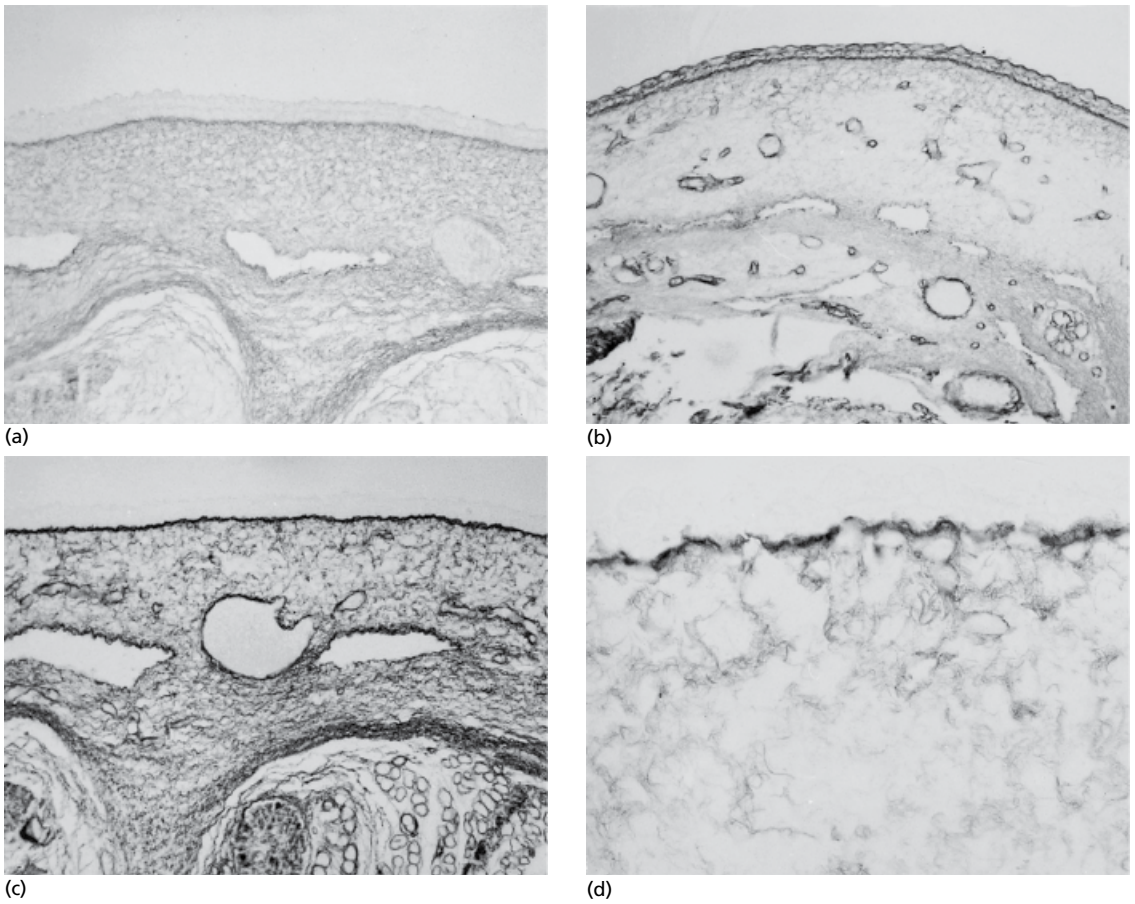


Fig. 1.11 Samples of embryonic skin immunostained with antibodies that recognize type I (a), III (b), V (c) and VI (d) collagens. Note that all of the collagens are concentrated beneath the DEJ but types III and V, especially, are found in association with all basement membranes. Types I, III and VI are found in the matrix throughout the dermal and subcutaneous tissue (a–c, ×150; d, ×300). Source: Immunostaining courtesy of Dr Lynne T. Smith.

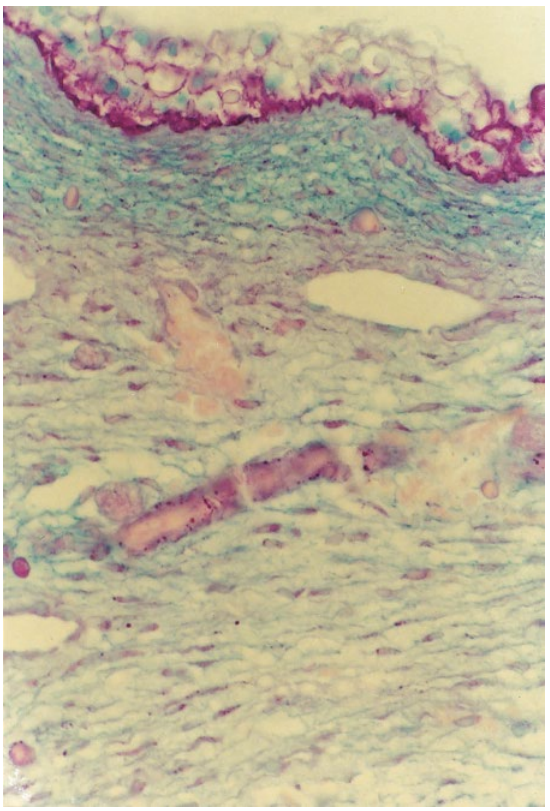


Fig. 1.12 Section of the body wall from a 57-day EGA embryo treated with the Alcian blue/periodic acid–Schiff (PAS) histochemical stains. The bright pink staining of the epidermis (glycogen) and DEJ (glycoproteins) indicates a PAS-positive reaction. The blue dermis reflects the high content of hyaluronic acid. The dermal–subcutaneous boundary is marked by a transition to a lighter slightly purple reaction indicating more of the collagen–glycosaminoglycan complex (×300). Source: Immunohistochemistry courtesy of Dr Richard Frederickson.

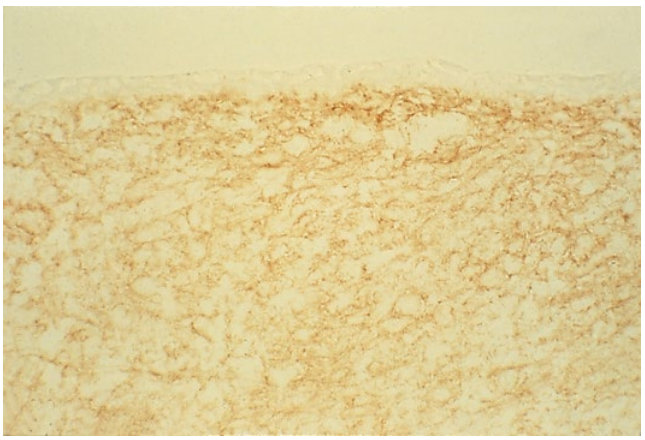
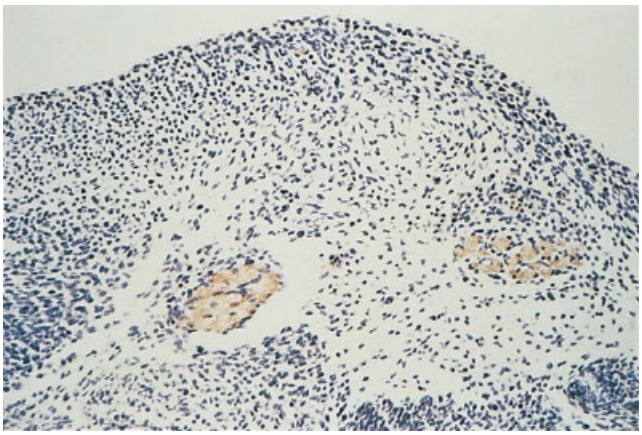


Fig. 1.13 Section of skin from a 57-day EGA human embryo immunostained with an antifibrillin antibody. Note staining throughout the dermis (×200). Source: Immunostaining courtesy of Dr Lynne T. Smith.



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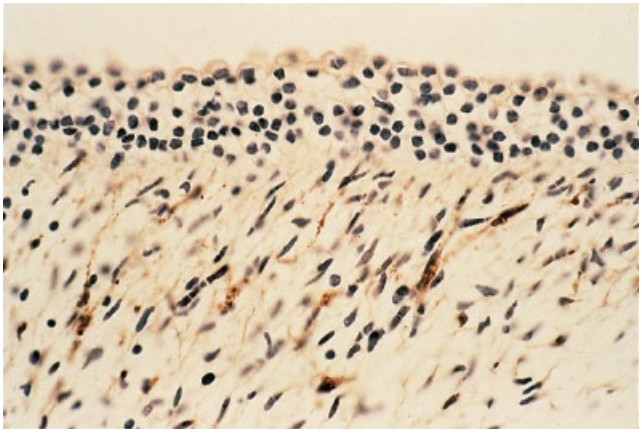
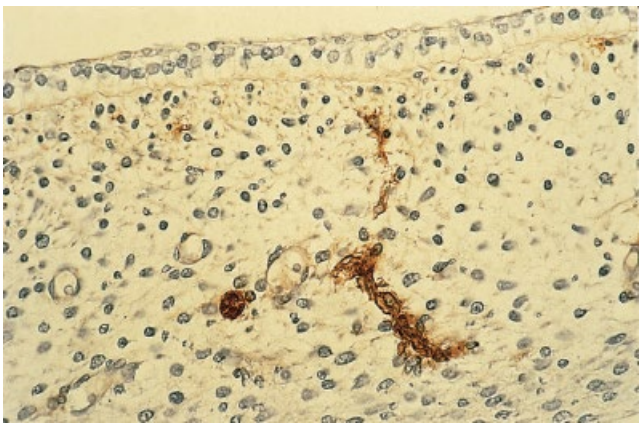


(b)



(c)

Fig. 1.14 Sections of human embryonic skin at 42 days EGA (a) and 59 days EGA (b) immunostained with PGP 9.5, which recognizes all cutaneous nerves, and of a sample of 52-day EGA embryonic skin (c) immunostained with p75 antibody, which recognizes the low-affinity NGFR. Note the large nerve trunks deep in the subcutaneous tissue (a), the significant density of the fine fibres in the tangential section of the dermis of (b) and the distribution of both nerves and vessels (c) (a, ×100; b, ×200; c, ×200).

skin and sections of skin immunostained with an antibody that demonstrates all cutaneous nerves (protein gene product 9.5 or PGP 9.5) [51,52] reveal finely beaded nerve filaments distributed in an impressive density in the subepidermal region and in association with blood vessels (Fig. 1.14b and c). The number of fibres recognized by this antibody increases during development as the

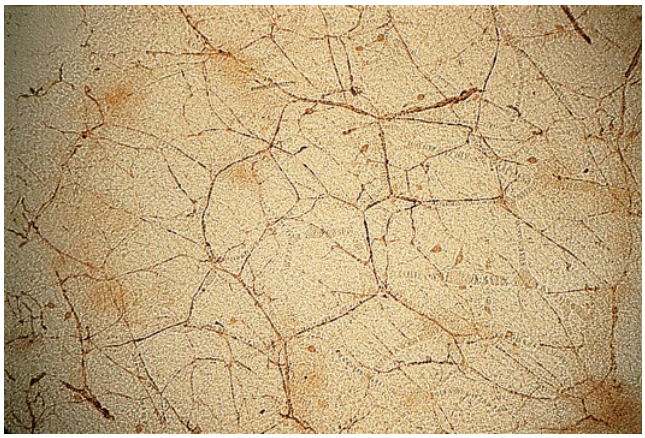


Fig. 1.15 Nerves and vessels in the skin of a 79-day EGA human fetus immunostained with an anti-neurofilament antibody. Note the positively stained nerve network, the immunopositive cells (presumably Merkel cells) and the vascular network (clear) (×25). Source: Immunostaining courtesy of Dr Mark Bressler.

fibres become organized in networks throughout the dermis and in relation to developing epidermal appendages [53]. At 7 weeks EGA, a few calcitonin gene-related product (CGRP)-immunopositive fibres, denoting sensory fibres, are also evident [53], but autonomic nerves are not yet recognized in the skin. Staining the tissue with the p75 low-affinity NGFR antibody also reveals the patterns of nerve fibres and specific concentrations of mesenchymal cells (e.g. around developing hair follicles) [54]. Both nerves and vessels are visible in stained, full-thickness samples of the nearly transparent skin (Fig. 1.15).

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Embryonic–fetal transition

The most remarkable time in skin development is the embryonic–fetal transition, which occurs at approximately 2 months' gestation, when the embryo measures about 31 mm in length (crown–rump), weighs about 2.5 g and has a humanoid appearance. The skin and the underlying tissues of the body wall are translucent, revealing the ribs and solid organs. The skin has a mucoid quality (Fig. 1.16). In spite of this structural simplicity, the cells in

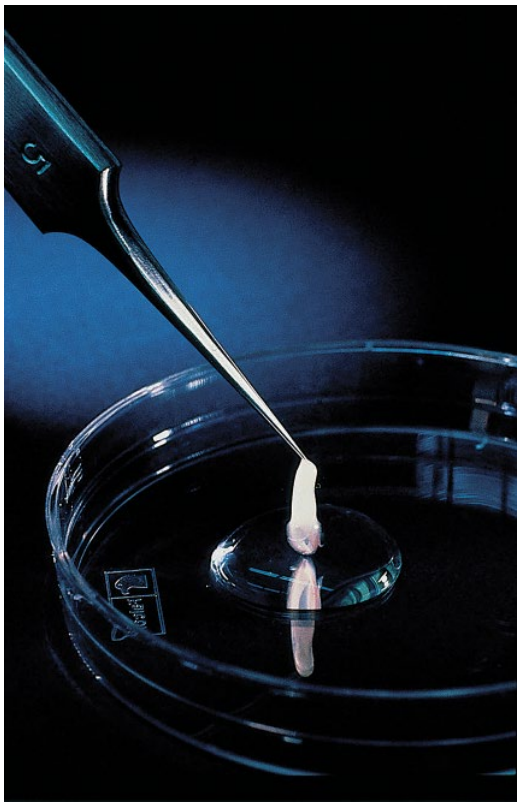
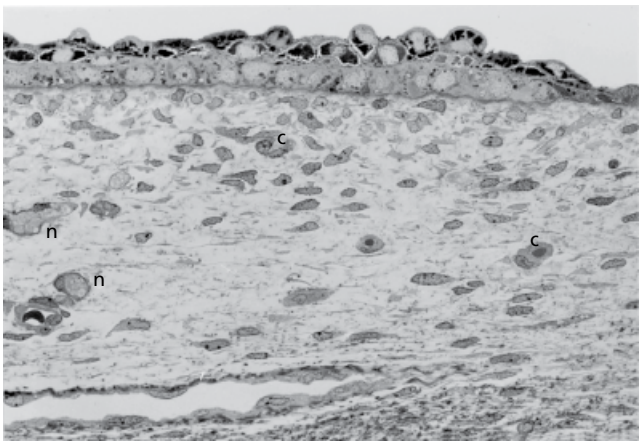
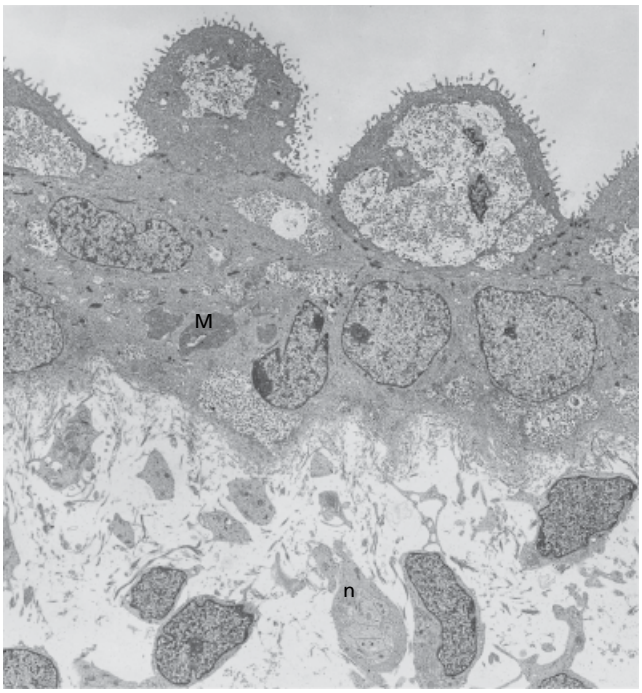


Fig. 1.16 Sample of human fetal skin of 80 days EGA held at the tips of a forceps and demonstrating the mucoid quality of the tissue.



(a)

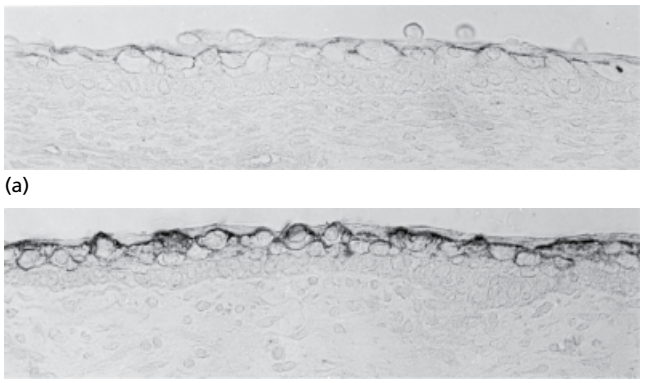


(b)

Fig. 1.17 Skin from a human fetus of approximately 70–89 days EGA shown at the light (a) and electron (b) microscopic levels. Note the intermediate layer of cells between basal and periderm epidermal layers, the distinction between dermis and subcutaneous tissue based on differences in the orientation of fibroblastic cells, the density of collagenous matrix and the subcutaneous vascular plane. Small nerves (n) and capillaries (c) are evident in the dermis. Segments of melanocytes (M) are evident within the basal layer and collagen is accumulated beneath the DEJ (×3675).

the skin begin to express characteristics of adult skin. The 2-month age is thus identified as an important landmark in skin development (see Fig. 1.1). Since this period of development begins the establishment of adult characteristics of the skin, it is a stage that is vulnerable to errors in development. The most apparent change in the skin is the stratification of the epidermis from two to three cell layers (Fig. 1.17a and b). An intermediate cell layer is added as the product of basal cell mitoses. Basal cells divide asynchronously to produce an epidermis that, initially, remains two-layered at some sites and at others becomes three layers thick.

Intermediate cells are both similar to and distinct from basal and periderm cells. Keratins are more abundant and distributed in a more specific distribution than in the cells in the basal and periderm layers; small bundles of keratin filaments associated with desmosomes outline the boundaries of the intermediate cells (Fig. 1.17b). The expression of the major keratin pairs in both basal- and intermediate-layer keratinocytes in the early fetal epidermis is now identical to the expression of the keratins in the fully keratinized adult epidermis. The K5 and K14 basal cell keratins are downregulated in the intermediate cells and a new keratin pair, K1 (56.5 kDa) and K10 (67 kDa), the high-molecular-weight differentiation-specific keratins, is synthesized [1,2] (Fig. 1.18). Other markers of keratinocyte differentiation (e.g. pemphigus antigen [3], cornified cell envelope proteins [4,5], blood group antigens [6] and cell-surface glycoproteins [7]; reviewed in [8–11]) are also expressed in the cytoplasm or on the surface of intermediate-layer cells. Like the periderm and basal cells, the first intermediate cells still contain glycogen as the primary cytoplasmic component (Fig. 1.17a and b). Thus, at this stage, when the epidermis is only a few cell layers thick, and has few similarities in morphology to adult epidermis, it possesses all of the keratins and many of the other markers that are typical of the epidermis throughout life. Therefore, genetic diseases that involve mutations in keratin proteins have the potential of being expressed as early as the first trimester in development.



(a)

(b)

Fig. 1.18 Keratin filaments in the cells of the intermediate layer of 77-day EGA fetal skin stain positively with the AE2 monoclonal antibody, which recognizes the K1 and K10 differentiation-specific keratins (a). The reaction pattern is even stronger when a second intermediate layer is added at the beginning of the second trimester (b) (a, ×120; b, ×120).

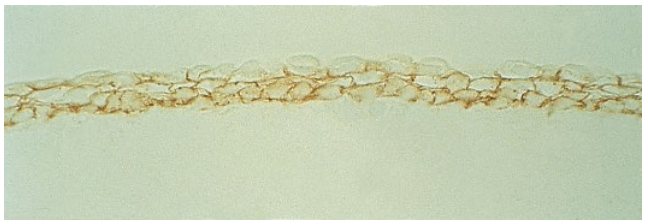


Fig. 1.19 Skin from a 72-day EGA fetus immunoreacted with an anti-epidermal growth factor receptor showing a reaction pattern on the membranes of basal and intermediate layer cells and on the basal and lateral borders of periderm cells (×120).

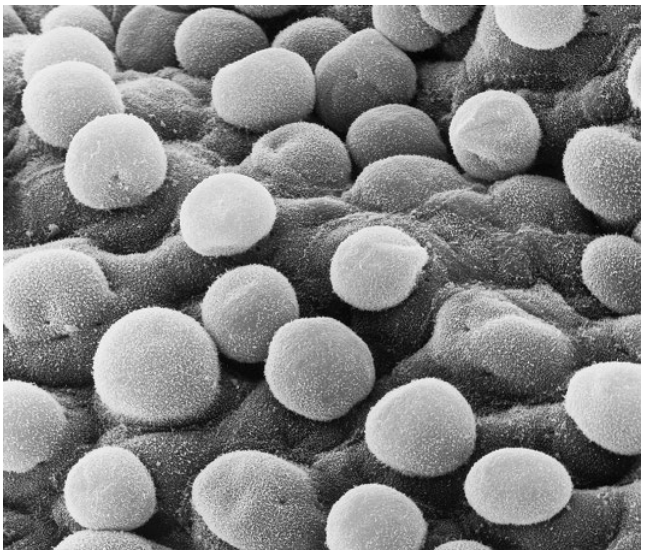


Fig. 1.20 Scanning electron micrograph of the periderm of a 60- to 70-day EGA human fetus showing the blebs and microvilli that modify the amniotic surface (×8000).

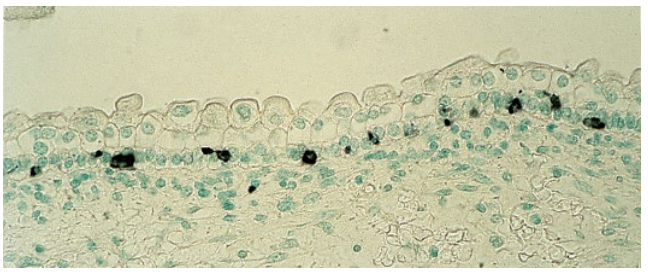


Fig. 1.21 Section of skin from an 82-day EGA fetus immunostained with the HMB-45 antibody, which recognizes melanocytes. Note the high density of the cells and their position within the basal epidermal layer (×200).

Initially, the keratinocytes of both basal and intermediate cell layers express epidermal growth factor (EGF) receptors [12] (Fig. 1.19), respond to EGF and retain the ability to proliferate [13,14]. Near the end of the first trimester, however, the proliferative cells become restricted primarily to the basal layer [13,14]; only the basal cells express P-cadherin [15], a marker of proliferative ability. Basal cells change in morphology and cell-surface properties after stratification. A greater volume of the cytoplasm is occupied by organelles and keratin filaments than with glycogen, and cell-surface carbohydrates that correlate with stratification and differentiation are differentially expressed by cells of the basal and intermediate layers [7,16]. Selected basal- and intermediate-layer keratinocytes participate in the formation of the epidermal appendages: the pilosebaceous structures, nails and teeth, and the eccrine sweat glands in thick skin. The morphogenesis of these structures is described under Unique features of developing human skin.

The cells of the periderm increase in size and develop microvilli-covered blebs that extend from the outermost surface of the cell into the amniotic cavity (Fig. 1.20). The molecular species of keratins remain the same as they were in the embryonic periderm cells, but the cells lose their ability to divide and to express P-cadherin [13,17]. Because the epidermal cells that are located in the more superficial layers express differentiation-related antigens, it is appealing to link stratification and the onset of differentiation. There must be more processes involved, however, than simply the addition of cell layers because embryonic skin maintained in suspension organ culture stratifies to become several layers thick but will not differentiate in the manner characteristic of early fetal skin *in vivo* [18,19].

Melanocytes are easily recognized in sections of fetal epidermis at 8 weeks EGA by their position along the basement membrane, dense cytoplasm, an absence of glycogen and a heterochromatic nucleus [20]. Around 80 days EGA, they are present in the epidermis in maximal density (~3000 cells/mm^2) [20] compared with all other stages of skin development, and in a nonrandom distribution among cells of the basal layer (Fig. 1.21). The numbers decrease towards birth then continue to decline over the decades of postnatal life. The high numbers of melanocytes around the embryonic–fetal transition may reflect the fact that these cells arrive early in the skin, proliferate and remain close together before there is substantial growth of the fetus. The labelling index of keratinocytes is also high [13] at this stage, suggesting that the paracrine interactions between melanocytes and keratinocytes that occur in the adult skin may be established early in development [20]. Melanosomes are recognized late in the third month of development (Fig. 1.22) and show some

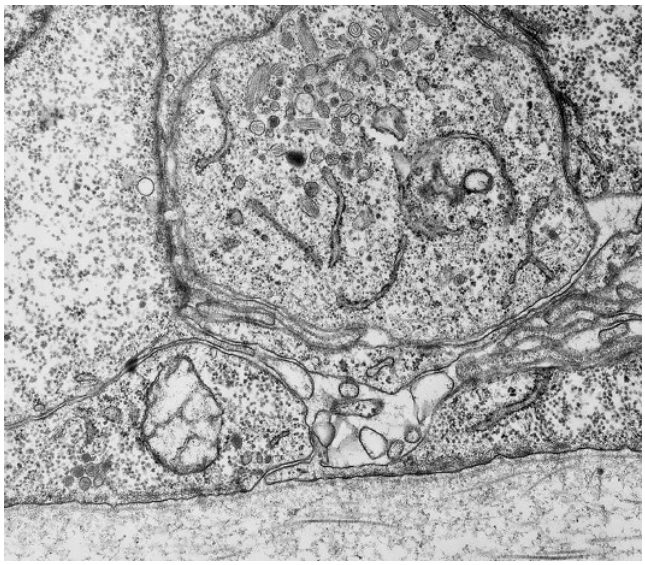


Fig. 1.22 Section through late embryonic–early fetal skin showing developing melanosomes in a melanocyte positioned between keratinocytes in the basal layer. The early age of the tissue is confirmed by the immature structure of the DEJ (×25 000).

Fig. 1.23 Epidermal sheet from an 80-day EGA fetus reacted to demonstrate ATPase. Note the regular distribution and density of these highly dendritic cells (×120).

evidence of pigment formation in selected sites of the body. Understanding the density of melanocytes and the onset of pigment synthesis had been previously useful in prenatal diagnosis of tyrosinase-negative oculocutaneous albinism, a technique no longer used [15,21].

Langerhans cells are also abundant in the epidermis at this stage (~50/mm^2) [22,23]. Unlike melanocytes, which migrate into the epidermis only during the embryonic period, the bone marrow-derived Langerhans cells migrate into the epidermis continually throughout life; their numbers do not increase significantly, however, until the third trimester and after birth [22,23]. Langerhans cells at 80 days EGA are highly dendritic (Fig. 1.23), begin to express CD1a at the surface [22–24] and develop Birbeck granules in the cytoplasm, suggesting that they may be capable of processing and presenting antigen *in utero*. The number of cells that are HLA-DR positive is significantly greater than the number that express CD1a at this stage;

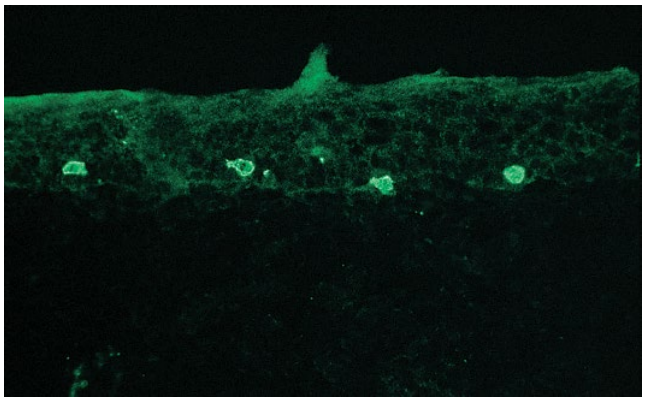


Fig. 1.24 Section of skin from the palm of an 83-day EGA fetus immunostained to recognize keratin 18 (green), an antibody that recognizes Merkel cells within the basal layer. Note the regular distribution of cells, presumably marking the sites of primary epidermal ridge location. The skin of the hand is more advanced in development than that of the trunk at an equivalent age (×300). Source: Immunostaining courtesy of Dr Dong-Kun Kim.

however, by about 13 weeks EGA Langerhans cells seem to express both markers with reasonable consistency.

By the end of the first trimester, Merkel cells are located along the primary epidermal ridges of palmar skin in regular alignment relative to the sites of origin of the sweat duct primordia and at a maximum density of ~1400 cells/mm^2 [25,26] (Fig. 1.24). In hair-bearing skin, they first become evident in association with the developing hair germs. At later stages in follicle development, they concentrate in the infundibulum and bulge regions of the hair pegs and bulbous hair pegs [27,28]. It is likely that the dermal Merkel cells originate in the follicular or interfollicular epidermis and migrate into the dermis, where they are suggested to play a role at early stages of development in attracting and organizing nerve fibres in the upper dermis and around developing appendages [29]. Merkel cells in the interfollicular epidermis lack NGF receptors (and produce NGF [30]), but dermal Merkel cells and Merkel cells of the developing follicle are immunopositive when the tissue is reacted with the p75 NGF antibody [29]. It must be recognized, however, that nerve fibres are already apparent in the embryonic dermis before dermal Merkel cells are detectable; thus, other factors must also attract or direct nerves into the skin. Other morphological markers that are characteristic for Merkel cells in postnatal skin, such as dense core granules, are not apparent in dermal Merkel cells at this stage. Merkel cells decrease in number during the later stages of fetal development [29].

The DEJ has acquired all of the adult features that are characteristic for this region (Fig. 1.25). Hemidesmosomes, anchoring filaments and anchoring fibrils are structurally complete, and the antigens related to these attachment structures, the skin-specific markers of the DEJ, are also expressed [31–33]. Immunostaining with an antibody to type VII collagen outlines the DEJ with high intensity [32] and stains basal cell cytoplasm (the primary source of this protein) with low intensity. Nonetheless, the structural organization of the DEJ appears delicate in contrast to the

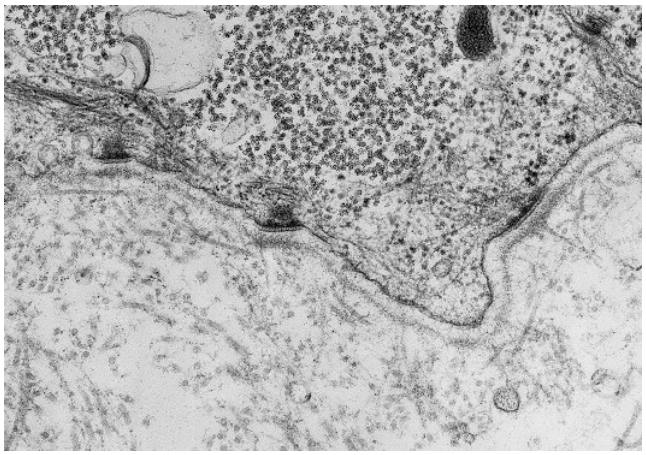


Fig. 1.25 Transmission electron micrograph of the DEJ of a 78-day EGA human fetus. Note the well-formed hemidesmosomes and associated keratin filaments, anchoring filaments within the lamina lucida and fine anchoring fibrils (×47 500).

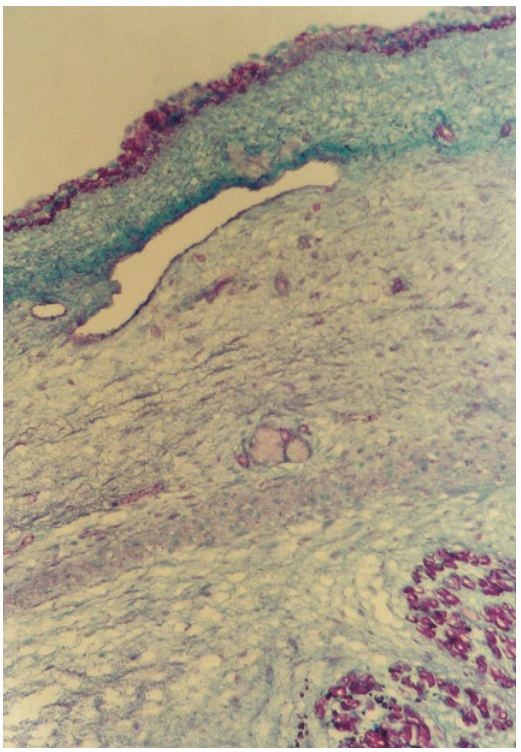
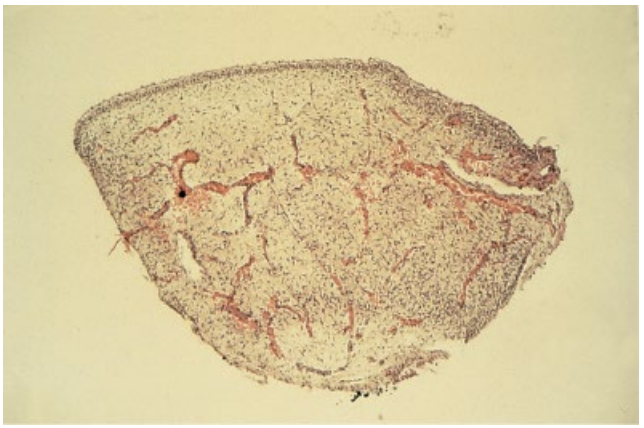
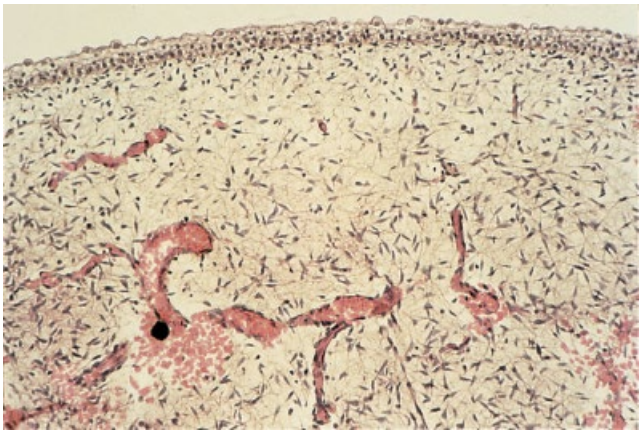


Fig. 1.26 Light micrograph of 72-day EGA fetal dermis stained with the Alcian blue/periodic acid–Schiff (PAS) histochemical stain. The low-magnification image shows the clear demarcation between the dermal and subcutaneous tissue and the different concentrations of fibrous and glycosaminoglycan matrix proteins in the two regions. A vascular plane also demarcates the two regions. Skeletal muscle is evident in the lower right corner of the micrograph (×200). Source: Histochemical staining courtesy of Dr Richard Frederickson.



(a)



(b)

Fig. 1.27 Light micrograph of 78-day EGA human skin showing the vascular pattern organized in a series of horizontal plexuses and vertical connecting vessels (a, b). Note that the diameters of the vessels become increasingly smaller towards the epidermal surface. In the higher-magnification image, the rounder cells of the papillary dermis are distinct from the more elongated fibroblastic cells of the reticular dermis and subcutaneous region (a, ×25; b, ×100). Source: Micrographs courtesy of Dr Greg Hébert.

robust structure of the basal lamina and anchoring fibrils in adult skin. The dermoepidermal interface is still flat although modifications of individual basal cells begin to alter the smoothness of this junction.

The dermis and subcutaneous tissue are distinguished on a morphological basis by differences in the organization and composition of the matrix (Fig. 1.26). Dermal and subcutaneous mesenchymal cells still retain glycogen in the cytoplasm, but they have assumed a distinctly fibroblastic morphology and are responsible for the synthesis of all of the matrix molecules that are characteristic of adult dermis. There is accumulation of small bundles of fibrous proteins within the interstitial space and papillary and reticular regions of the dermis are demarcated on the basis of increased cell density proximal to the epidermis (the papillary region) and larger collagen fibril diameter and fibre bundle size in the reticular region [9,34–36] (Fig. 1.27b). The position of the subpapillary vascular plexus of arterioles and postcapillary venules also forms an approximate boundary between these two dermal zones. In spite of the significant accumulation of matrix protein, the dermis remains highly cellular, with the matrix accounting for substantially less of the bulk of the skin than it does in the postnatal infant and the adult.

The skin is still transparent enough to permit the networks of vessels and nerves to be seen through the body wall of the fetus (Fig. 1.28). The vessels are organized in the dominant pattern of adult skin, with one plexus located at the dermosubcutaneous boundary and another

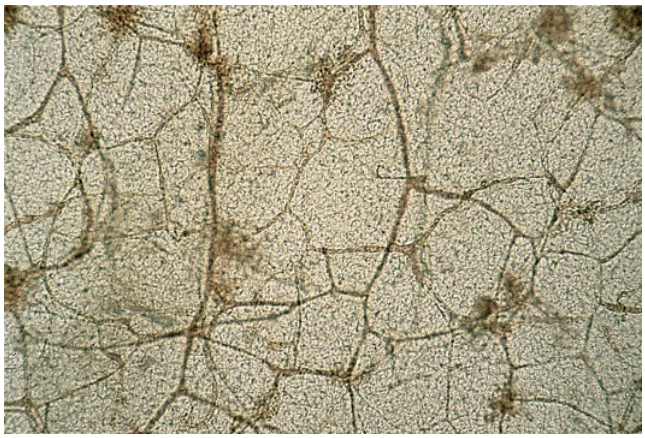


Fig. 1.28 Whole-mount sample of unfixed 74-day EGA fetal skin showing the vascular network of the skin through the translucent tissue of the body wall (×63). Source: Micrograph courtesy of Dr Carole Johnson.

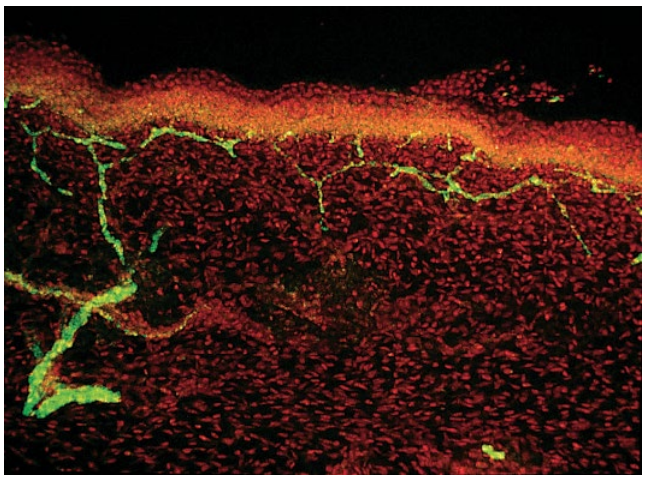


Fig. 1.29 Section of skin from a 77-day EGA fetus immunostained with the PGP 9.5 antibody, which recognizes all cutaneous nerves. Note the larger trunks deep in the dermis and the fine network of fibres that supplies the epidermis (×100). Source: Immunostaining courtesy of Dr Dong-Kun Kim.

at the boundary between the papillary and reticular dermis (Fig. 1.27a). Vertically orientated vessels connect the two horizontal plexuses, and fine capillaries extend into the papillary dermis [8–11]. Nerves are also readily apparent in both sections and whole-mount preparations of skin that are immunostained with the p75 antibody to NGFR [37], neurofilament protein, PGP 9.5 (Fig. 1.29), CGRP and neuropeptide Y (NPY). NPY recognition of certain fibres associated with blood vessels signifies the presence of autonomic fibres [38]. Like the vessels, large subcutaneous nerve trunks branch to finer and finer fibres that terminate beneath the DEJ. The nerve and vascular networks are at times parallel but are also separate from one another (see Fig. 1.15).

The hypodermis appears to have markedly fewer cells than either region of the dermis, and smaller bundles of fibrous matrix. Dilated channels course through the hypodermis, distinguishing it from deeper tissue of the body wall (see Figs 1.26 and 1.27). Red blood cells within the lumina of some of these structures suggest that they may belong to the venous side of the vasculature although the simplicity of the wall structure would suggest they could be lymphatics.

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Fetal skin

Conclusion of the first trimester

The first stages of fetal skin development occur from the time of the embryonic–fetal transition at 2 months to the end of the first trimester at 3 months, when a template of the adult skin is established. There are notable features of adult skin that are still lacking. The epidermis has yet to keratinize and one of the key proteins of keratinization, filaggrin, is not yet expressed in any region of the skin. The dermoepidermal interface lacks rete ridges and rete pegs. The dermis lacks fully formed elastic fibres and an elastic fibre network. Ectodermal appendages are just starting formation in limited areas; for example, sweat gland development is initiated only on the palms and soles and apocrine glands have not begun to develop. Hair and nails are not synthesized. Adipose tissue has not differentiated within the mesenchyme of the hypodermis. All of these features will be initiated and/or fully acquired during the second trimester, and changes will continue to occur in the structures and regions of the skin that have been established but not finalized.

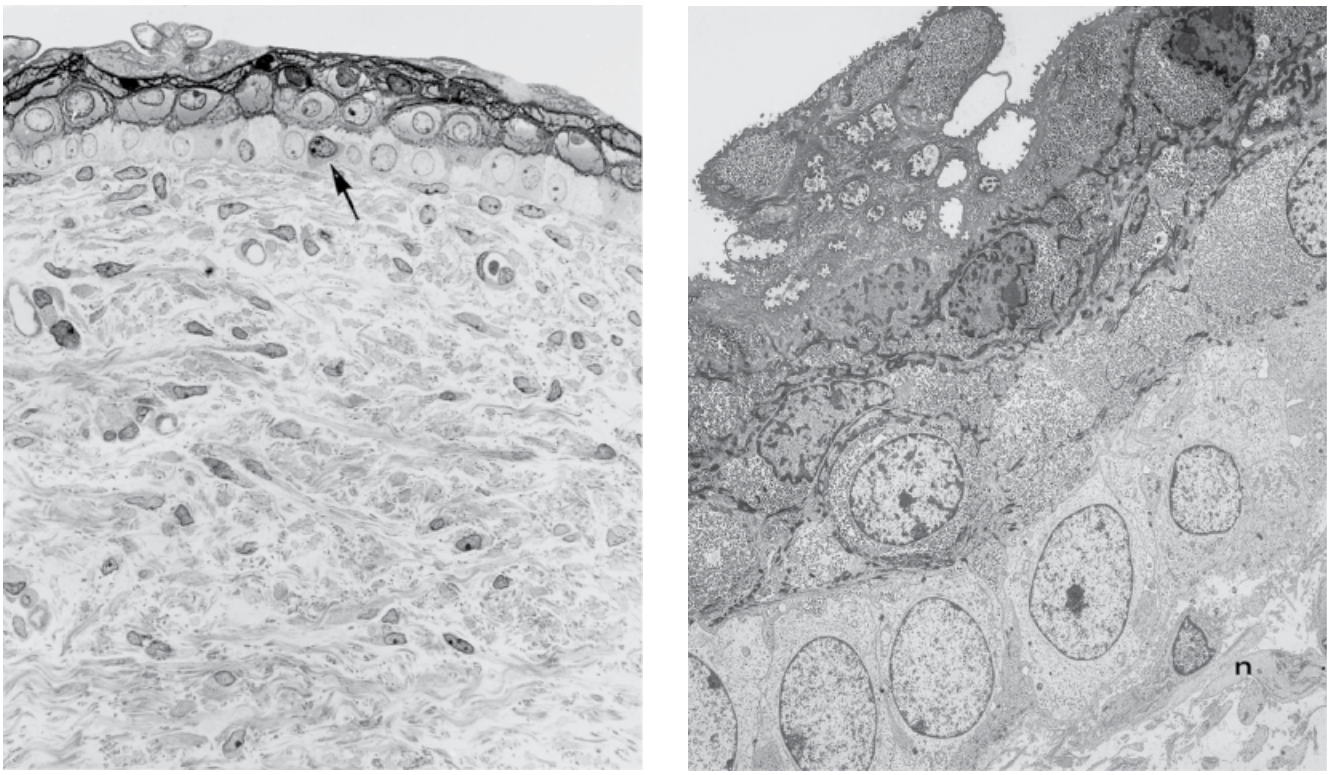
Second-trimester fetal skin

At 12 weeks' gestation, the fetus measures about 85 mm in length (crown–rump) and has a body form similar to that of the newborn. The skin and body wall are opaque. All of the events in skin morphogenesis initiated during the first trimester continue in the second trimester in parallel with the onset of formation of those structures that were identified as lacking in first-trimester skin. Landmark events in the second trimester include completion of the formation of the lanugo hair follicle and synthesis of the hair (around 17–19 weeks EGA), completion of the formation of the nail (around 20–22 weeks EGA) and keratinization of the interfollicular epidermis (around 22–24 weeks EGA). The timing is variable because the formation of the hair follicle and epidermal keratinization are regionally dependent. Sweat glands on the general body surface only begin to form around 17–18 weeks EGA (see Unique features of developing human skin). The 24-week-old fetus is fully formed and has hair on the scalp and body surface. The length is about 228 mm (crown–rump).

One or two additional intermediate cell layers are added to the epidermis by proliferation of basal keratinocytes and upward migration of the first intermediate cells. By 100–110 days EGA, there are typically three suprabasal, intermediate cell layers, which become progressively more flattened towards the epidermal surface (Fig. 1.30). The cells of the most superficial layer have large bundles of keratin filaments, which can be seen in stained specimens at the light microscopic level as a reticulate cytoskeleton (Fig. 1.30). Glycogen is still a major constituent of the cytoplasm.

As the epidermis thickens, the interface it forms with the dermis becomes less flattened and smooth, due largely to changes in the basal surface of each keratinocyte rather than to convolutions of the layer itself. Basal cells stain with less intensity than the intermediate-layer cells because there is less glycogen, the bundles of keratin filaments are smaller and the cytoplasm is more ribosome rich, dense and organelle filled (Fig. 1.30). At the end of the second trimester, the five-layered interfollicular epidermis keratinizes. Skin of the trunk shows signs of keratinization around 21 weeks EGA in the uppermost intermediate cell layers and the overlying periderm (Fig. 1.31).

Changes in the structure and composition of the plasma membrane mark the formation of a cornified cell envelope [1,2], and lamellar granules are identified in the cytoplasm (Fig. 1.31) and in the spaces between intermediate and periderm layers. The modified intermediate cells remain associated with the overlying periderm cells by infrequent and tenuous-appearing desmosomal attachments. Cells are also evident in which the nucleus is pyknotic and the cytoplasm contains dense bundles of filaments, vacuoles and other remnants of the cytoplasm. Cells beneath these seemingly incompletely keratinized



(a) (b)

Fig. 1.30 Light (a) and electron (b) micrographs of a section of skin from a 104-day EGA human fetus showing additional intermediate cell layers, changes in periderm morphology, the reticulate keratin cytoskeleton in upper intermediate cells, less intense staining of basal cells (a, b) and the smaller bundles of collagen fibrils in the papillary dermis (a). A melanocyte is evident (a, arrow). Several capillaries (a) and nerves (b, n) lie within the papillary region (a, ×300; b, ×3500).

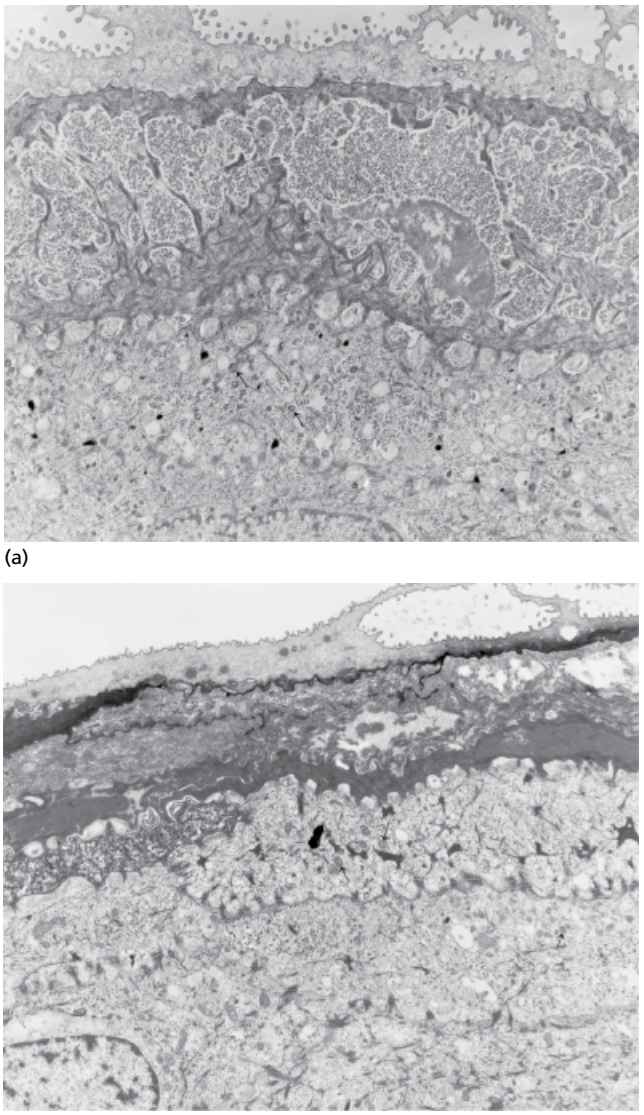
squames contain very small, stellate keratohyalin granules and react immunopositively with an antibody that recognizes profilaggrin and filaggrin proteins of the granule [3] (Fig. 1.31). The two or three subjacent intermediate cell layers can now be called spinous cells. At this stage, periderm cells are very large in diameter, flattened and also display a thickened cell envelope. Each cell covers a cluster of underlying epidermal cells. The periderm stains differently from the underlying epidermal cells, probably owing to a lesser amount of structural protein within the cytoplasm.

A few layers of thin, flattened, keratinized cells, organized in the manner of a true stratum corneum, are first apparent around 22–24 weeks EGA (Fig. 1.32). The granular cell layer is now more typical of an adult granular layer in that keratohyalin granules are larger and the cytoplasm contains less glycogen. The numbers of layers of the stratum corneum continue to increase in the third trimester to reach a more mature appearance by 34 weeks gestation [4]. Of note, premature birth hastens development of mature stratum corneum and epidermal thickness with histologically similar appearance to term infants seen within 2–3 weeks after birth regardless of gestational age [4].

By 22–24 weeks EGA, 1700 Merkel cells/mm^2 can be measured in the epidermis [5,6] and the number of Langerhans cells begins to increase (~200 cells/mm^2), although the adult level of approximately 650 cells/mm^2, or about 8500 cells/mm^3, is not achieved until after birth [7]. Melanosomes are transferred to keratinocytes in the fifth month of gestation.

All of the structures of the DEJ were formed in the first trimester, and only a few antigens of the DEJ (AF-1 and AF-2 associated with anchoring fibrils) remain to be recognized at this age [8]. By 19–21 weeks EGA, the hemidesmosomes are present at the basal keratinocyte plasma membrane with adult-like frequency and show a strong association with basal cell keratin filaments. Anchoring filaments and banded anchoring fibrils are well formed (reviewed in [9–12]).

Small bundles of interwoven, fibrous connective tissue occupy the interstitial space within the dermis (Fig. 1.33a), although they remain loosely organized because the sulphated proteoglycans and fibrous proteins of the interstitial matrix are still very hydrated. Elastin is detectable biochemically, and elastic fibres can be recognized as granular-appearing structures along the borders of collagen fibre bundles in immunostained samples of skin and by electron microscopy. The structure of the elastic fibres, however, even in the deepest portions of the reticular dermis, is similar to that of the elaunin fibres of adult skin, which have only sparse amounts of elastin associated with the microfibrillar bundles. The extent to which elastic fibres are developed is dependent upon the region



(a)

(b)

Fig. 1.31 Electron micrographs of the skin from two 21-week EGA fetuses showing early (a) and late (b) changes in the upper intermediate layers (spinous) at the onset of keratinization. Note the regressed periderm separating from the upper epidermal layers (a), lamellar granules in the top few layers (arrows), the small particulate (a) then stellate (b) keratohyalin granules, and the few layers of incompletely keratinized cells (black material) to demonstrate the permeability of the epidermis to tracers (a, ×12 150; b, ×9500). Source: Micrograph (b) courtesy of Dr Richard Frederickson.

of the skin. In addition to fibroblasts, mast cells, macrophages and smooth muscle cells are present in the dermis [9–12].

The hypodermis remains distinct from the dermis by its less dense matrix and cellularity. Around 15–16 weeks EGA, mesenchymal cells collect in globular arrays surrounded by a capsule-like assembly of matrix (Fig. 1.33b). This is the first stage of adipose tissue formation. Small vessels are present within these cellular aggregations. By 18 weeks EGA, lipid droplets are evident within some of the mesenchymal cells, and by 20 weeks lobules of fat are established.

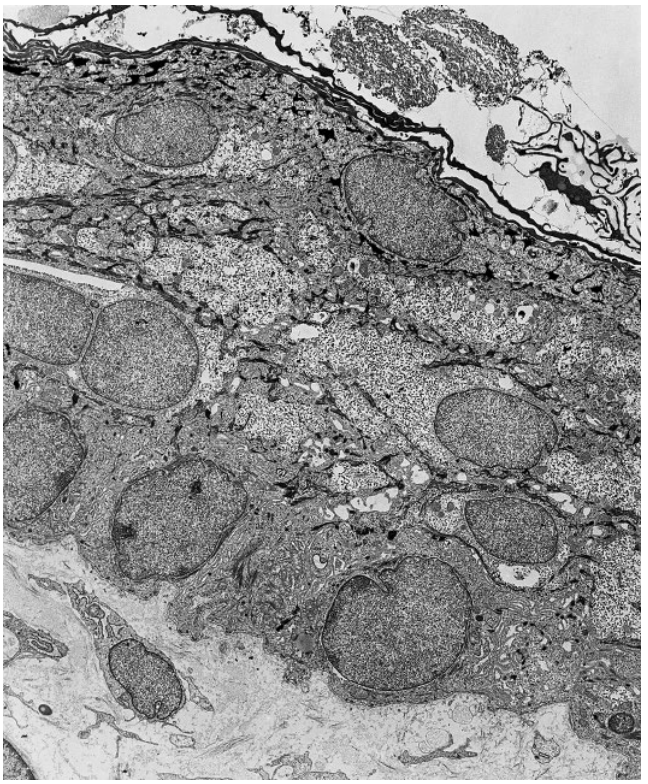


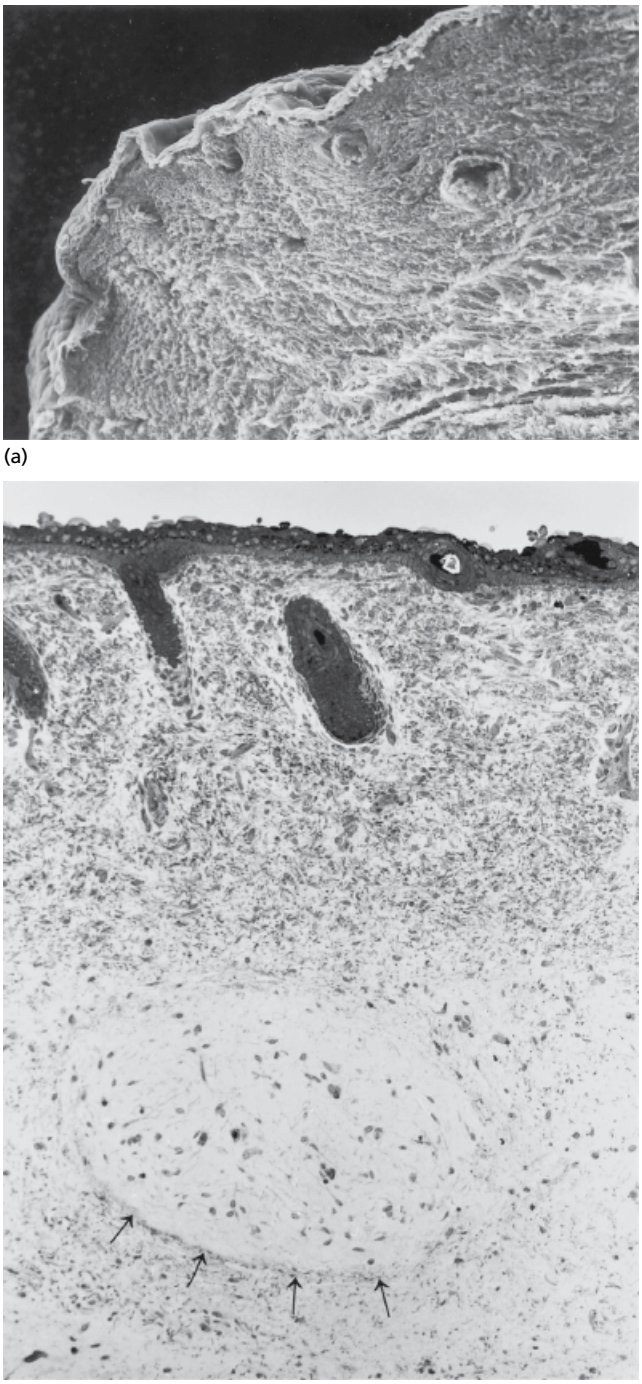
Fig. 1.32 Electron micrograph of the keratinized epidermis from a fetus at the end of the second trimester. There are a few layers of cornified cells, a single granular cell layer and three layers of spinous cells, which retain a significant quantity of glycogen. Note the greater irregularity in the basal border of the basal cells at the DEJ (×3000).

Third-trimester fetal skin

The skin in the third trimester appears structurally similar to postnatal skin (reviewed in [9–13]) (Fig. 1.34). The epidermis is fully keratinized, contours are beginning to form at the DEJ, the regions of the dermis are well defined, the adnexa are fully formed and reside deeply in the dermis, and large fat lobules fill the hypodermis. There are, however, some notable differences in structure in all regions: suprabasal epidermal cells retain a significant amount of glycogen in the cytoplasm and the dermis remains relatively thin. The bundles of collagen matrix are small elastic fibres and are immature in structure and composition, and the stratum corneum has fewer cell layers than infant or adult skin.

Studies of the function of skin of the premature infant provide some understanding of the status of the skin during the third trimester. In general, various functions of the skin, such as barrier properties, temperature regulation, sweating, response to tactile and mechanical stimuli [14], that have been measured reflect the gestational age more than the birthweight.

The epidermis, even though keratinized and possessing several layers of stratum corneum cells, is a less effective barrier than the infant epidermis. Transepidermal water loss, for example, decreases in a steep slope from 26 weeks EGA to 38 weeks EGA. It decreases even further and with a similarly rapid decline over the first 10–15 postnatal days (reviewed in [15,16]). Disorders of keratinization or



(a)

(b)

Fig. 1.33 Scanning electron (a) and light (b) micrographs of the human fetal dermis at 15 weeks EGA showing the density of the fibrous matrix, the differences in textures between papillary and reticular regions (a), and the boundary between the dermis and subcutaneous regions (a, b). The developing hair follicles are evident in cross-section in (a) and in longitudinal section in (b). A lobule of subcutaneous fat (arrows) is evident in (b) (a, ×80; b, ×100).

infection of the skin may give an even greater disadvantage to the premature newborn in its ability to regulate substances crossing the skin. The stratum corneum of the preterm infant is more permissive to absorption of substances from the external environment and those applied to skin to protect, treat or cleanse it in the neonatal nursery

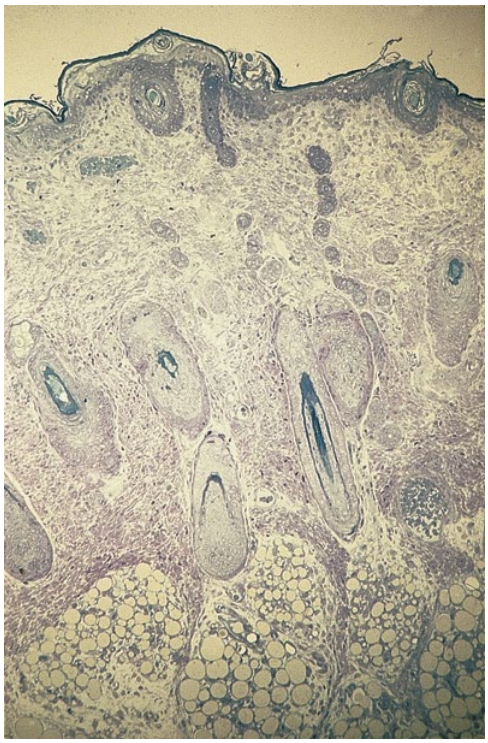


Fig. 1.34 A section through skin obtained from a third-trimester (34-week) human fetus showing all regions of the skin and the presence of hair follicles and eccrine sweat glands (×40).

(reviewed in [15,16]). These compromised epidermal barrier properties, coupled with the fact that the preterm/neonatal infant's body surface-to-volume ratio is very high, can place the premature infant at significant risk.

Although the structure and cellular differentiation of the sweat glands in the preterm infant and term newborn (see Unique features of developing human skin) appear little, if any, different from those of the infant, the sweating function requires a period of maturation after birth, presumably for the innervation to become fully established. The sweating response is limited or absent in the preterm infant to an extent that correlates with the gestational age (reviewed in [13]). Apocrine glands begin to secrete during this trimester [9–12].

Some aspects of the vasculature are less organized in the third-trimester fetus and the newborn than is characteristic in the infant. The marked redness of the newborn reflects the high density of superficial vessels in the dermis and the thinness of the epidermis. Remodelling of the microcirculation occurs as appendages and regions of the skin are completed. At birth, the capillary network is still disorganized and will stabilize only after birth (reviewed in [9–12]).

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Unique features of developing human skin

Periderm

The periderm is the outermost, transient cellular layer of the developing skin of some mammals and birds. These embryonic epidermal cells are larger than basal keratinocytes and cover the entire surface of the early epidermis. The origin of the periderm is not fully elucidated in humans but mouse studies may give some clue to its development. It is possible that the amnion contributes cells to periderm that grow over the single-layered epidermis. This is supported by the fact that the amnion and periderm are similar in keratin composition [1,2] and surface morphology and in the expression of other antigens [3,4]. Studies from early mouse embryos, however, suggest that a continuous sheet of tissue may not cover the epidermis as proposed by this model, because patches of periderm cells are present in some sites [5]. The single, ectodermal layer of the early embryo may divide and give rise to a second cell layer that becomes superficial to the basal layer [6]. This is supported by whole-mount studies showing expression of basal-layer keratinocytes K5 and K14 in both the single-layer ectoderm and the periderm [7].

The most remarkable features of the periderm are the morphological changes that the periderm cells undergo with progressive stages of development [8]. Studies of the surface of the developing skin using scanning electron microscopy, and of corresponding tissue sections examined by light and transmission electron microscopy from consistent regions of the body, have established the stages of human skin development [8] (Fig. 1.35).

The early embryo is covered by a thin, flattened pavement epithelium that is the periderm (Fig. 1.4). Around 8–11 weeks, when the epidermis stratifies, the periderm cells increase in volume and develop a rounded external surface. By 10–14 weeks, single blebs extend from the amniotic surface of each cell and the cell increases in diameter (see Fig. 1.20). All of the cell surface, including the blebs, is modified by microvilli. A network of microfilaments is organized beneath the plasma membrane. Later in the second trimester, the surfaces of the periderm cells project multiple blebs, the larger of which have the configuration of a blackberry (Figs 1.30 and 1.36). The cell diameter continues to increase as the cells become thinly stretched over the epidermis. By 16–23 weeks EGA, the blebs flatten and the periderm regresses (Fig. 1.37). It once again becomes a very thin layer of cells, which, at this stage, rarely contain a nucleus, have few if any organelles and are composed largely of disorganized, fine filaments [8].

The periderm cells do not undergo the events of differentiation that are typical for the keratinocyte. The composition of keratins in the periderm cells remains unchanged throughout development and, since neither the K1/K10 keratin proteins nor profilaggrin are present in the periderm cells of any stage, it is clear that they do not undergo full keratinization. The plasma membrane of the early second-trimester fetal periderm cell, however, appears similar to a cornified envelope (Fig. 1.38a) and the presence of several cornified cell envelope proteins, involucrin, loricrin, keratolinin (cystatin), small proline-rich proteins (SPRR) 1 and 2, and the transglutaminase 1 (TG1) enzyme, in the cytoplasm [9,10] of these cells has been demonstrated (Fig. 1.38b–d). The expression of cornified envelope proteins in conjunction with ultrastructural studies suggests that periderm cells do contribute to the cornified envelope.

Towards the end of the second trimester, individual periderm cells loosen from the underlying epidermal cells and are desquamated over the sites of elevated and exposed hair canals (follicular epidermis). They remain associated, however, with the interfollicular epidermis until the stratum corneum is formed. At this time, the periderm is mostly gone from the skin surface. The events that lead to disengagement of this layer are not known. Abnormality of periderm shedding is hypothesized to contribute to collodion formation.

The structural properties of periderm cells may provide clues as to the function of the layer. The blebs and microvilli increase the surface area of the periderm as it faces the amniotic fluid, suggesting that these cells may be important in the exchange of substances between the fetus and the amniotic fluid, across the skin, in one or

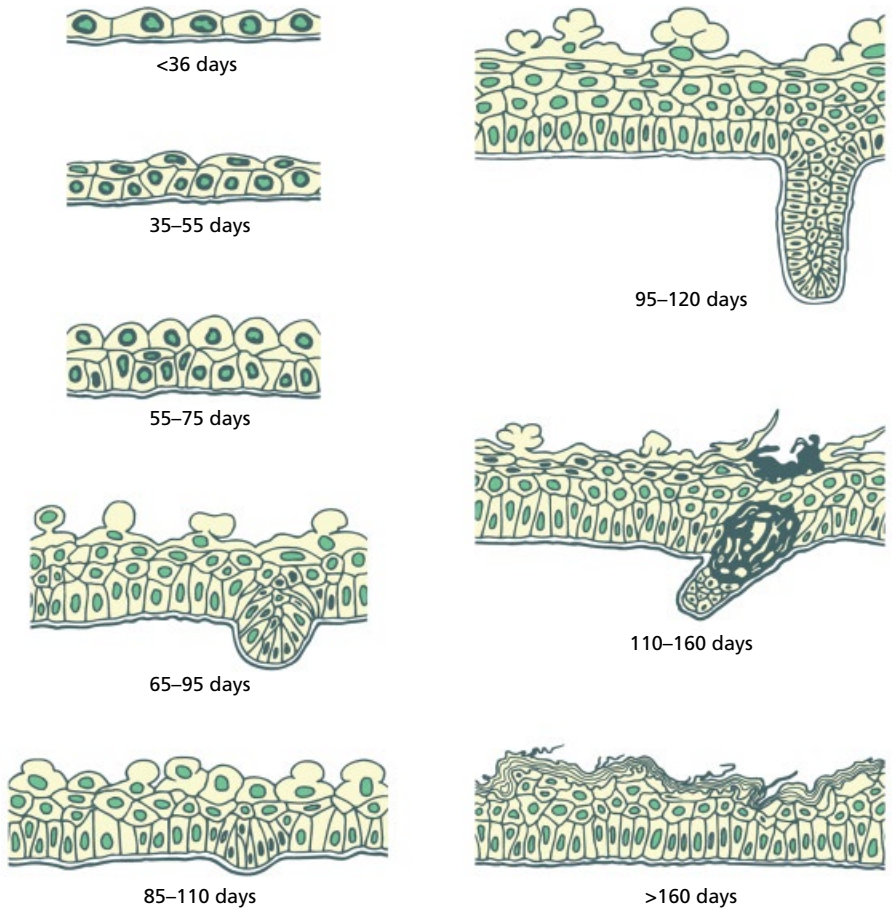


Fig. 1.35 Stages of epidermal development proposed on the basis of periderm structure.

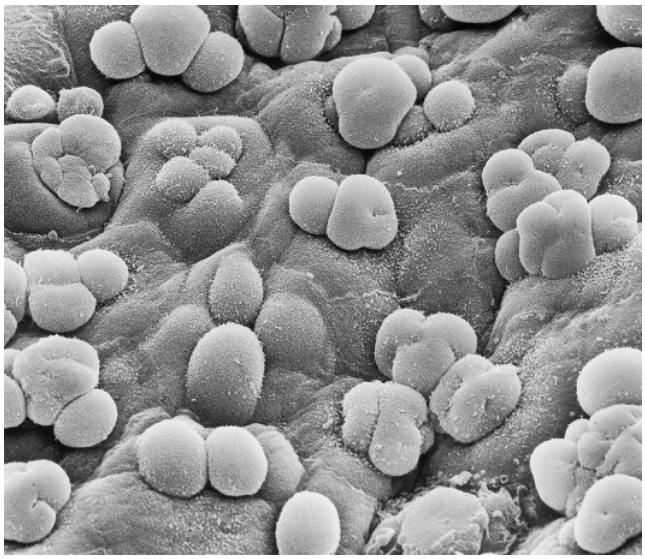


Fig. 1.36 Scanning electron micrograph of the periderm from a mid-second-trimester fetus showing multiple, complex blebs and microvilli extending from the amniotic surface (×1500).

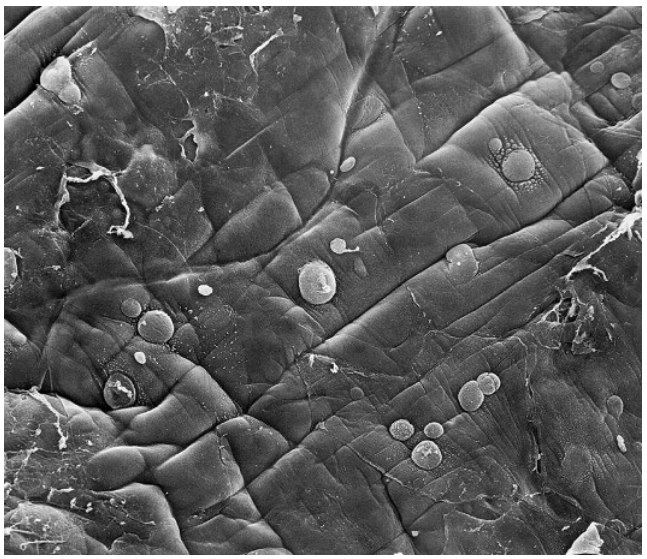


Fig. 1.37 Scanning electron micrograph of the surface of the flexor forearm of a late-second-trimester fetus showing the large, thin, regressed periderm cells (×800).

both directions. Direct evidence for this role in humans is limited. A morphological study of the intramembranous modifications of the periderm plasma membrane suggests that the cells have a role in regulating water transport [11], and Koren [12] has suggested that the skin absorbs nicotine dissolved in amniotic fluid; in the sheep fetus, the periderm has been shown to be involved in the uptake of drugs from the amniotic fluid [13]. It has also been postulated that the periderm is a secretory epithelium that adds material to the amniotic fluid [14] and that it serves as a protective layer for the developing epidermis (also reviewed in [15–17]). In mouse models of aberrant

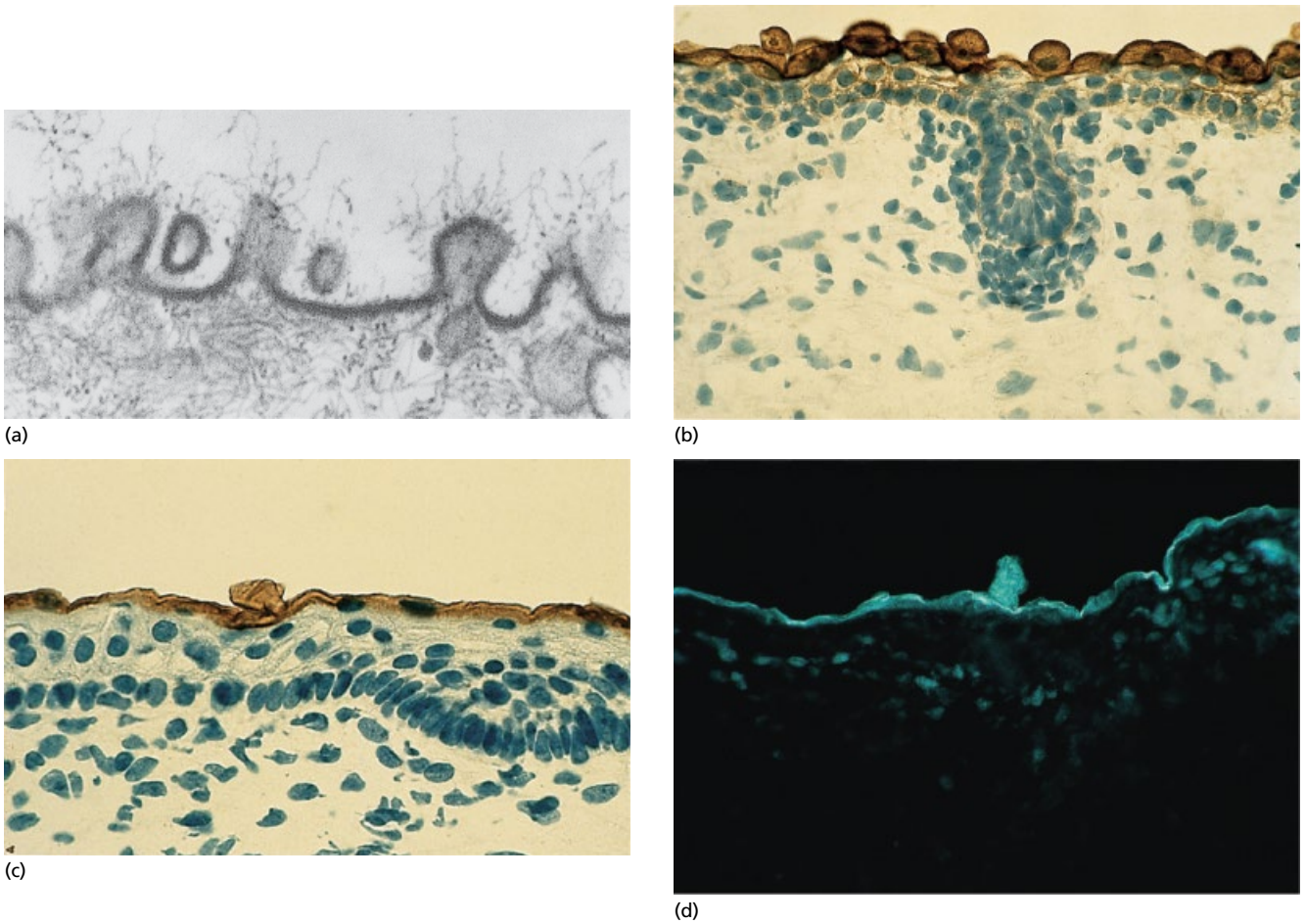


Fig. 1.38 Transmission image of the periderm cornified cell envelope from fetal skin of 21 weeks EGA (a). The periderm expresses involucrin early in the first trimester. (b) Data from a 98-day EGA fetus. Epidermal transglutaminase (c) is also expressed early in the periderm but does not appear to function until the end of the first trimester, when it first demonstrates cross-linking of dansyl cadaverine to substrate in the tissue (d) (a, ×41 250; b, ×300; c, ×300; d, ×300).

or absent periderm development, pathological adhesions developed at sites of apposed tissues [18]. This is likely the mechanism behind popliteal pterygium syndrome and cocoon or fetal encasement syndrome.

The periderm is regionally variable in its properties and timing of development [2]. Periderm of the plantar surface of the toe, for example, shows a very late stage of development at 70 days compared with trunk skin. At this time, the epidermis of the appendage is thicker and more differentiated than trunk skin. This suggests that the nature of the underlying epidermis determines the rate of modification of the periderm. This is supported by similarities in keratin patterning between the basal keratinocytes and the periderm cells [7]. The pattern of development of the mature stratum corneum follows periderm disaggregation, suggesting a functional relationship between these two processes which coincides with the limits of fetal viability between 22 and 26 weeks EGA [19].

The periderm is thus a distinct layer of cells that is defined early in development from the remainder of the epidermis. Many genetic disorders, for example, that modify cells of the basal and intermediate layers during development appear to have no direct or indirect consequences for periderm cells. Keratin filaments are aggregated in the second-trimester skin of fetuses affected with epidermolytic hyperkeratosis (EHK) [20] and epidermolysis bullosa simplex Dowling–Meara (EBS-DM) [21], but none of the filaments clump in periderm cells, nor are there other consequences for the periderm layer. The absence of clumping would be expected in the case of EHK since the keratins involved in this disorder are not keratins that are present in periderm cells, but basal cells and periderm cells do share keratins in common. The persistence of periderm cells with no adverse outcome in an environment of severe cell destruction in layers proximal to the periderm in EHK is surprising and argues for autonomy of this layer. To the contrary, however, animal models of disorders where mature epidermal barrier function is not achieved (e.g. ichthyosis) show incomplete shedding of the periderm suggesting a complex interplay that is not yet understood fully [22].

Regionalization in developing skin

Regional differences in properties of the skin are well documented in adult skin. Regionalization is also a phenomenon of developing skin even at very early ages of gestation. Few systematic studies have been done to

document these regional differences consistently throughout development [8,23]. Without a clear appreciation and accurate knowledge of differences in normal morphology at various sites, structural evaluation of skin samples that may be from unknown regions can be difficult. At the same time, it is essential to appreciate whether the disease of concern is also expressed with regional variation not only in the adult but also at the onset of expression of the disease during development [24,25]. In at least one situation, the understanding of regional variability of expression of a disease at its first presentation was gained from samples obtained for prenatal diagnosis [26] (Fig. 1.39). Systematic studies of affected fetal skin from multiple regions are valuable to undertake when tissue becomes available. Such efforts also expand our knowledge of the natural history of the disorder.

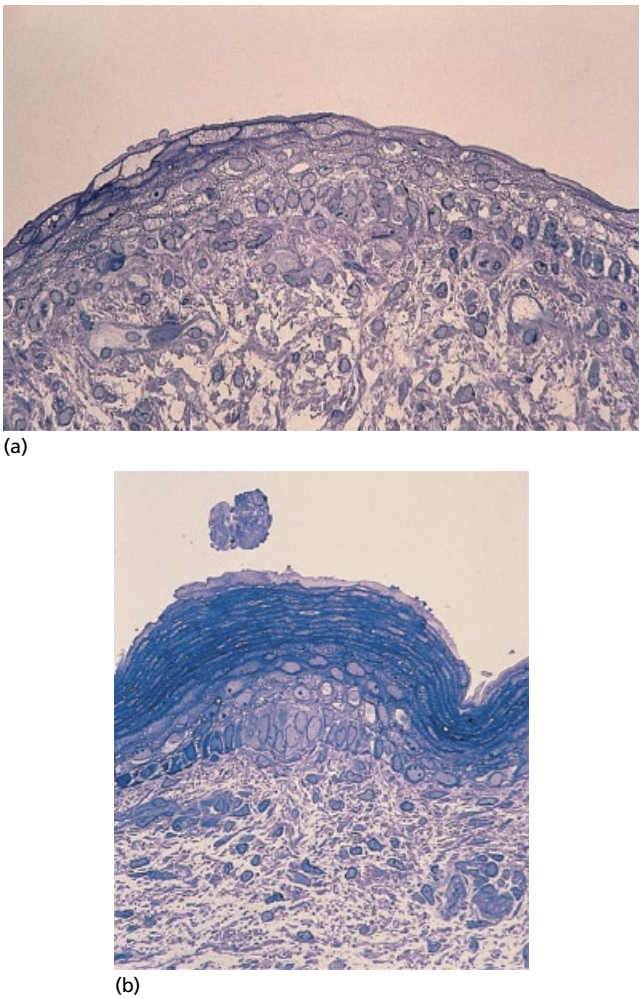


Fig. 1.39 Sections of skin obtained *in utero* by fetal skin biopsy from a fetus at risk of lamellar ichthyosis. Note the difference in morphology between the two samples. One sample shows an epidermis of normal thickness and state of development for 19 weeks EGA (a). The second sample shows a thickened epidermis, still covered by periderm (b). In both samples, hair canals were excessively keratinized. This disorder is expressed *in utero* with regional variation (a, ×300; b, ×300).

Keratinization

Keratinization of the nails, hair follicles (follicular epidermis), intraepidermal sweat ducts and the interfollicular epidermis occurs at different times during gestation. The nails are the earliest structures of the skin to keratinize *in utero*, with the appearance of cornified cells as early as 11–12 weeks EGA. The timing of keratinization of follicular epidermis is consistent with the cephalocaudal direction of follicle morphogenesis [25]; the interfollicular epidermis keratinizes first in thick skin and then in thin skin, with the latter also proceeding in a regionally dependent manner. The timing of keratinization for a given region appears to follow a rigidly specified programme during development. The molecular mediators of keratinization are beginning to be elucidated and in many instances disruption of these pathways causes diseases of abnormal keratinization [26]. Even in these situations of abnormal keratinization, for example in fetuses affected with lamellar ichthyosis, harlequin ichthyosis and EHK, there is no evidence for early or delayed onset of the keratinization process in either the follicular or interfollicular epidermis (reviewed in [27–30]).

Appendage formation

The embryonic epidermis gives rise to diverse structures collectively known as the ectodermal appendages. Despite their diverse structure and function in the mature skin, the hair follicles, nails, sweat glands, mammary glands and teeth have similar early inductive events. Interactions between the embryonic ectoderm and underlying dermis are important in the development of the skin appendages. Experimental studies of appendage formation have revealed the early molecular events that occur stepwise to control various aspects of appendage formation (reviewed in [31]). Epithelial and mesenchymal cells lie in close proximity at the sites of appendage formation, and in some cases make physical contact (Fig. 1.40). These structures are thought to develop in response to epithelial–mesenchymal interactions that initiate the process through instructive messages, sustain the process through permissive interactions and then support differentiation and maintenance of the fully developed appendage.

After the embryo–fetal transition, around 10–11 weeks EGA, basal epidermal cells, through various molecular events, undergo proliferation at specifically patterned sites to form buds that grow down into the dermis as hair germs and sweat ducts, or as a fold of tissue that establishes the nail fold. These earliest inductive events lead to the formation of an epidermal thickening, the placode. The epithelial layers then proliferate with growth into the mesenchyme, forming a bud. After bud formation, specific characteristics of the diverse appendages emerge. Nerves and vessels, cell adhesion molecules (CAMs), soluble mediators and homeobox genes (homeoproteins) have been implicated in the pattern formation of certain appendages (reviewed in [32–37). Several molecular pathways, including Wnt/β-catenin, fibroblast growth factor (FGF), transforming growth factor (TGF)β/bone morphogenetic protein (BMP) and hedgehog pathways, are well established as mediators of these early interactions (reviewed in [31]). The

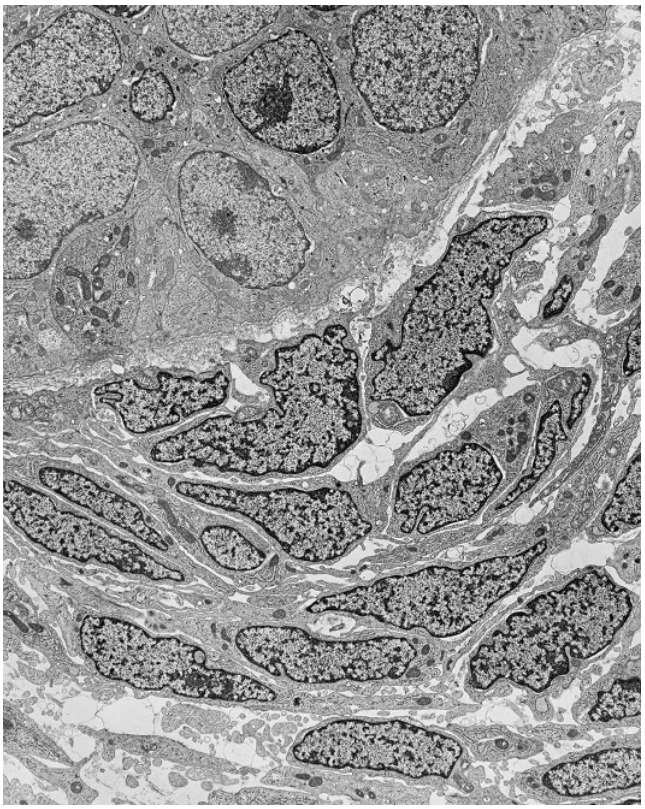


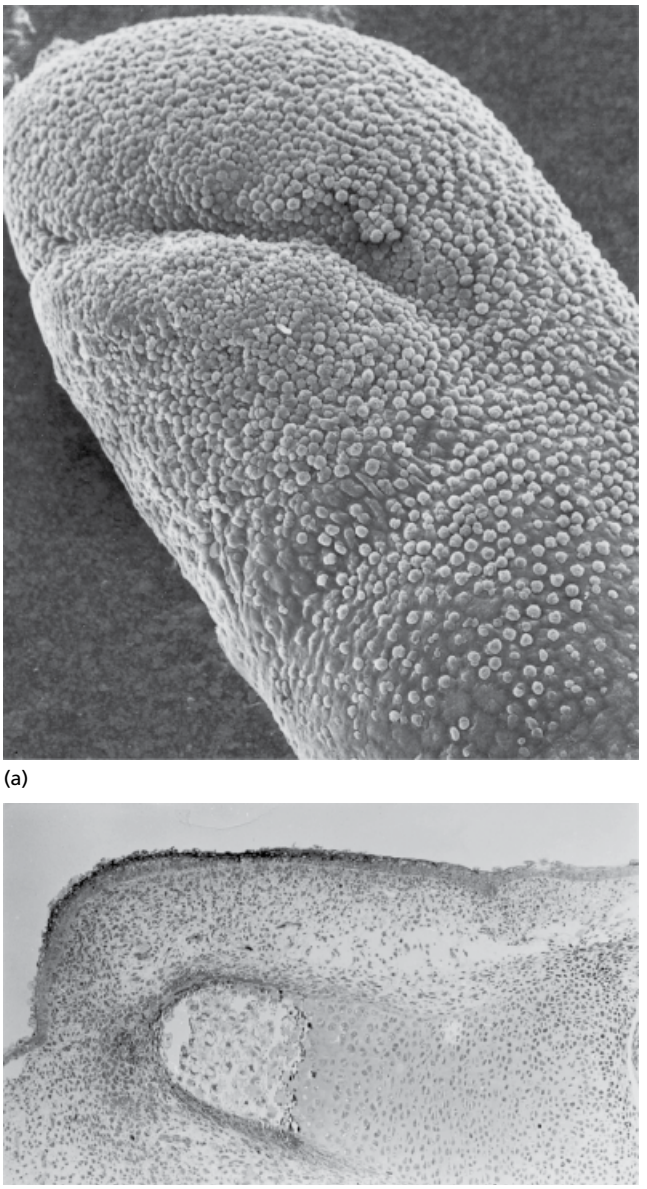
Fig. 1.40 Developing hair germ showing the close proximity between the epithelial cells of the germ and the mesenchymal cells of the dermis, which will follow the developing appendage and influence its development and differentiation (×8250).

evidence for these interactions in the development of the epidermal appendages in human skin is best documented for formation of the pilosebaceous apparatus [38–40].

Nail formation

The formation of the nail unit is highly dependent on dorsal–ventral patterning of the limb bud. As with other ectodermal appendages, epithelial–mesenchymal pathways are critical for proper patterning (reviewed in [41]). Mutations in factors critical in dorsal–ventral patterning of the mammalian limb, such as Wnt7a, engrailed 1 (EN1) and LIM homeobox transcription factor 1β (LMX1B), give rise to developmental nail anomalies such as nail–patella syndrome.

The distal rays of the digit are evident on the hand of the 50-day EGA embryo, and within the next 7 days the digits separate [42]. Formation of the nail on the dorsal surface of the digits and the eccrine sweat glands on the ventral surface is initiated at approximately the same time after embryo–fetal transition. By 70 days' gestation, the boundaries of the nail field are established externally by proximal, lateral and distal folds (Fig. 1.41a) and sections through the digit reveal a shallow nail fold (Fig. 1.41b). Development of the epidermis over the nail bed is furthest advanced at its distal-most margin [43,44].



(a)

(b)

Fig. 1.41 Developing nail. (a) Scanning electron micrograph of a digit from an 85-day EGA fetus showing the boundaries of the nail field recognized by proximal (identifying the position of the nail fold), lateral and distal folds. (b) A section through the digit of a 70-day EGA fetus shows the position of the nail fold and the thicker and more advanced epidermis over the nail bed (a, ×100; b, ×150).

By 90 days EGA, the dorsal ridge is evident superficially and is delineated from the plantar surface of the digital pad by a deep constriction (Fig. 1.42a). The nail fold has invaginated deeply into the dermis and organized into dorsal (roof of the fold) and ventral (floor of the fold) layers that are distinguished from one another morphologically and functionally (Fig. 1.42b). The ventral fold becomes the nail matrix, which is primarily responsible for the synthesis of the nail plate.

The earliest nail, which is formed late in the first trimester, consists of several layers of keratinized cells that are evident primarily at the distal margin of the nail bed and

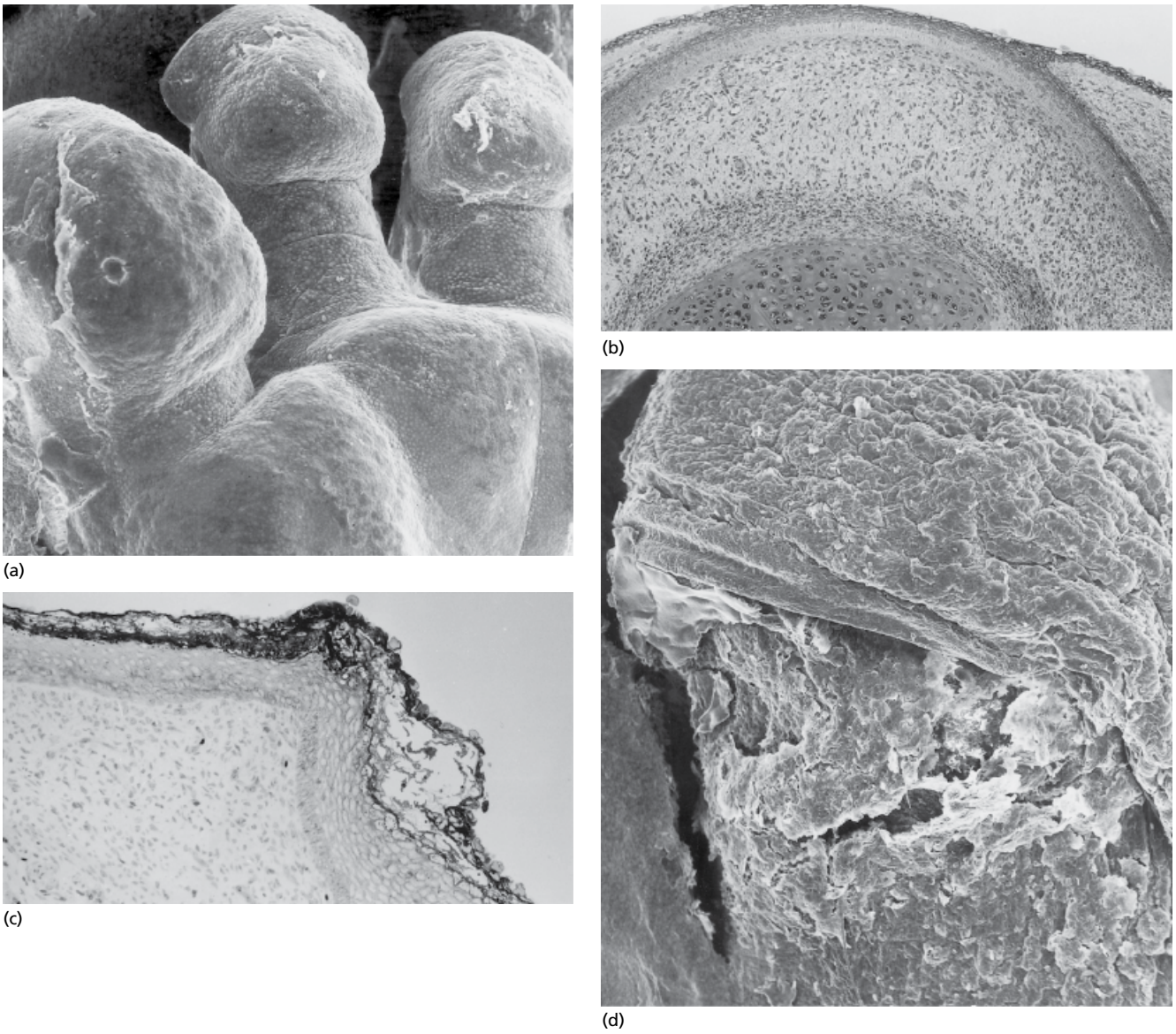


Fig. 1.42 Developing digit and nail. (a) Scanning electron micrograph of the ventral surface of the developing digit of an 80-day EGA fetus showing the dorsal ridge and the large volar pads. (b) Transverse section through the nail fold of a digit from an 85-day EGA fetus showing the distinction between dorsal and ventral (presumptive nail matrix) surfaces of the nail fold. The higher-magnification section shows more detail of the epidermal surface and the mesenchyme beneath the nail fold. (c) Transverse sections through the digit and distal-most tip of the nail bed of a digit from a 105-day fetus showing keratinization of the superficial cells forming the 'preliminary' nail. (d) Scanning electron micrograph of a nail of a 140-day EGA fetus showing the fragile nature of the nail plate (a, ×60; b, ×100; c, ×200; d, ×100).

over the dorsal ridge. By 15 weeks EGA, a thick cornified layer covers the nail bed (Fig. 1.42c). This 'preliminary' nail is easy to slough from the surface and thus may be composed to a greater extent of keratinized epidermal cells from the nail bed rather than derived from the matrix of the nail fold. The nail of a 19-week EGA fetus is established by both the nail matrix and the nail-bed epidermis, although the nail is still fragile (Fig. 1.42d). The nail that is present at birth is actually a composite of layers of cells derived from the dorsal nail fold (contributes the outermost layer of the nail) and the nail matrix (contributes the intermediate layer of the nail); the distal half to two-thirds of the nail bed contributes the inner layer of the nail. The layers are more evident in the fetus than in the postnatal individual [45].

Eccrine sweat gland formation

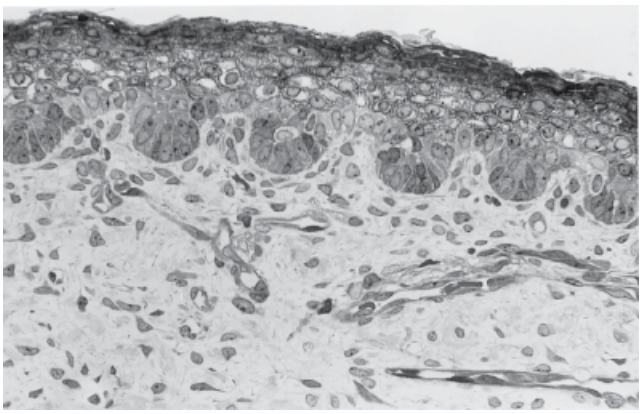
Human skin has the highest density of eccrine sweat glands amongst mammals. The development of the digits and the morphogenesis of eccrine sweat glands and nails occur on the hand in advance of the foot and on the distal pads ahead of the middle and proximal phalanges and the palm [32,42]. Eccrine sweat glands form on the general body surface at least 4–6 weeks later than on the palms and soles. They are the last of the epidermal appendages to be formed (reviewed in [32]). The structural events have been much better characterized for the ridged skin of the palmar plantar and digital skin than for trunk skin (reviewed in [15–17]). About 8.5–9 weeks EGA, the shape of the terminal digit is evident. Volar pads, transient mounds of mesenchyme that accumulate beneath

the epidermis on the ventral surface of the digits, are well formed (Fig. 1.42a). Their presence in the first trimester is presumed to influence the dermatoglyphic patterns [46,47] and the development of those flexion creases that are not considered to be dependent upon movements of the hand [42,48,49]. Interest in the development of flexion creases relates to the aberrant patterns they assume in certain congenital disorders. Volar pads begin to regress around 10.5–11 weeks EGA and are nearly gone by 12.5–13 weeks EGA [42] when, presumably, their influence is no longer needed for morphogenesis.

The primary epidermal ridges are first formed around 10–11 weeks EGA [50]. They are recognized in sectioned specimens as localized aggregations of basal epidermal cells on the digits, palms and soles (Fig. 1.43a); in sheets of epidermis viewed basally, they appear first as discontinuous then as continuous ridges [51]. At this stage, the epidermis on the plantar surface consists of five or six layers of intermediate cells and the periderm. Merkel cells containing the characteristic granules are distributed along the primary epidermal ridges, where they may attract periglandular nerve fibres to this position of the structure [52] (Fig. 1.43b). Electron microscopy has revealed nerve fibres associated with basal laminae underlying the ridges and Merkel cells, and occasionally extending into the epidermal tissue [32]. Merkel cell–nerve complexes are evident in digital skin before the primary ridges begin to form, and remain prominent in the primary ridges after the appearance of the sweat gland anlagen (Fig. 1.43a). They do not appear to migrate, however, into any region of the developing appendage.

The sweat gland primordia are recognized around 13–14 weeks at regular sites along the now flattened ridges as narrowed, solid, epithelial cords of cells that contain basal cell keratins and express classical carcinoembryonic antigen (CEA) on all cells [50,52]. There is no evidence of condensed mesenchyme associated with the onset of sweat gland development as there is in follicle development, thus suggesting that other sources of signalling molecules, possibly the volar pad mesenchyme, or nerves and/or other cell–cell interactions (perhaps within the epithelium) may instruct the sites for appendage formation and trigger the onset of gland development.

As the cords of epithelial cells elongate into the dermis, a thickening at the terminus defines the glandular segment from the duct [53] (Fig. 1.44a). Ductal, secretory, myoepithelial and acrosyringial cell types differentiate in the dermal and intraepidermal regions of the gland and duct, and are easily distinguished from one another by light and electron microscopy (Fig. 1.44) and by immunostaining patterns using antibodies to keratin intermediate filament proteins [53]. All cells continue to express CEA [52]. The secretory cells border a central lumen within the gland; myoepithelial cells are evident at the periphery of the structure. Cells of layers of the duct and of the gland are distinct from one another at 15 weeks EGA by their morphological properties and by differences in expression of keratins (duct) and vimentin (gland) and CAMs [53,54]. Coexpression of the two intermediate filament proteins



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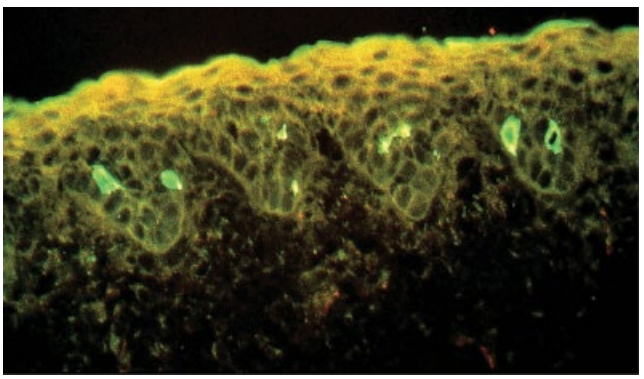

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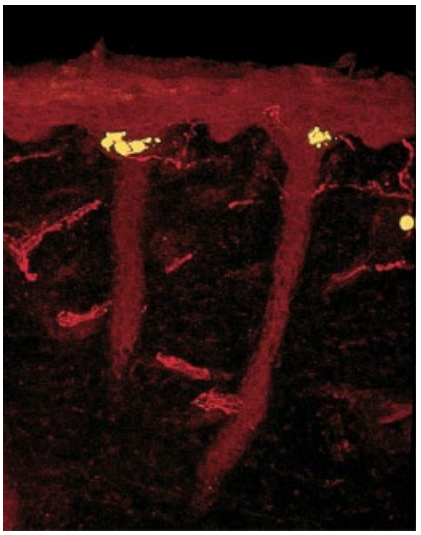
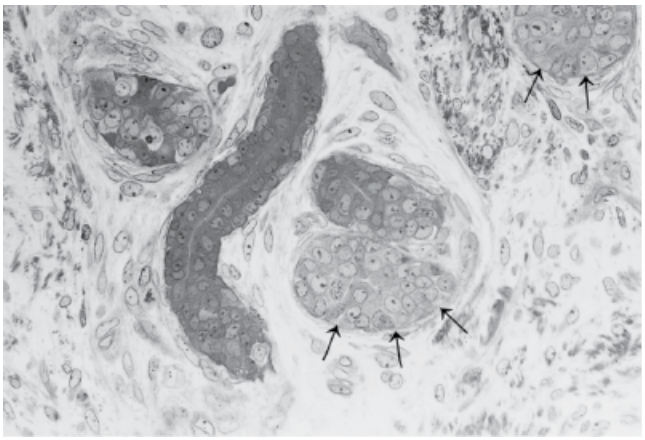


(c)

Fig. 1.43 Developing sweat glands on the ventral surfaces of the digit. (a) Cross-section through the digit of a 95-day EGA fetus showing the primary epidermis ridges organized from the basal layer of the epidermis. Note the abundance of nerves and vessels in the proximal dermis. (b) Immunolabelled section through the palm of a 105-day fetal hand showing the position of Merkel cells (green) marked by an antibody that recognizes keratin 18. The red labelling recognizes neurofilaments in dermal nerve fibres. (Source: Micrograph courtesy of Dr Dong Kun Kim.) (c) Immunolabelled section through the palm of a 163-day fetal hand showing the position of Merkel cells (green) marked by an antibody that recognizes keratin 20. The red labelling recognizes neurofilaments in dermal nerve fibres. Note the well-established sweat ducts and the secondary epidermal ridges alternating with the primary ridges from which the ducts are formed. (Source: Micrograph courtesy of Dr Dong-Kun Kim.) (a, ×300; b, ×300; c, ×100.)

(a)



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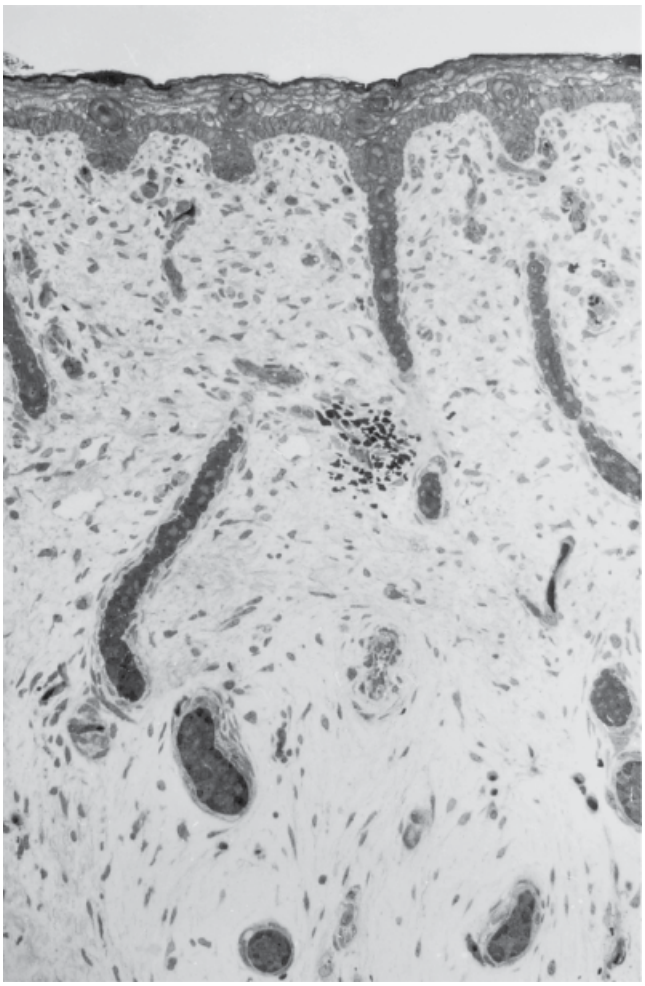

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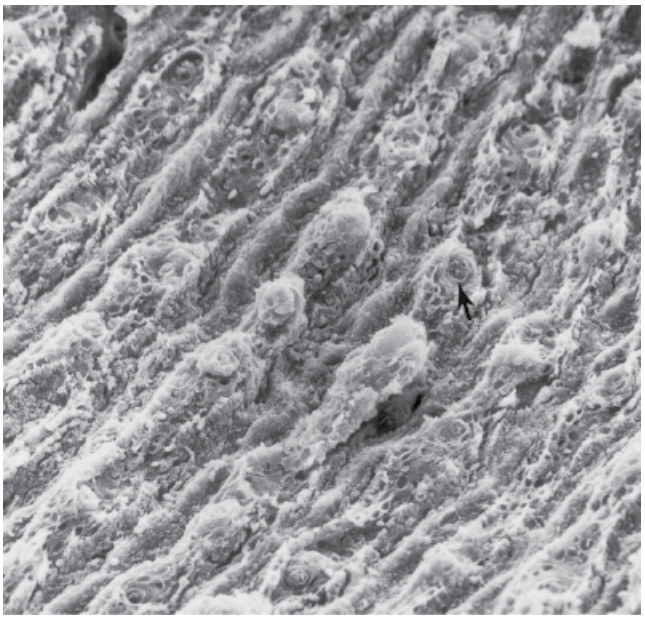


(d)

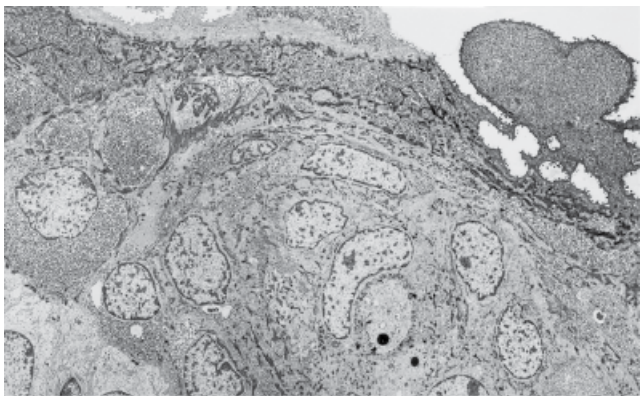
Fig. 1.44 Developing sweat glands and ducts. (a) Scanning electron micrograph of the palm of a 19-week EGA fetus showing the elongated ducts and the club-like terminal gland. (b) A section through the palm of a 147-day EGA fetus shows the position of the duct and gland in the dermis and the canalization (keratinization) of the intraepidermal portion of the duct. A higher-magnification image of the gland and duct in the palmar dermis of a 126-day EGA fetus (c) shows the cell layers of the duct and the cells of the gland (arrows). (d) Scanning electron micrograph of the undersurface of palmar epidermis of a 19-week EGA fetus showing the primary epidermal ridges with the remnants of torn sweat ducts spaced periodically and the secondary ridges that do not give rise to sweat ducts (a, ×80; b, ×120; c, ×300; d, ×80).

in the same cells of the secretory segment of the developing gland is unique to this appendage, but it is characteristic of other glandular tissues such as mammary and salivary glands [53].

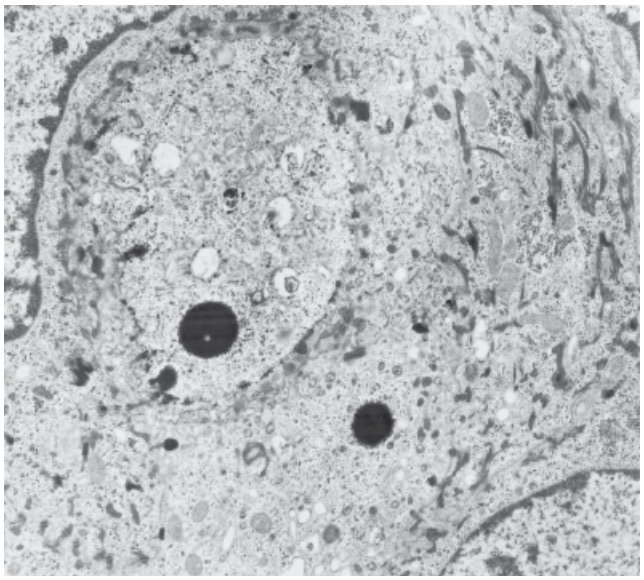
Secondary ridges form between the primary ridges (see Figs 1.43c and 1.44d). They do not give rise to sweat glands or contain Merkel cells. Globular keratohyalin granules are evident in the cytoplasm of the circumferentially

organized cells of the intraridge, the intraepidermal portion of the duct (the acrosyringium) at about 15 weeks EGA, signalling the onset of canalization in the acrosyringium (Fig. 1.45). The lumen of the duct forms by the fusion of cytoplasmic vesicles within ductal cells. The duct remains partially occluded even in the third trimester [55]. By 22–24 weeks EGA, the sweat glands on the palms and soles have attained the structure of the adult glands, with a coiled secretory gland.

The absence of sweat glands is a hallmark of the ectodermal dysplasias due to mutations in ectodysplasin pathway genes [56]. The ectodysplasin pathway interacts with the Wnt/β-catenin pathway, amongst others, to control eccrine gland formation from induction through secretory duct differentiation [57]. While these pathways are shared with other skin appendages, the molecular mechanisms leading to specification of eccrine glands specifically is not well established.



(a)



(b)

Fig. 1.45 Formation of the intraepidermal sweat duct by the development and coalescence of vesicles and subsequent keratinization of the lining cells (a). Note the globular keratohyalin granules that are characteristic of acrosyringial keratinization (×9100). Source: Micrograph originally published in Odland G, Holbrook K. Curr Prob Derm 1981;9:29–49. Reproduced with permission of Karger Publishers.

Pilosebaceous apparatus formation

The pilosebaceous apparatus is best described as a composite epithelial–mesenchymal structure with critical molecular 'cross-talk' between the two. Morphogenesis of the hair follicle begins on the head and face at around 70–80 days EGA, shortly after the epidermis stratifies, then proceeds in a cephalocaudal direction [20]. The process is completed at around 19–20 weeks EGA, when hairs extend from the lanugo follicle through the periderm-covered surface of the skin. Follicles form in regular patterns in all body regions, with the distances separating each dependent upon the specific site (Fig. 1.46). The stages of follicle development, including hair germ, hair peg, bulbous hair peg and lanugo follicle stages of follicle formation (Fig. 1.47), are based on the vellus hairs on the trunk [58]. Follicles form only during development and decline in numbers as a function of ageing.

The induction of follicles, their stages of development, maintenance in the adult and the cyclical growth and regression of the scalp follicles are all dependent upon an association of the follicle epithelium with dermal mesenchymal cells that form a cellular and matrix sheath around the developing and mature follicle and establish the dermal papilla as a special collection of mesenchymal cells that modulate the production and elongation of hairs [59].

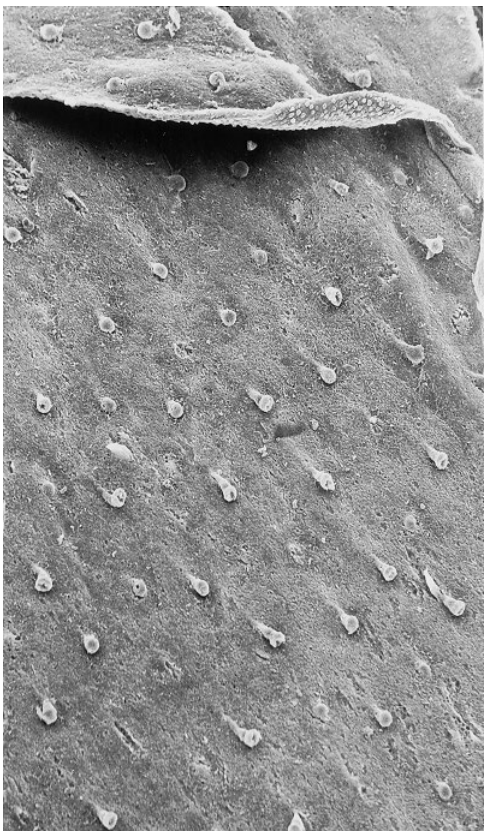


Fig. 1.46 Scanning electron micrograph of the undersurface of the epidermis from a 15-week EGA fetus showing the pattern of hair follicles in the hair peg and hair germ stages of development. Note the longitudinal grooves in the epidermis that mark the position of intraepidermal hair canals (×100).

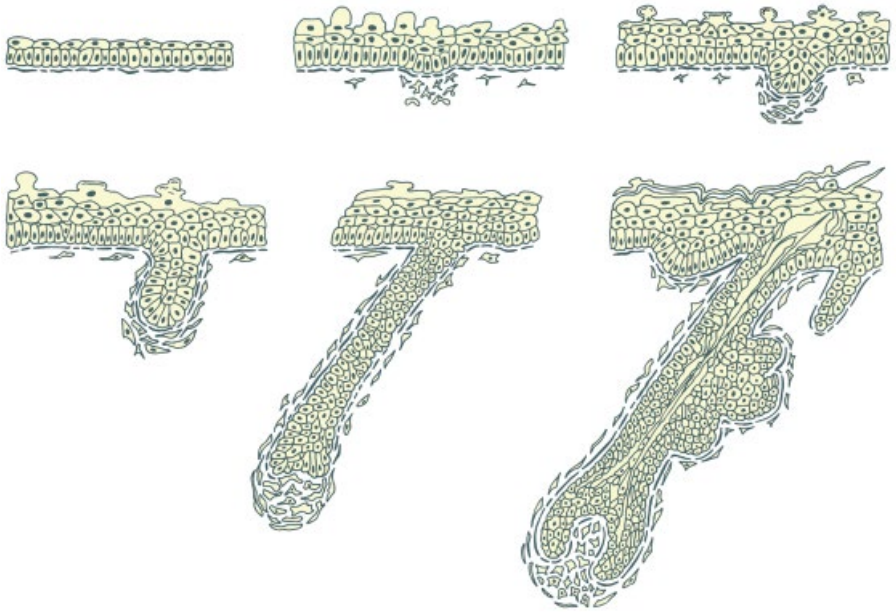


Fig. 1.47 Diagram of the stages of hair follicle formation including the prefollicle two-layered epidermis, pregerm, hair germ, early hair peg, late hair peg/early bulbous hair peg and lanugo follicle stages.

The epithelial and mesenchymal cells have been extensively characterized at each stage of follicle formation with regard to the expression of growth factors, growth factor receptors, cytokines, other signalling molecules and growth regulators, and structural proteins and enzymes (reviewed in [60–63]). Experimental studies in animal models, transgenic animals, tissue recombination preparations and various cell and organ culture systems have revealed the functions of specific populations of cells in developing follicles (e.g. dermal papilla and cells of the bulge) and events that signal early and sequential steps in the induction of other appendage primordia [31,36,37]. Thus, the data can be used only to infer what may be occurring *in utero* at the time the human follicles form de novo as most studies are done on postnatal human hair follicles.

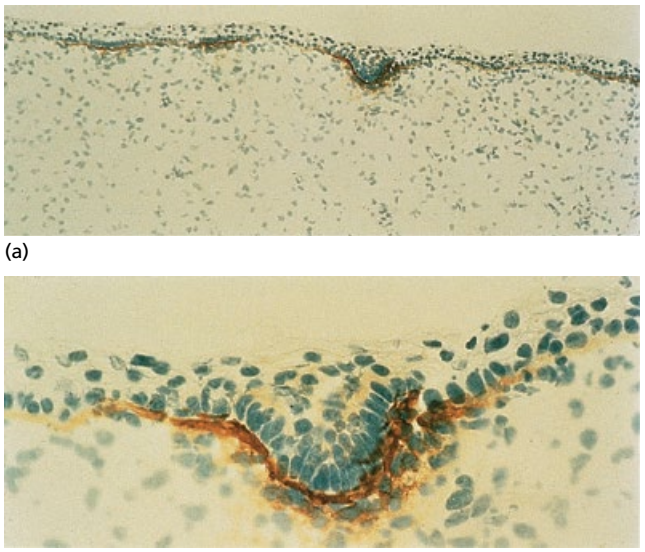
The sites of follicle formation can be recognized, even before the hair germs are visible, in sections of fetal skin by immunostaining the tissue with an antibody that recognizes the matrix molecule tenascin [33,34]. Patches of reaction product at the basement membrane zone correspond to pregerms, or sites where basal keratinocyte nuclei are closely spaced and mesenchymal cells are aggregated (Fig. 1.48) [64,65]. Cells from the basal epidermal layer bud into the dermis to become hair germs (Fig. 1.48b). Condensed mesenchymal cells associate closely with the germs, often extending processes that contact the basal lamina (Fig. 1.40); this collection of mesenchymal cells is intensely immunoreactive with antibodies to NGFR (p75) (Fig. 1.49), NCAM and other growth factor receptors. Little if any collagenous matrix is present around the cells as a consequence of either downregulated production or enhanced degradation (reviewed in [33,34]). Merkel cells are recognized in some of the developing germs. As is the case with the other appendages in which Merkel cells are prominent, they may play a role in targeting nerve fibres towards the developing appendage.

At around 13–14 weeks EGA, the hair germs elongate into the dermis as cords of cells called hair pegs (Figs 1.46 and 1.50). The hair peg consists of an inner core of cuboidal cells and outer layer of columnar cells that is associated with the basal lamina surrounding the follicle and continuous with that of the interfollicular epidermis. Cells of the outer layer contain the same keratins as the basal epidermal keratinocytes and the cells of the inner core contain intermediate cell keratins (Fig. 1.50a), thus implicating the origins of follicle cells from two epidermal layers. Merkel cells are distributed among the outer root sheath keratinocytes.

Early hair pegs are cylindrical, but as they elongate further they develop three regions: (i) a constricted, neck-like connection with the epidermis (the presumptive infundibulum); (ii) a central, cylindrical region (the presumptive isthmus); and (iii) a terminal zone that becomes widened at its most distal end (the lower follicle and the presumptive bulb) (Fig. 1.50). The length of the hair peg and the three zones of the developing follicle are exaggerated in some regions of the skin but more subtle in others.

Changes occur in all three regions, with the first notable events taking place at the proximal and distal ends. Elongated core cells in the neck of the follicle continue into the epidermis, where they form a strand of cells that lies between the basal and intermediate cell layers (see Fig. 1.50). This is the hair tract that marks the position and pathway of the presumptive hair canal (Fig. 1.50c) [66].

The distal end of the hair peg flattens and the epithelial cells along this basal border elongate to form a distinct layer that establishes the matrix (Fig. 1.50a and b). The flattened end of the follicle begins to invaginate into the cord, shaping the bulb with the matrix as the roof of the bulb. Mitotic figures are evident in matrix cells, and longitudinally orientated cells, presumably the progeny of the dividing matrix cells, move out of the matrix into the



(a)

(b)

Fig. 1.48 Immunolabelled section of human fetal skin at 70–75 days EGA showing tenascin-positive sites where hair germs have formed or are expected to form (a and b). The clustering of basal keratinocytes is apparent at sites where tenascin is strongly expressed in the basement membrane zone (a) (a, ×100; b, ×350). Source: Micrograph courtesy of Dr Beth Kaplan.

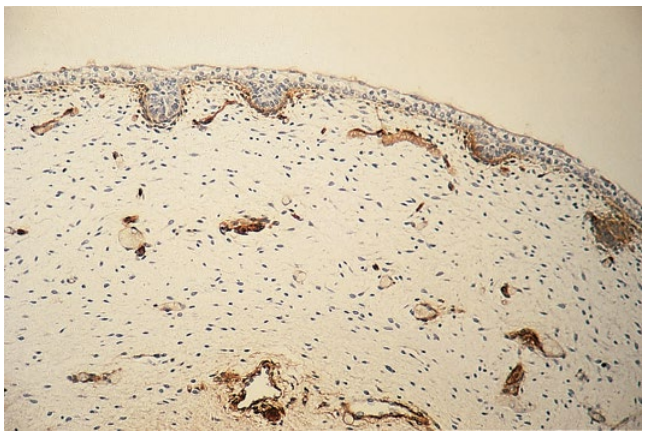
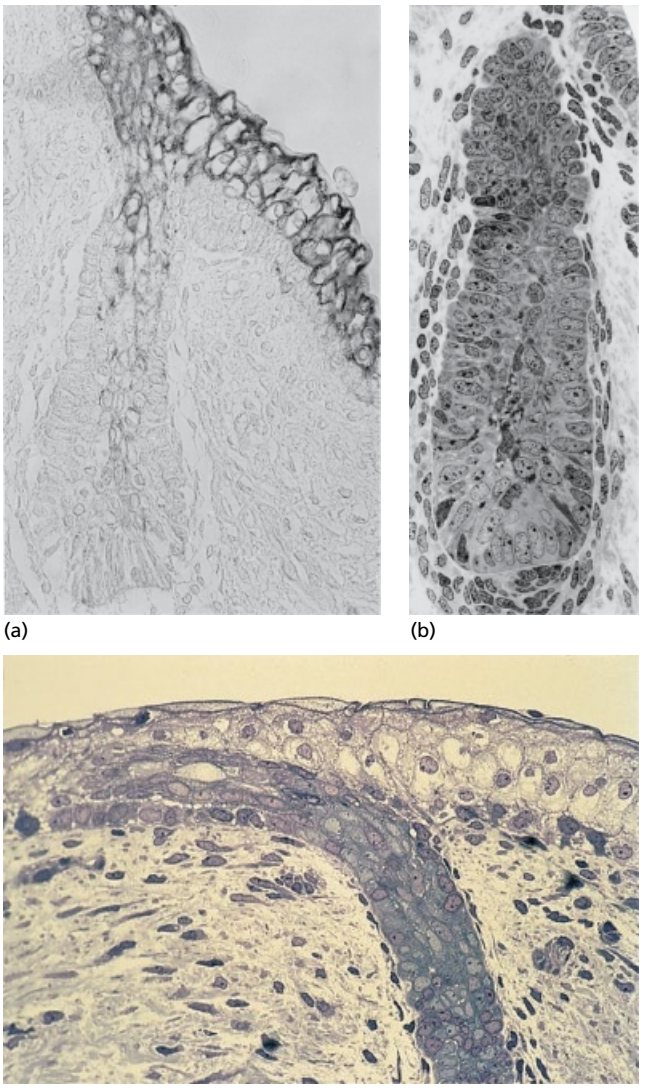


Fig. 1.49 Hair germs in the skin of a 97-day fetus immunostained to recognize the p75 neurotrophic receptor, which recognizes NGFR. Note the concentration of this immunoreactive material within the mesenchymal cells surrounding the hair germ (×120).



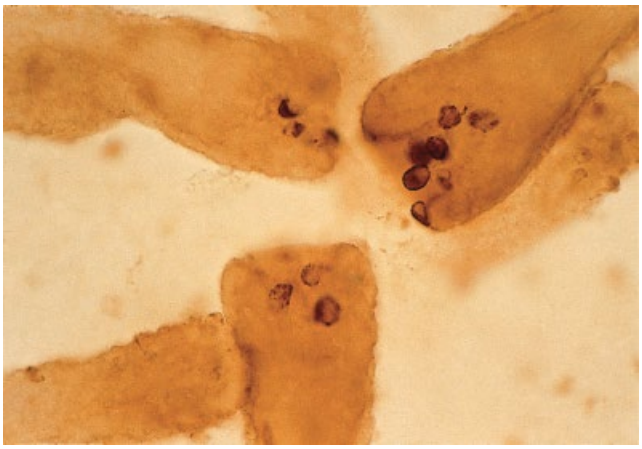
(a) (b)

(c)

Fig. 1.50 Late-stage hair pegs (a, b) showing the continuity of the intermediate-layer keratins into the upper core cells of the follicle (a), the regions of the peg and the mesenchymal cells surrounding the peg and aggregated at its tip (the presumptive dermal papilla) in association with the presumptive matrix of the follicle. Note the differences in cell orientation in the inner and outer and the distal and proximal regions of the peg (b). (c) Section through the upper end of a hair peg showing the continuation of cells into the epidermis as the hair track (a, ×300; b, ×300; c, ×300).

centre of the cord, thereby establishing the first layers of the inner root sheath and the hair (the hair cone). Cells of the outer layer of the follicle located adjacent and lateral to the matrix appear to become more loosely associated with one another, perhaps permitting the inward migration of the cells derived from the matrix. Melanocytes aggregate in the matrix and produce melanin ahead of melanocytes in the general body skin, thus making the bulbs of developing hair follicles ideal sites to examine when evaluating skin biopsy samples from a fetus at risk of tyrosinase-negative oculocutaneous albinism (reviewed in [27]) (Fig. 1.51). Such samples can be induced to synthesize melanin by the dihydroxyphenylalanine (DOPA) reaction if the fetus is normal [67,68].

During these events, the cord is surrounded in its entirety by several layers of elongated mesenchymal cells that form a sheath. The connective tissue matrix is sparse within this cellular sheath and appears to be devoid of the fibrillar collagens that are present in the surrounding subepidermal and interstitial matrix (Fig. 1.52). Differences in matrix molecules are observed at different levels



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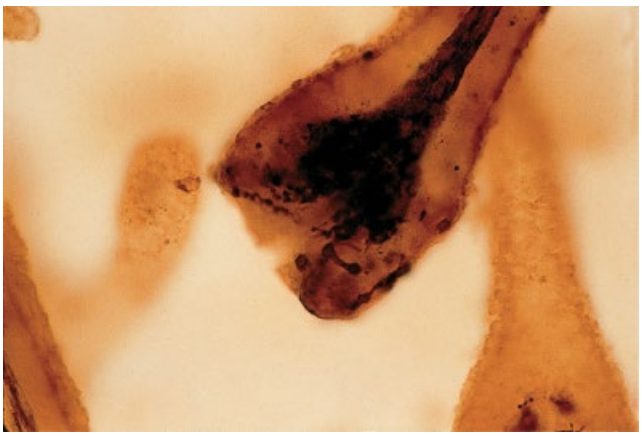


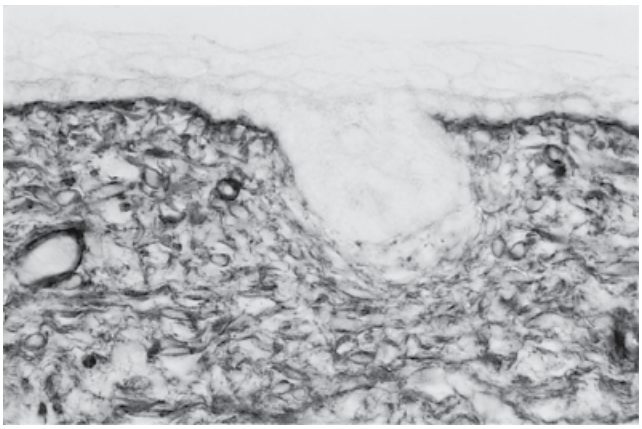
(b)

Fig. 1.51 The bulb of a hair peg (a) and lanugo follicle (b) in skin from different regions obtained at ages 115 days and 125 days EGA. Note the concentration of melanocytes in the matrix of the follicle (a, ×300; b, ×300).

of the hair peg and when comparing the dermal papilla with the follicle sheath [33,34].

Between 15 and 17 weeks EGA, bulges of epithelial cells begin to grow out from the epithelial cord on the posterior surface of the follicle and the adult layers of the follicle differentiate into the hair and internal root sheath. Once these bulges form, the follicle is called a bulbous hair peg (Fig. 1.53a). The factors that stimulate the development of these structures to arise from the follicle, at a precise stage in the hair peg formation and at precise sites along the hair peg, are unknown. There are no obvious landmarks along the hair peg that provide morphological clues as to how the bulges might originate.

The most superior bulge is the primordium of the sebaceous gland (Fig. 1.53a). Cells begin to produce sebum soon after this structure is evident. Analysis of epidermal lipids from fetal skin at this stage reveals a sterol/wax ester content, which suggests that the material is similar to adult sebum [69]. The second bulge, the 'true bulge', forms concurrently with and slightly distal to the sebaceous gland. It is the site of follicular stem cells



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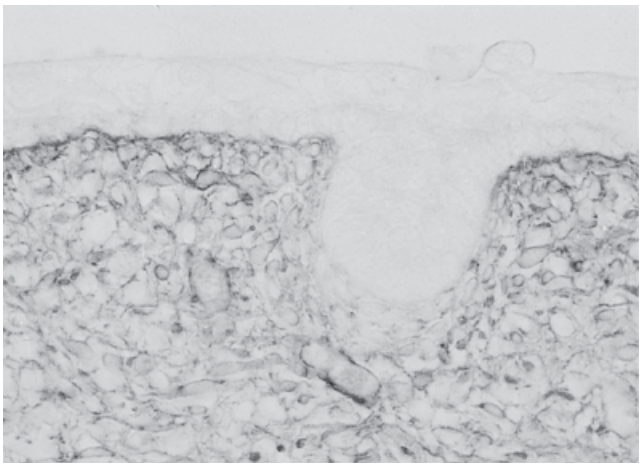

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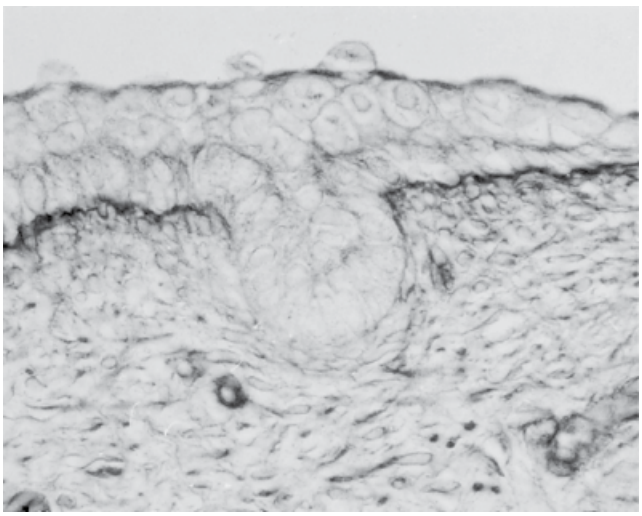
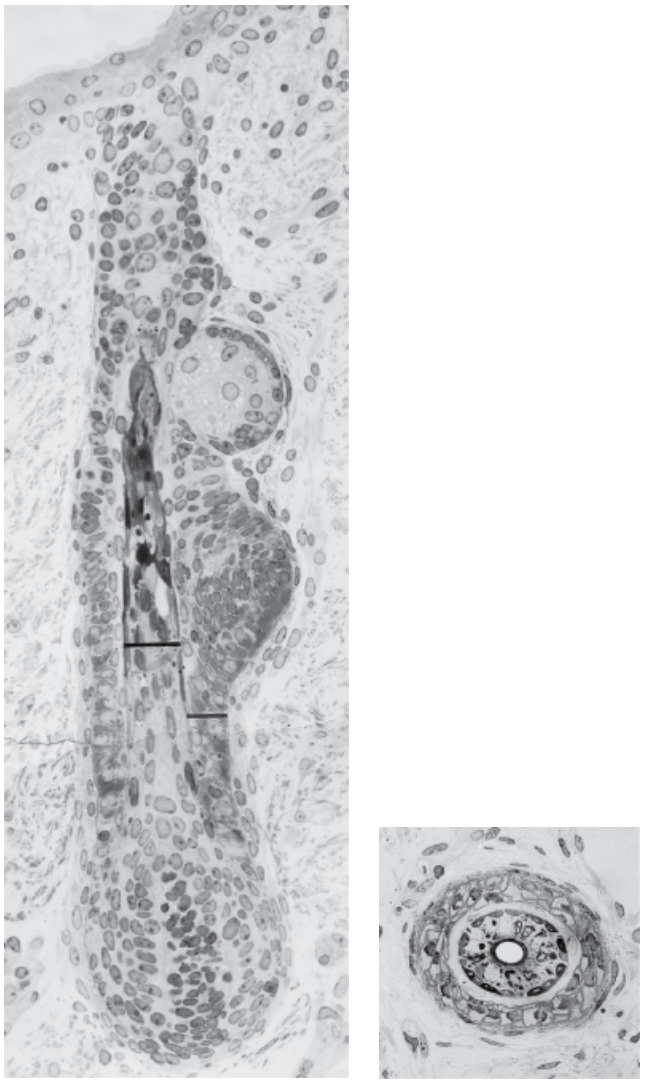


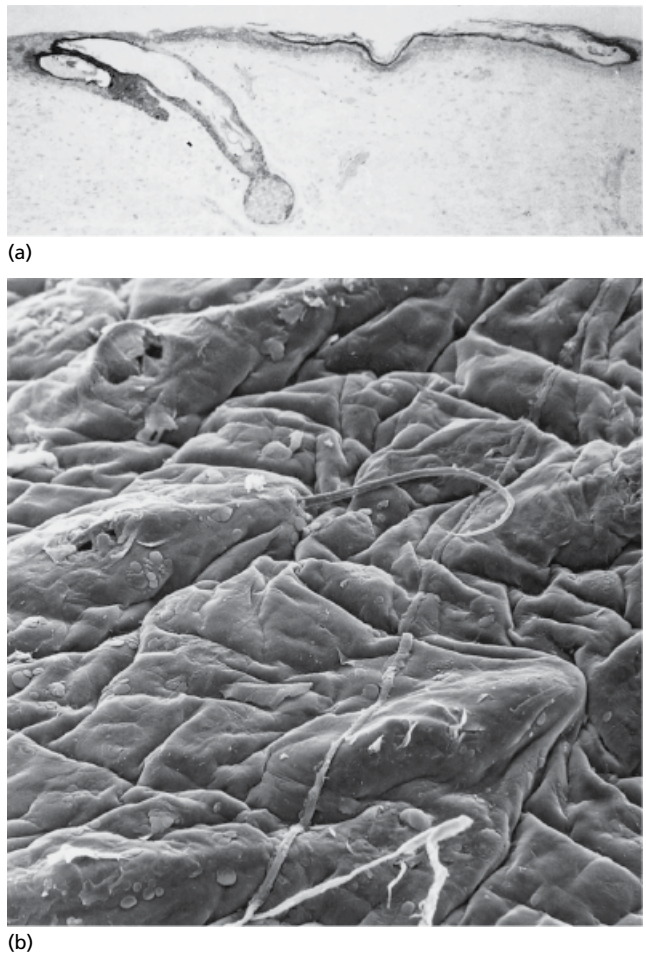
(c)

Fig. 1.52 Sections of fetal skin immunolabelled with antibodies to collagens of the dermis. Note the decreased staining for types I (a), III (b) and V (c) collagens in the developing hair germs (a, ×300; b, ×300; c, ×300). Source: Immunolabelling studies by Dr Lynne T. Smith.



(a) (b)

Fig. 1.53 Bulbous hair peg in the skin of a 15-week EGA fetus. (a) In the longitudinal section through the follicle, note the sebaceous gland, the bulge located just distal to the sebaceous gland, the cell layers of the inner root sheath (inner bar) and the outer layers of the outer root sheath (outer bar). The infundibulum is the region of the follicle that lies between the sebaceous gland and the epidermis. Note the cells of the dermal papilla within the bulb. (b) A cross-section through a region between the two bars shows the layers of the outer root sheath and the inner root sheath (a, ×300; b, ×300).



(a) (b)

Fig. 1.54 The hair canal. (a) Section through the skin of a 138-day EGA fetus showing the floor of an opened hair canal. Keratinization of this structure stands out in contrast to the non-keratinized epidermis. (b) Scanning electron micrograph of the skin of a 21-week EGA fetus showing hair canals within (beneath the surface of) the epidermis. Note that one hair has emerged and others are evident through the thinned epidermal layers above the canal (a, ×100; b, ×185).

[70] and the point of attachment of the arrector pili muscle (Fig. 1.53a). Multipotent epithelial stem cells reside in the bulge [71]. The stem cell population is maintained throughout development and postnatal life, giving rise to the cells responsible for regenerating the cycling follicle as well as having an important role in wound healing. Merkel cells also concentrate in the bulge at early stages of bulge formation. They may be important in establishing this structure, stimulating proliferation or attracting nerve fibres and smooth muscle cells to the site. A third bulge may form superior to the sebaceous gland as the primordium of the apocrine sweat gland. These structures are located in the restricted sites of the body where apocrine sweat glands are present in the postnatal infant (axilla, areola, scalp, external eyelid, auditory meatus and anogenital regions).

The cylindrical layers of the follicle differentiate and keratinization begins in several different structures of the follicle concurrently: the outer layer of cells of the inner root sheath (layer of Henle); the cuticle and cortex of the hair (Fig. 1.53b); the sebaceous duct; and the hair canal. Continued production of cells of the three layers of the inner root sheath and the hair gradually creates the keratinized tube of the inner root sheath and the hair. Keratinization within the hair tract canalizes the cord of cells and forms a keratin-lined channel that courses diagonally through the epidermis (Figs 1.50c and 1.54a) [69]. The granular and cornified cell layers of the hair canal form a sharp contrast with the remainder of the, as yet non-keratinized, epidermis (Fig. 1.54a). The angle of the canal with the epidermis and the intraepidermal length of the canal are regionally variable. On the eyebrow, for example, the hair canals are closely spaced and their paths are very short. In other regions of the body, such as the appendages, the canals can be very long. By examining