Baran & Dawber's
Diseases of the Nails and their Management

Baran & Dawber's Diseases of the Nails and their Management

FOURTH EDITION

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Preface

Since our first edition appeared in 1984, *Baran & Dawber's Diseases of the Nails and their Management* has been one of the most important sources for nail specialists. This fourth edition has been enriched by numerous additional contributions, and now constitutes a true encyclopedia of the nail.

We owe this to the young collaborators who were entrusted with either writing new chapters such as 'Imaging the Nail Unit', or with thorough updating, as in the chapter on 'Hereditary and Congenital Nail Disorders'. Finally, the all-important addition of an original appendix providing differential diagnosis of nail findings in both color and shape by anatomic site ensures overall continuity.

I would like to express my particular gratitude to Martin Sugden, the commissioning editor, for his support and encouragement all along, and my heartfelt thanks to Nicole Baran, my wife, for her unflagging energy and dedication in taking up and sharing the challenge.

Robert Baran

List of Abbreviations

3D	three-dimensional
ACA	anticardiolipin antibody
ACTH	adrenocorticotropic hormone
ADFK	acquired digital fibrokeratoma
ADL	activities of daily living
AER	apical ectodermal ridge
AIDS	acquired immunodeficiency syndrome
ALHE	angiolymphoid hyperplasia with eosinophilia
ALM	acrolentiginous melanoma
AORN	Association of Operating Room Nurses
APACHE	acral pseudolymphomatous angiokeratoma of children
APES	aminopropyltriethoxysilane
AVA	arteriovenous anastomoses
AVF	arteriovenous fistula
AZT	azidothymidine
BDD	blistering distal dactylitis
BMP	bone morphogenetic protein
BMZ	basement membrane zone
BPNH	bilateral periventricular nodular heterotopia
CA	cyanoacrylate
CARI	congenital autosomal recessive ichthyosis
CDC	Centers for Disease Control
CEA	carcinoembryonic antigen
CMC	chronic mucocutaneous candidiasis
CMV	cytomegalovirus
COIF	congenital onychodysplasia of the index fingers
CT	computed tomography
DBP	dibutyl phthalate
DEB	dystrophic epidermolysis bullosa
DIP	distal interphalangeal
DLSO	distal and lateral subungual onychomycosis
DMPS	dimercapto-propane-sulfonate
DMSA	dimercaptosuccinic acid
DMSO	dimethyl sulfoxide
EB	epidermolysis bullosa
EBA	epidermolysis bullosa acquisita
ED	ectodermal dysplasia
EGFR	epidermal growth factor receptor
EM	electron microscopy
EMA	epithelial membrane antigen
EO	endonyx onychomycosis
FDA	Food and Drug Administration
FEDL	flashlamp excited dye laser
FEF	forced expiratory flow
FEV_1	forced expiratory volume in 1 sec
GVHD	graft-versus-host disease
H&E	hematoxylin and eosin
HEMA	hydroxy-ethylmethacrylate
HFMD	hand, foot and mouth disease
HIV	human immunodeficiency virus
HOOD	hereditary osteoonychodysplasia
HPV	human papillomavirus
HSR	high spatial resolution
HSV	herpes simplex virus
HTLV	human T-cell leukemia virus
IDS	International Society for Dermoscopy
ILM	incident light microscopy
ILVEN	inflammatory linear verrucous epidermal nevus
IP	incontinentia pigmenti
IU	international units
IVT	ischemic venous thrombosis
KA	keratoacanthoma
KID	keratosis, ichthyosis and deafness
LE	lupus erythematosus
LED	light-emitting diode
LM	longitudinal melanonychia
MES	multiple exostoses syndrome
MIC	minimum inhibitory concentration
MIM	Mendelian Inheritance in Man
MIP	maximum intensity projection
MMA	methylmethacrylate
MRI	magnetic resonance imaging
MSH	melanocyte-stimulating hormone
NAPSI	Nail Psoriasis Severity Index
NTOM	nerve territory-orientated macrodactyly
PA	posteroanterior

PAI	plasminogen activator inhibitor
PaO_2	partial pressure of oxygen in arterial blood
PAS	periodic acid-Schiff
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
PIU	pterygium inversum unguis
PNF	proximal nail fold
PRP	pityriasis rubra pilaris
PSO	proximal subungual onychomycosis
PUVA	psoralen ultraviolet A
PVC	polyvinyl chloride
PWSO	proximal white subungual onychomycosis
RA	rheumatoid arthritis
ROS	reactive oxygen species
RV	residual volume
SCC	squamous cell carcinoma
SE	spin echo
SLE	systemic lupus erythematosus
SLN	sentinel lymph node
SLR	single lens reflex
SM	subungual melanoma
SNR	signal-to-noise
SO	subungual onychomycosis
SSM	superficial spreading melanoma
STIR	short time inversion recovery
SWO	superficial white onychomycosis
T	tesla
TAR	thrombocytopenia absent radius
TDO	total dystrophic onychomycosis
TGF	transforming growth factor
TNF	tumor necrosis factor
TOWL	transonychial water loss
TTD	trichothiodystrophy
TUDDS	transungual drug delivery system
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
US	ultrasonography
UV	ultraviolet
UVB	ultraviolet B
VEGF	vascular endothelial growth factor

CHAPTER 1
Science of the Nail Apparatus

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Gross anatomy and terminology

Knowledge of nail unit anatomy and terms is important for clinical and scientific work [1]. The nail is an opalescent window through to the vascular nail bed. It is held in place by the nail folds, origin at the matrix and attachment to the nail bed. It ends at a free edge distally, overlying the hyponychium. These structures are illustrated in Figures 1.1 and 1.2. Definitions of the components of the nail unit are as follows.

- **Nail plate (nail)**: durable keratinized structure which continues growing throughout life.
- **Lateral nail folds**: the cutaneous folded structures providing the lateral borders to the nail.
- **Proximal nail fold (posterior nail fold)**: cutaneous folded structure providing the visible proximal border of the nail, continuous with the cuticle. On the undersurface this becomes the dorsal matrix.
- **Cuticle (eponychium)**: the layer of epidermis extending from the proximal nail fold and adhering to the dorsal aspect of the nail plate.
- **Nail matrix (nail root)**: traditionally, this can be split into three parts [2]. The dorsal matrix is synonymous with the ventral aspect of the proximal nail fold. The intermediate matrix (germinative matrix) is the epithelial structure starting at the point where the dorsal matrix folds back on itself to underlie the proximal nail. The ventral matrix is synonymous with the nail bed and starts at the border of the lunula, where the intermediate matrix stops. It is limited distally by the hyponychium.
- **Lunula (half moon)**: the convex margin of the intermediate matrix seen through the nail. It is paler than the adjacent nail bed. It is most commonly visible on the thumbs and great toes. It may be concealed by the proximal nail fold.
- **Nail bed (ventral matrix, sterile matrix)**: the vascular bed upon which the nail rests, extending from the lunula to the hyponychium. This is the major territory seen through the nail plate.
- **Onychodermal band**: the distal margin of the nail bed has a contrasting hue in comparison with the rest of the nail bed [3]. Normally, this is a transverse band of 1–1.5 mm of a deeper pink (Caucasian) or brown (Afro-Carribean). Its colour, or presence, may vary

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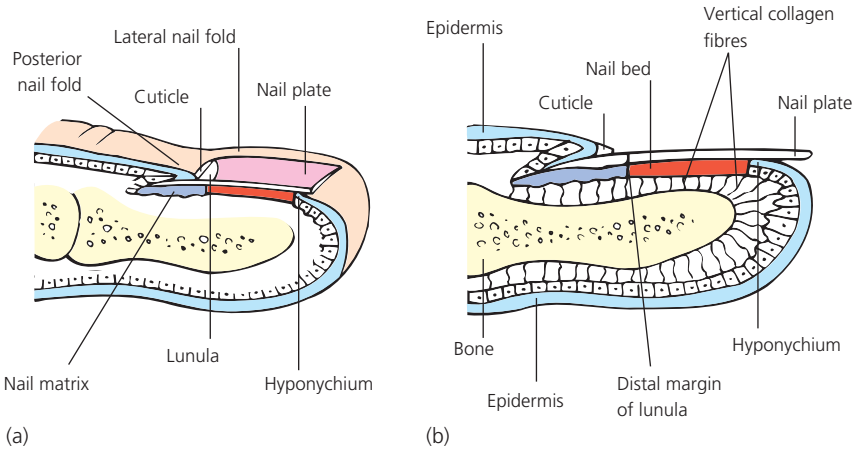


Figure 1.1 Longitudinal section of a digit showing the dorsal nail apparatus.

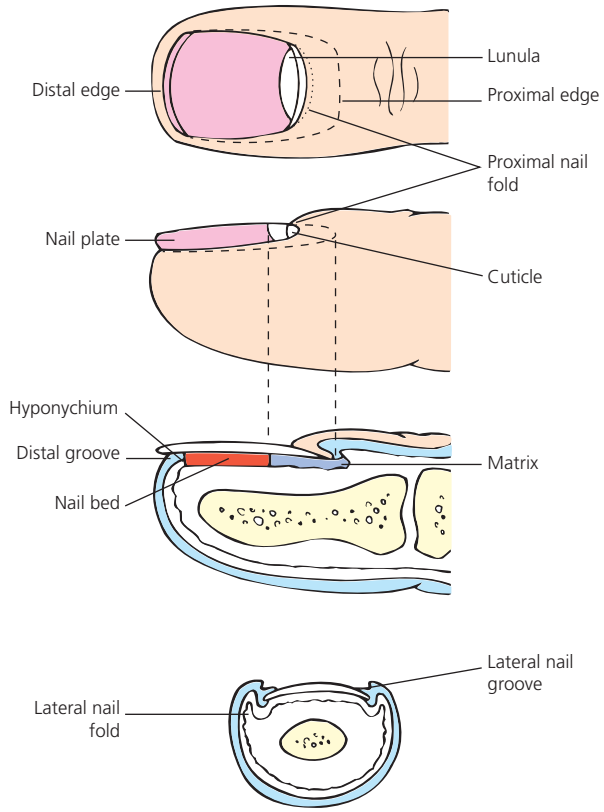


Figure 1.2 The tip of a digit showing the component parts of the nail apparatus.

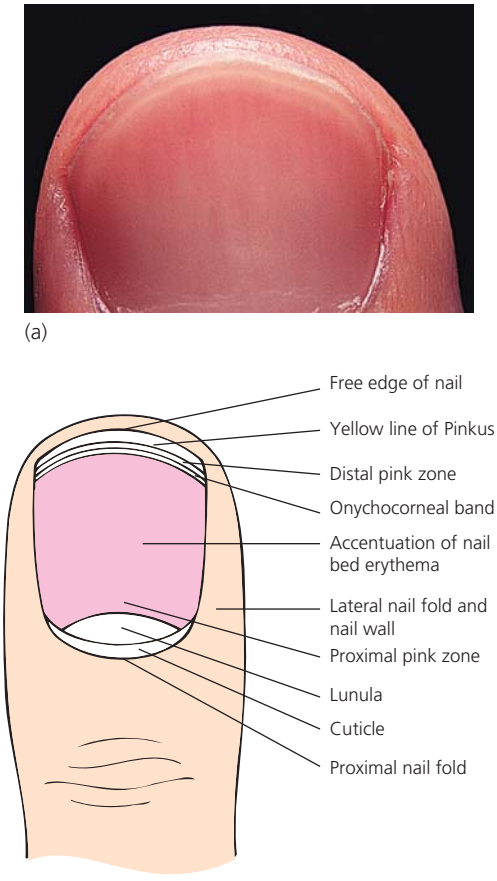


Figure 1.3 (a) Onychodermal band. (b) Diagrammatic representation of the morphological features of the normal nail; detail of the distal physiological color bands is shown. Courtesy of T.S. Sonnex and W.A.D. Griffiths.

with disease or compression which influences the vascular supply (Fig. 1.3). Sonnex *et al*. [4] examined 1000 nails from thumbs and fingers in 100 subjects, alive and dead. In addition to clinical observation, they obtained histology from cadavers. Their findings are summarized in Table 1.1. The onychocorneal band represents the first barrier to penetration of materials beyond the nail plate. Disruption of this barrier by disease or trauma precipitates a range of further events affecting the nail bed. The white appearance of the central band represents the transmission of light from the digit tip through the stratum corneum and up through the nail. If the digit is placed against a black surface, the band appears dark.

- **Hyponychium (contains the solenhorn)**: the cutaneous margin underlying free nail, bordered distally by the distal groove.
- **Distal groove (limiting furrow)**: a cutaneous ridge demarcating the border between subungual structures and the finger pulp.

Embryology

Morphogenesis

8–12 weeks

Individual digits are discernible from the 8th week of gestation [5]. The first embryonic element of the nail unit is the nail anlage, present from 9 weeks as the epidermis overlying the dorsal tip of the digit. At 10 weeks, a distinct region can be seen and is described as the primary nail field. This almost overlies the tip of the terminal phalanx, with clear proximal and lateral grooves in addition to a well-defined distal groove. The prominence of this groove is partly due to the distal ridge, thrown up proximally, accentuating the contour. The primary nail field grows proximally by a wedge of germinative matrix cells extending back from the tip of the digit. These cells are proximal to both the distal groove and ridge. The spatial relationship of these two latter structures remains relatively constant as the former becomes the vestigial distal groove and the latter the hyponychium (Fig. 1.4).

Table 1.1 Clinical appearance of distal zones of the nail bed.

Zone	Subzone	Appearance
Free edge	–	Clear gray
Onychocorneal band		
I	Distal pink zone	0.5–2 mm distal pink margin, may merge with free edge
II	Central white band	0.1–1 mm distal white band representing the point of attachment of the stratum corneum arising from the digit pulp
III	Proximal pink gradient	Merging with nail bed

13–14 weeks

Differential growth of the slowly developing primary nail field and surrounding tissue results in the emergence of overhanging proximal and lateral nail folds. Depending on the point of reference, the nail folds may be interpreted as overhanging [6] or the matrix as invaginating. By 13 weeks the nail field is well defined in the finger, with the matrix primordium underlying a proximal nail fold. By 14 weeks the nail plate is seen emerging from beneath the proximal nail fold, with elements arising from the lunula as well as more proximal matrix.

17 weeks to birth

At 17 weeks, the nail plate covers most of the nail bed and the distal ridge has flattened. From 20 weeks, the nail unit and finger grow in tandem, with the nail plate abutting the distal ridge. This is now termed the hyponychium. The nail bed epithelium no longer produces keratohyalin, with a more parakeratotic appearance. By birth the nail plate

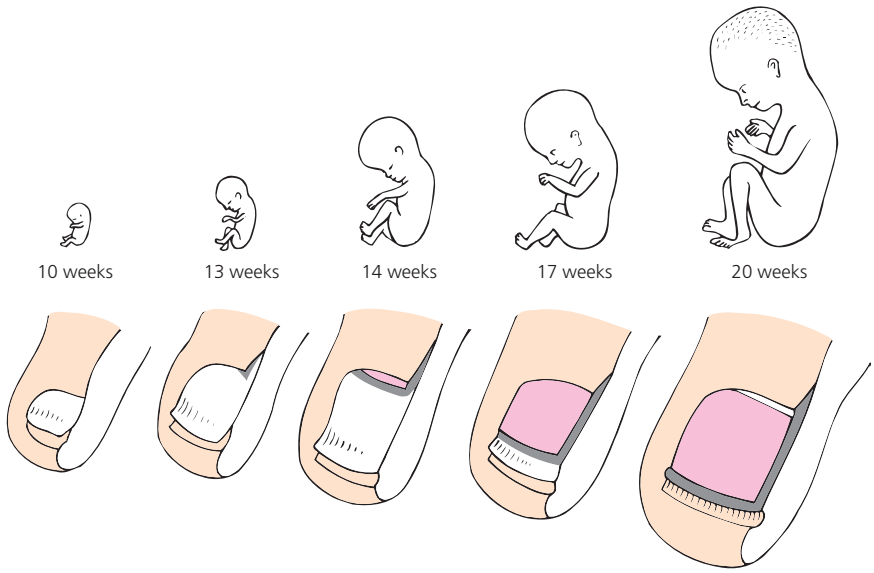


Figure 1.4 Embryogenesis of the nail apparatus. *Ten weeks*: the primary nail field can be seen with proximal, lateral and distal grooves. The latter is accentuated by a distal ridge. *Thirteen weeks*: a wedge of matrix primordium moves proximally, with the invagination of the proximal nail fold above. *Fourteen weeks*: the nail plate emerges. *Seventeen weeks*: the nail plate covers most of the nail bed and the distal ridge starts to flatten. *Twenty weeks*: the nail plate extends to the distal ridge, now termed hyponychium. Finger and nail grow roughly in tandem from now on. Fetuses are one-fifth of actual size.

extends to the distal groove, which becomes progressively less prominent. The nail may curve over the volar surface of the finger. It may also demonstrate koilonychia. This deformity is normal in the very young and a function of the thinness of the nail plate. It reverses with age.

Tissue differentiation

Keratins are filament-forming proteins of epithelial cells. They are found within the cytoplasm. There are 54 human keratins and their genes are divided into three categories:

- epithelial keratins/genes
- hair keratins/genes
- keratin pseudogenes.

Schweizer devised the reclassification of keratins according to the system described below to accommodate the changing knowledge of keratins in the context of the previous system (Table 1.2) [7].

The element of common ground between hair and nail biology is reflected in many shared keratins that lend physical characteristics to the tissue. Hence, although nail biology is not acknowledged in this scheme, where there is a designation of hair keratin, it is common for it also to be a nail keratin and for the higher level of sulfur amino acids in the keratin to afford a larger number of intramolecular cross-links and greater physical stability and strength.

Keratin synthesis can be identified in the nail unit from the earliest stages of its differentiation [8]. In 12- and 13-week embryos, the nail-matrix anlage is a thin epithelial wedge penetrating from the dorsal epidermis into the dermis. This wedge is thought to represent the "ventral matrix primordium." By week 15, hard keratins are seen throughout the nail bed and matrix. This could have significance concerning theories of nail embryogenesis and growth, where debate exists as to the contribution made by the nail bed to nail growth [5,9–12]. However, at 22 weeks, the layer of hard keratin positive cells remains very thin in the nail bed, whereas it is considerably thickened in the matrix. In the adult nail, there have been reports of both the presence [13] and absence [8,14–16] of hard keratins in the nail bed.

Histological observation at 13 and 14 weeks reveals parakeratotic cells just distal to this nail plate primordium staining for disulfydryl groups. This contrasts to adjacent epithelium, suggesting the start of nail plate differentiation. This early differentiation represents matrix formation and Merkel cells have been detected in the matrix primordium of human fetuses between weeks 9 and 15 [17]. Merkel cells may play a role in the development of epidermal appendages and are detectable using monoclonal antibodies specific to keratin 20. Their role in ontogenesis would explain their disappearance from the nail matrix after week 22 [17]. However, this is not a universal finding, with an abundance of Merkel cells identified in the matrix of young adult and cadaver nail specimens in one study [18].

At the 13–22-week stage there is coincident increase in the expression of hard keratins and the development of keratohyalin granules.

By 25 weeks, most features of nail unit differentiation are complete. Changes may still occur in the chemical constitution of the nail plate after this date. A decrease in sulfur and aluminum and a rise in chlorine have been noted as features of full-term newborns in comparison with the nail plate of premature babies [19]. An elevated aluminum level may correspond to bone abnormalities which lead to osteopenia.

Table 1.2 Keratins and their former designations (www.interfil.org/proteinsTypeInII.php).

Category	Number range
Human type I epithelial keratins	9–28
Human type I hair keratins	31–40
Non-human type I epithelial and hair keratins	41–70
Human type II epithelial keratins	1–8 and 71–80
Human type II hair keratins	81–86
Non-human type II epithelial and hair keratins	87–120
Type II keratin pseudogene	121–220
Type I keratin pseudogenes	221 →

Factors in embryogenesis

The nail plate grows from the 15th week of gestation until death. Many factors act upon it in this time and influence its appearance. Because it is a rugged structure, growing over a cycle of 4–18 months, it provides a record of the effects of these influences. To consider the different formative mechanisms, it is important to distinguish between:

- embryogenesis
- regrowth
- growth.

There is overlap between all these processes, with the main clues concerning embryogenesis deriving from fetal studies and analysis of congenital abnormalities. Regrowth is the growth of the nail plate following its removal. This may be for therapeutic reasons or following accidental trauma with associated damage. Observation of this process adds to our understanding of both growth and embryogenesis. Growth is the continuous process of nail plate generation over a fully differentiated nail bed and hyponychium. Embryogenesis is the subject of this section.

In the chick limb bud formation, there is a complex interaction between mesoderm and ectoderm. Initially, the mesoderm induces the development of the apical ectodermal ridge (AER). The mesoderm then becomes dependent upon the AER for the creation of the limb. Removal of the AER results in a halt of mesodermal differentiation. Replacing the underlying mesoderm with mesoderm from another part of the limb primordium still results in normal differentiation [20]. However, the AER continues to be dependent upon the mesoderm, which must be of limb type. Replacement of limb mesoderm with somite mesoderm causes flattening of the AER. These morphogenetic interactions occur prior to cytodifferentiation [21]. In the human, cases of anonychia secondary to phenytoin [22] might implicate the drug at this stage, prior to 8 weeks. Drugs have been suggested as contributing to congenital nail dystrophies mainly affecting the index finger [23].

Subsequent work on limb bud biology has explored the significance of the transcription factor LIM1B in the mouse embryo limb formation. This factor is implicated in the dorsal/ventral polarity of the evolving limb and has been confirmed to have a similar role in humans. Loss of effective LIM1X function results in duplication of structures such that there might be a ventral ventral digit rather than dorsal ventral where the finger pulp is repeated on both sides of the digit [24]. The LIM1X system also acts on genes determining development of the eye and urogenital tract, which is the basis for involvement of all these systems in nail patella syndrome. In this pathology, the differentiation messages from the mesenchyme to the ectoderm appear to be communicated in a manner that might formally be described in observational limb bud experiments.

LMX1B is thought to be mediated through the spondin pathway where spondins are a family of proteins contributing to intracellular communication. In hereditary anonychia, it has been demonstrated that there is a defect in R-spondin 4 secretion where this protein would normally determine the activity of the Wnt/β catenin signaling system that is thought in turn to play a part in the initiation of nail unit formation [25, 26]. R-spondin 2 is expressed in the AER in normal mouse limb development [27]. Mice bred to be deficient in this spondin have substantial congenital limb anomalies, with lack of phalangeal development and no nail unit [27]. Consistent with the model of mesenchyme inducing the overlying ectoderm, spondins have been identified in fibroblast cultures but not keratinocytre cultures [28].

Multiple other biological pathways appear relevant to the formation of a normal nail unit. Transgenic mice with changes to the *Akt* gene demonstrate absent nail and distal bone. Akt is a serine/threonine protein kinase implicated in cell signaling [29]. Although the spondins reside in the mesenchyme and appear relevant to the interaction between mesenchyme and ectoderm, Akt is epithelial and is thought to play a part in the action of bone morphogenetic protein (BMP). BMP is part of the transforming growth factor (TGF)-β family of mediators. It is found in many different forms with a range of morphogenetic roles. In relation to the formation of the nail unit, it has been proposed that there is a two-way process whereby it is supportive of nail unit development, but equally that the nail unit plays a part in the regeneration of the distal phalanx when it is lost through trauma in infancy [30] and these processes may in part be mediated through BMP4.

Congenital abnormalities provide clinical examples of instances where the role of a BMP or similar factor appears central. Congenital onychodysplasia of the index fingers (COIF) is frequently associated with abnormalities of the terminal phalanges and interphalangeal joints [31]. The nail may be absent, small or composed of several small nails on the dorsal tip of the affected finger. The bony abnormality varies, with the most marked change being bifurcation of the terminal phalanx on lateral x-ray [32]. However, a bony abnormality is not mandatory in this condition or other conditions with ectopic nail [33]. A normal nail may overlie an abnormal bone on other than the index finger [34]. COIF appears to demonstrate an association between abnormalities of bone and nail, rather than the presence of a strict relationship. It may represent a fault of mesoderm/ectoderm interaction at the stage when these layers are mutually dependent. It has been suggested that a vascular abnormality may provide the common factor between pathology in the two embryonic layers [35]. This would also be consistent with the part played by BMP in vascular development in embryogenesis [36]. If this is the case, it appears likely that any vascular abnormality arises due to a defect of patterned embryogenesis rather than a random event, given that a form of COIF can occur in the big toe of individuals with involved fingers [37].

An interpretation based upon a mutual mesodermal and ectodermal fault would fit with the observation of two cases of congenital anonychia and hypoplastic nails combined with hypoplastic phalanges [38]. These cases were used as a foil for the suggestion of a mechanism of "bone-dependent nail formation." It might also be argued in reverse that the bone was dependent upon the nail.

Regional anatomy

Histological preparation

High-quality sections of the nail unit are difficult to obtain. Nails are very hard and tend to split or tear. In biopsies containing nail plate and soft subungual and

periungual tissue, the nail plate is often torn from the matrix and other adjacent structures by the microtome. Laboratories unused to nail histology will often have problems, contact the clinician for advice, be slow to provide a result and have sections of mixed quality. This is in large part due to the hardness of nail, which does not soften adequately with the normal decalcification processes used in bone histology, the other hard material laboratories are used to handling. Problems can be diminished using a range of techniques to soften the nail which may be less practical if there are soft tissue attachments requiring histological examination.

When obtaining a specimen for histology, it is useful to ensure that it is oriented. In samples with indicative structures such as a nail fold or the digit pulp, this may be relatively easy, although it can be valuable to ink the edge of the biopsy most closely related to the pathology. This is particularly true for the lateral longitudinal biopsy where typically the edge abutting the lateral nail fold will hold less information than the opposite inner edge. For punch biopsies or other small samples, it may be helpful to ink the upper surface so that sections are cut perpendicular to this edge and clear histological assessment is possible [39].

Nail softening techniques

Nail alone

There are several different techniques to soften the nail plate. Lewis [5] recommended routine fixation in 10% formalin and processing as usual. Earlier methods employed fixation with potassium bichromate, sodium sulfate or sodium bisulfite and water. The section is then decalcified with nitric acid and embedded in collodion. Alkiewicz and Pfister [40] recommended softening the nail with thioglycollate or hydrogen peroxide. Nail fragments are kept in 10% potassium thioglycollate at 37°C for 5 days or in 20–30% hydrogen peroxide for 5–6 days. The nail is then fixed by boiling in formalin for 1 min before cutting 10–15 mm sections.

Although softening of nail clippings for histology is not mandatory, it is possible and may be helpful. Suarez *et al*. [41] suggest soaking the clipping for 2 days in a mix of mercuric chloride, chromic acid, nitric acid and 95% alcohol. The specimen is then transferred to absolute alcohol, xylene and successive paraffin mixtures, sectioned at 4 mm and placed on gelatinized slides. An alternative method, described for preserving histological detail in the nail plate, entails fixation in a mix of 5% trichloracetic acid and 10% formalin for the initial 24 h [42]. This is followed by a modified polyethylene glycol-pyroxylin embedding method. Ultra-thin sections can be provided by embedding the nail in plastic such as 2-hydroxyethyl methacrylate [43].

Nail and soft tissue

In nail biopsies containing soft tissue, more gentle methods of preparation are necessary. The specimen can be soaked in distilled water for a few hours before placing in formalin [44]. Twelve hours in 10% formalin followed by 3 days in 3% phenol prior to embedding is reported to achieve good results [45]. After routine fixation and embedding, permanent wave solution (of the type used in hairdressing), thioglycollate or 10% potassium hydroxide solution can be applied with a cotton swab to the surface of the paraffin block every two or three sections. Lewin *et al.* [46] suggest applying 1% aqueous polysorbate 40 to the cut surface of the block for 1 h at 4°C.

Sections will sometimes adhere to normal slides but when there is nail alone, the material tends to curl as it dries and may fall off. This means that it may be necessary to use gelatinized or 3-aminopropyltriethoxysilane (APES) slides. Given the difficulty of obtaining high-quality sections, it is worth cutting many at different levels to maximize the chance of getting what is needed.

Routine staining with hematoxylin and eosin is sufficient for most cases. Periodic acid-Schiff (PAS) and Grocott's silver stain can be used to demonstrate fungi; a blancophore fluorochromation selectively delineates fungal walls [47]. More recently, Gomori methanamine silver stain has been advocated following pretreatment with chromic acid and sodium bisulfite [48]. Some of the more representative material in a nail sample for histology for fungus may be in the crumbling substance on the ventral aspect. This can be examined separately but requires a container such as a paper lens container to prevent dispersal of the material and to avoid problems with preparing sections [43]. Toluidine blue at pH 5 allows better visualization of the details of the nail plate [49, 50]. Fontana's argentaffin reaction demonstrates melanin. Hemoglobin is identified using a peroxidase reaction. Prussian blue and Perl stains are not helpful in the identification of blood in the nail as they are specific to the hemosiderin product of hemoglobin breakdown caused by macrophages, which does not occur in the nail [40, 51, 52].

Masson-Goldner's trichrome stain is very useful to study the keratinization process and Giemsa stain reveals slight changes in the nail keratin.

Standard techniques for microwave antigen retrieval for immunohistochemistry, routine polymerase chain reaction studies and TUNEL assays all appear feasible in combined soft tissue and nail specimens [425].

Polarization microscopy shows the regular arrangement of keratin filaments and birefringence is said to be absent in disorders of nail formation such as leuconychia.

Nail matrix and lunula

For simplicity, the nail matrix (syn. intermediate matrix) will be defined as the most proximal region of the nail bed extending to the lunula. This is commonly considered to be the source of the bulk of the nail plate, although further contributions may come from other parts of the nail unit (such as nail growth). Contrast with these other regions helps to characterize the matrix.

The matrix is vulnerable to surgical and accidental trauma; a longitudinal biopsy of greater than 3 mm width is likely to leave a permanent dystrophy [53] (Fig. 1.5). Once matrix damage has occurred, it is difficult to effectively repair it [54–56]. This accounts for the relatively small amount of histological information on normal nail matrix.

It is possible to make distinctions between distal and proximal matrix on functional grounds, given that 81% of cell numbers in the nail plate is provided by the proximal 50% of the nail matrix [57] and surgery to distal matrix is less likely to cause scarring than more proximal surgery. Clinically, the matrix is synonymous with the lunula, or half moon, which can be seen through the nail emerging from beneath the proximal nail fold as a pale convex structure. This is most prominent on the thumb, becoming less prominent in a gradient towards the little finger. It is rarely seen on the toes. The absence of a clinically identifiable lunula may mean that the vascular tone of the nail bed and matrix has obscured it or that the proximal nail fold extends so far along the nail plate that it lies over the entire matrix.

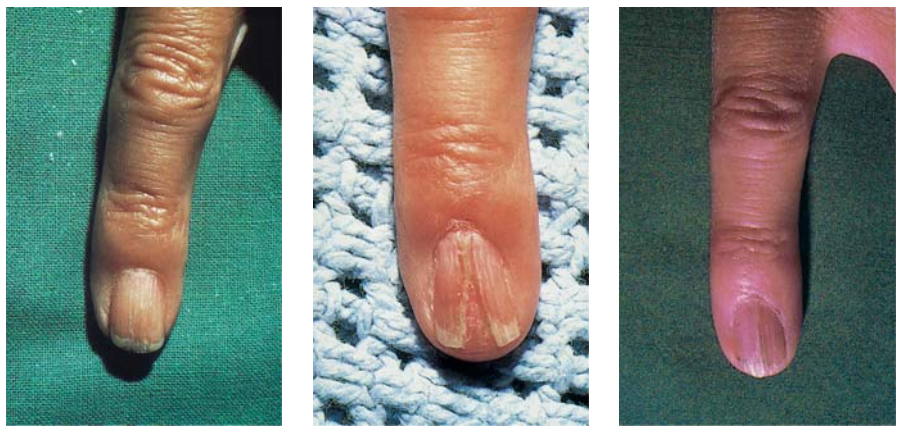
High-resolution magnetic resonance imaging idenitifies the matrix and dermal zones beneath. Drapé *et al*. [58] described a zone beneath the distal matrix where there is loose connective tissue and a dense microvascular network. It may be the presence of this network that accounts for the variable sign of red lunulae in some systemic conditions [59, 60]. However, the histological observations of Lewin suggested that there is diminished vascularity and increased dermal collagen beneath the matrix contributing to the pallor which helps identify the area [61]. This has been confirmed in a more recent study utilizing injection of gelatinized Indian ink into amputation specimens [62]. The close association between the nail matrix and joint apparatus results in magnetic resonance imaging changes in tendon sheath and matrix coincidentally [63] and may demonstrate changes in matrix prior to the onset of any clinical nail disease [64].

The thinner epidermis of the nail bed may account for the contrast between the white and pink appearance of the lunula and bed, respectively [65]. Many suggestions have been made to account for the appearance of the lunula [49, 61, 65–68] (Box 1.1).

Macroscopically, the distal margin of the matrix is convex and is easily distinguished from the contiguous nail bed once the nail is removed, even if the difference is not clear prior to avulsion. The nail bed is a more deep red and has surface corrugations absent from the matrix. At the proximal margin of the matrix, the contour of the lunula is repeated. At the lateral apices, a subtle ligamentous attachment has been described, arising as a dorsal expansion of the lateral ligament of the distal interphalangeal joint [69]. Lack of balance between the symmetrical tension on these attachments may explain some forms of acquired and congenital malalignment [70].

Box 1.1 Possible causes for the pale appearance of the lunula

- The surface of the nail is smoother and more shiny proximally.
- The thicker epidermis of the lunula obscures the underlying vasculature.
- The nail attachment at the lunula is less firm, allowing greater refraction and reflection at the nail/soft tissue interface.
- The underlying dermis has fewer capillaries in it.
- The underlying dermis is of looser texture.
- The matrix epithelium in the lunula has more nuclei than the nail bed, making it appear parakeratotic with an altered color.



(a) (b) (c)

Figure 1.5 Longitudinal nail biopsy of Zaias: (a) before biopsy; (b) 5 weeks after; (c) 3 months later.

Routine histology

The cells of the nail matrix are distinct from the adjacent nail bed distally and the ventral surface of the nail fold, lying at an angle above. The nail matrix is the thickest area of stratified squamous epithelium in the midline of the nail unit, comparable with the hyponychium. There are long rete ridges characteristically descending at a slightly oblique angle, their tips pointing distally. Laterally, the matrix rete ridges are less marked, whereas those of the nail bed nail folds become prominent.

Unlike the overlying nail fold, but like the nail bed, the matrix has no granular layer (Fig. 1.6). The demarcation between overlying nail fold and matrix is enhanced by the altered morphology of the rete ridges. At their junction at the apex of the matrix and origin of the nail, the first matrix epithelial ridge may have a bobbed appearance like a lopped sheep's tail. PAS staining is marked at both the distal and proximal margins of the intermediate matrix (Fig. 1.7). Distally, there is often a step reduction in the epithelial thickness at the transition of the matrix with the nail bed. This represents the edge of the lunula.

Nail is formed from the matrix as cells become larger and paler and eventually the nucleus disintegrates. There is progression with flattening, elongation and further pallor. Occasionally, retained shrunken or fragmented nuclei persist to be included into the nail plate. Lewis [5] called these "pertinax bodies." They can give an impression of the longitudinal progression of growth in the nail plate (Fig. 1.8).

Melanocytes are present in the matrix where they reach a density of up to 300/mm^2 [71–75]. This can also be expressed as number of melanoncytes per linear millimeter of matrix epidermis examined. Figures for this are a mean of 7.5, median of 7.7 and range of 4–9 [76] (Table 1.3).

Dendritic cells are found in the epibasal layers and most prominent in the distal matrix [73–75]. This point can

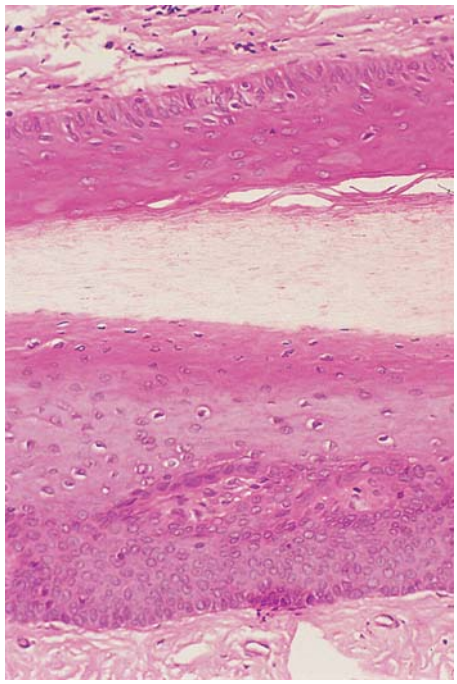


Figure 1.6 A granular layer is absent from the germinal matrix (*lower part*) and the ventral aspect of the proximal nail fold (*upper part*).

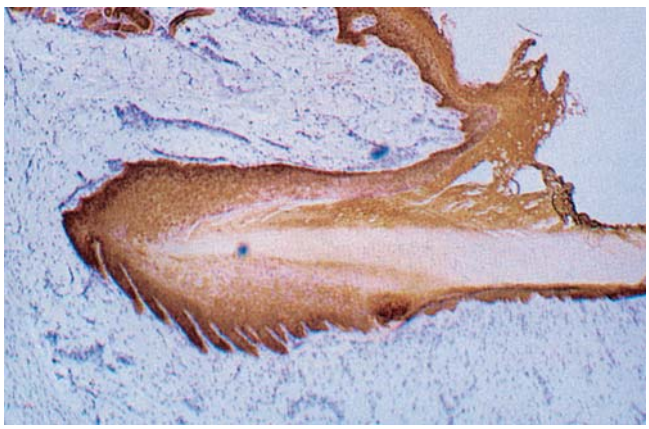


Figure 1.7 Keratin stain of the nail apparatus delineating the epithelial structures of the matrix and proximal nail fold.

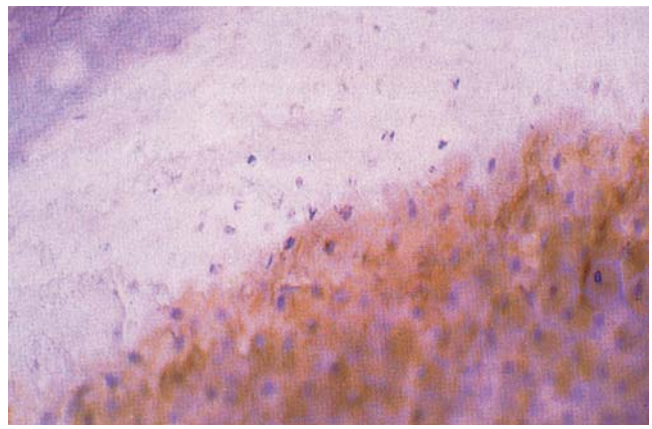


Figure 1.8 Pertinax bodies can be seen as the nuclear remnants within the nail plate.

Table 1.3 Number of melanocytes found per mm of matrix in normal and pathological states.

Pathology	Mean	Median	Range
Invasive melanoma	102	92.5	52–212
In situ melanoma	58.9	51	39–136
Melanotic macule	15.3	14	5–31
Normal control	7.7	7.5	4–9

Reproduced from Amin [76] with permission from Lippincott, Williams and Wilkins.

be refined in terms of the functional status of the melanocytes. Ortonne described melanocytes of the proximal matrix as being in a single compartment of largely dormant cells. Those in the distal matrix are in two compartments, with both a dormant and functionally differentiated population. Longitudinal melanonychia most commonly arises from pigment contributed to the nail plate by these differentiated distal melanocytes. Ortonne also defined a smaller population of nail bed melanocytes, with approximately 25% of the number found in the matrix and none of these were differentiated in terms of DOPA staining. This differs from the observations of de Berker *et al*. [74] where the nail bed was noted to lack melanocyte markers.

The suprabasal location of nail matrix melanocytes can lead to difficulties in the interpretation of histological specimens obtained to exclude dysplasia in instances of melanonychia, given that ascending melanocytes are a sign of dysplasia in normal epidermis. This complication may be related to the fact that the differentiation of melanocytes in the matrix is different from that found elsewhere, given that they typically do not produce pigment in Caucasians and they are detected by the antibody HMB-45, which recognizes melanoma cells and fetal melanocytes but not mature melanocytes [73]. Both HMB-45 and Melan-A are useful markers of nail matrix melanocytes. They are best supplemented with S100 as a means of increasing sensitivity to dermal melanoncytes and, in particular, desmoplastic melanoma [77]. In spite of these difficulties in interpretation, melanoma is a relatively rare cause of subungual pigmentation, although it is usually considered necessary to exclude it histologically, particularly in white adults [73, 78].

Melanin in the nail plate is composed of granules derived from matrix melanocytes [9]. Longitudinal melanonychia may be a benign phenomenon, particularly in Afro-Caribbeans: 77% of black people will have a melanonychia by the age of 20 and almost 100% by 50 [79, 80]. The Japanese also have a high prevalence of longitudinal melanonychia, being present in 10–20% of adults [81]. In a study of 15 benign melanonychia cases in Japanese patients, they were found to arise from an increase in activity and number of DOPA-positive melanocytes in the matrix, not a melanocytic nevus [71]. A survey of fingers and toes of 2457 Chinese patients found none with melanonychia beneath the age of 20, 0.6% of those between 20 and 29, increasing to 1.7% in those over 50 [82]. A French study of Caucasians found a 1.4% prevalence in the community and 12.6% prevalence in hospitalized patients [83]. The difference may have in part reflected different clinical sensitivity amongst community and hospital clinicians. In all studies, where mentioned, the thumb and big toe are the most commonly affected digit. Longitudinal melanonychia in Caucasians is more sinister; Oropeza [84] stated that a subungual pigmented lesion in this group has a higher chance of being malignant than benign.

There is only a thin layer of dermis dividing the matrix from the terminal phalanx. This has a rich vascular supply (see "Vascular supply" below) and an elastin and collagen infrastructure giving attachment to periosteum.

Electron microscopy

Transmission electron microscopy confirms that in many respects, matrix epithelium is similar to normal cutaneous epithelium [85–88]. The basal cells contain desmosomes and hemidesmosomes and interdigitate freely. Differentiating cells are rich in ribosomes and polysomes and contain more RNA than equivalent cutaneous epidermal cells. As cell differentiation proceeds towards the nail plate, there is an accumulation of cytoplasmic microfibrils (7.5–10 nm). These fibrils are haphazardly arranged within the cells up to the transitional zone. Beyond this, they become aligned with the axis of nail plate growth.

Membrane-coating granules (Odland bodies) are formed within the differentiating cells. They are discharged onto the cell surface in the transitional zone and have been thought to contribute to the thickness of the plasma membrane. They may also have a role in the firm adherence of the squamous cells within the nail plate, which is a notable characteristic [89]. The glycoprotein characteristics of cell membrane complexes isolated from nail plate may reflect the constituents of these granules [90].

Mitochondria are degraded during the transitional phase, whilst RNA-containing ribosomes are evident up to the stage of plasma membrane thickening. Vacuoles containing lipid and other products of cytolysis are seen at the transitional stage. Dorsal matrix cells start to show nuclear shrinkage at this point, whereas the nuclei in the matrix remain intact to a higher level.

Electron microscopy has been used to examine the nail plate in detail in fungal disease [91], alopecia areata [92], connective tissue diseases [93] and psoriasis [94].

Nail bed and hyponychium

The nail bed extends from the distal margin of the lunula to the hyponychium. It is also called the ventral matrix, depending on whether or not you believe that it contributes to the substance of the nail plate (see "Nail growth" below). Avulsion of the nail plate reveals a pattern of longitudinal epidermal ridges stretching to the lunula (Fig. 1.9). On the underside of the nail plate is a complementary set of ridges, which has led to the description of the nail being led up the nail bed as if on rails (Fig. 1.10). The small vessels of the nail bed are orientated in the same axis. This can be demonstrated by using corrosion casting from cadaver digits [95] and is

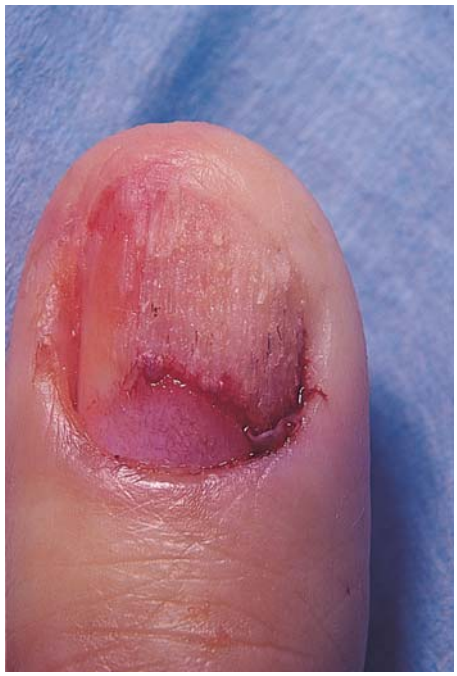


Figure 1.9 The epidermis of the nail bed has longitudinal ridges visible after nail avulsion.

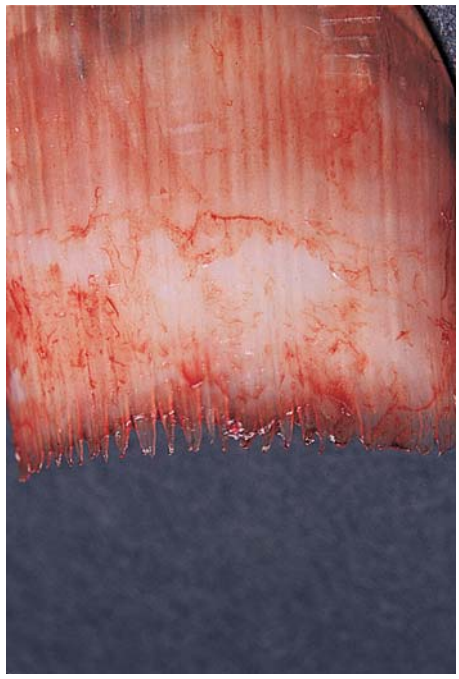


Figure 1.10 The undersurface of the nail plate shows longitudinal ridging which matches that seen on the nail bed. This pattern is lost at the margin of the lunula, where the nail is in continuity with the matrix from which it arises.

clinically manifested by splinter hemorrhages (Figs 1.11, 1.12), where heme is deposited on the undersurface of the nail plate and grows out with it. The free edge of a nail loses the ridges, suggesting that they are softer than

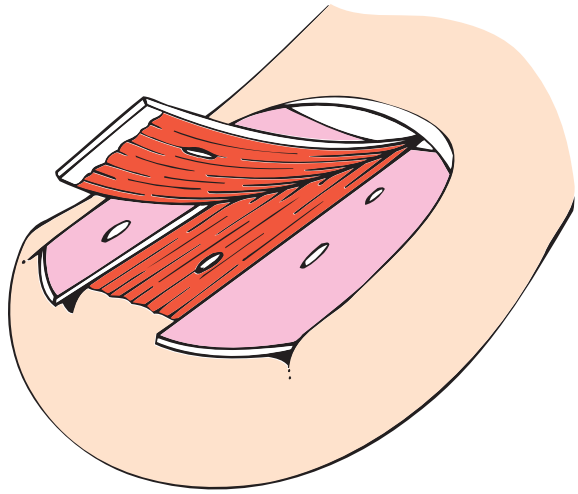


Figure 1.11 The appearance of splinter hemorrhages. Heme from longitudinal nail bed vessels is deposited on the underside of the nail plate. This grows out in the shape of a splinter.

Figure 1.12 The undersurface of the nail has dark-stained blood in the longitudinal grooves corresponding to splinter hemorrhages.

the main nail plate structure. The nail bed also loses these ridges shortly after loss of the overlying nail. It is likely that the ridges are generated at the margin of the lunula on the ventral surface of the nail to be imprinted upon the nail bed.

The epidermis of the nail bed is thin over the bulk of its territory. It becomes thicker at the nail folds where it develops rete ridges. It has no granular layer except in disease states. The dermis is sparse, with little fat, firm collagenous adherence to the underlying periosteum and no sebaceous or follicular appendages [61]. Sweat ducts can be seen at the distal margin of the nail bed using *in vivo* magnification (Fig. 1.13) [96].

The hyponychium lies between the distal ridge and the nail plate and represents a space as much as a surface. Perrin [97] has described an analog of the hair

Figure 1.13 Sweat pores in the distal nail bed. Reproduced from Maricq [96] with permission from Wiley-Blackwell.

follicle isthmus at the junction of the hyponychium and nail bed, referred to as the nail isthmus, leading on to the nail infundibulum, which he proposed would replace the term hyponychium. The distal ridge (see "Factors in embryogenesis" above) is seen from the 10th week of gestation onwards. The hyponychium and onychocorneal band may be the focus or origin of subungual hyperkeratosis in some diseases such as pityriasis rubra pilaris (see Table 1.8) or pachyonychia congenita. In these instances, and in some elderly people, it can be thought of as the solenhorn described by Pinkus [98].

Pterygium inversum unguis is a further condition characterized by changes in the distal nail bed and hyponychium [99]. There is tough, fibrotic tissue tethering the free edge of the nail plate to the underlying soft structures. It is found in both congenital [100] and acquired forms [101]. The etiology is not clear. Patterson proposed that it was a combination of a genetic predisposition and microvascular ischemia.

The hyponychium and overhanging free nail provide a crevice which is a reservoir for microbes, relevant in surgery and the dissemination of infection. After 10 min of scrubbing the fingers with povidone-iodine, nail clippings were cultured for bacteria, yeasts and molds [102]. In 19 out of 20 patients, *Staphylococcus epidermidis* was isolated, seven patients had an additional bacteria, eight had molds and three had yeasts. These findings could have significance for both surgeons and patients. However, in a randomized trial of chlorhexidine scrub used with or without a nail brush, the nail brush did statistically diminish the number of colony-forming units obtained from the scrubbed hand [103].

The hand-to-mouth transfer of bacteria is suggested by the high incidence of *Helicobacter pylori* beneath the nails of those who are seropositive for antibodies and have oral carriage. Dowsett *et al*. [104] found that 58% of those with tongue *H. pylori* had it beneath the index fingernail, representing a significant (P = 0.002) association.

Nail folds

The proximal and lateral nail folds give purchase to the nail plate by enclosing more than 75% of its periphery. They also provide a physical seal against the penetration of materials to vulnerable subungual and proximal regions.

The epidermal structure of the lateral nail folds is unremarkable, and comparable with normal skin. There is a tendency to hyperkeratosis, sometimes associated with trauma. When the trauma arises from the ingrowth of the nail, considerable soft tissue hypertrophy can result, with repeated infection (such as ingrowing nails).

The proximal nail fold has three parts. Its upper aspect is normal glabrous skin, providing no direct influence upon the nail plate. At the point where its distal margin meets the nail plate, it forms the cuticle (eponychium). In health, the cuticle adheres firmly to the dorsal aspect of the nail plate, achieving a seal. Its disruption may be associated with systemic disorders (collagen vascular) or local dermatoses. In the latter, it may be the avenue for contact allergens or microbes. The ventral aspect of the proximal nail fold is apposed to the dorsal aspect of the nail. It contrasts with the adjacent matrix by being thinner, with shorter rete ridges, and having a granular layer. Keratins expressed in the proximal nail fold may differ on its dorsal and ventral aspects and can contrast with expression elsewhere in the nail unit [15] (see "Nail growth" below).

The proximal nail fold has significance in four main areas.

- It may contribute to the generation of the nail plate through a putative dorsal matrix on its ventral aspect.
- It may influence the direction of growth of the nail plate by directing it obliquely over the nail bed.
- Nail fold microvasculature can provide useful information in some pathological conditions.
- When inflamed, it can influence nail plate morphology as seen in eczema, psoriasis, habit tic deformity and paronychia.

The first two issues are dealt with in the section on nail growth (see "Nail growth" below), the latter under vasculature (see "Vascular supply" below) and "The Nail in Dermatological Disease" (see Chapter 6).

Immunohistochemistry of periungual tissues

Keratins

The most extensive immunohistological investigations of the nail unit have utilized keratin antibodies. The nail plate [14, 105], human embryonic nail unit [8, 14, 106], accessory digit nail unit [107, 108] and adult nail unit [15, 47, 106] have all been examined (Table 1.4).

Table 1.4 Keratins in the nail unit.

Type II keratins	Type I keratins	Nail fold	Nail bed	Matrix
K1	K10	+	–	+
K5	K14	+	+	–
K6a		–	+	+
K6b	K16	–	+	+
	K17	–	–	+
K81 (Hb1)	K31 (Ha1)	–	–	+
K85 (Hb5)	K32 (Ha2)	–	–	+
K86 (Hb6)	K34 (Ha4)	–	–	+
	K38 (Ha8)	–	–	+
Other keratins not found or not tested for in the nail unit				
K6c (K6e/h)	K15			
K2 (K2e)	K9			
K3	K12			
K4	K13			
K7	K18			
K8	K19			
K71 (K6irs1)	K20			
K72 (K6irs2)	K23			
K73 (K6irs3)	K24			
K74 (K6irs4)	K25 (K25irs1)			
K75 (K6hf)	K26 (K25irs2)			
K76 (K2p)	K27 (K25irs3)			
K77 (K1b)	K28 (K25irs4)			
K78 (K5b)	K33a (Ha3-I)			
K79 (K6l)	K33b (Ha3-II)			
K80 (Kb20)	K35 (Ha5)			
K82 (Hb2)	K36 (Ha6)			
K83 (Hb3)	K37 (Ha7)			
K84 (Hb4)	K39 (Ka35)			
	K40 (Ka36)			

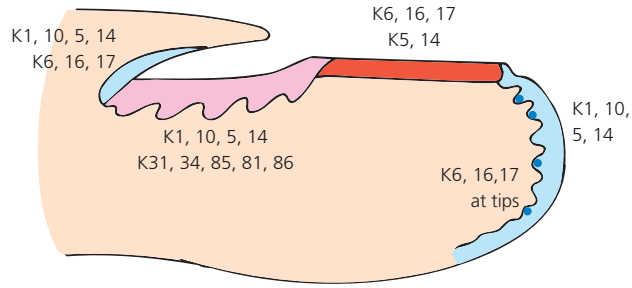


Figure 1.14 Distribution of keratins in the human periungual and subungual tissues.

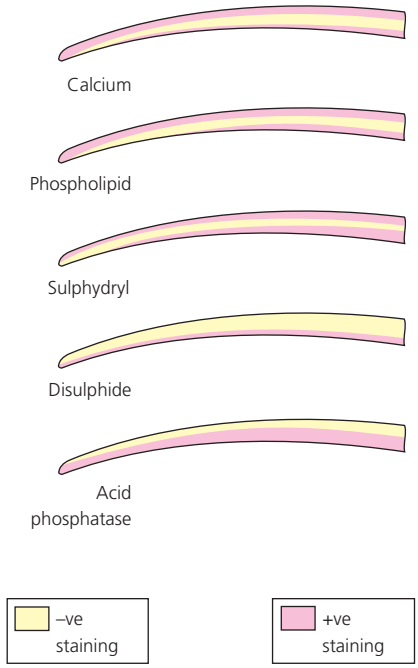


Figure 1.15 The histochemistry of the human nail plate. Nail plates were sectioned and stained. Index, calcium; middle, phospholipid; ring, sulfydryl; little, disulfide; thumb, acid phosphatase. Reproduced from Jarrett and Spearman [138] with permission from the American Medical Association.

Using monospecific antibodies, de Berker *et al*. [15, 107] detected keratins 1 and 10 in a suprabasal location in the matrix and noted their absence from the nail bed (Fig. 1.14) (see "Nail growth" and "Nail plate" below). Keratins 1 and 10 are "soft" epithelial keratins found suprabasally in normal skin [109] and characteristic of cornification with terminal keratinocyte differentiation. Their absence from normal nail bed is reversed in disease where nail bed cornification is often seen, alongside development of a granular layer and expression of keratins 1 and 10 [110]. The development of a granular layer in subungual tissues can be interpreted as a pathological sign in nail histology, seen in a range of diseases and probably associated with changes in keratin expression [111].

Ha-1 (K31), a "hard" keratin, is found in the matrix. Keratin 7 has been found at other sites in the nail unit and hair follicle, whereas Ha-1, detected by the monoclonal antikeratin antibody LH TRIC 1, is limited to the matrix of the nail (Fig. 1.15) and the germinal matrix of the hair follicle [16, 107]. Other hard hair/nail keratins have been highlighted as limited to the matrix where K85 (hHb5), K34 (hHa4), K81 (hHb1) and K86 (hHb6) have all been found

within the conventional boundaries of the matrix. Keratin 19 is probably not found in the adult matrix [8, 15, 47]. However, Moll *et al*. [8] did detect keratin 19 at this site in 15-week embryo nail units. Keratin 19 is also found in the outer root sheath of the hair follicle and lingual papilla [14].

The colocalization of hard and soft keratins within single cells of the matrix has been observed by several workers in bovine hoof [112] and human nail [15, 113, 114], suggesting that these cells are contributing both forms of keratin to the nail plate. This dual differentiation continues into *in vitro* culture of bovine hoof matrix cells [113]. Culture of human nail matrix confirms the persistence of hard keratin expression [115, 116].

Markers for keratins 8 and 20 are thought to be specific to Merkel cells in the epidermis. Positive immunostaining for these keratins has been noted by Lacour *et al*. [106] in adult nail matrix and de Berker *et al*. [15] in infant accessory digits. Some workers have failed to detect Merkel cells and while it seems likely that they are present in fetal and young adult matrix, it may be that the cells are less common or absent as people age [117].

The nail bed appears to have a distinct identity with respect to keratin expression. Keratins 6, 16 and, to a lesser degree, 17 are all found in the nail bed and are largely absent from the matrix [15]. This finding has gained clinical significance with the characterization of the underlying fault in some variants of pachyonychia congenita where abnormalities of nail bed keratin lead to a grossly thickened nail plate. Mutations in the gene for keratin 17 have been reported in a large Scottish kindred with the PC-2, or Jackson-Lawlor, phenotype [118, 119]. There is a cross-over with steatocystoma multiplex where the same mutation of keratin 17 may cause this phenotype which appears to be independent of the specific keratin 17 mutation [120–122]. Mutations in the gene coding for K6b produce a phenotype seen with K17 gene mutations [123]. Mutations in the K6a [124] and K16 [119] genes have been reported in PC-1, originally described as the Jadassohn–Lewandsky variant of pachyonychia congenita.

Expression of keratins 6, 16 and 17 extend beyond the nail bed onto the digit pulp and are thought to match the physical characteristics of this skin which is adapted to high degrees of physical stress [125]. In particular, expression of keratin 17 is found at the base of epidermal ridges, which might also support the idea that this keratin is associated with stem cell function.

It is important to recognize that the hard keratins responsible for the characteristics of nail tissue are the product of an interaction between underlying mesenchyme fibroblasts and the overlying epithelium. Hard nail keratins can be induced both *in vivo* and *in vitro* using nail matrix mesenchyme and non-nail epithelium [126, 127]. Induced expression of hard keratin is not the same as producing a nail, as the product of these experiments can be a poorly organized structure only recognizable as nail in immunohistochemical terms [128]. The specific nature of the nail mesenchyme may correspond to the presence of nail mesenchyme versican, where versican is a chondroitin sulfate proteoglycan and a member of the lecticans family [129].

Non-keratin immunohistochemistry

Haneke [47] has provided a review of other important immunohistochemically detectable antigens. Involucrin is a protein necessary for the formation of the cellular envelope in keratinizing epithelia. It is strongly positive in the upper two-thirds of the matrix and elsewhere in the nail unit [130] and weakly detected in the suprabasal layers. Pancornulin and sciellin are also detected in the matrix [130]. The antibody HHF35 is considered specific to actin. It has been found to show a strong membranous staining and weak cytoplasmic staining of matrix cells [47].

In the dermis, vimentin was strongly positive in fibroblasts and vascular endothelial cells. Vimentin and desmin were expressed in the smooth muscle wall of some vessels. S100 stain, for cells of neural crest origin, revealed perivascular nerves, glomus bodies and Meissner's corpuscles distally.

Filaggrin could not be demonstrated in the matrix in Haneke's work or by electron microscopy [14]. However, Manabe and O'Guin [131] have detected the coexistence of trichohyalin and filaggrin in monkey nail, located in the area they term the "dorsal matrix" which is likely to correspond to the most proximal aspect of the human nail matrix as it merges with the undersurface of the proximal nail fold. Kitahara and Ogawa [114] have identified filaggrin in the human nail in the same location and O'Keefe *et al.* [132] have found trichohyalin in the "ventral matrix" of human nail, which is synonymous with the nail bed. Manabe noted that these two proteins coexist with keratins 6 and 16, which are more characteristic of nail bed than matrix. It is argued that filaggrin and trichohyalin may stabilize the intermediate filament network of K6 and K16, which are normally associated with unstable or hyperproliferative states. Where pathological mutations of the filaggrin gene and those for keratin 16 coexist, the phenotype may be more severe than in the parent with the original isolated keratin gene mutation [133].

The plasminogen activator inhibitor (PAI) type 2 has been detected in the nail bed and matrix where it has been argued that it may have a role in protecting against programmed cell death [134]. The basement membrane zone of the entire nail unit has been examined, employing a wide range of monoclonal and polyclonal antibodies [108]. Collagen VII, fibronectin, chondroitin sulfate and tenascin were among the antigens detected.

Table 1.5 Analysis of nail unit basement membrane zone using monoclonal and polyclonal antibodies.

	Digit 1				Digit 2					Digit 3	
	Nail apparatus				Nail apparatus						
	Fold	Matrix	Bed	HN	Proximal phalangeal skin	Fold	Matrix	Bed	HN	Split skin	Intact skin
Monoclonal antibody											
LH7:2	+	+	+	+	+	+	+	+	+	Epi	+
L3d	+	+	+	+	+	+	+	+	+	Epi	+
Co1 IV	+	+	+	+	+	+	+	+	+	Epi	+
GB3	+	+	+	+	+	+	+	+	+	Epi	+
LH24	+	+	+	+	+	+	+	+	+	Epi	+
LH39	+	+	+	+	+	+	+	+	+	Epi	+
GDA	+	+	+	+	+	+	+	+	+	Epi	+
Tenascin	+	+	+	+	+	+	+	+	+	Epi	+
a6	+	+	+	+	+	+	+	+	+	Epi	+
G71	+	+	+	+	+	+	+	+	+	Epi	+
Polyclonal antibody											
Fibronectin	–	–	–	–	–	–	–	–	–	–	–
Laminin	+	+	+	+	+	+	+	+	+	Derm	+
BP 220 kDa	+	+	+	+	+	+	+	+	+	Epi	+
EBA 250 kDa	+	+	+	+	+	+	+	+	+	Derm	+
LAD 285 kDa	+	+	+	+	+	+	+	+	+	Epi	+
LAD ?kDa	+	+	+	+	+	+	+	+	+	Derm	+

Derm, dermis; Epi, epithelium; HN, hyponychium.

All except tenascin were present in a quantity and pattern indistinguishable from normal skin. Tenascin was absent from the nail bed, which was attributed to the fact that the dermal papillae are altered or considered absent (Table 1.5).

Nail plate

The nail plate is composed of compacted keratinized epithelial cells. It covers the nail bed and intermediate matrix and is curved in both the longitudinal and transverse axes. This allows it to be embedded in nail folds at its proximal and lateral margins, which provide strong attachment and make the free edge a useful tool. This feature is more marked in the toes than the fingers. In the great toe, the lateral margins of the matrix and nail extend almost halfway around the terminal phalanx. This provides strength appropriate to the foot (Fig. 1.16). The nail appears as a layered structure when examined histologically with silver stain [5], with ultrasound [135], using optimal coherence tomography [136] or scanning electron microscopy [137]. The different orientation of keratin fibrils within these layers appears to lend characteristics of both toughness and flexibility.

Lewis [5] described a silver stain that delineates the nail plate zones. Three regions of nail plate have been histochemically defined [138] (see Fig. 1.15). The dorsal plate has a relatively high calcium, phospholipid and sulfydryl group content. It has little acid phosphatase activity and is physically hard. The phospholipid content may provide some water resistance. The intermediate nail plate has a high acid phosphatase activity, probably corresponding to the number of retained nuclear remnants. There is a high number of disulfide bonds and low content of bound sulfydryl groups, phospholipid and calcium. Controversy suggests that the ventral nail plate may be a variable entity [139]. Jarrett and Spearman [138] described it as a layer only one or two cells thick. These cells are eosinophilic and move both upwards and forward with nail growth. With respect to calcium, phospholipid and sulfydryl groups, it is the same as the dorsal nail plate. It shares a high acid phosphatase and frequency of disulfide bonds with the intermediate nail plate.

Ultrasound examination of *in vivo* and avulsed nail plate suggests that it has the physical characteristics of a bilamellar structure [140]. There is a superficial dry compartment and a deep humid one. This has been given as evidence against the existence of a ventral matrix contribution to the nail plate. Synchrotron x-ray microdiffraction has been used to identify a trilamellar structure, where the dorsal and ventral fibers run transversely and the central fibers run in the longitudinal axis of the nail plate, occupying 70% of nail plate thickness. This lamination enhances nail resistance to tear and fracture forces in multiple axes [141].

The upper surface of the nail plate is smooth and may have a variable number of longitudinal ridges that change with age. These ridges are sufficiently specific to allow forensic identification and the distinction between identical twins [142]. Lyonization studies suggest that there is a sustained pattern of X-inactivation within the progenitor cells of single longitudinal nail ridges [143]. The ventral surface also has longitudinal ridges that correspond to complementary ridges on the upper aspect of the nail bed (see "Nail bed and hyponychium" above) to which it is bonded (Fig. 1.17). These nail ridges may be best examined using polarized light. They can also be used for forensic identification [144], as may blood groups from fragments of nail plate [145].

The nail plate gains thickness and density as it grows distally [12] according to analysis of surgical specimens. *In vivo* ultrasound suggests that there may be an 8.8%

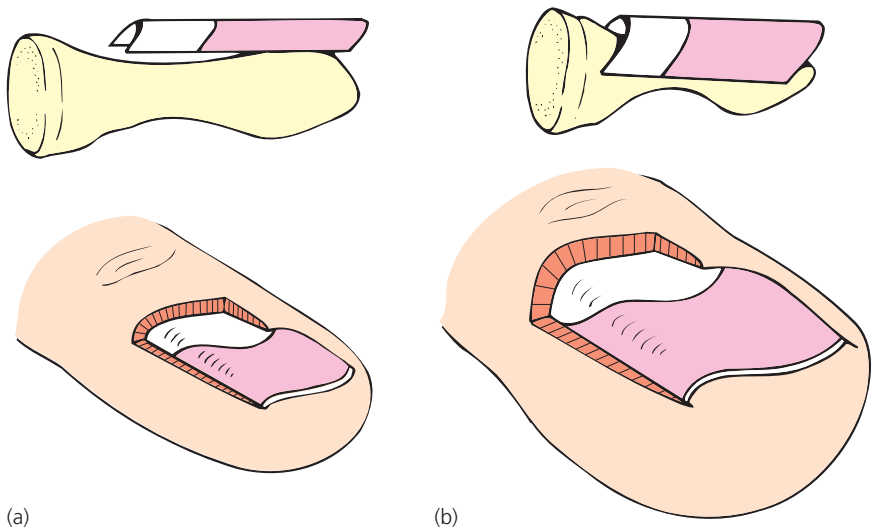


Figure 1.16 Nail plate association with soft tissue and bone in the finger and toe. (a) In the finger, the nail plate has modest transverse curvature and shallow association with soft tissues. (b) In the great toe, the nail plate has more marked transverse curvature and deep soft tissue association. This makes it appropriate to the foot but also accounts for the tendency to ingrow and the need for deep lateral extirpation at lateral matricectomy.

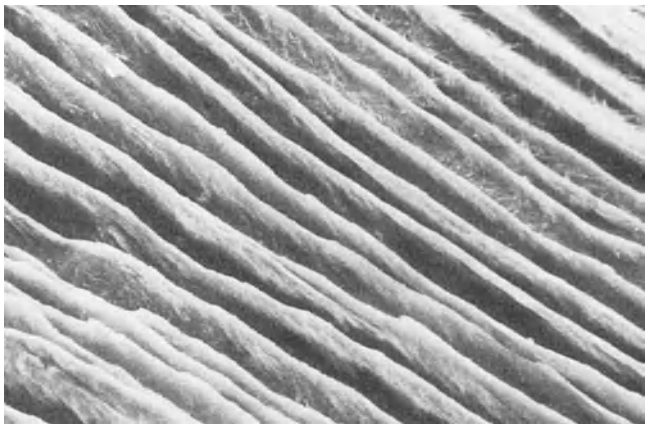


Figure 1.17 Scanning electron micrograph of the nail bed demonstrating longitudinal ridges.

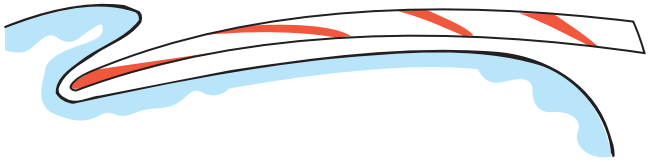


Figure 1.18 Shaded areas represent 7-day periods of nail growth, separated by 1 month with transition of nail from horizontal to oblique axis over 4 months.

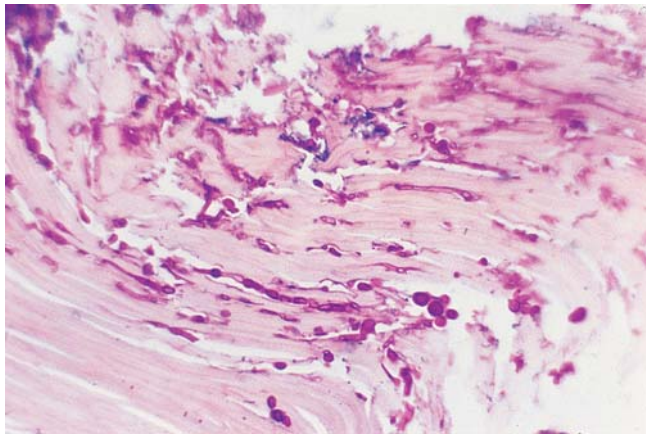


Figure 1.19 Fungal spores and hyphae can be seen in the stained section of a nail clipping taken from a nail with onychomycosis.

reduction in thickness distally [146]. A thick nail plate may imply a long intermediate matrix. This stems from the process whereby the longitudinal axis of the intermediate matrix becomes the vertical axis of the nail plate (Fig. 1.18). Other factors, like linear rate of nail growth [147], vascular supply, subungual hyperkeratosis and drugs, also influence thickness.

In clinical practice, histology of the nail plate may be useful in the identification of fungal infections in culture-negative specimens [41, 47] (Fig. 1.19). It may also be used to identify the dorsoventral location of melanin in the nail clipping of a longitudinal melanonychia and hence allow prediction of the site of melanocyte activity in the intermediate matrix [148, 149]. Sonnex *et al*. [4] describe the histology of transverse white lines in the nail.

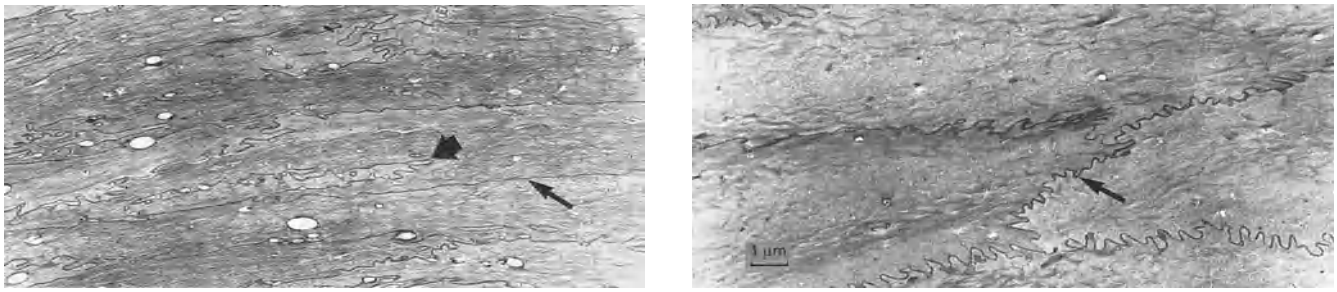
Germann *et al*. [150] utilized a form of tape-stripping in conjunction with light microscopy to examine dorsal nail plate corneocyte morphology in disease and health. They found that conditions of rapid nail growth (psoriasis and infancy) resulted in smaller cell size. Nail keratin protein has been sampled and quantified using a similar tape-stripping method followed by colorimetric quantification [151].

Electron microscopy

Scanning electron microscopy has added to our understanding of onychoschizia [152, 153] as well as basic nail plate structure [154, 155]. In the normal nail, corneocytes can be seen adherent to the dorsal aspect of the nail plate. In cross-section, the compaction of the lamellar structure is visible. Both these features can be seen to be disrupted in onychoschizia following repeated immersion and drying of the nail plates. Scanning electron microscopy has also been used for assessing the location of fungal invasion into the nail plate [156, 157] although the lack of differential staining seen in routine light microscopy may mean that the latter is usually more useful.

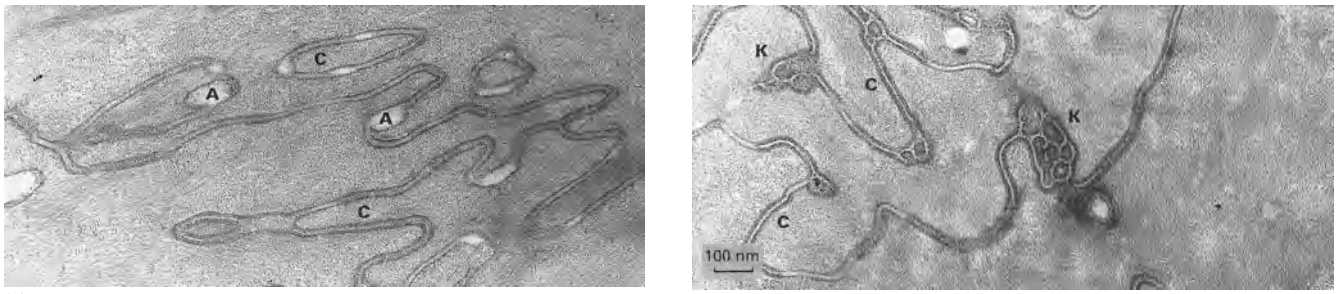
Transmission electron microscopy has been used to identify the relationship between the corneocytes of the nail plate [89]. Using Thierry's tissue-processing techniques, material for the following description has been provided. Cell membranes and intercellular junctions are easily discernible (Fig. 1.20). Even though at low magnification one can differentiate the dorsal and intermediate layers of the nail plate, the exact boundary is unclear using transmission electron microscopy. Cells on the dorsal aspect ($34 \times 60 \times 2.2\,\mu m$) are half as thick as ventral cells ($40 \times 50 \times 5.5\,\mu m$), with a gradation of sizes in between. In the dorsal nail plate, large intercellular spaces are present corresponding to ampullar dilations (Figs 1.21, 1.22). These gradually diminish in the deeper layers and are absent in the ventral region. At this site, cells are joined by complete folds, membranes of adjacent cells appearing to penetrate each other to form "anchoring knots."

Corneocytes of the dorsal nail plate are joined laterally by infrequent deep interdigitations. The plasma membranes between adjacent cell layers are more discretely indented, often with no invaginations (see Fig. 1.20). In the deeper parts of the nail plate, the interdigitations are more numerous but more shallow (see Fig. 1.20). No tight or gap junctions are seen in either of the major nail layers in this series [89] although they were identified previously



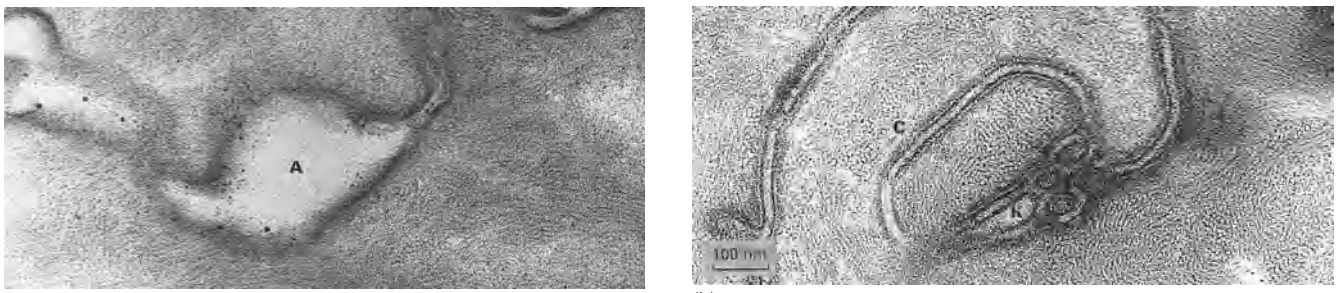
(a) (b)

Figure 1.20 (a) Transmission electron micrograph of the upper part of the nail plate. The corneocytes are flattened and joined laterally by infrequent deep interdigitations (*broad arrow*). (b) The cell membranes between adjacent cell layers are discretely indented and in parts without invaginations: Thiery's technique. Courtesy of G. Achten.



(a) (b)

Figure 1.21 (a) Upper part of the nail plate showing ampullar dilations (A). (b) Lower part of the nail plate showing anchoring knots (K). The only cell-to-cell coupling observed (C) is a desmosome. Courtesy of G. Achten.



(a) (b)

Figure 1.22 (a,b) Upper part of the nail plate as shown in Fig. 1.20, in greater detail. Courtesy of G. Achten.

by Forslind and Thyresson [154]. The intercellular material is homogeneous and separated from the cell membrane by two thin electron-dense lines. The space between the cell membranes varies from 25 nm to 35 nm (see Figs 1.20, 1.22). No complete desmosomal structures are seen.

Nail bed cells show considerable infolding and interdigitation at their junction with the nail plate cells (Fig. 1.23). They are polygonal and show no specific alignment. They are between 6 and 20 µm across and show neither tight nor gap junctions. They do, however, have desmosomal connections of the type seen in normal epidermis (Fig. 1.24).

Cryoelectron microscopy allows examination of fractured intracellular components in great detail without the artefacts normally associated with the chemical processing and coating of traditional electron microscopy (EM) techniques. Using this approach, the trichocyte intermediate filaments have been examined in rat vibrissae. Although these may differ from human nail in some respects, they will share the designation of hard keratins and so allow some transferable observations. The main observation was that the classic keratin fibril structure is the same, but a further arrangement of satellite proteins "decorates" the keratin. These proteins are suspected of being high sulfur amino acid proteins lending the keratin

some of its rugged character [158], possibly through enhancing cross-linking between keratins and increasing their stability [159].

Using different preparation techniques, other workers have demonstrated other anatomical details. On the cytoplasmic side of the cell membranes of nail plate cells lies a layer of protein particles [85, 86, 160]. Other staining techniques suggest that the single type of intercellular bond described by Parent *et al*. [89] may be a spot desmosome [161].

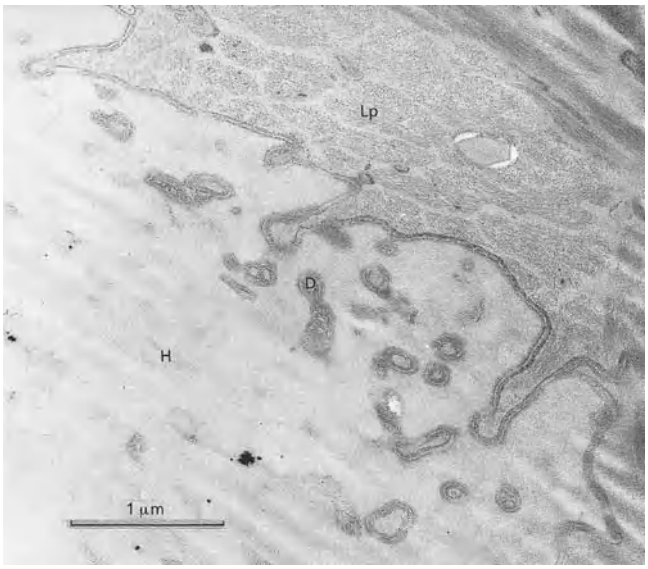


Figure 1.23 Corneocytes of the lowest part of the nail plate (Lp) sending out numerous digitations (D) penetrating the hyponychial nail bed cells (H). Courtesy of G. Achten.

Vascular supply

Arterial supply

The vascular supply of the finger is considered in detail here. Many of the anatomical principles may be extended to the anatomy of the foot and toe, whilst details can be sought elsewhere [6].

The radial and ulnar arteries supply deep and superficial palmar arcades that act as large anastomoses between the two vessels. From these arcades extend branches aligned with the phalanges. Four arteries supply each digit, two on either side. The dorsal digital arteries are small and arise as branches of the radial artery. They undertake anastomoses with the superficial and deep palmar arches and the palmar digital vessels before passing distally into the finger. The palmar digital arteries provide the main blood supply to the fingers. They receive contributions from the deep and superficial palmar arcades. Although paired, one is normally dominant

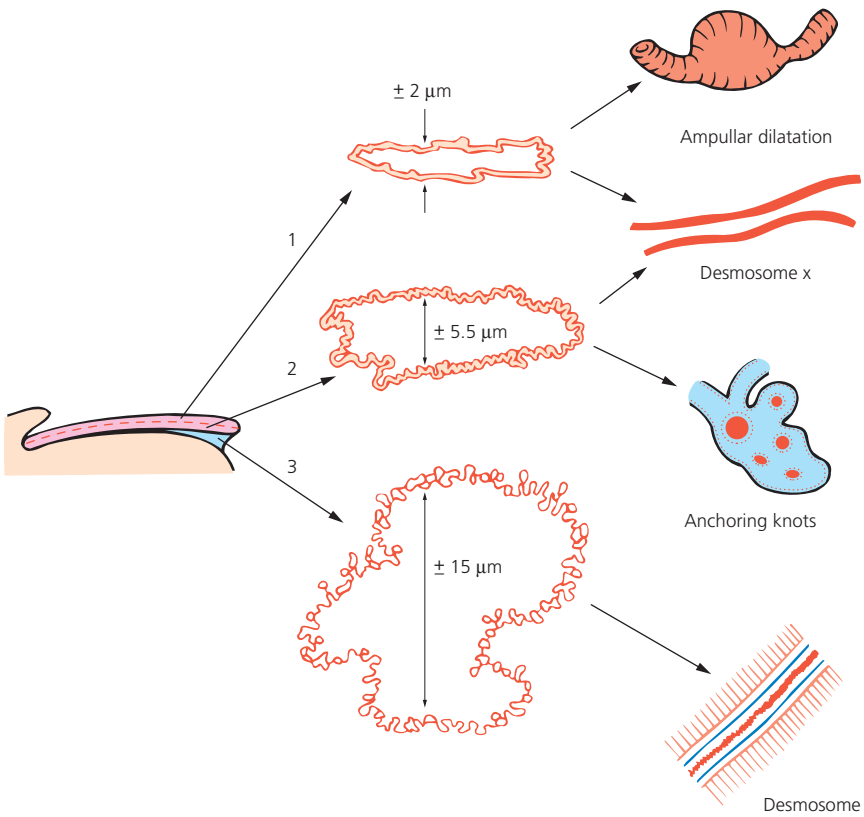


Figure 1.24 Intercellular junctions of the three parts of the nail: 1, upper plate; 2, lower plate; 3, hyponychial ventral nail with desmosome as seen by Thiery's technique. Courtesy of G. Achten.

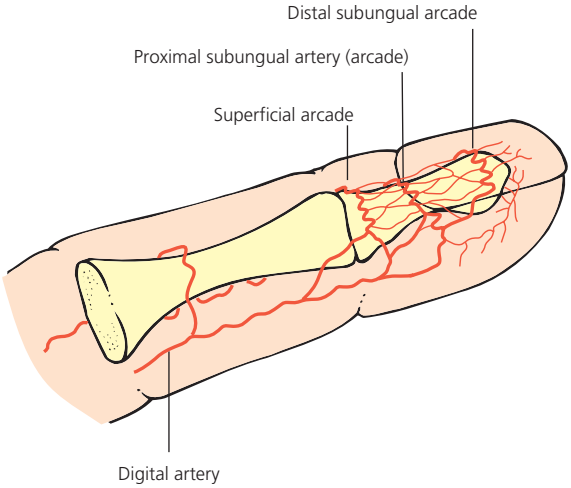


Figure 1.25 Arterial supply of the distal finger.

[162]. They anastomose via dorsal and palmar arches around the distal phalanx. The palmar arch is located in a protected position, beneath the maximal padding of the finger pulp and tucked into a recess behind the protruberant phalangeal boss (Fig. 1.25). This is of functional value as it protects against occlusion of the blood supply when the fingers exert maintained grip.

The dorsal nail fold arch (superficial arcade) lies just distal to the distal interphalangeal joint. It supplies the nail fold and extensor tendon insertion. It is tortuous, with numerous branches to the intermediate nail matrix. Its transverse passage across the finger can be roughly located by pushing proximally on the free edge of the nail plate. This produces a faint crease about 5 mm proximal to the cuticle and is both the cul de sac of the proximal nail fold and the line of the dorsal nail fold arch.

The subungual region is supplied by distal and proximal subungual arcades, arising in turn from an anastomosis of the palmar arch and the dorsal nail fold arch. Helpful studies on adults and fetuses have been performed by Flint [163], Wolfram-Gabel and Sick [62] and Sangiorgi [95]. The latter made use of corrosion casting on cadaver digits to demonstrate the complex microvasculature.

The tortuosity of the main vessels in the finger is a notable feature. Vessels may turn through 270° and resemble a coiled spring [162]. Functionally, this can be interpreted as protection against occlusion by kinking in an articulated longitudinal structure.

Venous drainage

Venous drainage of the finger is by deep and superficial systems. The deep system corresponds to the arterial supply. Superficially, there exist the dorsal and palmar digital veins, which are in a prominent branching network, particularly on the dorsal aspect. However, in the microsurgical techniques needed to restore amputations, it appears that distally, the palmar superficial veins are largest [164].

Although the arterial supply to the nail unit is substantial, the matrix will tolerate only limited trauma before scarring [53]. A longitudinal biopsy of greater than 3 mm is likely to leave a permanent dystrophy. Equally, it appears to need a precise, not just abundant, blood supply. Non-vascularized split-thickness nail bed grafting is moderately successful for the nail bed, but not for intermediate matrix [55]. This is in the presence of otherwise adequate local blood supply at sites of previous trauma. Toenail matrix grafts can be made successfully if they are transplanted with associated soft tissue and a venous pedicle [54]. The local arterial supply is then anastomosed through this pedicle.

Effects of altered vascular supply

Impaired arterial supply can have a considerable effect upon the finger pulp and nail unit. Lynn *et al*. [165] claimed that there was almost complete correlation between occluded arteriographic findings and the presence of paronychial infection or ulceration, ridged brittle fingernails or phlyctenular gangrene. Samman and Strickland [166] reviewed the nail dystrophies of 41 patients with features of peripheral vascular disease. In this uncontrolled study, they observed that onycholysis, Beau's lines, thin brittle nails and yellow discoloration were all attributable to ischemia in the absence of other causes. It has also been suggested that congenital onychodysplasias may result from digital ischemia *in utero* [35]. Immobilization might be associated with diminished local blood supply and has been noted to reduce nail growth [167]. Conversely, the increased growth associated with arteriovenous shunts may reflect the role of greater blood flow [168]. Clubbing constitutes a change in both the nail and nail bed. It is believed that it arises secondary to neurovascular pathology. Postmortem studies suggest that it is due to increased blood flow with vasodilation rather than vessel hyperplasia [169].

Nail fold vessels

The nail fold capillary network [170] is seen easily with a ×4 magnifying lens, dermatoscope [171] or ophthalmoscope. The latter should be set at +40 and the lens held very close to a drop of oil on the nail fold. The network is similar to the normal cutaneous plexus in health, except that the capillary loops are more horizontal and visible throughout their length. The loops are in tiers of uniform size, with peaks equidistant from the base of the cuticle (Fig. 1.26) [172]. The venous arm is more dilated and tortuous than the arterial arm. There is a wide range of morphologies within the normal population [173]. Features in some disorders may be sufficiently gross to be useful without magnification, erythema and hemorrhages being the most obvious.

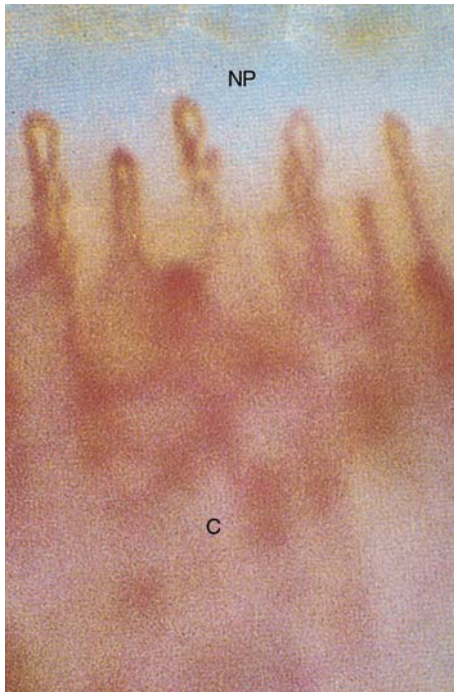


Figure 1.26 Capillary loops visible in the proximal nail fold. NP, nail plate; C, cuticle.

In the first 10 years of life, the pattern of nail fold vessels is immature [174]. Microscopy of small vessels in adulthood can be of diagnostic value in some connective tissue diseases [175, 176]. Pathological features include venous plexus visibility, density of capillary population, avascular fields, hemorrhages, giant capillaries and cessation of blood flow following cooling. When determined quantitatively, using television microscopy, Studer found it possible to distinguish between systemic and disseminated cutaneous lupus erythematosus, and between localized and systemic sclerosis [177]. In patients with undifferentiated connective tissue disease, it may be possible to predict which will progress to systemic sclerosis by undertaking quantitative analysis of nail fold vessel dimensions. The larger the vessels, the more likely that the condition is going to progress [178]. The mechanism of dilated vessel evolution may in part arise from impaired fibrinolysis, macroglobulinemia and cryoglobulinemia [172].

Fibrinogen may increase in subjects in renal failure on continuous ambulatory peritoneal dialysis. This has been proposed as a cause for the changes seen in nail fold vessels of such patients in proportion to abnormalities of urea and uric acid clearances [179]. Nail fold vessel changes may also occur in psoriasis and appear to correlate with nail pitting, onycholysis and periungual psoriatic plaques [180]. However, it can be imagined that clinical or subclinical elements of cutaneous psoriasis may represent the underlying change in vessel pattern.

The capillary networks in the normal nail fold of toes and fingers have been compared using videomicroscopy. This has revealed a greater density of capillaries in the toenail fold but with a reduced rate of flow [181]. The exact pattern of an individual's nail fold vessels can be used as an identifying characteristic [182].

Intravenous bolus doses of Na-fluorescein dye have been followed through nail fold microscopy [183]. There is rapid and uniform leakage from the capillaries in normal subjects to within 10 μm of the capillaries. It is suggested that a sheath of collagen may prevent diffusion beyond this point. The same procedure has been followed in patients with rheumatoid arthritis, demonstrating decreased flow rates and abnormal flow patterns, but no change in vessel leakage [184]. Static nail fold microscopy has been used for the investigation of Raynaud phenomenon [185]. It is possible to assess vascular toxicity affecting nail fold vessels following chemotherapy, using the same method [186]. A small number of laboratories are also able to employ *in vivo* capillary pressure measurement for nail fold vessels [187]. Video studies can be used to measure red cell velocity in nail fold capillaries [188] which has been used as a means of quantifying vascular damage in subjects with systemic sclerosis [189]. Systemic sclerosis and other microvascular disorders can be usefully assessed using laser Doppler, which can be combined with videocapillaroscopy for further detail [190].

Ultimately, histological information on the vessels and tissue of the nail folds may be helpful. The technique and benefits of nail fold biopsy have been described [191]. Amyloid deposits, subintimal hyalinosis and severe dermal fibrosis are cited as useful supplementary information yielded by biopsy.

Glomus bodies

A glomus is defined as a ball, tuft or cluster, a small conglomeration or plexus of cavernous blood vessels. In the skin it is an end-organ apparatus in which there is an arteriovenous anastomosis bypassing the intermediary capillary bed. This anastomosis includes the afferent artery and the Sucquet–Hoyer canal. The latter is surrounded by structures including cuboidal epithelioid cells and cells possibly of smooth muscle or pericyte origin (Zimmerman type). These are surrounded by a rich nerve supply and then the efferent vein which connects with the venous system outside the glomus capsule.

The nail bed is richly supplied with glomus bodies and their presence in histological specimens should be interpreted in this context, rather than assuming that their abundance has some pathological significance. These are neurovascular bodies which act as arteriovenous anastomoses (AVA). AVAs are connections between the arterial

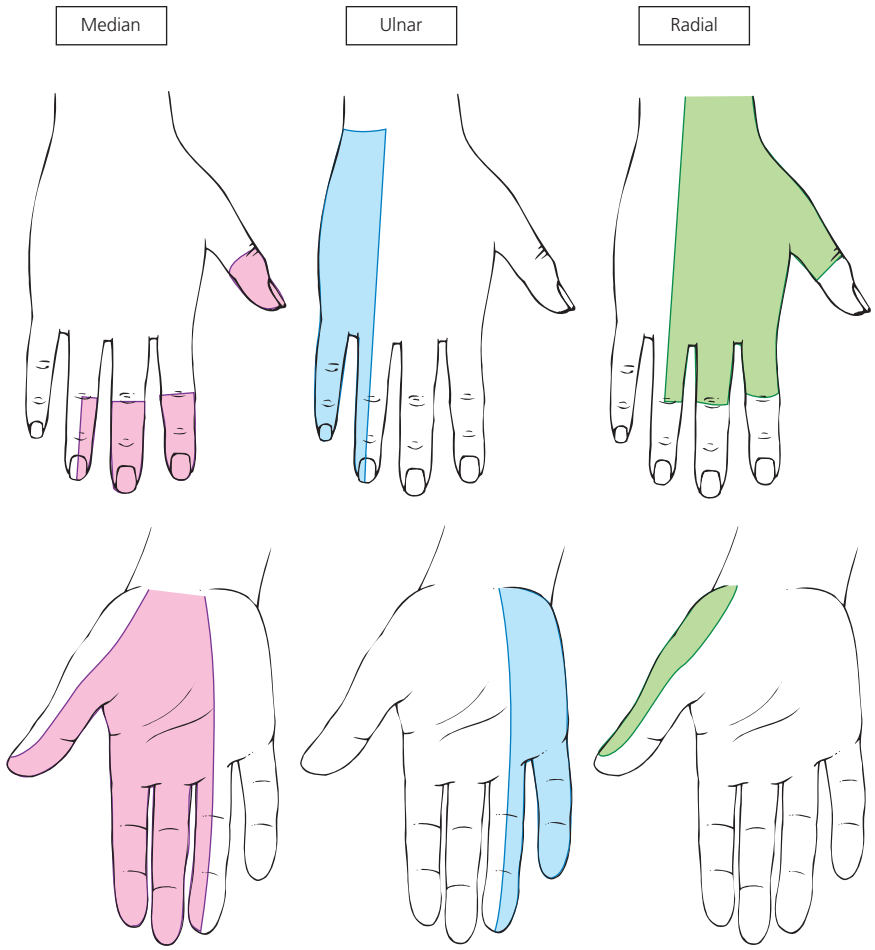


Figure 1.27 Sensory supply of the hand.

and venous side of the circulation with no intervening capillaries. Each glomus body is an encapsulated oval organ 300 µm long composed of a tortuous vessel uniting an artery and venule, a nerve supply and a capsule. It contains many modified large muscle cells, resembling epithelioid cells, and cholinergic nerves. Digital nail beds contain 93–501 glomus bodies per cm^3. They lie parallel to the capillary resevoirs which they bypass. They are able to contract asynchronously with their associated arterioles such that in the cold, arterioles constrict and glomus bodies dilate. They can thus serve as regulators of capillary circulation, acquiring the name "the peripheral heart of Masson" [192]. They are particularly important in the preservation of blood supply to the peripheries in cold conditions.

Nerve supply

The periungual soft tissues are innervated by dorsal branches of paired digital nerves. Wilgis and Maxwell [193] stated that the digital nerve is composed of three major fascicles supplying the digit tip, with the main branch passing under the nail bed and innervating both nail bed and matrix [194]. Winkelmann [195] showed many nerve endings adjacent to the epithelial surface, mainly in the nail folds. Serial dissections of cadaver hands demonstrate that there is often dual sensory innervation of the nail unit on the dorsal aspect of the digit which is relevant when attempting anesthesia with ring block [196] (Fig.1.27).

Comparative anatomy and function

The comparative anatomy of the nail unit can be considered from two aspects. There is the comparison of the nail with other ectodermal structures and most particularly hair and its follicle. The nail can also be viewed in an evolutionary setting alongside the hoof and claw. In this respect, the functional qualities of the nail or its equivalent are exemplified by the morphological differences in different species.

The human nail can be considered to have many mechanical and social functions, the most prominent of which are:

- fine manipulation
- scratching

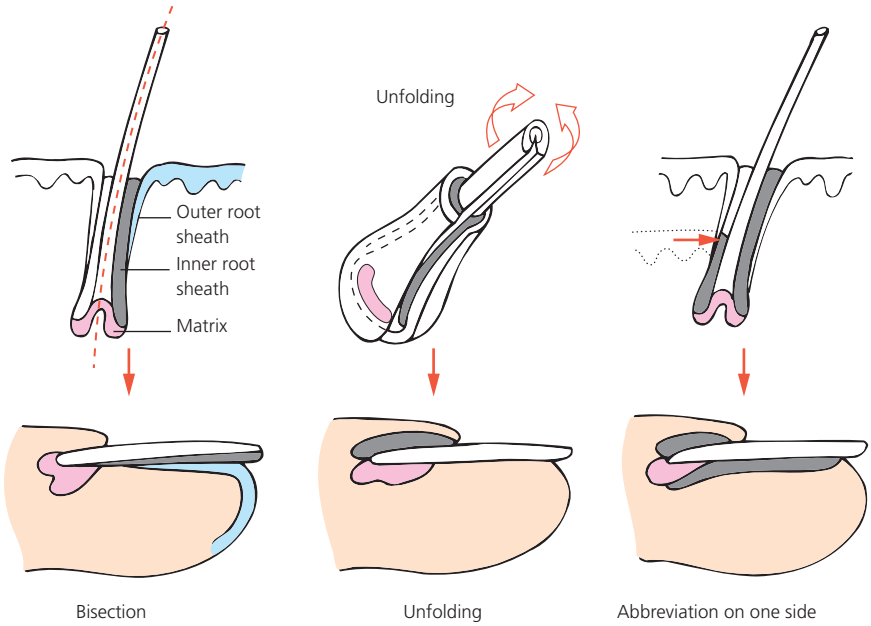


Figure 1.28 Models of hair follicle/nail unit homology.

- physical protection of the extremity
- as a vehicle for cosmetics and esthetic manipulation.

In comparison with other species, the first three functions have evolved with detailed physical modifications in the form of the hoof, claw and nail.

The nail and other appendages

An appendage is formed through the interaction of mesoderm and ectoderm, which in differentiated states usually means the interaction between dermis and epidermis. Those appendages most closely related to nail include hair and tooth. There are many shared aspects of different appendages, illustrated by diseases, morphology and analysis of the biological constituents.

Congenital abnormalities of hair, tooth and nail coexist in several conditions, underlining their common ground. Ectodermal dysplasias represent a group of disorders in which these appendages, as well as eccrine sweat glands, may be affected in association with skin changes.

In some conditions, only two of the appendages seem to be affected, such as the hair and nail changes described by Barbareschi *et al*. [197] or tooth and nail changes in the hypodontia and nail dysplasia syndrome (Witkop tooth and nail syndrome) [198, 199]. Alternatively, the same genetic defect, such as a mutation in the gene for keratin 17, may underlie two separate diseases where the nail is abnormal in one phenotype and the hair follicle in the other [200]. Presumably an additional factor determines which of the possible phenotypes prevails.

While diseases illustrate interrelationships between appendages, further common ground can be defined in terms of morphology. Achten [201] noted that the nail unit was comparable in some respects to a hair follicle, sectioned longitudinally and laid on its side (Fig. 1.28). Perrin has also described the area between the nail bed and hyponychium as the nail isthmus, to emphasize the resemblance to the isthmus of the hair follicle [202]. The hair bulb was considered analogous to the intermediate nail matrix and the cortex to the nail plate. As a model to stimulate thought, this idea is helpful. It also encourages the consideration of other manipulations of the hair follicle that might fit the analogy more tightly. The nail unit could be seen, as in Figure 1.28, as an unfolded form of the hair follicle, producing a hair with no cortex, just hard cuticle. Scanning electron microscopy of the nail confirms that its structure is more similar to compacted cuticular cells than cortical fibers. A third model could represent the nail unit as a form of follicle abbreviated on one side, providing a modified form of outer root sheath to mold and direct nail growth in the manner of the proximal nail fold (see Fig. 1.28). The matrix and other epithelial components of tooth can be seen in a similar comparative light and even the lingual papilla, which shares some keratin expression with the nail, shows some morphological similarities with the nail and hair follicle [131].

In pachyonychia congenita where alopecia is found, transverse sections of scalp follicles reveal dyskeratosis of the outer root sheath, attracting comparisons with the nail bed [203]. In some diseases immunological focus is shared between nail matrix and hair follicle, as in lichen planus and alopecia areata. This suggests common ground in immunological identity, which could be related to specific keratins. Ito [204] described a pattern of relative immune privilege in the proximal nail matrix similar to that seen in the hair follicle which might normally play a part in blocking the autoimmune attention of white cells. Defects

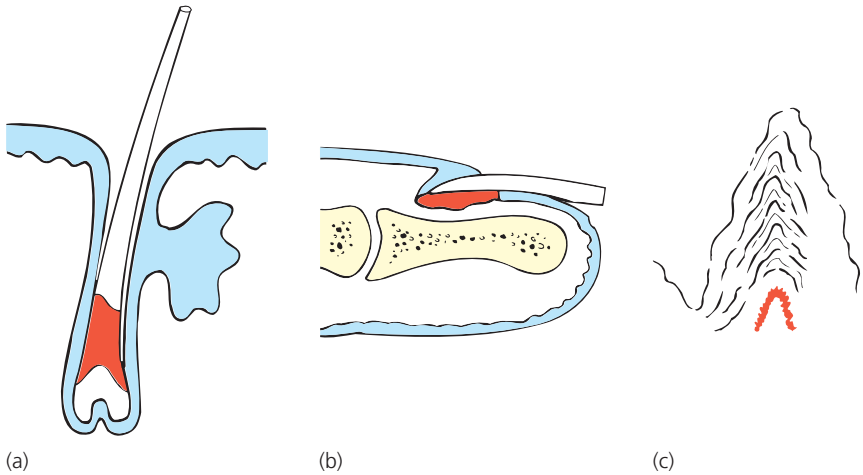


Figure 1.29 Localization of immunoreactivity of the hard keratin Ha-1 in (a) the anagen hair follicle (×200), (b) the nail unit (×10) and (c) the human lingual papilla (×500). After Westgate *et al.* [16] with permission from Wiley-Blackwell.

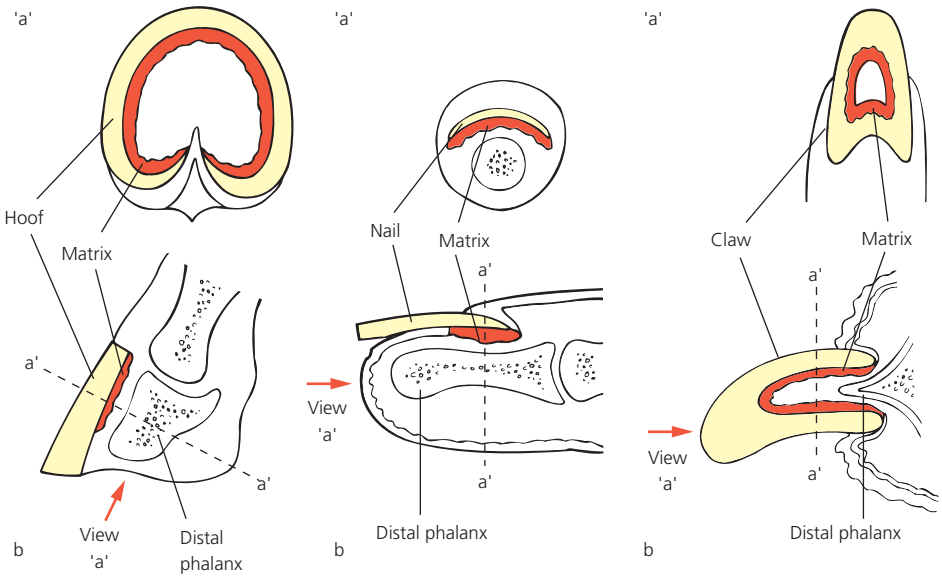


Figure 1.30 Comparison of hoof, nail and claw and their matrix (red) origins.

in this could open the way to a common path and manifestation of disease (Fig. 1.29).

The character of the nail plate and hair has led to their use in assays of circulating metabolites. They both lend themselves to this because they are long-lasting structures that may afford historical information. Additionally, their protein constituents bind metabolites and they provide accessible specimens. This allows both hair and nail to be used in the detection of systemic metabolites which may have disappeared from the blood many weeks previously (see "Exogenous materials in nail analysis" below).

Phylogenetic comparisons

The structure of claws and hooves and their evolutionary relationship to the human nail have been well reviewed [205]. In higher primates, nails have developed with the acquisition of manual dexterity. Other mammals do not possess such flattened claws from which nails have evolved (Fig. 1.30).

The lowest evolutionary level at which claws are seen is in the amphibia [206]. The matrix contributes the greatest mass to the nail plate in humans and other primates, with a lesser contribution from the dorsal and nail bed matrices. Claws are formed from an extensive germinal matrix, which occupies the territory of the nail bed in primates [207]. It is sometimes described as comprising a dorsal and ventral component [208], where differential growth of these components is responsible for the curve. The orientation of the matrix and hence growth of nail may be influenced by the shape of the underlying phalanx [207]. It is postulated that their sharp tip is produced by a dominant midline matrix.

Claws are significantly more three-dimensional than nails and this is achieved by the coronal distribution of

matrix tissue around the terminal phalanx. If this is recognized, the comparisons between other hard keratinized animal appendages such as horns and beaks become obvious. All these structures share physical and biochemical attributes specific to their biological character and function. In some respects, the upper beak has more in common with the morphology of the nail than do claws and comparisons have been made in both structure and constituents between beak and claw [209]. The disorders of claws presenting to one university veterinary service demonstrated a preponderance of trauma and bacterial infection [210] (Table 1.6). This differs from dermatological experience in humans where complaints are usually attributable to dermatoses such as psoriasis or eczema or to fungal infection.

Claws and talons are harder than nails, probably because of the content of calcium as crystalline hydroxyapatite within keratinocytes (cf. human nails) [211]. A study of onychomadesis (nail shedding) in dogs looked at mineral constituents of normal claws, human nails and the hooves of cows and pigs [212]. It appears that there is no particular pattern of homology between different species in this respect (Table 1.7).

Keratin immunohistochemistry for epithelial and hair/nail keratins has made it easier to identify the types of keratins found in animal appendages. Such studies illustrate substantial homology between the canine claw and human nail [213] and, to a lesser extent, similarities with the reptilian claw [214]. The anatomy of the equine hoof allows comparison with human nails but there are also substantial differences, including the presence of some keratins, K42 and K124, not found in human tissue [215]. In some instances, animal mutants have helped corroborate the role of a specific keratin in the human phenotype. Mice with mutation of the gene expressing K75 (K6hf) demonstrate features similar to humans with pachyonychia congenita where the human K75 gene is also known to be at fault [216].

Orientation of keratin microfibrils may contribute to their strength. Fourier-transform Raman spectroscopy shows that bird and reptile claws are made up mainly of β-sheeted keratin in contrast to the predominantly α-helical keratin conformation of human nail keratin [217].

Claws and nails have more in common with each other than they do with hooves. However, the bovine hoof has provided a useful source of research tissue for experiments on colocalization of hard and soft keratin expression in matrix cells and the characteristics of matrix cells in tissue culture [113]. Hooves have evolved to provide a "bulky claw" for weight bearing and locomotion over hard ground [218]. It is interesting that among the prosimians, tarsiers have nails on all digits apart from the second and third digits of the hindlimb which bear claws [205]. In hooves, the nail fold and root have been displaced backwards with a forwards extension of the nail bed. The hard "soft plate" under hooves

Table 1.6 Proportion of diagnoses of dogs with disorders of the claws from a study of 196 affected dogs.

Diagnosis	% of cases
Bacterial paronychia	35.5
Trauma	22
Neoplasia	14
Fungal	4
Lupoid	4
Bullous disorder	4
Demodicosis	1
Systemic illness	0.5
Idiopathic	15

Adapted from Scott and Miller [210] with permission from Lingua.

Table 1.7 Mineral content (expressed as mg/kg, standard error in parentheses).

Mineral	Dog claw	Porcine hoof	Bovine hoof	Human nail
Calcium	771 (83)	1699 (50)	1481 (25)	671 (806)
Magnesium	238 (21)	220 (10)	300 (11)	100 (121)
Iron	268 (31)	73 (8)	17 (1.1)	29 (64)
Potassium	430 (53)	1050 (30)	785 (53)	–
Sodium	676 (50)	–	523 (16)	2400 (1800)
Copper	6.3 (0.5)	4.6 (0.13)	8.3 (0.3)	29 (89)
Zinc	129 (5)	160 (4)	128 (1.7)	106 (154)

Adapted from Harvey and Markwell [212] with permission from Wiley-Blackwell.

is produced from an area equivalent to the subungual part of the claw. In some animals, cloven hooves have only developed on the digits that touch the floor. In horses, the single large hoof is produced from the third digit. The typical hoof shape is due to a deep, backwardly placed root matrix with the ventral plate formed from the subungual epidermis. The microfibrils in hooves are from 25 to 100 nm in diameter. The orientation of the fibrils is along the main axis of the hoof, similar to the hair cortex.

Physiology

Nail production

Definition of the nail matrix

In the first section, we have attempted to define the matrix in anatomical terms, assisted by histology and immunohistochemistry illustrating regional differentiation within the nail unit and in particular with respect to keratin expression. These measures provide indirect information on aspects of nail production and help us to address the central question of which tissues produce nail plate and which simply support and surround it. There is considerable biological and clinical relevance to this point, given that the focus of embryogenesis, damage repair and disease processes are better understood if the exact location of nail formation is established.

The location or existence of nail matrix tumors is often poorly defined because there is a lack of awareness of the site and pivotal role of nail matrix disturbance in the creation of abnormal nail morphology. Equally, diagnostic biopsies or sampling can be misdirected if the likely source of nail abnormalities is not recognized at the outset; a clear prognosis following surgery or trauma cannot be given unless the clinician understands the relative contributions of the nail matrix and nail bed.

In spite of the importance of the question, controversy remains as to the relative contributions of the three putative nail matrices to the nail plate. The three contenders are the dorsal, intermediate and ventral matrices (Fig. 1.31). The first is part of the proximal nail fold, the last is the nail bed. Lewis [5] claimed that the nail plate demonstrated a three-layer structure on silver staining and that each layer derived from one of the possible matrices. This remains one of the indirect histological methods of defining the matrix which have been supplemented by more direct measures of nail plate production.

Markers of matrix and nail bed proliferation

Zaias and Alvarez [11] disagreed with Lewis on the basis of *in vivo* autoradiographic work on squirrel monkeys, where dynamic aspects of the process were being examined. Tritiated thymidine injected into experimental animals was only incorporated into classic matrix (or intermediate matrix, to use Lewis's terminology). Norton used human subjects in further autoradiographic studies

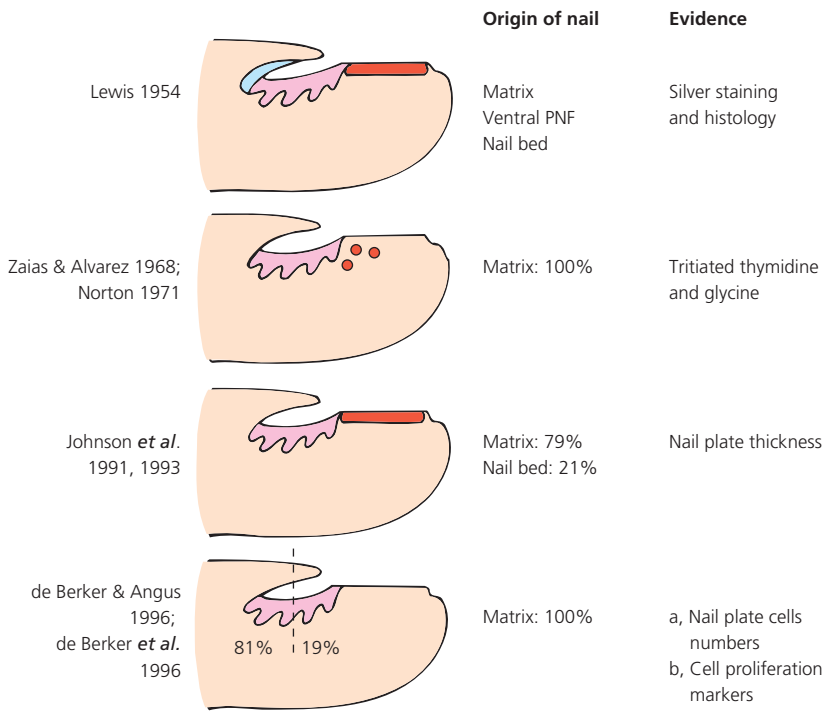


Figure 1.31 Theories of nail plate origin.

[219]. Although there was some incorporation of radiolabeled glycine in the area of the nail bed, it was in a poorly defined location, making clear statements impossible.

Immunohistochemical techniques allow us to examine proliferation markers in human tissue, without the drawbacks of autoradiography. Antibodies to proliferating cell nuclear antigen and to the antigen K1-67 associated with cell cycling have been used on longitudinal sections of healthy and diseased nail units [202, 220]. Both markers demonstrate labeling indices in excess of 20% for the nail matrix, in contrast with 1% or less for the nail bed in healthy tissue. The differences are less marked according to Perrin [202], with an index of 21% in the matrix and 5% in the nail bed. In psoriatic nail and onychomycosis, the labeling index of nail bed rises to >29%. While these indices do not directly measure nail plate production, a very low index for normal nail bed is consistent with other studies suggesting that the nail bed is an insignificant player in normal nail production. The situation may change in disease and the definition of nail plate becomes difficult when substantial subungual hyperkeratosis produces a ventral nail of indeterminate character [139].

Nail plate indicators of matrix location

Johnson *et al*. [12, 221] dismissed the evidence of Zaias, claiming that the methodology was flawed. They examined nail growth by the measurement of change in nail thickness along a proximal to distal longitudinal axis. They demonstrated that 21% of nail plate thickness in traumatically lost big toenails was gained as the nail grew over the nail bed. This was taken as evidence of nail bed contribution to the nail plate.

A similar study developed this observation with histology of the nail plate taken at fixed reference points along the longitudinal nail axis and comparing nail plate thickness at these sites with numbers of corneocytes in the dorsoventral axis of the nail [57]. The result of this was to confirm the observation that the nail plate thickens over the nail bed but that this is not matched by an increase in nail cells. In fact, the number of cells reduces by 10%, but this was not of statistical significance. These combined studies may be reconciled if we propose that the shape of cells within the big toenail becomes altered with compaction as the nail grows. This is likely where clinical experience shows that the nail develops transverse rippling where there is habitual distal trauma.

If the loss of nail cell numbers along the nail bed is a genuine observation, it might suggest that cells are being shed from the nail surface. This is compatible with the status of nail plate as a modified form of stratum corneum. Heikkilä *et al*. [222] provide evidence in support of this where nail growth was measured by making indentations on the nail surface and measuring the change in the volume of these grooves as they reach the free edge. There was a reduction of volume by 30–35%, which was taken as evidence of a nail bed contribution to the nail plate. However, this interpretation is less believable than the possibility that the nail is losing cells from the surface, and histology of grooves in a similar study shows that this is likely to be the case [223].

Flow cytometry of matrix cells

Haneke and Kiesewetter (unpublished data) have performed flow cytometry on matrix cells obtained during surgical lateral matrixectomy for ingrowing nail. This demonstrated that 94% of the matrix cells were in G0/1 phase, 3.4% in S phase and 2% in G2+M phase. The corresponding values for matrix connective tissue cells were 96.6% for G0/1, 2.3% for S and 1.1% for G2+M phases. The differences between matrix cells and associated connective tissue were statistically significant. This suggests that the percentage of cells in the phase of DNA synthesis and mitosis (S plus G2+M phases) in the nail matrix is much lower than that of hair matrix cells and equals that of the cells in the hair root sheath. However, the values may have been underestimated in this experiment if the matrixectomies failed to sample the most basal matrix cells, as can happen in this operation. Also, this technique was not applied to distinguish nail bed from matrix and does not directly address the issue of which tissues are primarily involved in nail plate production.

Clinical markers of matrix location

The clearest demonstration of nail generation is the effect of digit amputation at different levels. Trauma within the lunula is more likely to cause irreparable nail changes than that of the nail bed [224]. This observation is true for adults and children alike, although the likelihood of normal regrowth is greater in children with similar trauma [225]. Longitudinal biopsies of the entire nail unit within the midzone of the nail are said to cause a chronic split if the width of the biopsy exceeds 3 mm [53]. However, there are several factors in addition to the width of the biopsy that can contribute to scarring with longitudinal biopsies and smaller biopsies in the midzone can also give long-term problems.

In some circumstances, most commonly old age, there is a pattern of subungual hyperkeratosis associated with nail thickening whch gives the impression of a nail bed contribution to the nail plate. Historically, this has been referred to as the solenhorn (Fig. 1.32) and considered a germinal element of the hyponychium. Samman considered this issue in the context of a patient with pustular psoriasis [139] and concluded that the ventral nail is a movable feast, manifesting itself in certain pathological circumstances.