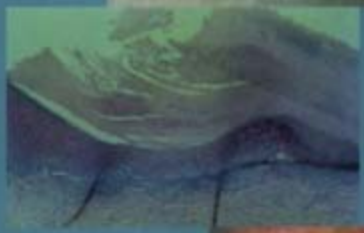
Baran and Dawber's
Diseases of the Nails and their Management

Third Edition

edited by
R. Baran
R.P.R. Dawber
D.A.R. de Berker
E. Haneke &
A. Tosti



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Preface to the Third Edition

It is now over 20 years since we first conceived the idea of a detailed reference book on all aspects of nail science, nail disorders and their treatment. By that time in our lecturing and teaching commitments around the world we had been privileged to work with a vast array of great experts in the field, all beginning to move across traditional specialty boundaries—one saw podiatrists with great operating skills working with dermatological and plastic surgeons; beauticians showing doctors that aesthetic and 'functional' remedies may at times be more relevant than formal medical therapeutics; geneticists seeing that the nail apparatus may give more specific insight into mechanisms of hereditary diseases than other sites . . . and so on. Blackwell Science accepted the project and the first edition appeared in 1984.

The 'cross-fertilization' between specialists has continued apace during the last 17 years to the extent that expanding knowledge and better medical and surgical treatments have led to the Third Edition being 50% larger than the First Edition. This expansion has been controlled and enhanced by the editorial team increasing to five—David de Berker, Antonella Tosti and Eckart Haneke have great breadth of experience in the scientific and clinical aspects of the nail apparatus and those used to the Second Edition will recognize this input well beyond their specific contributions to many chapters. There has been considerable enlargement of the sections on nail apparatus tumours and nail surgery; this is due almost entirely to the new contributions by Elvin Zook and Jean-Luc Drapé. Last but not least we welcome Bernard Richert; his clinical experience has been carefully applied to the physical signs section. Significant changes have occurred in the understanding and treatment of onychomycosis since the Second Edition and this is reflected in the fungal diseases section.

Many clinical figures included in this edition have been contributed by colleagues from all over the world and we offer them our most grateful thanks; we hope that all their contributions have been acknowledged in the text and apologise if inadvertently we have failed to do so at any point. After three editions of this book it is time for a very particular acknowledgement to be made to Nicole Baran. Those directly involved will know of her immense administrative commitment far beyond the call of duty! One can truly say that she is the great catalyst that has made the book gel.

Robert Baran
Rodney Dawber

Preface to the First Edition

Since the earliest publication by Heller there have been several books written on diseases of the nail: in particular the works of Alkiewicz and Pfister; Pardo-Castello and Pardo; Samman, Sertoli and Zaïas must be mentioned as they are of high quality and extremely useful, mainly to dermatologists.

For many years we have felt that there is a need for a comprehensive reference book on all aspects of the nail in health and disease. It is evident that in different cultures nail abnormalities are often seen by a variety of specialists, e.g. traumatic and genetic dystrophies are rarely seen initially by dermatologists whilst cosmetic and industrial problems may be handled by dermatologists, industrial health experts, cosmetologists or chiropodists. These are a few examples of the need for a reference book to 'cross' speciality, and even more important, parochial, national medical barriers. We believe that a satisfactory book on the nail must do this. The world is small! We have both travelled widely in recent years and do hope that the content and style of the book succeeds in this aim.

Some people may be surprised to find a Frenchman and Englishman apparently having agreed with each other for long enough to produce a book of this nature—not all French and English are enemies! We have worked diligently to benefit from our language differences and to combine the differences in training and interests and hope that this first truly international book, including authors from France, Germany, Sweden, UK and USA, will be of use world-wide.

Though the chapters have been contributed by specific authors, we must point out that the book is very much a group activity; in particular, the editors have contributed much from their own files to every section. This applies to the script, references and figures, and therefore any errors of fact, emphasis or quality of picture may be the fault of the editors rather than the named chapter writers!

The inclusion of colour pictures has obviously made the book more expensive than with black and white pictures alone. We gave considerable thought to this and decided to include them as important diagnostic aids because of the photogenic nature of the nail; and the fact that between the ten authors we had a unique opportunity to pool material collected over many years.

Acknowledgements

An undertaking of this kind is quite impossible without the help of a vast number of colleagues the world over who have encouraged, cajoled and constructively disagreed with us over the many years that we have been interested in nails; and more specifically we must thank those who have provided details and pictures of their patients—these are acknowledged in the script.

We are deeply indebted to Georges Achten, Peter Samman and Nardo Zaïas who at various times have stimulated our interest in this field; without their help in our careers this book would not have materialized in any shape or form.

We are very grateful to Dr Gerald Godfrey and Chris Gummer who gave great assistance in formulating the final text.

Robert Baran
Rodney Dawber

CHAPTER 1

Science of the nail apparatus

R.P.R. Dawber, D.A.R. de Berker & R. Baran

Gross anatomy and terminology

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 Figs 1.1 and 1.2. The definition 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 (Lewis 1954). The dorsal matrix is synonymous with the ventral aspect of the proximal nail fold. Intermediate matrix (germinative matrix) is the epithelial structure starting at the point that 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 more pale than 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 (Terry 1955). 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 with disease or compression which influences the vascular supply (Fig. 1.3). Sonnex *et al.* (1991) 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 can be summarized in Table 1.1. The onychocorneal band represents the first barrier to penetration of materials to beneath 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.

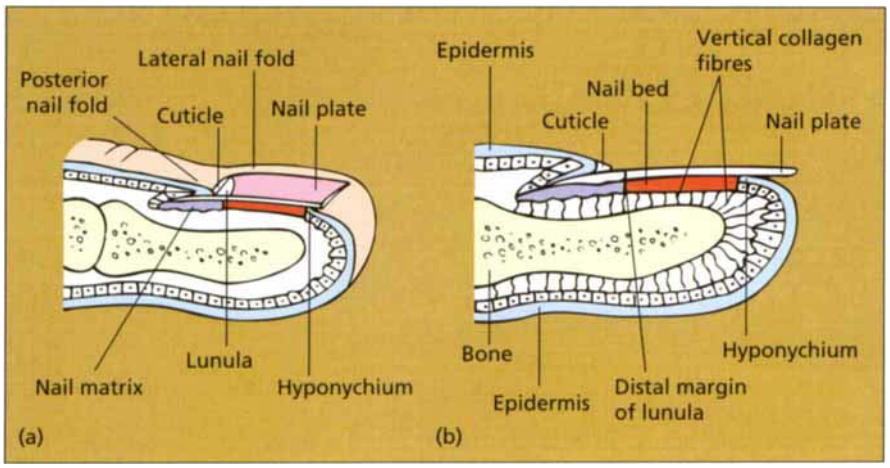


Fig. 1.1 Longitudinal section of a digit showing the dorsal nail apparatus

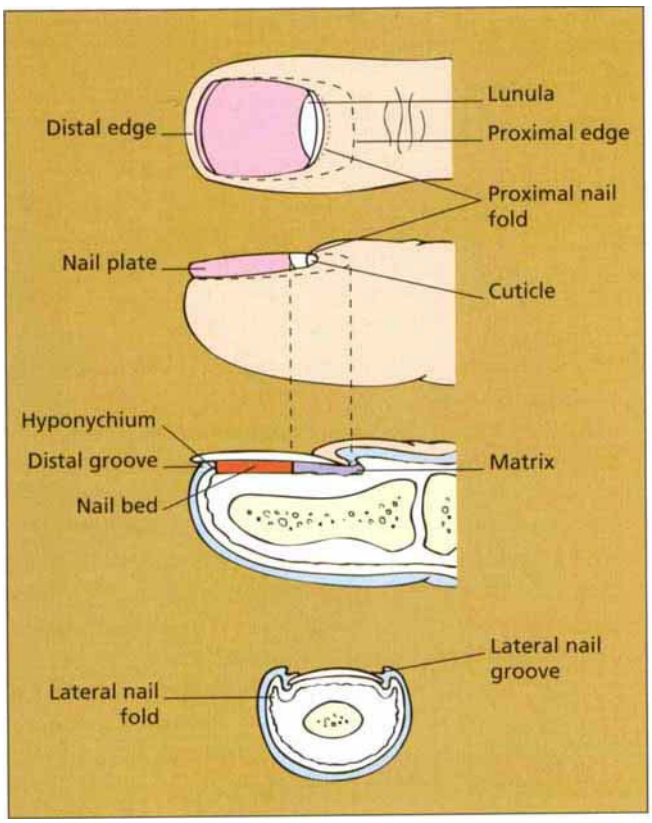
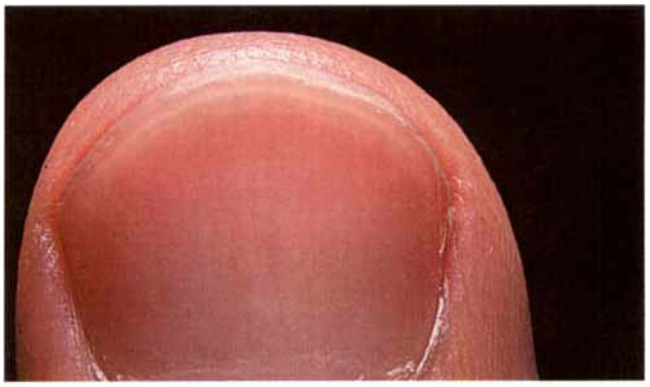


Fig. 1.2 The tip of a digit showing the component parts of the nail apparatus



(a)

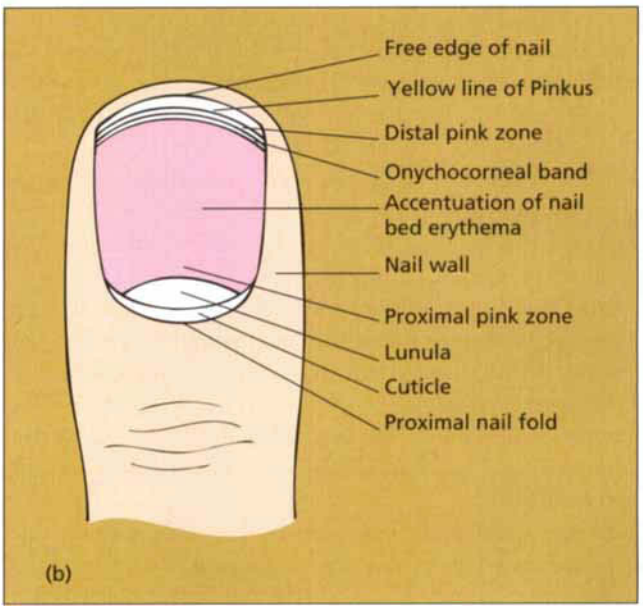


Fig. 1.3 (a) Onychodermal band. (b) Diagrammatic representation of the morphological features of the normal nail, detail of the distal physiological colour bands are shown. (Courtesy of T.S. Sonnex and W.A.D. Griffiths, St John's Institute of Dermatology.)

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.

References

Lewis, B.I. (1954) Microscopic studies of fetal and mature nail and surrounding soft tissue. *Archives of Dermatology and Syphilis* 70, 732–744.

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

Zone	Sub-zone	Appearance
Free edge		Clear grey
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

Sonnex, T.S., Griffiths, W.A.D. & Nicol, W.J. (1991) The nature and significance of the transverse white band of human nails. *Seminars in Dermatology* 10, 12–16.

Terry, R.B. (1955) The onychodermal band in health and disease. *Lancet* i, 179–181.

Embryology

Morphogenesis

8–12 weeks

Individual digits are discernible from the 8th week of gestation (Lewis 1954). 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).

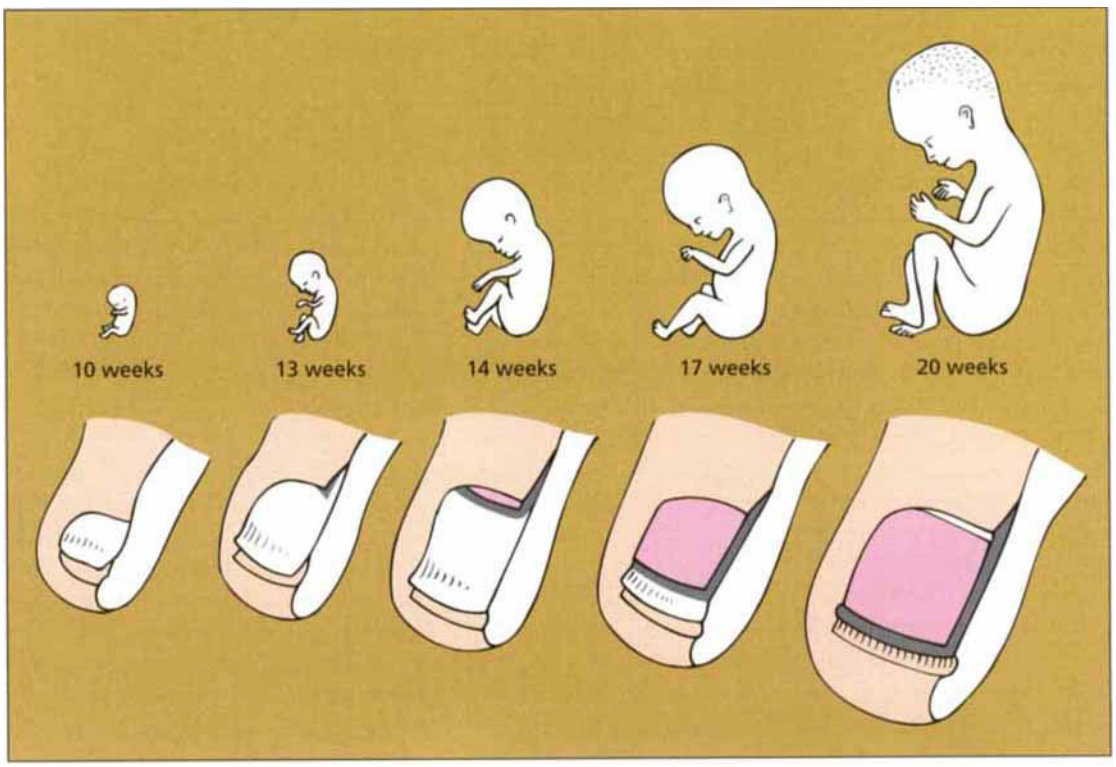


Fig. 1.4 Embryogenesis of the nail apparatus. 10 weeks: the primary nail field can be seen with proximal, lateral and distal grooves. The latter is accentuated by a distal ridge. 13 weeks: a wedge of matrix primordium moves proximally, with the invagination of the proximal nail fold above. 14 weeks: the nail plate emerges. 17 weeks: the nail plate covers most of the nail bed and the distal ridge starts to flatten. 20 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 the actual size.

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 (Warwick & Williams 1973) 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 now becomes termed the hyponychium. The nail bed epithelium no longer produces keratohyalin, with a more parakeratotic appearance. By birth the nail plate 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

Keratin synthesis can be identified in the nail unit from the earliest stages of its differentiation (Moll *et al.* 1988). 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'. Keratin represents about 80% of the intracellular structural protein of epithelial cells. It belongs to the family of intermediate filaments. There are many different keratins with varied structural properties and localization within animals. They are divided into two groups, the first of which are 'soft', epithelial keratins commonly found in the skin. The second is 'hard', trichocyte or hair/nail keratins found in hair, nail, thymus, tooth primordia and tongue. Unfortunately, the funding for research and the access of researchers to scalp rather than nail matrix as a substrate for study means that these keratins are most commonly referred to in the literature as hair keratins. We feel that it is more precise to refer to them by their physical characteristics as 'hard' keratins rather than by a misleading epithet defining only one of their origins.

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 (Lewis 1954; Zaias 1963; Hashimoto *et al.* 1966; Zaias & Alvarez 1968; Johnson *et al.* 1991). 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 (Baden & Kubilus 1984) and absence (Heid *et al.* 1988; Moll *et al.* 1988; de Berker *et al.* 1992; Westgate *et al.* 1997) 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 disulphydryl 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 (Moll & Moll 1993). 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 (Moll & Moll 1993). 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 (Cameli *et al.* 1998).

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 sulphur and aluminium and a rise in chlorine has been noted as a feature of full-term newborns in comparison with the nail plate of premature babies (Sirota *et al.* 1988). An elevated aluminium level may correspond to bone abnormalities which lead to osteopenia.

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:

1 embryogenesis;
2 regrowth;
3 growth.

There is overlap in 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. Observations of this process add 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 (Zwilling 1968). 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 (Grant 1978). In the human, cases of anonychia secondary to phenytoin (Hanson & Smith 1975) might implicate the drug at this stage, prior to 8 weeks. Drugs have been argued as being contributory to congenital nail dystrophies mainly affecting the index finger (Higashi *et al.* 1975).

Dermal–epidermal interactions in appendage formation have been closely examined using the hair follicle, where the role of the dermal papilla is central in the induction of epidermal differentiation, both *in vivo* and *in vitro* (Jahoda 1984; Reynolds *et al.* 1993).

Other congenital abnormalities highlight the debate that spans embryogenesis, growth and regrowth. Congenital onychodysplasia of the index fingers (COIF) is frequently associated with abnormalities of the terminal phalanges and interphalangeal joints (Baran 1980). 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 (Millman & Strier 1982). However, a bony abnormality is not mandatory in this condition or other conditions with ectopic nail (Aoki & Suzuki 1984). A normal nail may overly an abnormal bone on other than the index finger (Kinoshita & Nagano 1976). 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 (Kitayama & Tsukada 1983). 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 (Koizumi *et al.* 1998).

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 (Baran & Juhlin 1986). 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.

References

Aoki, K. & Suzuki, H. (1984) The morphology and hardness of the nail in two cases of congenital onychoheterotopia. *British Journal of Dermatology* **110**, 717–723.

Baden, H.P. & Kubilus, J. (1984) A comparative study of the immunologic properties of hoof and nail fibrous proteins. *Journal of Investigative Dermatology* **83**, 327–331.

Baran, R. (1980) Syndrome d'Iso et Kikuchi (COIF syndrome); 2 cas avec revue de la littérature (44 cas). *Annales de Dermatologie et de Vénéréologie* **107**, 431–435.

Baran, R. & Juhlin, L. (1986) Bone dependent nail formation. *British Journal of Dermatology* **114**, 371–375.

de Berker, D., Leigh, I. & Wojnarowska, F. (1992) Patterns of keratin expression in the nail unit—an indicator of regional matrix differentiation. *British Journal of Dermatology* **127**, 423.

Cameli, N., Ortonne, J.P., Picardo, M., Peluso, A.M. & Tosti, A. (1998) Distribution of Merkel cells in adult human nail matrix (letter). *British Journal of Dermatology* **139**, 541.

Grant, P. (ed.) (1978) Limb morphogenesis: a dialog between an ectodermal sheet and mesoderm. In: *Biology of Developing Systems*, pp. 414–421. Holt, Rinehart and Winston, New York.

Hanson, J.W. & Smith, D.W. (1975) The fetal hydantoin syndrome. *Journal of Pediatrics* **87**, 285–290.

Hashimoto, K., Gross, B.G., Nelson, R. & Lever, W.F. (1966) The ultrastructure of the skin of human embryos. III. The formation of the nail in 16–18 week old embryos. *Journal of Investigative Dermatology* **47**, 205–207.

Heid, H.W., Moll, I. & Franke, W.W. (1988) Patterns of trichocytic and epithelial cytokeratins in mammalian tissues. II. Concomitant and mutually exclusive synthesis of trichocytic and epithelial cytokeratins in diverse human and bovine tissues. *Differentiation* **37**, 215–230.

Higashi, N., Ikegami, T. & Asada, Y. (1975) Congenital nail defects of the index finger. *Japanese Journal of Clinical Dermatology* **29**, 699–701.

Jahoda, C.A.B. (1984) Induction of hair growth by implantation of cultured dermal papilla cells. *Nature* **311**, 560–562.

Johnson, M., Comaish, J.S. & Shuster, S. (1991) Nail is produced by the normal nail bed: a controversy resolved. *British Journal of Dermatology* **125**, 27–29.

Kinoshita, Y. & Nagano, T. (1976) Congenital defects of the index finger. *Japanese Journal of Plastic and Reconstructive Surgery* **19**, 23.

Kitayama, Y. & Tsukada, S. (1983) Congenital onychodysplasia. Report of 11 cases. *Archives of Dermatology* **119**, 8–12.

Koizumi, H., Tomoyori, T. & Ohkawara, A. (1998) Congenital onychodysplasia of the index fingers with anomaly of the great toe. *Acta Dermato-Venereologica* **78**, 478–479.

Lewis, B.L. (1954) Microscopic studies of fetal and mature nail and surrounding soft tissue. *Archives of Dermatology and Syphilis* **70**, 732–744.

Millman, A.J. & Strier, R.P. (1982) Congenital onychodysplasia of the index fingers. *Journal of the American Academy of Dermatology* **7**, 57–65.

Moll, I., Heid, H.W., Franke, W.W. & Moll, R. (1988) Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. *Differentiation* **39**, 167–184.

Moll, I. & Moll, R. (1993) Merkel cells in ontogenesis of human nails. *Archives of Dermatological Research* **285**, 366–371.

Reynolds, A.J., Lawrence, C.M. & Jahoda, C.A. (1993) Human hair follicle germinative epidermal cell culture. *Journal of Investigative Dermatology* **101**, 634–638.

Sirota, L., Straussberg, R., Fishman, P. *et al.* (1988) X-ray microanalysis of the fingernails in term and preterm infants. *Pediatric Dermatology* **5**, 184–186.

Warwick, R. & Williams, P.L. (eds) (1973) *Gray's Anatomy*, 35th edn. Longman, Harlow, Essex.

Westgate, G.E., Tidman, N., de Berker, D., Blount, M.A., Philpott, M.P. & Leigh, I.M. (1997) Characterisation of LH Tric 1, a new monospecific monoclonal antibody to the hair keratin Ha 1 Br. *Journal of Dermatology* **137**, 24–31.

Zaias, N. (1963) Embryology of the human nail. *Archives of Dermatology* **87**, 37–53.

Zaias, N. & Alvarez, J. (1968) The formation of the primate nail plate. An autoradiographic study in the squirrel monkey. *Journal of Investigative Dermatology* **51**, 120–136.

Zwilling, E. (1968) Morphogenetic phases in development. In: *The Emergence of Order in Developing Systems*. (eds M. Locke), pp. 184–207. Academic Press, New York.

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. This effect can be diminished by softening the nail, which may be less practical if there are soft tissue attachments requiring histological examination.

Nail softening techniques

Nail alone

There are several different techniques to soften the nail plate. Lewis (1954) recommended routine fixation in 10% formalin and processing as usual. Earlier methods employed used fixation with potassium bichromate, sodium sulphate and water. The section is then decalcified with nitric acid and embedded in collodion. Alkiewicz and Pfister (1976) 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 μm sections.

Although softening of nail clippings for histology is not mandatory, it is possible and may be helpful. Suarez *et al.* (1991) 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, successive paraffin mixtures, sectioned at 4 μm 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 (Alvarez & Zaias 1967). This is followed by a modified polyethylene glycol-pyroxylin embedding method.

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 (Bennett 1976). Good results are obtained with routine fixation and embedding if permanent wave solution, thioglycollate or 10% potassium hydroxide solution, is applied with a cotton swab to the surface of the paraffin block every two or three sections. Lewin *et al.* (1973) suggests 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 in obtaining high quality sections it is worth cutting many at different levels to maximize the chance of getting what you need.

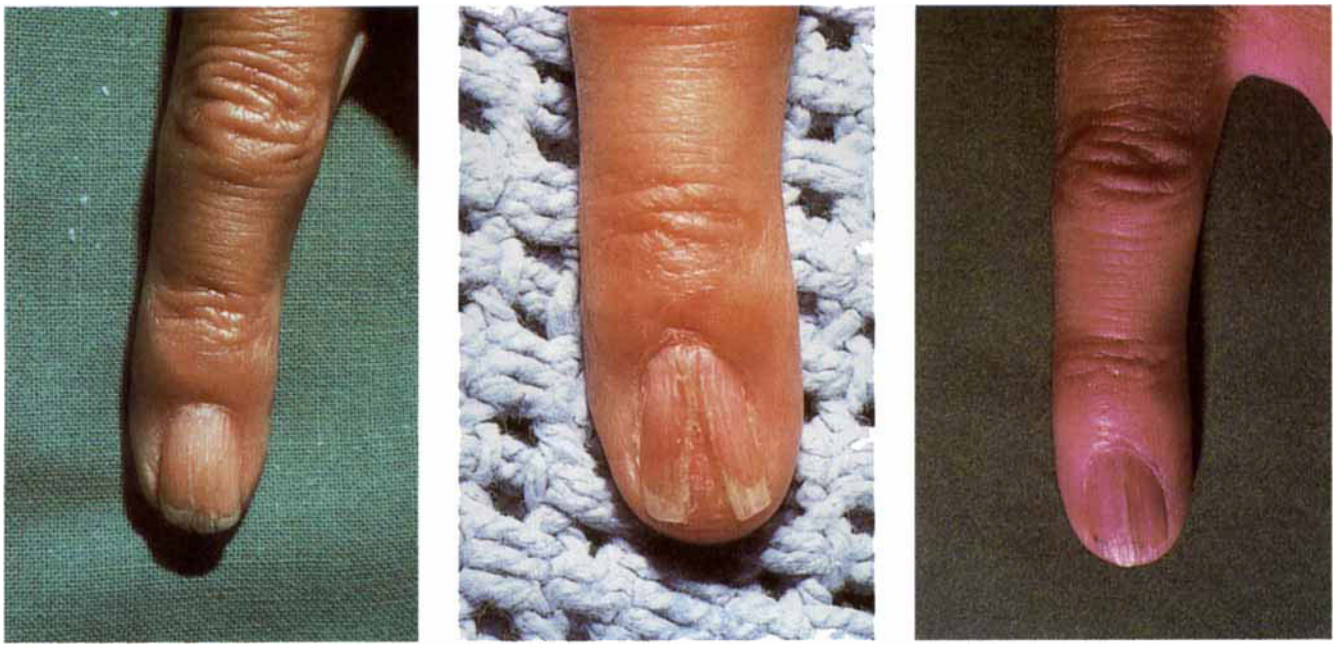
Routine staining with haematoxylin and eosin is sufficient for most cases. Periodic acid Schiff (PAS) and Grocott's silver stain can be used to demonstrate fungi; a blancophore fluorochromation selectively delineates fungal walls (Haneke 1991). Toluidine blue at pH 5 allows better visualization of the details of the nail plate (Achten 1963; Achten *et al.* 1991). Fontana's argentaffin reaction demonstrates melanin. Haemoglobin is identified using a peroxidase reaction. Prussian Blue and Perl stains are not helpful in the identification of blood in the nail. They are specific to the haemosiderin product of haemoglobin breakdown caused by macrophages. This does not occur in the nail (Achten & Wanet 1973; Alkiewicz & Pfister 1976; Baran & Haneke 1984).

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

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 (qv. nail growth). Contrast with these other regions helps characterize the matrix.



(a) (b) (c)

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

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 (Zaias 1967) (Fig. 1.5). Once matrix damage has occurred, it is difficult to effectively repair it (Nakayama *et al.* 1990; Pessa *et al.* 1990). 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 are provided by the proximal 50% of the nail matrix (de Berker *et al.* 1996b) 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 have 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.* (1996) 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 (Wilkerson & Wilkin 1989; Cohen 1996). 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 (Lewin 1965). This has been confirmed in a more recent study utilizing injection of gelatinized Indian ink into amputation specimens (Wolfram-Gabel & Sick 1995).

The thinner epidermis of the nail bed may account for the contrast between white and pink appearance of the lunula and bed, respectively (Burrows 1917). Many suggestions have been made to account for the appearance of the lunula (Burrows 1917, 1919; Ham & Leeson 1961; Achten 1963; Lewin 1965; Baran & Gioanni 1969):

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

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 (Guero *et al.* 1994). Lack of balance between the symmetrical tension on these attachments may

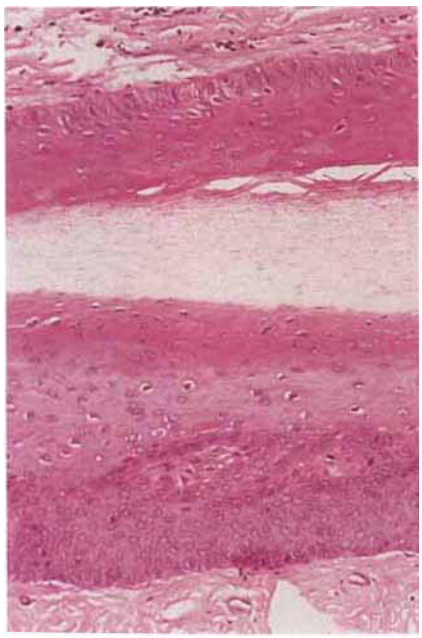


Fig. 1.6 A granular layer is absent from the germinal matrix (lower part) and the ventral aspect of the proximal nail fold (upper part).

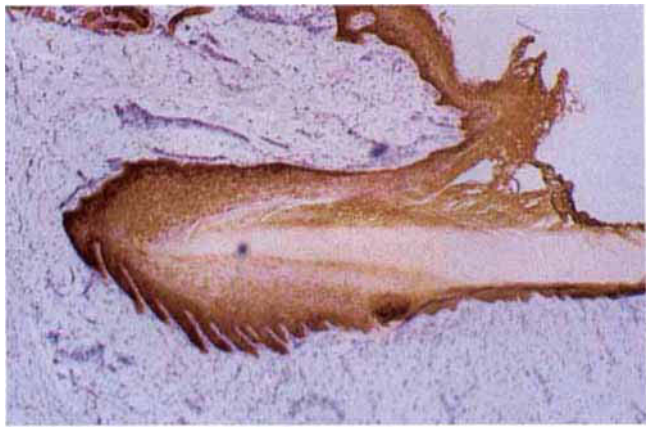


Fig. 1.7 Keratin stain of the nail apparatus delineating the epithelial structures of the matrix and proximal nail fold.

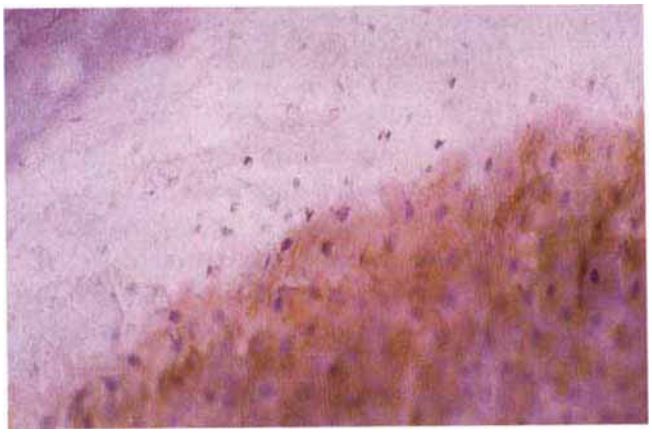


Fig. 1.8 Pertinax bodies can be seen as the nuclear remnants within the nail plate.

explain some forms of acquired and congenital malalignment (de Berker & Baran 1998).

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. Periodic acid Schiff 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 more pale 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 (1954) 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 (Higashi 1968; Higashi & Saito 1969; Tosti *et al.* 1994; de Berker *et al.* 1996a; Perrin *et al.* 1997). They are dendritic cells found in the epibasal layers and most prominent in the distal matrix (Tosti *et al.* 1994; de Berker *et al.* 1996a; Perrin *et al.* 1997). This point can 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.* (1996a) 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 is 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 (Tosti *et al.* 1994). In spite of these difficulties of interpretation, melanoma is a relatively rare cause of subungual pigmentation, although it is usually considered necessary to exclude it histologically, particularly in white adults (Tosti *et al.* 1994; Molina & Sanchez 1995).

Melanin in the nail plate is composed of granules derived from matrix melanocytes (Zaias 1963). 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 (Monash 1932; Leyden *et al.* 1972). The Japanese also have a high prevalence of longitudinal melanonychia, being present in 10–20% of adults (Kopf & Waldo 1980). In a study of 15 benign melanonychia 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 naevus (Higashi 1968). Longitudinal melanonychia in Caucasians is more sinister. Oropeza (1986) stated that a subungual pigmented lesion in this group has a higher chance of being malignant than of being benign.

There is only a thin layer of dermis dividing the matrix from the terminal phalanx. This has a rich vascular supply (see 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. (Hashimoto 1971a,b,c,d). 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 (Parent *et al.* 1985). The glycoprotein characteristics of cell membrane complexes isolated from nail plate may reflect the constituents of these granules (Allen *et al.* 1991).

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.

References

Achten, G. (1963) L'ongle normal et pathologique. *Dermatologica* **126**, 229–245.

Achten, G. & Wanet, J. (1973) Pathologie der nagel. In: *Spezielle Pathologische Anatomie*, Vol. 7 (eds W. Doerr, G. Seifert & E. Uehlinger), pp. 487–528. Springer Verlag, New York.

Achten, G., Andre, J. & Laporte, M. (1991) Nails in light and electron microscopy. *Seminars in Dermatology* **10**, 54–64.

Alkiewicz, J. & Pfister, R. (1976) *Atlas der Nagelkrankheiten*, p. 8. Schattauer-Verlag, Stuttgart.

Allen, A.K., Ellis, J. & Rivett, D.E. (1991) The presence of glycoproteins in the cell membrane complex of variety of keratin fibres. *Biochimica et Biophysica Acta* **1074**, 331–333.

Alvarez, R. & Zaias, N. (1967) A modified polyethylene glycol-pyroxylin embedding method specially suited for nails. *Journal of Investigative Dermatology* **49**, 409–410.

Baran, R. & Gioanni, T. (1969) Les dyschromies ungueales. *Hospital (Paris)* **57**, 101–107.

Baran, R. & Haneke, E. (1984) Diagnostik und Therapie der streifenformigen nagelpigmentierung. *Hautarzt* **35**, 359–365.

Bennett, J. (1976) Technique of biopsy of nails. *Dermatologic Surgery and Oncology* **2**, 325–326.

de Berker, D.A.R. & Baran, R. (1998) Acquired malalignment: a complication of lateral longitudinal biopsy. *Acta Dermato-Venereologica* **78**, 468–470.

de Berker, D., Dawber, R.P.R., Thody, A. & Graham, A. (1996a) Melanocytes are absent from normal nail bed: the basis of a clinical dictum. *British Journal of Dermatology* **134**, 564.

de Berker, D.A.R., MaWhinney, B. & Sviland, L. (1996b) Quantification of regional matrix nail production. *British Journal of Dermatology* **134**, 1083–1086.

Burrows, M.T. (1917) The significance of the lunula of the nail. *Anatomical Record* **12**, 161–166.

Burrows, M.T. (1919) The significance of the lunula of the nail. *Johns Hopkins Medical Journal* **18**, 357–361.

Cohen, P.R. (1996) The lunula. *Journal of the American Academy of Dermatology* **34**, 943–953.

Drapé, J.L., Wolfram-Gabel, R., Idy-Peretti, I. *et al.* (1996) The lunula: a magnetic resonance imaging approach to the subnail matrix area. *Journal of Investigative Dermatology* **106**, 1081–1085.

Guero, S., Guichard, S. & Fraitag, S.R. (1994) Ligamentary structure at the base of the nail. *Surgical and Radiological Anatomy* **16**, 47–52.

Ham, A.W. & Leeson, T.S. (eds) (1961) *Histology*, 4th edn. Pitman Medical, London.

Haneke, E. (1991) Fungal infections of the nail. *Seminars in Dermatology* **10**, 41–53.

Hashimoto, K. (1971a) The marginal band: a demonstration of the thickened cellular envelope of the human nail. *Archives of Dermatology* **103**, 387–393.

Hashimoto, K. (1971b) Ultrastructure of the human toenail. II. *Journal of Ultrastructural Research* **36**, 391–410.
Hashimoto, K. (1971c) Ultrastructure of the human toenail: I proximal nail matrix. *Journal of Investigative Dermatology* **56**, 235–246.
Hashimoto, K. (1971d) Ultrastructure of the human toenail. Cell migration, keratinization and formation of the intercellular cement. *Archiv für Dermatologische Forschung* **240**, 1–22.
Higashi, N. (1968) Melanocytes of nail matrix and nail pigmentation. *Archives of Dermatology* **97**, 570–574.
Higashi, N. & Saito, T. (1969) Horizontal distribution of the DOPA-positive melanocytes in the nail matrix. *Journal of Investigative Dermatology* **53**, 163–165.
Kopf, A.W. & Waldo, F. (1980) Melanonychia striata. *Australasian Journal of Dermatology* **21**, 59–70.
Lewin, K. (1965) The normal finger nail. *British Journal of Dermatology* **77**, 421–430.
Lewin, K., Dewitt, S. & Lawson, R. (1973) Softening techniques for nail biopsy. *Archives of Dermatology* **107**, 223–224.
Lewis, B.L. (1954) Microscopic studies of fetal and mature nail and the surrounding soft tissue. *Archives of Dermatological Syphilology* **70**, 732–744.
Leyden, J.J., Spot, D.A. & Goldsmith, H. (1972) Diffuse banded melanin pigmentation in nails. *Archives of Dermatology* **105**, 548–550.
Molina, D. & Sanchez, J.L. (1995) Pigmented longitudinal bands of the nail. A clinicopathologic study. *American Journal of Dermatopathology* **17**, 539–541.
Monash, S. (1932) Normal pigmentation in the nails of negroes. *Archives of Dermatology* **25**, 876–881.
Nakayama, Y., Iino, T., Uchida, A. *et al.* (1990) Vascularised free nail grafts nourished by arterial inflow from the venous system. *Plastic and Reconstructive Surgery* **85**, 239–245.
Oropeza, R. (1986) Melanomas of special sites. In: *Cancer of the Skin: Biology, Diagnosis, Management*, Vol. 2. pp. 974–987. Blackwell Scientific Publications, Oxford.
Parent, D., Achten, G. & Stouffs-Vanhoof, F. (1985) Ultrastructure of the normal human nail. *American Journal of Dermatopathology* **7**, 529–535.
Perrin, C., Michiels, J.F., Pisani, A. & Ortonne, J.P. (1997) Anatomic distribution of melanocytes in normal nail unit: an immunohistochemical investigation. *American Journal of Dermatopathology* **19**, 462–467.
Pessa, J.E., Tsai, T.M., Li, Y. & Kleinert, H.E. (1990) The repair of nail deformities with the non-vascularised nail bed graft. Indications and results. *Journal of Hand Surgery* **15A**, 466–470.
Suarez, S.M., Silvers, D.N. & Scher, R.K. (1991) Histologic evaluation of nail clippings for diagnosing onychomycosis. *Archives of Dermatology* **127**, 1517–1519.
Tosti, A., Cameli, N., Piraccini, B.M. *et al.* (1994) Characterization of nail matrix melanocytes with anti-PEP1, anti-PEP8, TMH-1, and HMB-45 antibodies. *Journal of the American Academy of Dermatology* **31** (2 Part 1), 193–196.
Wilkerson, M.G. & Wilkin, J.K. (1989) Red lunulae revisited: a clinical and histopathologic examination. *Journal of the American Academy of Dermatology* **20**, 453–457.
Wolfram-Gabel, R. & Sick, H. (1995) Vascular networks of the periphery of the fingernail. *Journal of Hand Surgery [Br]* **20B**, 488–492.
Zaias, N. (1963) Embryology of the human nail. *Archives of Dermatology* **87**, 37–53.

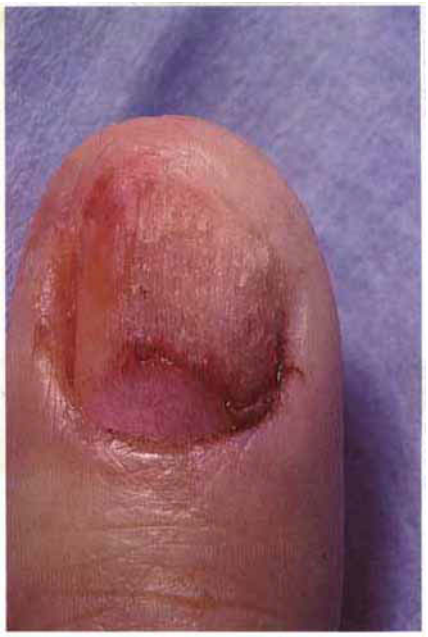


Fig. 1.9 The epidermis of the nail bed has longitudinal ridges visible after nail avulsion.

Fig. 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.

Zaias, N. (1967) The longitudinal nail biopsy. *Journal of Investigative Dermatology* **49**, 406–408.

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

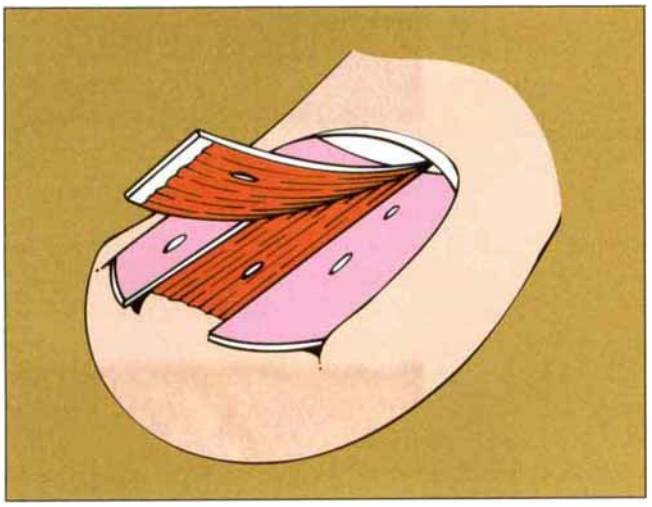


Fig. 1.11 The appearance of splinter haemorrhages. Haem from longitudinal nail bed vessels is deposited on the underside of the nail plate. This grows out in the shape of a splinter.

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

small vessels of the nail bed are orientated in the same axis. This is demonstrated by splinter haemorrhages (Figs 1.11 & 1.12), where haem 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 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 (Lewin 1965). Sweat ducts can be seen at the distal margin of the nail bed using *in vivo* magnification (Fig. 1.13) (Maricq 1967).

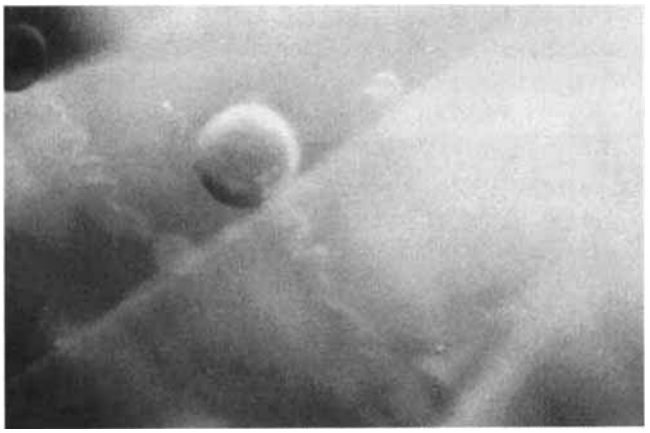


Fig. 1.13 Sweat pores in the distal nail bed (Maricq 1967).

The hyponychium lies between the distal ridge and the nail plate and represents a space as much as a surface. 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 below) or pachyonychia congenita (see below). In these instances, and in some elderly people, it can be thought of as the solenhorn described by Pinkus (1927).

Pterygium inversum unguis is a further condition characterized by changes in the distal nail bed and hyponychium (Caputo & Prandi 1973). There is tough, fibrotic tissue tethering the free edge of the nail plate to the underlying soft structures. It is found in both congenital (Odom *et al.* 1974) and acquired forms (Patterson 1977). The aetiology is not clear. Patterson proposed that it was a combination of a genetic predisposition and microvascular ischaemia.

The hyponychium and overhanging free nail provide a crevice. This 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 moulds (Rayan & Flournoy 1987). In 19 out of 20 patients *Staphylococcus epidermidis* was isolated, seven patients had an additional bacteria, eight had moulds and three had yeasts. These findings could have significance to both surgeons and patients.

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.* (1999) 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 (qv. 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 of 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 (de Berker *et al.* 2000; see 'Nail growth' below).

The proximal nail fold has significance in four main areas:

1 It may contribute to the generation of nail plate through a putative dorsal matrix on its ventral aspect.

2 It may influence the direction of growth of the nail plate by directing it obliquely over the nail bed.

3 Nail fold microvasculature can provide useful information in some pathological conditions.

4 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 'Vasculature' below) and dermatological diseases and the nail (see Chapter 5).

References

de Berker, D., Wojnarowska, F., Sviland, L. *et al.* (2000) Keratin expression in the normal nail unit: markers of regional differentiation. *British Journal of Dermatology* **142**, 89–96.

Caputo, R. & Prandi, G. (1973) Pterygium inversum unguis. *Archives of Dermatology* **108**, 817–818.

Dowsett, S.A., Archila, L., Segreto, V.A. *et al.* (1999) *Helicobacter pylori* infection in indigenous families of Central America: serostatus and oral and fingernail carriage. *Journal of Clinical Microbiology* **37**, 2456–2460.

Lewin, K. (1965) The normal finger nail. *British Journal of Dermatology* **77**, 421–430.

Maricq, H.R. (1967) Observation and photography of sweat ducts of the fingers *in vivo*. *Journal of Investigative Dermatology* **48**, 399–401.

Odom, R.B., Stein, K.M. & Maibach, H.I. (1974) Congenital, painful aberrant hyponychium. *Archives of Dermatology* **110**, 89–90.

Patterson, J.W. (1977) Pterygium inversum unguis-like changes in scleroderma. *Archives of Dermatology* **113**, 1429–1430.

Pinkus, F. (1927) In: *Handbuch der Haut und Geschlechtskrankeiten* (eds J.J. Jadassohn), pp. 267–289. Springer, Berlin.

Rayan, G.M. & Flournoy, D.J. (1987) Microbiologic flora of human fingernails. *Journal of Hand Surgery* **12A**, 605–607.

Immunohistochemistry of periungual tissues

Keratins

The most extensive immunohistological investigations of the nail unit have utilized keratin antibodies. The nail plate (Lynch *et al.* 1986; Heid *et al.* 1988), human embryonic nail unit (Heid *et al.* 1988; Moll *et al.* 1988; Lacour *et al.* 1991), accessory digit nail unit (de Berker *et al.* 1992, 1999; Sinclair *et al.* 1994) and adult nail unit (Haneke 1990; Lacour *et al.* 1991; de Berker *et al.* 2000) have all been examined.

Using the antibody 34βE12, Haneke (1990) demonstrated positivity of the basal two-thirds of the matrix. This antibody detects keratins 5, 10 and 11, indicating the presence of one or more of these at this location. Using monospecific antibodies, de Berker *et al.* (1992, 2000) 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' above and 'Nail plate' below). Keratins 1 and 10 are 'soft' epithelial keratins found suprabasally in normal skin (Purkis *et al.* 1990) and characteristic of cornification with terminal keratinocyte differentiation. Their absence from normal nail bed is reversed in disease where nail bed cornificaton is often seen, alongside development of a granular layer and expression of keratins 1 and 10 (de Berker *et al.* 1995). 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 (Fanti *et al.* 1994).

Ha-1 is found in the matrix. Ha-1 is a 'hard' keratin. Keratin 7 has been found at other sites in the nail unit and hair follicle, whereas Ha-1, detected by the monoclonal anti-keratin antibody LH TRIC 1, is limited to the matrix of the nail (Fig. 1.15) and the germinal matrix of the hair follicle (de Berker *et al.* 1992; Westgate *et al.* 1997). Keratin 19 is probably not found in the adult matrix (Moll *et al.* 1988; Haneke 1990; de Berker *et al.* 2000). However, Moll *et al.* (1988) did detect keratin 19 at this site in 15-week embryo nail units. Keratin 19 is also

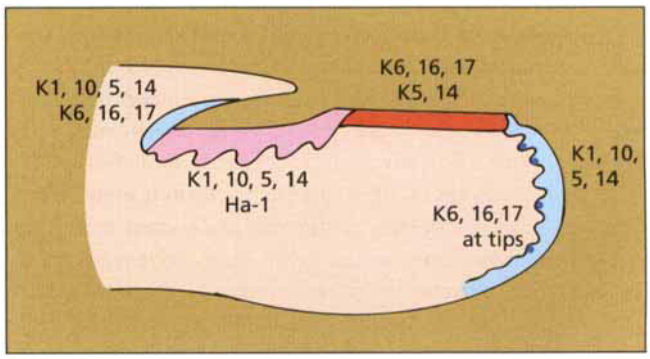


Fig. 1.14 Distribution of keratins in the human periungual and subungual tissues.

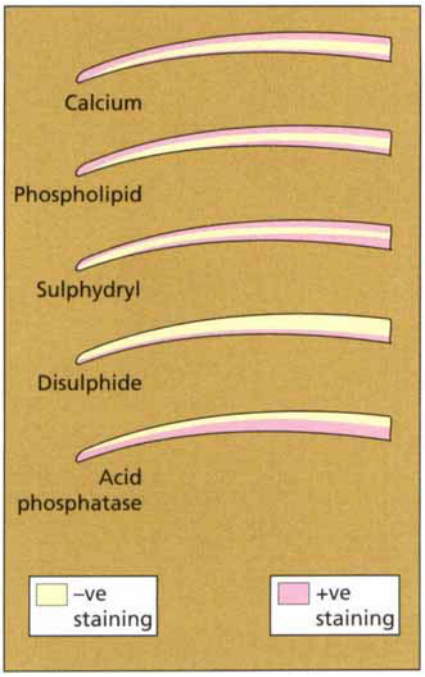


Fig. 1.15 The histochemistry of the human nail plate (after Jarrett & Spearman 1966). Nail plates were sectioned and stained. Index, calcium; middle, phospholipid; ring, sulphydryl; little, disulphide; thumb, acid phosphatase.

found in the outer root sheath of the hair follicle and lingual papilla (Heid *et al.* 1988).

The co-localization of hard and soft keratins within single cells of the matrix has been observed by several workers in bovine hoof (Kitahara & Ogawa 1993) and human nail (Kitahara & Ogawa 1994, 1997; de Berker *et al.* 2000), 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 (Kitahara & Ogawa 1994). Culture of human nail matrix confirms the persistence of hard keratin expression (Picardo *et al.* 1994; Nagae *et al.* 1995).

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.* (1991) in adult nail matrix and de Berker *et al.* (2000) in infant accessory digits. Some workers have failed to detect Merkel cells and whilst 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 (Boot *et al.* 1992).

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 (de Berker *et al.* 2000). 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 thicknened 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 (Munro *et al.* 1994; McLean *et al.* 1995). 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 (Corden & McLean 1996; Hohl 1997; Covello *et al.* 1998). Mutations in the gene coding for K6b produce a phenotype seen with K17 gene mutations (Smith *et al.* 1998). Mutations in the K6a (Bowden *et al.* 1995) and K16 (McLean *et al.* 1995) 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 (Swensson *et al.* 1998). 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.

Non-keratin immunohistochemistry

Haneke (1990) 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 (Baden 1994) and weakly detected in the suprabasal layers. Pancornulin and sciellin are also detected in the matrix (Baden 1994) 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 (Haneke 1990).

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 (Heid *et al.* 1988). However, Manabe and O'Guin (1994) 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 (1997) have identified filaggrin in the human nail in the same location and O'Keefe *et al.* (1993) 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 act to stabilize the intermediate filament network of K6 and K16, which are normally associated with unstable or hyperproliferative states.

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 (Lavker *et al.* 1998).

The basement membrane zone of the entire nail unit has been examined, employing a wide range of monoclonal and

Table 1.2 Analysis of nail unit basement membrane zone using monoclonal and polyclonal antibodies.

	Digit 1					Digit 2					
	Nail apparatus					Nail apparatus				Digit 3	
	Fold	Matrix	Bed	HN	Proximal phalangeal skin	Fold	Matrix	Bed	HN	Split skin	Intact skin
Mono. Ab											
LH7:2	+	+	+	+	+	+	+	+	+	epi	+
L3d	+	+	+	+	+	+	+	+	+	epi	+
Col IV	+	+	+	+	+	+	+	+	+	epi	+
GB3	+	+	+	+	+	+	+	+	+	epi	+
LH24	+	+	+	+	+	+	+	+	+	epi	+
LH39	+	+	+	+	+	+	+	+	+	epi	+
GDA	+	+	+	+	+	+	+	+	+	epi	+
Tenascin	+	+	+	+	+	+	+	+	+	epi	+
a6	+	+	+	+	+	+	+	+	+	epi	+
G71	+	+	+	+	+	+	+	+	+	epi	+
Poly. Ab											
Fibronectin	–	–	–	–	–	–	–	–	–	–	–
Laminin	+	+	+	+	+	+	+	+	+	derm	+
BP 220 kDa	+	+	+	+	+	+	+	+	+	epi	+
EBA 250 kDa	+	+	+	+	+	+	+	+	+	derm	+
LAD 285 kDa	+	+	+	+	+	+	+	+	+	epi	+
LAD ? kDa	+	+	+	+	+	+	+	+	+	derm	+

HN, Hyponychium

polyclonal antibodies (Sinclair *et al.* 1994). Collagen VII, fibronectin, chondroitin sulphate and tenascin were among the antigens detected. 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 1.2).

References

Baden, H. (1994) Common transglutaminase substrates shared by hair, epidermis and nail and their function. *Journal of Dermatologic Science* 7 (Suppl.), S20–S26.

de Berker, D., Leigh, I. & Wojnarowska, F. (1992) Patterns of keratin expression in the nail unit—an indicator of regional matrix differentiation. *British Journal of Dermatology* **127**, 423.

de Berker, D., Sviland, L. & Angus, B.A. (1995) Suprabasal keratin expression in the nail bed: a marker of dystrophic nail differentiation. *British Journal of Dermatology* **133** (Suppl. 45), 16.

de Berker, D., Wojnarowska, F., Sviland, L. *et al.* (2000) Keratin expression in the normal nail unit: markers of regional differentiation. *British Journal of Dermatology* **142**, 89–96.

Boot, P.M., Rowden, G. & Walsh, N. (1992) The distribution of Merkel cells in human fetal and adult skin. *American Journal of Dermatopathology* **14**, 391–396.

Bowden, P.E., Haley, J.L., Kansky, A. *et al.* (1995) Mutation of a type II keratin gene (K6a) in pachyonychia congenita. *Nature Genetics* **10**, 363–365.

Corden, L.D. & McLean, W.H. (1996) Human keratin disease: hereditary fragility of specific epithelial tissues. *Experimental Dermatology* 5, 297–307.

Covello, S.P., Smith, F.J.D., Sillevis Smitt, J.H. *et al.* (1998) Keratin 17 mutations cause either steatocystoma multiplex or pachyonychia congenita type 2. *British Journal of Dermatology* **139**, 475–480.

Fanti, P.A., Tosti, A., Cameli, N. & Varotti, C. (1994) Nail matrix hypergranulosis. *American Journal of Dermatopathology* **16**, 607–610.

Hancke, E. (1990) The human nail matrix—flow cytometric and immunohistochemical studies. In: *Clinical Dermatology in the Year 2000*. Book of Abstracts, May.

Heid, W.H., Moll, I. & Franke, W.W. (1988) Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. II. Concomitant and mutually exclusive synthesis of trichocytic and epithelial cytokeratins in diverse human and bovine tissues. *Differentiation* **37**, 215–230.

Hohl, D. (1997) Steatocystoma multiplex and oligosymptomatic pachyonychia congenita of the Jackson–Lawler type. *Dermatology* **195**, 86–88.

Kitahara, T. & Ogawa, H. (1993) Coexpression of keratins characteristic of skin and nail differentiation in nail cells. *Journal of Investigative Dermatology* **100**, 171–175.

Kitahara, T. & Ogawa, H. (1994) Variation of differentiation in nail and bovine hoof cells. *Journal of Investigative Dermatology* **102**, 725–729.

Kitahara, T. & Ogawa, H. (1997) Cellular features of differentiation in the nail. *Microscopic Research Techniques* **38**, 436–442.

Lacour, J.P., Dubois, D., Pisani, A. & Ortonne, J.P. (1991) Anatomical mapping of Merkel cells in normal human adult epidermis. *British Journal of Dermatology* **125**, 535–542.

Lavker, R.M., Risse, B., Brown, H. *et al.* (1998) Localisation of plasminogen activator inhibitor Type 2 (PAI-2) in hair and nail: implications for terminal differentiation. *Journal of Investigative Dermatology* **110**, 917–922.

Lynch, M.H., O'Guin, W.M., Hardy, C. *et al.* (1986) Acid and basic hair/nail ('hard') keratins: their co-localisation in upper corticle and cuticle cells of the human hair follicle and their relationship to 'soft' keratins. *Journal of Cell Biology* **103**, 2593–2606.

McLean, W.H.I., Rugg, E.L., Luny, D.P. *et al.* (1995) Keratin 16 and 17 mutations cause pachyonychia congenita. *Nature Genetics* **9**, 273–278.

Manabe, M. & O'Guin, W.M. (1994) Existence of trichohyalin-keratohyalin hybrid granules: co-localisation of 2 major intermediate filament-associated proteins in non-follicular epithelia. *Differentiation* **58**, 65–75.

Moll, I., Heid, H.W., Franke, W.W. & Moll, R. (1988) Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. *Differentiation* **39**, 167–184.

Munro, C.S., Carter, S., Bryce, S. *et al.* (1994) A gene for pachyonychia congenita is closely linked with the gene cluster on 17q12-q21. *Journal of Medical Genetics* **31**, 675–678.

Nagae, H., Nakanishi, H., Urano, Y. & Arase, S. (1995) Serial cultivation of human nail matrix cells under serum-free conditions. *Journal of Dermatology* **22**, 560–566.

O'Keefe, E.J., Hamilton, E.H., Lee, S.C. & Steiner, P. (1993) Trichohyalin: a structural protein of hair, tongue, nail and epidermis. *Journal of Investigative Dermatology* **101**, 65s–71s.

Picardo, M., Tosti, A., Marchese, C. *et al.* (1994) Characterisation of cultured nail matrix cells. *Journal of the American Academy of Dermatology* **30**, 434–440.

Purkis, P.E., Steel, J.B., Mackenzie, I.C. *et al.* (1990) Antibody markers of basal cells in complex epithelia. *Journal of Cellular Science* **97**, 39–50.

Sinclair, R.D., Wojnarowska, F. & Dawber, R.P.R. (1994) The basement membrane zone of the nail. *British Journal of Dermatology* **131**, 499–505.

Smith, F.J., Corden, L.D., Rugg, E.L. *et al.* (1997) Missense mutations in keratin 17 cause either pachyonychia congenita type 2 or a phenotype resembling steatocystoma multiplex. *Journal of Investigative Dermatology* **108**, 220–223.

Smith, F.J., Jonkman, M.F., van Goor, H. *et al.* (1998) A mutation in human keratin K6b produces a phenocopy of the K17 disorder pachyonychia congenita type 2. *Human Molecular Genetics* **7**, 1143–1148.

Swensson, O., Langbein, L., McMillan, J.R. *et al.* (1998) Specialised keratin expression pattern in human ridged skin as an adaptation to high physical stress. *British Journal of Dermatology* **139**, 767–775.

Wesrgate, G.E., Tidman, N., de Berker, D. *et al.* (1997) Characterization of LH Tric-1, a new monospecific monoclonal antibody to the trichocyte keratin Ha1. *British Journal of Dermatology* **137**, 24–30.

Nail plate

The nail plate is composed of compacted keratinized epithelial cells. It covers the nail bed and intermediate matrix. It 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 half way around the terminal phalanx. This provides strength appropriate to the foot (Fig. 1.16).

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 (Diaz *et al.* 1990). The ventral surface also has longitudinal ridges that correspond to complementary ridges on the upper aspect of the nail bed (see 'Nail bed' 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 (Apolinar & Rowe 1980), as may blood groups from fragments of nail plate (Garg 1983).

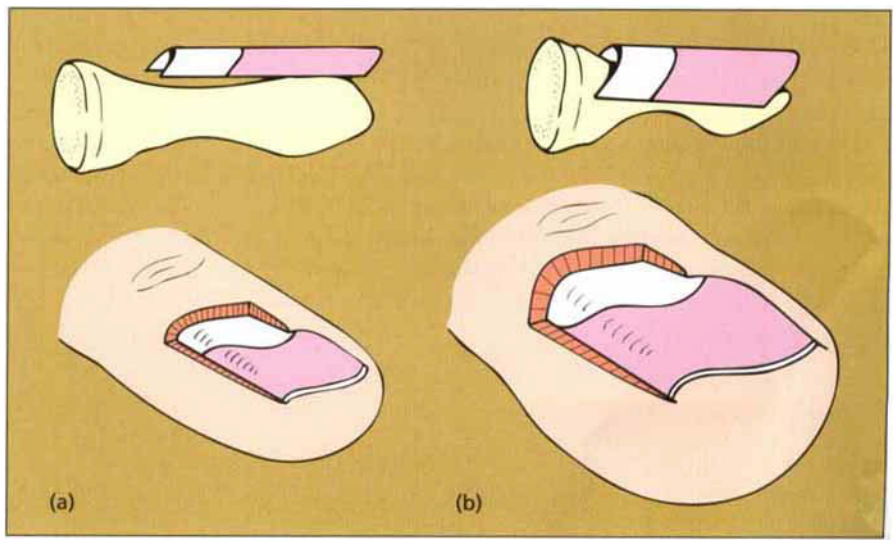


Fig. 1.16 Nail plate association with soft tissue and bone in the finger and toe. (a) In the finger, the nail plate has modest transverse curvature and shallow association with soft tissues. (b) In the great toe, the nail plate has more marked transverse curvature and deep soft tissue association. This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

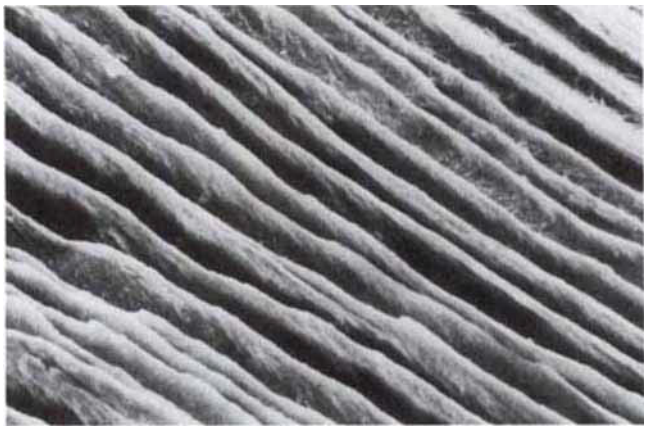


Fig. 1.17 Scanning electron micrograph of the nail bed demonstrating longitudinal ridges.

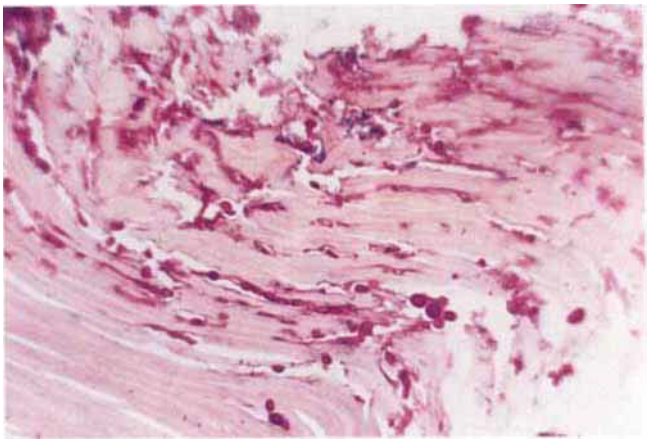


Fig. 1.19 Fungal spores and hyphae can be seen in the stained section of a nail clipping taken from a nail with onychomycosis.

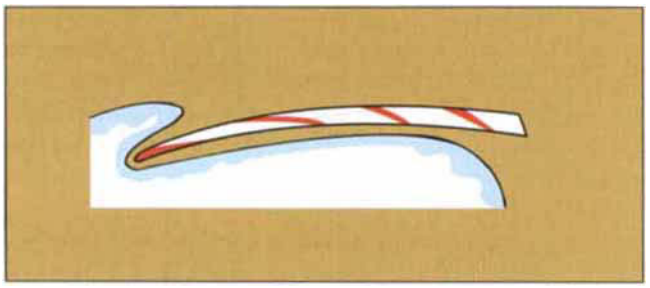


Fig. 1.18 Shaded areas represent 7-day periods of nail growth, separated by 1 month with transition of nail from horizontal to oblique axis over 4 months.

The nail plate gains thickness and density as it grows distally (Johnson *et al.* 1991) according to analysis of surgical specimens. *In vivo* ultrasound suggests that there may be an 8.8% reduction in thickness distally (Finlay *et al.* 1987). 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 (Samman & White 1964), vascular supply, subungual hyperkeratosis and drugs also influence thickness.

The tendency to describe a dorsal, intermediate and ventral matrix, has generated description of corresponding layers of the nail plate deriving from the different matrix zones. Whether or not the three demarcations of the nail matrix exist, it is important to recognize the basic principle that proximal regions of matrix produce dorsal nail plate and distal matrix produces ventral nail plate.

Light microscopy

Lewis (1954) described a silver stain that delineates the nail plate zones. Three regions of nail plate have been histochemically defined (Jarrett & Spearman 1966) (Fig. 1.15). The dorsal plate has a relatively high calcium, phospholipid and sulphydryl 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 disulphide bonds, low content of bound sulphydryl groups, phospholipid and calcium. Controversy allows that the ventral nail plate may be a variable entity (Samman 1961). Jarrett and Spearman (1966) 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 sulphydryl groups it is the same as the dorsal nail plate. It shares a high acid phosphatase and frequency of disulphide bonds with the intermediate nail plate.

Ultrasound examination of *in vivo* and avulsed nail plates suggests that it has the physical characteristics of a bilamellar structure (Jemec & Serup 1989). 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.

In clinical practice, histology of the nail plate may be useful in the identification of fungal infections in culture-negative specimens (Haneke 1991; Suarez *et al.* 1991) (Fig. 1.19). It may also be used to identify the dorso-ventral 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 (Baran & Kechijian 1989; Dawber & Colver 1991).

Germann *et al.* (1980) utilized a form of tape-stripping in conjunction with light microscopy to examine dorsal nail plate corneocyte morphology in disease and health. She found that conditions of rapid nail growth (psoriasis and infancy) resulted in smaller cell size. Sonnex *et al.* (1991) describes the histology of transverse white lines in the nail.

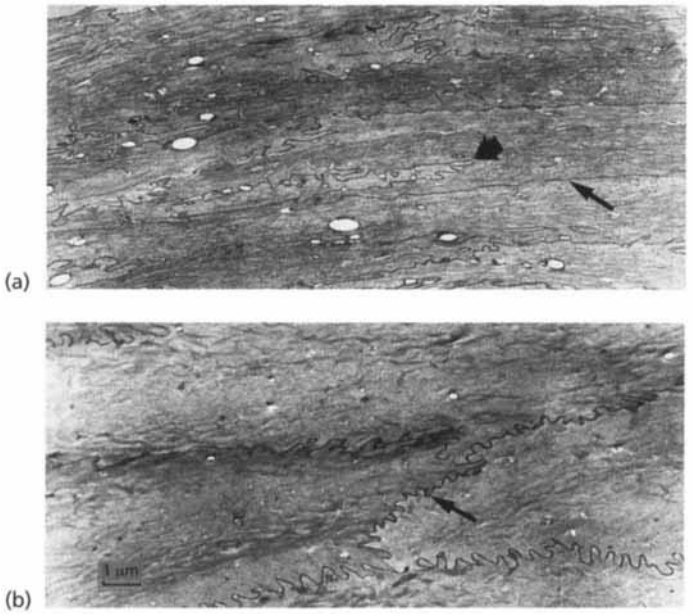


Fig. 1.20 (a) Transmission electron micrograph of the upper part of the nail plate. The corneocytes are flattened and joined laterally by infrequent deep interdigitations (broad arrow). (b) The cell membranes between adjacent cell layers are discretely indented and in parts without invaginations—Thiery's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

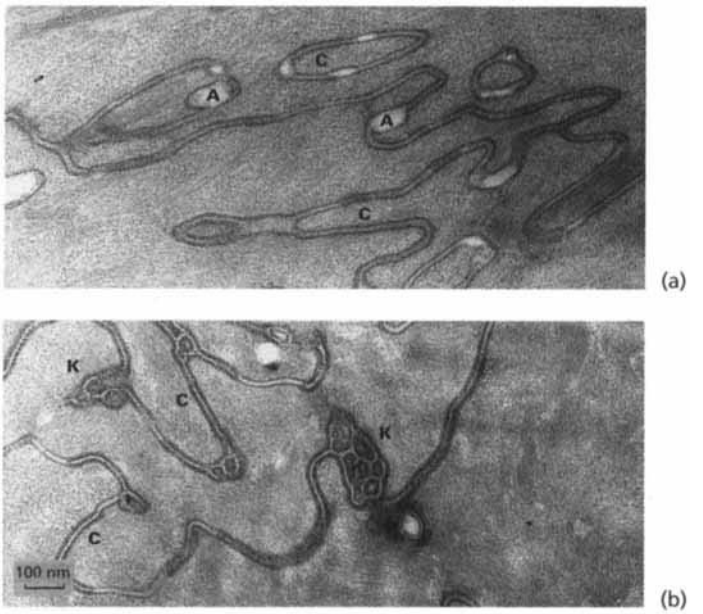


Fig. 1.21 (a) Upper part of the nail plate showing ampullar dilatations (A). (b) Lower part of the nail plate showing anchoring knots (K). The only cell-to cell coupling observed (C) is a desmosome. (Courtesy of Professor G. Achten.)

Electron microscopy

Scanning electron microscopy has added to our understanding of onychoschizia (Shelley & Shelley 1984; Wallis *et al.* 1991) as well as basic nail plate structure (Forslind & Thyresson 1975; Dawber 1980). 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.

Transmission electron microscopy has been used to identify the relationship between the corneocytes of the nail plate (Parent *et al.* 1985). 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 ($34 \times 60 \times 2.2$ μm) aspect are half as thick as ventral cells ($40 \times 50 \times 5.5$ μm), with a gradation of sizes in between. In the dorsal nail plate, large intercellular spaces are present corresponding to ampullar dilatations (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

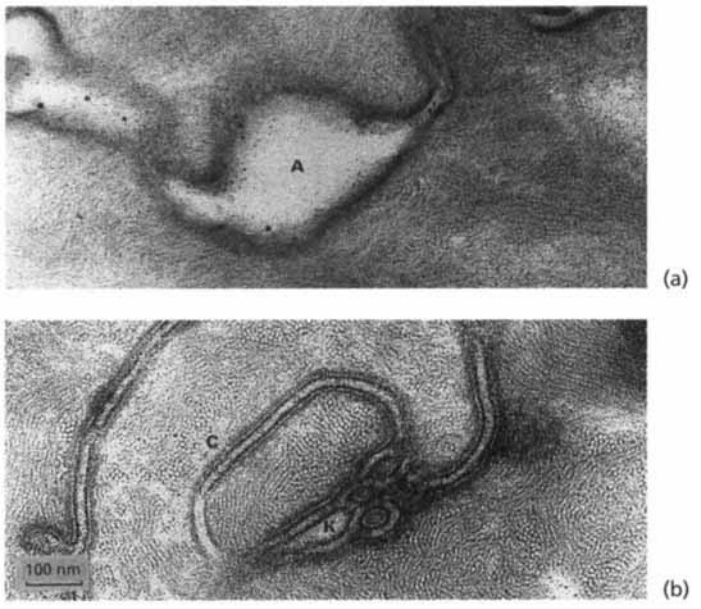


Fig. 1.22 Upper part of the nail plate as in Fig. 1.20, in greater detail. (Courtesy of Professor G. Achten.)

invaginations (Fig. 1.20). In the deeper parts of the nail plate the interdigitations are more numerous, but more shallow (Fig. 1.20). No tight gap junctions are seen in either of the major nail layers in this series (Parent *et al.* 1985), although they were identified previously by Forslind and Thyresson (1975). The intercellular material is homogeneous and separated from

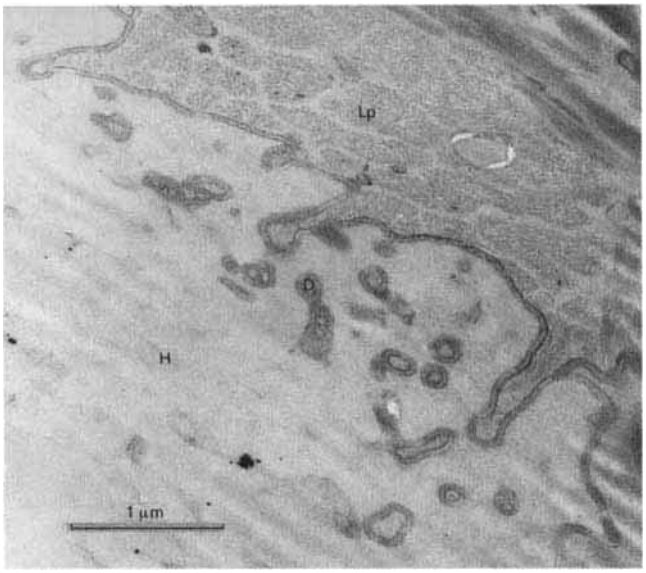


Fig. 1.23 Corneocytes of the lowest part of the nail plate (Lp) sending out numerous digitations (D) penetrating the hyponychial nail bed cells (H). (Courtesy of Professor G. Achten.)

the cell membrane by two thin electron-dense lines. The space between the cell membranes varies from 2.5 nm to 3.5 nm (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).

Cells of the hyponychium are distinguished from the nail plate on the basis of morphology, staining affinities and size.

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 (Hashimoto 1971a,b; Caputo *et al.* 1982). Other staining techniques suggest that the single type of intercellular bond described by Parent *et al.* (1985) may be a spot desmosome (Arnn & Stoehelin 1981).

References

Apolinar, E. & Rowe, W.F. (1980) Examination of human fingernail ridges by means of polarized light. *Journal of Forensic Science CA* 25, 154–161.

Arnn, Y. & Stoehelin, L.A. (1981) The structure and function of spot desmosomes. *International Journal of Dermatology* 20, 331–339.

Baran, R. & Kechijian, P. (1989) Longitudinal melanonychia diagnosis and management. *Journal of the American Academy of Dermatology* 21, 1165–1175.

Caputo, R., Gasparini, G. & Contini, D. (1982) A freeze-fracture study of the human nail plate. *Archives of Dermatological Research* 272, 117–125.

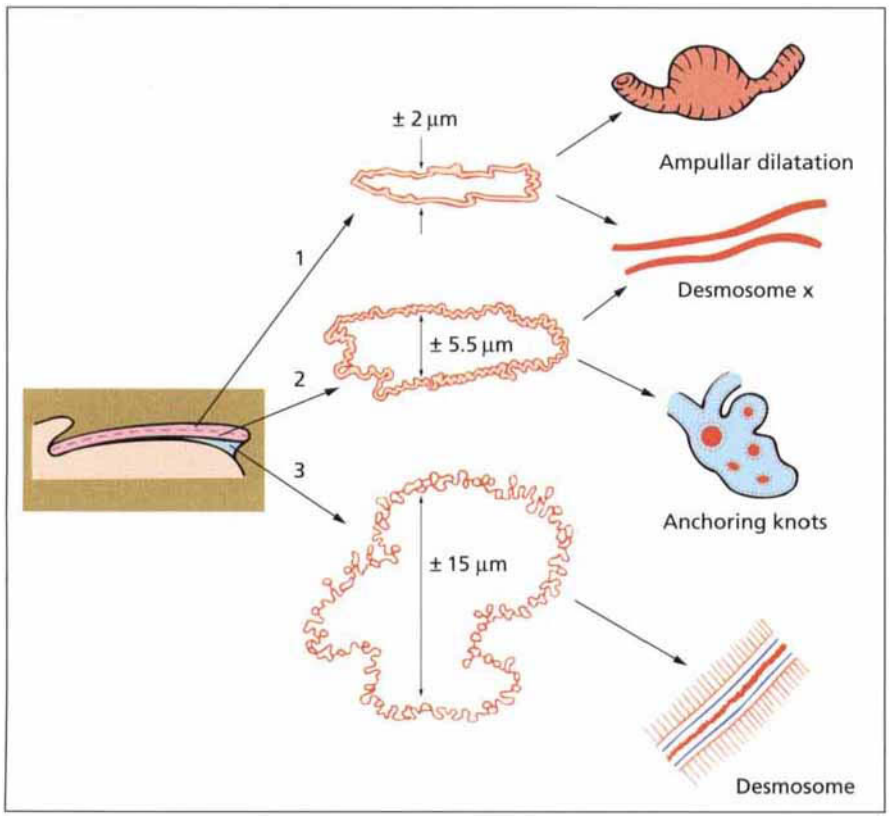


Fig. 1.24 Intercellular junctions of the three parts of the nail: 1, upper plate; 2, lower plate; 3, hyponychial ventral nail with desmosome as seen by Thiery's technique. (Courtesy of Professor G. Achten.)

Dawber, R.P.R. (1980) The ultrastructure and growth of human nails. *Archives of Dermatological Research* **269**, 197–204.

Dawber, R.P.R. & Colver, G.B. (1991) The spectrum of malignant melanoma of the nail apparatus. *Seminars in Dermatology* **10**, 82–87.

Diaz, A.A., Boehm, A.F. & Rowe, W.F. (1990) Comparison of fingernail ridge patterns of monozygotic twins. *Journal of Forensic Science CA* **35**, 97–102.

Finlay, A.Y., Moseley, H. & Duggan, T.C. (1987) Ultrasound transmission time: an *in vivo* guide to nail thickness. *British Journal of Dermatology* **117**, 765–770.

Forslind, B. & Thyresson, N. (1975) On the structures of the normal nail. *Archiv für Dermatologische Forschung* **251**, 199–204.

Garg, R.K. (1983) Determination of ABO (H) blood group substances from finger and toenails. *Zeitschrift für Rechtsmedizin* **91**, 17–19.

Germann, H., Barran, W. & Plewig, G. (1980) Morphology of corneocytes from human nail plates. *Journal of Investigative Dermatology* **74**, 115–118.

Haneke, E. (1991) Fungal infections of the nail. *Seminars in Dermatology* **10**, 41–53.

Hashimoto, K. (1971a) The marginal band: a demonstration of the thickened cellular envelope of the human nail. *Archives of Dermatology* **103**, 387–393.

Hashimoto, K. (1971b) Ultrastructure of the human toenail. II. *Journal of Ultrastructural Research* **36**, 391–410.

Jarrett, A. & Spearman, R.I.C. (1966) The histochemistry of the human nail. *Archives of Dermatology* **94**, 652–657.

Jemec, G.B.E. & Serup, J. (1989) Ultrasound structure of the human nail plate. *Archives of Dermatology* **125**, 643–646.

Johnson, M., Comaish, J.S. & Shuster, S. (1991) Nail is produced by the normal nail bed: a controversy resolved. *British Journal of Dermatology* **125**, 27–29.

Lewis, B.L. (1954) Microscopic studies of fetal and mature nail and surrounding soft tissue. *Archives of Dermatology and Syphilis* **70**, 732–744.

Parent, D., Achten, G. & Stouffs-Vamhoof, F. (1985) Ultrastructure of the normal human nail. *American Journal of Dermatopathology* **7**, 529–535.

Samman, P.D. (1961) The ventral nail. *Archives of Dermatology* **84**, 1030–1033.

Samman, P.D. & White, W.F. (1964) The 'yellow nail' syndrome. *British Journal of Dermatology* **76**, 153–157.

Shelley, W.B. & Shelley, D. (1984) Onychschizia: scanning electron microscopy. *Journal of the American Academy of Dermatology* **10**, 623–627.

Sonnex, T.S., Griffiths, W.A.D. & Nicol, W.J. (1991) The nature and significance of the transverse white band of human nails. *Seminars in Dermatology* **10**, 12–16.

Suarez, S.M., Silvers, D.N., Scher, R.K. *et al.* (1991) Histologic evaluation of nail clippings for diagnosing onychomycosis. *Archives of Dermatology* **127**, 1517–1519.

Wallis, M.S., Bowen, W.R. & Guin, J.D. (1991) Pathogenesis of onychoschizia (lamellar dystrophy). *Journal of the American Academy of Dermatology* **24**, 44–48.

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 (Warwick & Williams 1973).

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 (Smith *et al.* 1991a). 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.

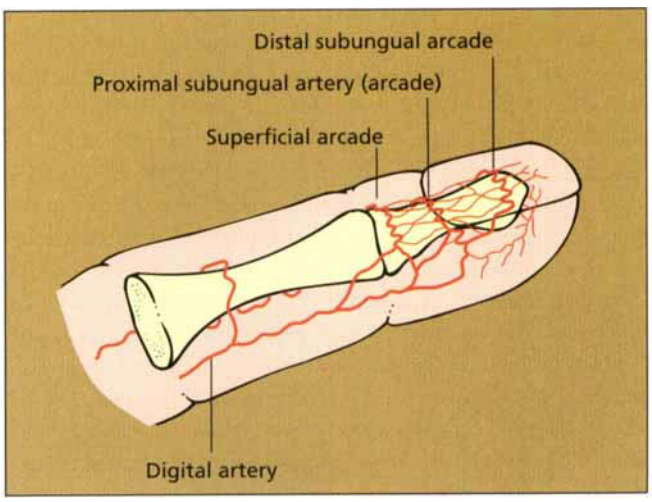


Fig. 1.25 Arterial supply of the distal finger.

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. A helpful study on adults and fetuses was performed by Flint (1955) and more recently by Wolfram-Gabel and Sick (1995).

The tortuosity of the main vessels in the finger is a notable feature. Vessels may turn through 270° and resemble a coiled spring (Smith *et al.* 1991a). 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. These 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 (Smith *et al.* 1991b).

Although the arterial supply to the nail unit is substantial, the matrix will tolerate only limited trauma before scarring (Zaias 1967). 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 (Pessa *et al.* 1990). This is in the presence of otherwise adequate local blood supply at sites of previous trauma. Toenail matrix grafts can be made successful if they are transplanted with associated soft tissue and a venous pedicle (Nakayama *et al.* 1990). 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.* (1955) 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 (1962) reviewed the nail dystrophies of 41 patients with features of peripheral vascular disease. In this uncontrolled study, he observed that onycholysis, Beau's lines, thin brittle nails and yellow discoloration were all attributable to ischaemia in the absence of other causes. It has also been suggested that congenital onychodysplasias may result from digital ischaemia *in utero* (Kitayama & Tsukada 1983). Immobilization might be associated with diminished local blood supply and has been noted to reduce nail growth (Dawber 1981). Conversely, the increased growth associated with arteriovenous shunts may reflect the role of greater blood flow (Orentreich *et al.* 1979). Clubbing constitutes a change in both the nail and nail bed. It is believed that it arises secondary to neurovascular pathology. Post-mortem studies suggest that it is due to increased blood flow with vasodilatation rather than vessel hyperplasia (Currie & Gallagher 1988).

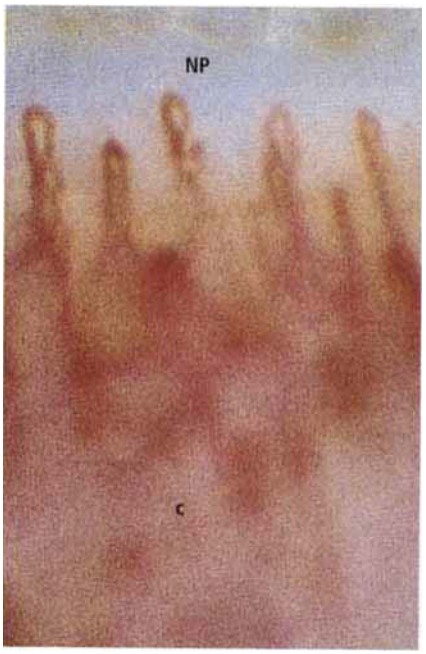


Fig. 1.26 Capillary loops visible in the proximal nail fold. NP, nail plate; c, cuticle.

Nail fold vessels

The nail fold capillary network (Gilje *et al.* 1974) is seen easily with a ×4 magnifying lens, dermatoscope or an ophthalmoscope. With the latter it should be set at +40 and the lens held very close to a drop of oil on the nail fold. It 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) (Ryan 1973). The venous arm is more dilated and tortuous than the arterial arm. There is a wide range of morphologies within the normal population (Davies & Landau 1966). Features in some disorders may be sufficiently gross to be useful without magnification; erythema and haemorrhages being the most obvious.

In the first 10 years of life, the pattern of nail fold vessels is immature (Basserga *et al.* 1996). Microscopy of small vessels in adulthood can be of diagnostic value in some connective tissue diseases (Buchanan & Humpston 1968; Granier *et al.* 1986). Pathological features include venous plexus visibility, density of capillary population, avascular fields, haemorrhages, 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 (Studer *et al.* 1991). 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 (Ohtsuka *et al.* 1998). The mechanism of dilated vessel evolution may in part arise from impaired fibrinolysis, macroglobulinaemia and cryoglobulinaemia (Ryan 1973).

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 (Schumann *et al.* 1996). Nail fold vessel changes may also occur in psoriasis and appear to correlate with nail pitting, onycholyis and periungual psoriatic plaques (Ohtsuka *et al.* 1994). 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 video-microscopy. It has revealed a greater density of capillaries in the toe nail fold, but with a reduced rate of flow (Richardson & Schwartz 1984). The exact pattern of an individual's nail fold vessels can be used as an identifying characteristic (Krylova & Soboleva 1995).

Intravenous bolus doses of Na-fluorescein dye have been followed through nail fold microscopy (Bollinger *et al.* 1979). 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 (Grassi *et al.* 1989).

Nail fold microscopy has been used for the investigation of Raynaud's phenomenon (Mahler *et al.* 1987). It is possible to assess vascular toxicity affecting nail fold vessels following chemotherapy, using the same method (Hansen *et al.* 1990).

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 (Schnitzler *et al.* 1976). Amyloid deposits, subintimal hyalinosis and severe dermal fibrosis are cited as useful supplementary information yielded by biopsy.

Glomus bodies

The term 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 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' (Masson 1937). They are particularly important in the preservation of blood supply to the peripheries in cold conditions.

Nail bed infarcts and splinter haemorrhage can be seen in a range of systemic disorders and local mechanical and dermatological abnormalities (see below).

Nerve supply (Fig. 1.27)

The periungual soft tissues are innervated by dorsal branches of paired digital nerves. Wilgis and Maxwell (1979) 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 (Zook 1988). Winkelmann (1960) showed many nerve endings adjacent to the epithelial surface, mainly in the nail folds.

References

Basserga, M., Bonacci, E., Cammarota, M.G. & d'Amico, N. (1996) La cappilaroscopia periungueale nello studio del microcircolo in età pediatrica. *Minerva Pediatrics* **48**, 297–301.

Bollinger, A., Jäger, K., Roten, A., Timeus, C. & Mahler, F. (1979) Diffusion, pericapillary distribution and clearance of Na-fluorescein in the human nail fold. *Pflügers Archives* **382**, 137–143.

Buchanan, I. & Humpston, D.J. (1968) Nail fold capillaries in connective tissue disorders. *Lancet* **41**, 845–847.

Currie, A.E. & Gallagher, P.J. (1988) The pathology of clubbing: vascular changes in the nail bed. *British Journal of the Chest* **82**, 382–385.

Davies, E. & Landau, J. (eds) (1966) *Clinical Capillary Microscopy*. C.C. Thomas, Springfield, IL.

Dawber, R.P.R. (1981) The effect of immobilization upon finger nail growth. *Clinical and Experimental Dermatology* **6**, 533–535.

Flint, M.H. (1955) Some observations on the vascular supply of the nail bed and terminal segments of the finger. *British Journal of Plastic Surgery* **8**, 186–195.

Gilje, O., Kierland, R. & Baldes, E.J. (1974) Capillary microscopy in the diagnosis of dermatologic diseases. *Journal of Investigative Dermatology* **22**, 199–206.

Granier, F., Vayssairat, M., Priollet, P. *et al.* (1986) Nail fold capillary microsopy in mixed connective tissue disease. *Arthritis and Rheumatism* **29**, 189–195.

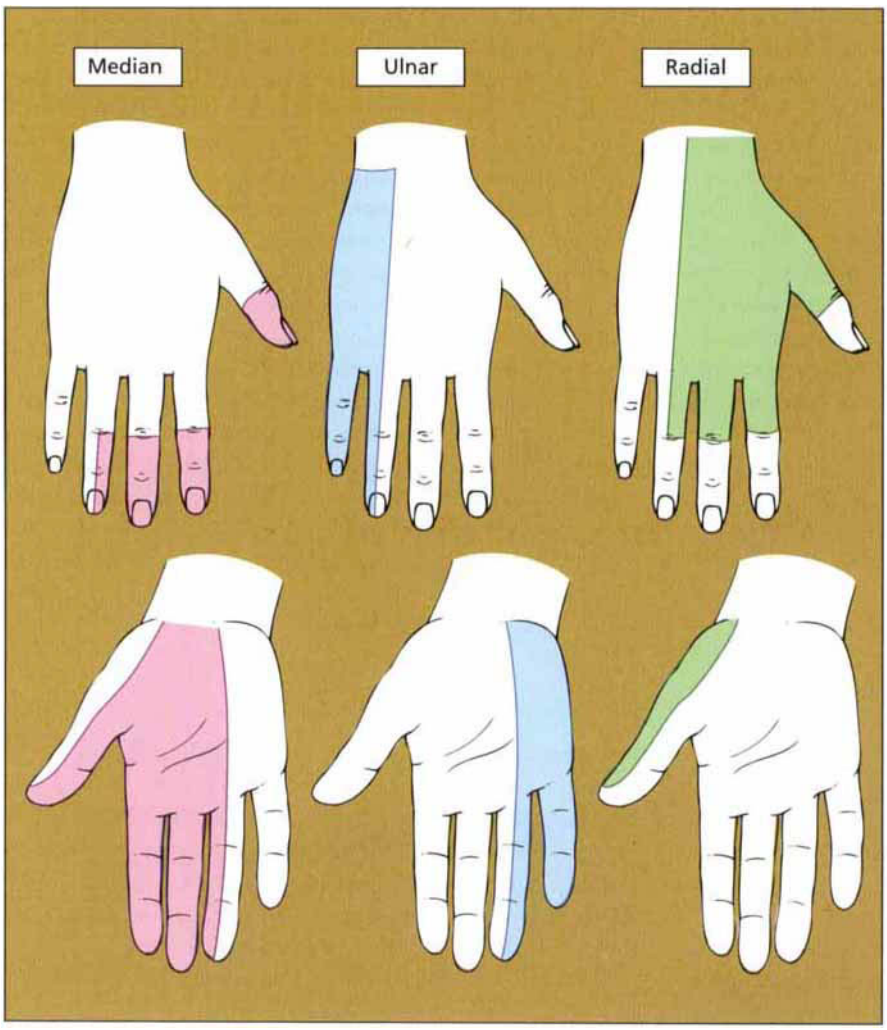


Fig. 1.27 Sensory supply of the hand.

Grassi, W., Felder, M., Thüring-Vollenweider, U. & Bollinger, A. (1989) Microvascular dynamics at the nail fold in rheumatoid arthritis. *Clinical and Experimental Rheumatology* 7, 47–53.

Hansen, S.W., Olsen, N., Rossing, N. & Rorth, M. (1990) Vascular toxicity and the mechanism underlying Raynaud's phenomenon in patients treated with cisplatin, vinblastine and bleomycin. *Annals of Oncology* 1, 289–292.

Kitayama, Y. & Tsukada, S. (1983) Congenital onychodysplasia. Report of 11 cases. *Archives of Dermatology* 119, 8–12.

Krylova, N.V. & Soboleva, T.M. (1995) The use of capillaroscopy of the skin of the nail wall in personal identification. *Sudebno-Meditsinskaia Ekspertiza* 38, 16–19.

Lynn, R.B., Steiner, R.E. & Van Wyck, F.A.F. (1955) Arteriographic appearances of the digital arteries of the hands in Raynaud's disease. *Lancet* i, 471–474.

Mahler, F., Saner, H., Boss, C. *et al.* (1987) Local cold exposure test for capillaroscopic examination of patients with Raynaud's syndrome. *Microvascular Research* 33, 422–427.

Masson, P. (ed.) (1937) *Les Glomus neurovasculaires*. Hermann et Cie, Paris.

Nakayama, Y., Iino, T., Uchida, A., Kiyosawa, T. & Soeda, S. (1990) Vascularised free nail grafts nourished by arterial inflow from the venous system. *Plastic and Reconstructive Surgery* 85, 239–245.

Ohtsuka, T., Tamura, T., Yamakage, A. & Yamazaki, S. (1998) The predictive value of quantitative nail fold capillary microscopy in patients with undifferentiated connective tissue disease. *British Journal of Dermatology* 139, 622–629.

Ohtsuka, T., Yamakage, A. & Miyachi, Y. (1994) Statistical definition of nail fold capillary pattern in patients with psoriasis. *International Journal of Dermatology* 33, 779–782.

Orentreich, N., Markofsky, J. & Vogelman, J.H. (1979) The effect of ageing on linear nail growth. *Journal of Investigative Dermatology* 73, 126–130.

Pessa, J.E., Tsai, T.M., Li, Y. & Kleinert, H.E. (1990) The repair of nail deformities with the non-vascularised nail bed graft: indications and results. *Journal of Hand Surgery* 15A, 466–470.

Richardson, D. & Schwartz, R. (1984) Comparison of resting capillary flow dynamics in the finger and toe nail folds. *Microcirculation, Endothelium and Lymphatics* 1, 645–656.

Ryan, T.J. (1973) Direct observations of blood vessels in the superficial vasculature system of the skin. In: *The Physiology and Pathophysiology of the Skin*, Vol. 2 (ed. A. Jarrett), pp. 658–659. Academic Press, London.

Samman, P. & Strickland, B. (1962) Abnormalities of the finger nails associated with impaired peripheral blood supply. *British Journal of Dermatology* 74, 165–173.

Schumann, L., Korten, G., Holdt, B. & Holtz, M. (1996) Microcirculation of the fingernail fold in CAPD patients: preliminary observations. *Peritoneal Dialysis International* **16**, 412–416.

Schnitzler, L., Baran, R., Civatte, J., Schubert, B., Verret, J.L. & Hurez, D. (1976) Biopsy of the proximal nail fold in collagen diseases. *Journal of Dermatologic Surgery* **2**, 313–315.

Smith, D.O., Oura, C., Kimura, C. & Toshimori, K. (1991a) Artery anatomy and tortuosity in the distal finger. *Journal of Hand Surgery* **16A**, 297–302.

Smith, D.O., Oura, C., Kimura, C. & Toshimori, K. (1991b) The distal venous anatomy of the finger. *Journal of Hand Surgery* **16A**, 303–307.

Studer, A., Hunziker, T., Lutolf, O. *et al.* (1991) Quantitative nail fold capillary microscopy in cutaneous and systemic lupus erythematosus and localised and systemic scleroderma. *Journal of the American Academy of Dermatology* **24**, 941–945.

Warwick, R. & Williams, P.L. (1973) *Gray's Anatomy*, 35th edn. Longman, Harlow, Essex.

Wilgis, E.F.S. & Maxwell, G.P. (1979) Distal digital nerve graft. *Journal of Hand Surgery* **4**, 439–443.

Winkelmann, R.K. (1960) Similarities in cutaneous nerve end-organs. In: *Advances in Biology of Skin: Cutaneous Innervation* (ed. W. Montagna), pp. 48–62. Pergamon, New York.

Wolfram-Gabel, R. & Sick, H. (1995) Vascular networks of the periphery of the fingernail. *Journal of Hand Surgery [Br]* **20**, 488–492.

Zaias, N. (1967) The longitudinal nail biopsy. *Journal of Investigative Dermatology* **49**, 406–408.

Zook, E.G. (1988) Injuries of the fingernail. In: *Operative Hand Surgery* (ed. D.P. Green), 2nd edn. Churchill Livingstone, New York.

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;
- physical protection of the extremity;
- a vehicle for cosmetics and aesthetic 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.* (1997) or tooth and nail changes in the hypodontia and nail dysplasia syndrome (Witkop tooth and nail syndrome) (Murdoch-Kinch *et al.* 1993; Garzon & Paller 1996). 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 hair follicle in the other (Smith *et al.* 1997). Presumably an additional factor determines which of the possible phenotypes prevails.

Whilst diseases illustrate inter-relationships between appendages, further common ground can be defined in terms of morphology. Achten (1968) noted that the nail unit was comparable in some respects to a hair follicle, sectioned longitudinally and laid on its side (Fig. 1.28). 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 Fig. 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 fibres. 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 mould and direct nail growth in the manner of the proximal nail fold (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 (Manabe & O'Guin 1994). 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 (Templeton & Wiegand 1997).

Constituents of some appendages, such as keratin and trichohyalin, are both specific in their physical attributes and specific to certain appendages. A considerable amount of biochemical work on hair and nail illustrates this point. In one study (Dekio & Jidoi 1989) two-dimensional electrophoresis was used to determine the presence of nine keratins in human hair and nail. Those of molecular weights 76, 73, 64, 61 and 55 kDa were common to hair and nail. One component of 61 kDa was specific to hair, and two components, both with a molecular weight of 50 kDa, were specific to nail. Further definition of these proteins was given by Heid *et al.* (1988) who employed gel electrophoresis, immunoblotting, peptide mapping and

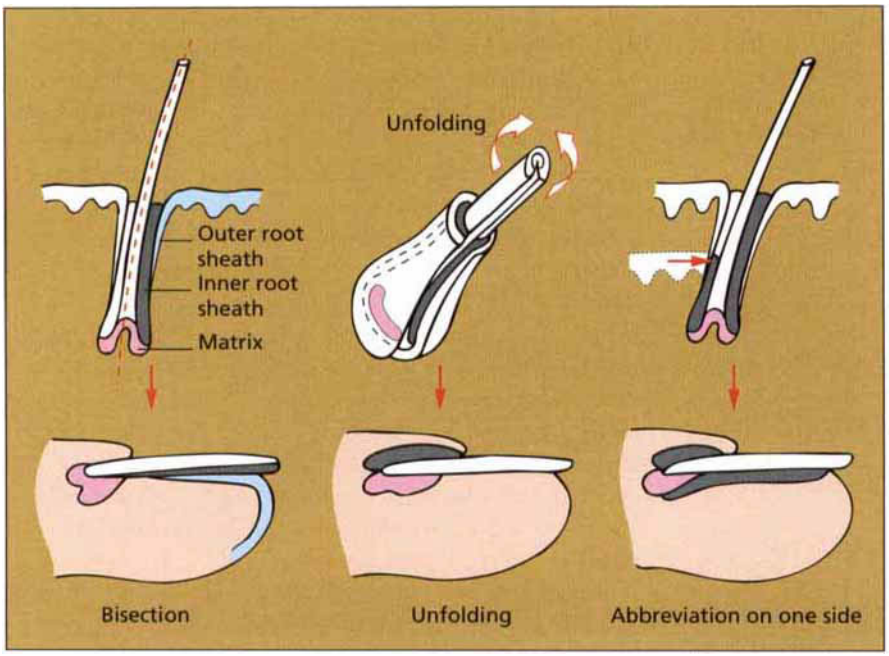


Fig. 1.28 Models of hair follicle/nail unit homology.

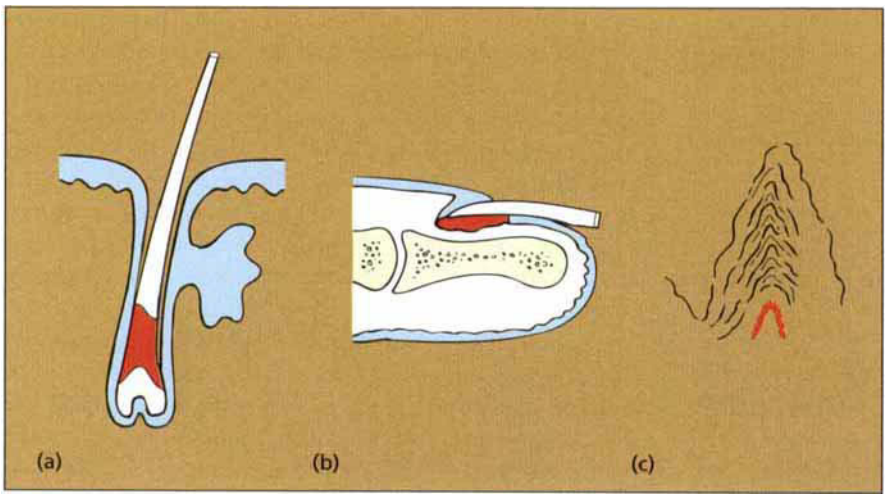


Fig. 1.29 Localization of immunoreactivity of the hard keratin Ha-1 in (a) the anagen hair follicle (×200), (b) the nail unit (×10) and (c) the human lingual papilla (×500). (After Westgate *et al.* 1997.)

complementary keratin binding analysis. Heid found that whilst nail plate contained both 'soft' epithelial and 'hard' hair/nail keratins, plucked hairs contained only the latter. By contrast, 'soft' epithelial keratins could be detected in the hair follicle and coexpressed with 'hard' keratins in a pattern also seen in the nail matrix. Although these 'hard' keratins are found in small amounts in the embryonic thymus and lingual papilla, they can generally be thought to be a feature of the hair/nail differentiation. Common ground between the lingual papilla and nail is demonstrated in the nail dystrophy of dyskeratosis congenita, where there are oral changes including changes in expression of keratins of the lingual papillae (Ogden *et al.* 1992).

The proposal that different appendages have common paths of early differentiation was pursued further by Lynch *et al.* (1986) who suggested that the precursor cells of hair cortex and nail plate share a major pathway of epithelial differentiation. She felt that the acidic 44 kDa/46 kDa and basic 56–60 kDa 'hard' keratins represent a coexpressed keratin pair that defines hair/nail tissue.

More recently, immunohistochemistry with monospecific antibodies has facilitated mapping out the distribution of certain hard keratins such as Ha-1, found in hair follicle, nail matrix and lingual papilla (Westgate *et al.* 1997) (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

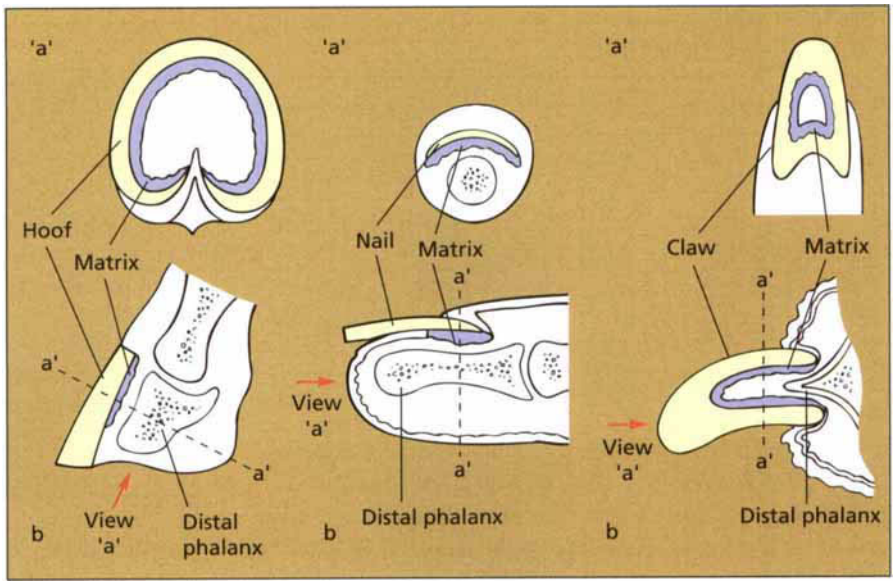


Fig. 1.30 Comparison of hoof, nail and claw and their matrix (red) origins.

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 has been well reviewed (Spearman 1978). 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 (Lucas & Strettenheim 1972). The matrix contributes the greatest mass to the nail plate in man and other primates, with a lesser contribution from the dorsal and nail bed matrices. Claws (Kato 1977) are formed from an extensive germinal matrix, which occupies the territory of the nail bed in primates. It is sometimes described as comprising a dorsal and ventral component (Mueller *et al.* 1993), 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 (Kato 1977). 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 becomes 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 (Gillespie *et al.* 1982). The disorders of claws presenting to one university veterinary service demonstrated a preponderance of trauma and bacterial infection (Scott & Miller 1992) (Table 1.3). 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; Pautard 1964). A study of onychomadesis (nail shedding) in dogs looked at mineral constituents of normal claws, human nails and the hooves of cows and pigs (Harvey & Markwell 1996). It appears that there is no particular pattern of homology between different species in this respect (Table 1.4).

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 (Akhtar & Edwards 1997).

Table 1.3 Proportion of diagnoses of dogs with disorders of the claws from a study of 196 affected dogs. (Adapted from Scott & Miller 1992.)

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

Table 1.4 Mineral content (expressed as mg/kg, standard error in parentheses). (Adapted from Harvey & Markwell 1996.)

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)

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 (Kitahara & Ogawa 1994). Hooves have evolved to provide a 'bulky claw' for weight bearing and locomotion over hard ground (Sisson & Grossman 1953). It is interesting that among the prosimians, tarsiers have nails on all digits apart from the 2nd and 3rd digits of the hindlimb which bear claws (Spearman 1978). 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 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 3rd 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 μm in diameter. The orientation of the fibrils is along the main axis of the hoof, similar to the hair cortex.

References

Achten, G. (1968) Normale histologie und histochemie des nagels. In: *Handbuch der Haut- und Geschlechtskrankeiten*, Vol. 1 (ed. J. Jadassohn), pp. 339–376. Springer-Verlag, Berlin.

Akhtar, W. & Edwards, H.G. (1997) Fourier-transform Raman spectroscopy of mammalian and avian keratotic biopolymers. *Spectrochemica Acta A, Molecular and Biomolecular Spectroscopy* 53A, 81–90.

Barbareschi, M., Cambiaghi, S., Crupi, A.C. & Tadini, G. (1997) Family with 'pure' hair-nail ectodermal dysplasia. *American Journal of Medical Genetics* 72, 91–93.

Dekio, S. & Jidoi, J. (1989) Comparison of human hair and nail low-sulfur protein compositions on two-dimensional electrophoresis. *Journal of Dermatology* 16, 284–288.

Garzon, M.C. & Paller, A.S. (1996) What syndrome is this? Witkop tooth and nail syndrome. *Pediatric Dermatology* 13, 63–65.

Gillespie, J.M., Marshall, R.C. & Woods, E.F. (1982) A comparison of lizard claw keratin proteins with those of avian beak and claw. *Journal of Molecular Evolution* 18, 121–129.

Harvey, R.G. & Markwell, P.J. (1996) The mineral composition of nails in normal dogs and comparison with shed nails in canine idiopathic onychomadesis. *Veterinary Dermatology* 7, 29–34.

Heid, H.W., Moll, I. & Franke, W.W. (1988) Patterns of expression of trichocytic and epithelial cytokeratins in mammalian tissues. *Differentiation* 37, 215–230.

Kato, T. (1977) A study on the development of the cat claw. *Hiroshima Journal of Medical Science* 26, 103–126.

Kitahara, T. & Ogawa, H. (1994) Variation of differentiation in nail and bovine hoof cells. *Journal of Investigative Dermatology* 102, 725–729.

Lucas, A.M. & Strettenheim, P.R. (1972) *Avian Anatomy Integument. Part 2. Agricultural Handbook, No. 362*. US Government Printing Office, Washington DC.

Lynch, M.H., O'Guin, W.M., Hardy, C. *et al.* (1986) Acidic and basic hair/nail ('hard') keratins: their co-localization in upper cortical and cuticle cells and the human hair follicle and their relationship to 'soft' keratins. *Journal of Cell Biology* 103, 2593–2606.

Manabe, M. & O'Guin, W.M. (1994) Existence of trichohyalin-keratohyalin hybrid granules: co-localisation of 2 major intermediate filament-associated proteins in non-follicular epithelia. *Differentiation* 58, 65–75.

Mueller, R.S., Sterner-Kock, A. & Stannard, A.A. (1993) Microanatomy of the canine claw. *Veterinary Dermatology* 4, 5–11.

Murdoch-Kinch, C.A., Miles, D.A. & Poon, C.K. (1993) Hypodontia and nail dysplasia syndrome. Report of a case. *Oral Surgery, Oral Medicine and Oral Pathology* 75, 403–406.

Ogden, G.R., Chisholm, D.M., Leigh, I.M. & Lane, E.B. (1992) Cytokeratin profiles in dyskeratosis congenita: an immunocytochemical investigation of lingual hyperkeratosis. *Journal of Oral Pathology and Medicine* 21, 353–357.

Pautard, F.G.E. (1964) Calcification of keratin. In: *Progress in Biological Sciences in Relation to Dermatology*, Vol. 2 (eds A.J. Rook & R.E. Champion), p. 227. Cambridge University Press.

Scott, D.W. & Miller, W.H. (1992) Disorders of the claw and clawbed in dogs. *Compendium* 14, 1448–1458.

Sisson, S. & Grossman, J.D. (eds) (1953) *The Anatomy of the Domestic Animals*. W.B. Saunders, Philadelphia.

Smith, F.J.D., Corden, L.D., Rugg, E.L. *et al.* (1997) Missense mutations in keratin 17 cause either pachyonychia congenita type 2 or a phenotype resembling steatocystoma multiplex. *Journal of Investigative Dermatology* 108, 220–223.

Spearman, R.I.C. (1978) The physiology of the nail. In: *The Physiology and Pathophysiology of the Skin*, Vol. 5 (ed. A. Jarrett), p. 1827. Academic Press, New York.

Templeton, S.F. & Wiegand, S.E. (1997) Pachyonychia congenita-associated alopecia. A microscopic analysis using transverse section technique. *American Journal of Dermatopathology* 19, 180–184.

Westgate, G.E., Tidman, N., de Berker, D., Blount, M.A., Philpott, M.P. & Leigh, I.M. (1997) Characterisation of LH Tric 1, a new monospecific monoclonal antibody to the hair keratin IIa 1. *British Journal of Dermatology* 137, 24–31.

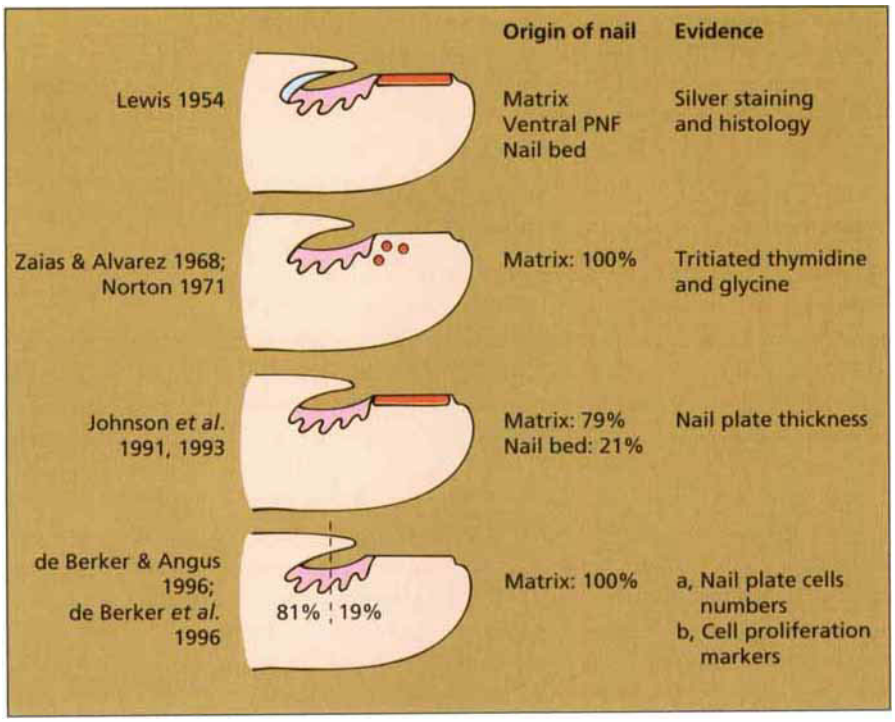


Fig. 1.31 Theories of nail plate origin.

Physiology

Nail growth

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 tumours 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 matrix (Fig. 1.31). The first is part of the proximal nail fold, the latter is the nail bed. Lewis (1954) 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 productions.

Markers of matrix and nail bed proliferation

Zaias and Alvarez (1968) 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 classical matrix (or intermediate matrix to use Lewis's terminology). Norton used human subjects in further autoradiographic studies (Norton 1971). Although there was some incorporation of radiolabelled glycine in the area of the nail bed, it was in a poorly defined location, making clear statements impossible.

More recent immunohistochemical techniques have allowed 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 (de Berker & Angus 1996). Both markers demonstrated labelling indices in excess of 20% for the nail matrix in contrast with 1% or less for the nail bed in healthy tissue. In psoriatic nail and onychomycosis, the labelling index of nail bed rises to >29%. Whilst 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 (Samman 1961).

Nail plate indicators of matrix location

Johnson *et al.* (1991, 1993) dismissed the evidence of Zaias, claiming that the methodology was flawed. She examined nail growth by the measurement of change in nail thickness along a proximal to distal longitudinal axis. She 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 dorso-ventral axis of the nail (de Berker *et al.* 1996). 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 they 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.* (1996) provides 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 (de Berker 1997).

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 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. It 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.

Ultrasound as a tool to define nail matrix

Ultrasound studies of the nail plate have done little to support the notion that the nail bed contributes significantly to its substance (Finlay *et al.* 1987; Jemec & Serup 1989). Jemec claimed that the nail plate had a clear two-part structure, none of which appeared to come from the nail bed. Finlay observed that the nail plate had a more rapid ultrasound transmission distally; paradoxical if one imagines a nail bed contribution. This last comment is almost diametrically opposite that of Johnson *et al.* (1991).

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 (Nishi *et al.* 1996). This observation is true for adults and children alike, although the likelihood of normal regrowth is greater in children with similar trauma (Libbin & Neufeld 1988). 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 (Zaias 1967). 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 which gives the impression of a nail bed contribution to the nail plate. Historically, this has been referred to as the solehorn (Fig. 1.32) and considered a germinal element of the hyponychium. Samman considered this issue in the context of a patient with pustular psoriasis (Samman 1961). He concluded that the ventral nail is a movable feast, manifesting itself in certain pathological circumstances.

Normal nail morphology

The main issues in normal nail morphology are: why is it flat? and why is the free edge rounded and not pointed? Factors influencing nail plate thickness are dealt with earlier (see 'Nail plate' above).

Why does nail grow out straight?

The first question was addressed in an article by Kligman

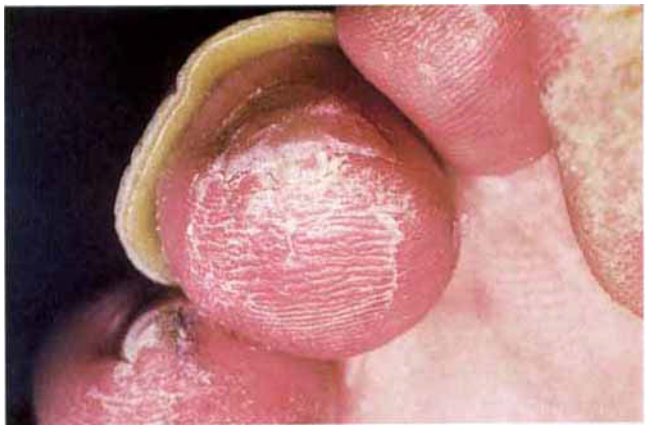


Fig. 1.32 Vestigial solenhorn seen as focal subungual hyperkeratosis.

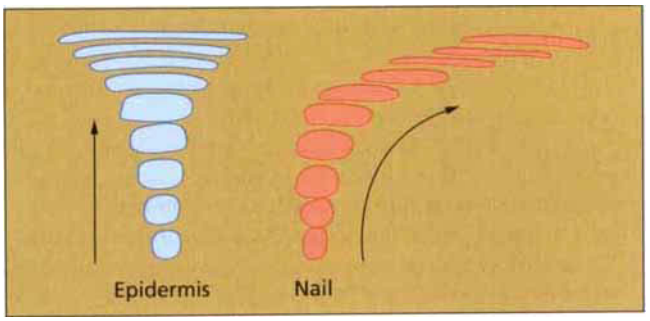


Fig. 1.33 Change in shape and direction of the cells within the epidermis and the nail matrix. (After Kligman 1961.)

(1961) entitled, 'Why do nails grow out instead of up?'. His hypothesis was that the proximal nail fold acts to mould the nail as it moves away from the matrix giving an oblique growth path. From observing other keratinizing epithelia, he noted that growth is normally parallel to the axis of keratinization. From this, he considerd it anomalous that nails grow out along the nail bed and not upwards (Fig. 1.33). A patient with the nervous habit of chewing off the proximal nail fold did not provide an adequate experiment to demonstrate its function. However, when given the opportunity to autograft 5-mm matrix punch biopsies from digit to forearm, nail tissue was seen to grow upwards like a cutaneous horn. This was presented as proof of the hypothesis.

Baran was in disagreement (Baran 1981), and presented evidence from surgical experience in the removal of the proximal nail fold and the lack of subsequent change in the nail. He also challenged the validity of Kligman's experiment on the basis that the underlying terminal phalanx has a great influence upon nail growth (Baran & Juhlin 1986) and this was lost in transplanting the graft to the arm. Further examples of ectopic nail growth do not resolve the issue (Kikuchi *et al.* 1984) and the relevance of acquired bone and nail changes occurring in tandem has its own literature. Carpal tunnel syndrome can result in abnormal nails alongside acroosteolysis and ischaemic skin lesions (Tosti *et al.* 1993). The reversal of many of the skin and nail features on treatment of the carpal tunnel compression suggests a neurovascular origin to both nail and bone changes in this pattern of acroosteolysis. Where the aetiology of the osteolysis is termed idiopathic, there are also nail changes (Todd & Saxe 1994). It seems unlikely that these cases represent a specific influence of bone upon nail formation, but rather that both structures are responding to some undefined agent. There are a wide range of primary disorders in which secondary osteolysis and altered nails are recognized complications (Todd & Saxe 1994).

All the models demonstrating the influence of the different periungual tissues and bone upon the nail are flawed. Those above do not acknowledge the adherent quality of the nail bed as an influential factor, or the the guiding influence of the lateral nail being embedded in the lateral nail folds. The role of the nail bed becomes manifest in psoriasis affecting the toes where the combination of subungual hyperkeratosis and trauma can produce upward growing nails in the presence of an apparently normal proximal nail fold. It is reasonable with our present knowledge to consider horizontal nail growth as being attributable to more than one part of the nail unit (Fig. 1.34).

What determines the contour of the free edge?

The second issue is why are nails rounded and not pointed? This has generally been accepted as being a function of the shape of the lunula, as illustrated in Fig. 1.35. The mechanism of this is seen in Fig. 1.34. Given that nails are growing continuously throughout life, it is possible to argue that we rarely see the true free edge, but observe the eroded or manicured outline. However, there are two instances when we see the genuine free edge; at birth and with regrowth following avulsion (Fig. 1.36). These appear to follow the margin of the lunula. Finally, the nail bed may have some role in determining the shape of the free edge. Trauma to the nail bed can result in nail plate dystrophies giving the free edge a scalloped contour. This can be corrected with nail bed grafts (Pessa *et al.* 1990).

References

Baran, R. (1981) Nail growth direction revisited. *Journal of the American Academy of Dermatology* **4**, 78–83.

Baran, R. & Juhlin, L. (1986) Bone dependent nail formation. *British Journal of Dermatology* **114**, 371–375.

de Berker, D. (1997) Nail growth measurement by indentation. *Clinical and Experimental Dermatology* **22**, 109.

de Berker, D. & Angus, B. (1996) Proliferative compartments in the normal nail unit. *British Journal of Dermatology* **135**, 555–559.

de Berker, D.A.R., MaWhinney, B. & Sviland, L. (1996) Quantification of regional matrix nail production. *British Journal of Dermatology* **134**, 1083–1086.

Finlay, A.Y., Moseley, H. & Duggan, T.C. (1987) Ultrasound transmission time: an *in vivo* guide to nail thickness. *British Journal of Dermatology* **117**, 765–770.

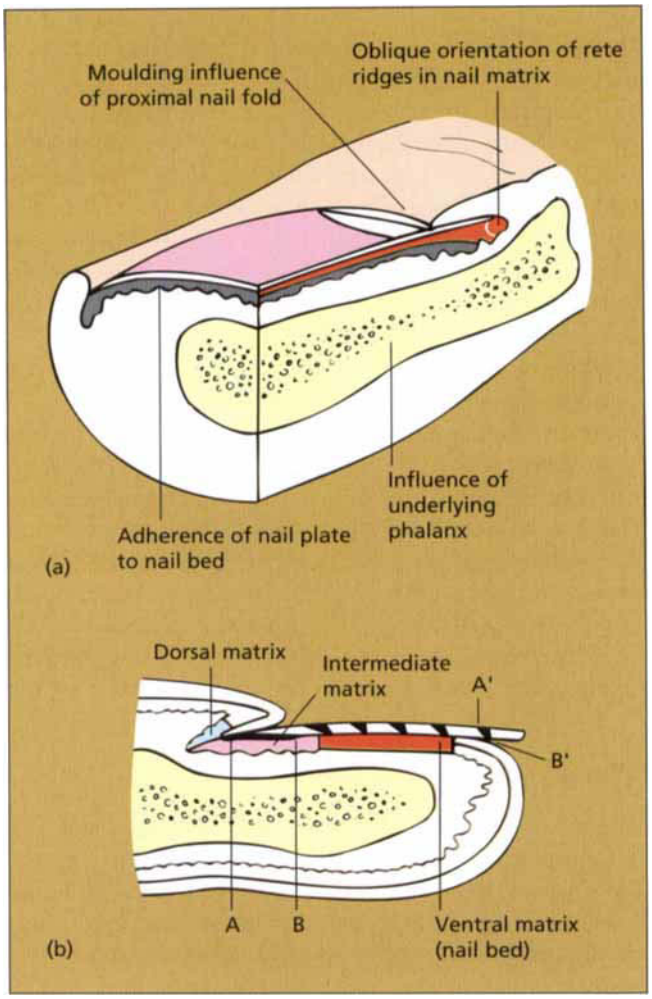


Fig. 1.34 Why do nails grow out instead of up? (i) Guiding restraint of proximal nail fold; (ii) inductive influence of underlying phalanx; (iii) containment by lateral nail folds; (iv) adherence to nail bed. (b) Generation of nail plate marked at monthly intervals. The horizontal axis of the intermediate axis is transformed into an oblique axis. Proximal matrix, A, generates dorsal nail A'. Distal intermediate matrix, B, generates ventral nail B'.

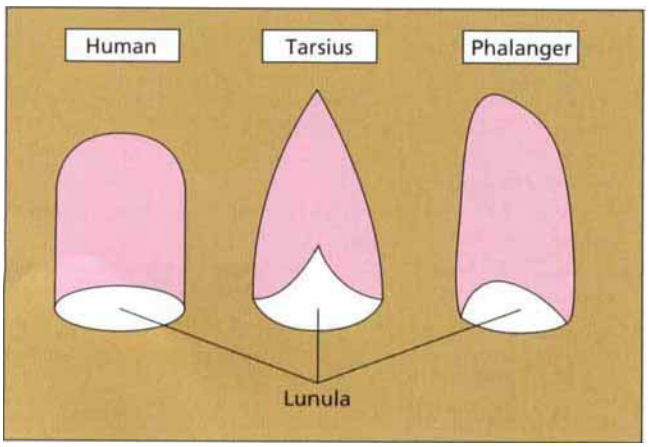


Fig. 1.35 Relationship of lunula to nail tip shape.

Heikkilä, H., Stubb, S. & Kiistala, U. (1996) Nail growth measurement employing nail indentation—an experimental follow-up study of nail growth *in situ*. *Clinical and Experimental Dermatology* **21**, 96–99.

Jemec, G.B.E. & Serup, J. (1989) Ultrasound structure of the human nail plate. *Archives of Dermatology* **125**, 643–646.

Johnson, M. & Schuster, S. (1993) Continuous formation of nail along the nail bed. *British Journal of Dermatology* **128**, 277–280.

Johnson, M., Comaish, J.S. & Shuster, S. (1991) Nail is produced by the normal nail bed: a controversy resolved. *British Journal of Dermatology* **125**, 27–28.

Kikuchi, I., Ogata, K. & Idemori, M. (1984) Vertically growing ectopic nail. *Journal of the American Academy of Dermatology* **10**, 114–116.

Kligman, A. (1961) Why do nails grow out instead of up? *Archives of Dermatology* **84**, 181–183.

Lewis, B.L. (1954) Microscopic studies of fetal and mature nail and the surrounding soft tissue. *Archives of Dermatology and Syphilis* **70**, 732–744.

Libbin, R.M. & Neufeld, D.A. (1988) Regeneration of the nail bed. *Plastic and Reconstructive Surgery* **81**, 1001–1002.

Nishi, G., Shibata, Y., Tago, K. *et al.* (1996) Nail regeneration in digits replanted after amputation through the distal phalanx. *Journal of Hand Surgery* **21A**, 229–233.

Norton, L.A. (1971) Incorporation of thymidine-methyl-H^3 and glycine-2-H^3 in the nail matrix and bed of humans. *Journal of Investigative Dermatology* **56**, 61–68.

Pessa, J.E., Tsai, T.M., Li, Y. & Kleinert, H.E. (1990) The repair of nail bed deformities with the nonvascularised nail bed graft. Indications and results. *Journal of Hand Surgery* **15A**, 466–470.

Samman, P. (1961) The ventral nail. *Archives of Dermatology* **84**, 192–195.

Todd, G. & Saxe, N. (1994) Idiopathic phalangeal osteolysis. *Archives of Dermatology* **130**, 759–762.

Tosti, A., Morelli, R., D'Alessandro, R. & Bassi, F. (1993) Carpal tunnel syndrome presenting with ischemic skin lesions, acroosteolysis and nail changes. *Journal of the American Academy of Dermatology* **29**, 287–290.

Zaias, N. (1967) The longitudinal nail biopsy. *Journal of Investigative Dermatology* **49**, 406–408.

Zaias, N. & Alvarez, J. (1968) The formation of the primate nail plate. An autoradiographic study in the squirrel monkey. *Journal of Investigative Dermatology* **51**, 120–136.

Nail growth measurement

The literature on nail growth has relied on quantification. A range of methods have been employed, mostly requiring the imprint of a fixed reference mark on the nail and measuring its change in location relative to a fixed structure separate from the nail after a study period. Gilchrist and Dudley Buxton (1938) made a transverse scratch about 2 mm from the most distal margin of the lunula. This distance was then measured using a rule and magnifier. Changes in the distance with time provided a record of growth rate. There have been variants of this, with the scratch being made at the convex apogee of the lunula and subsequent measurements made with reference to the lunula (Hillman 1955), or alternatively making a scratch a fixed 3 mm from the cuticle and noting the change with time (Dawber

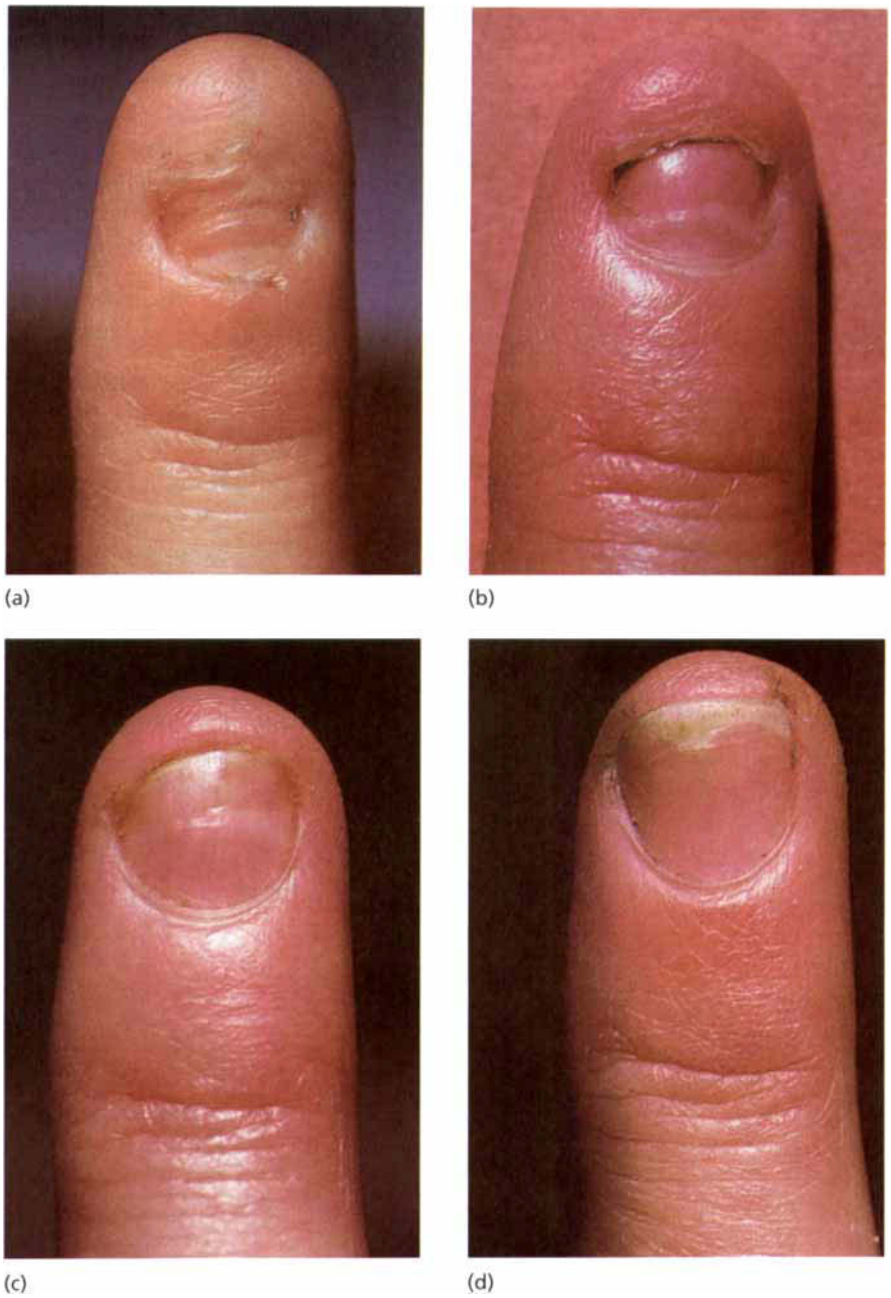


Fig. 1.36 (a–d) Regrowth of the fingernail following traumatic avulsion. The free edge is parallel to the lunula.

1970a) (Fig. 1.37). The precision of these methods was increased by the introduction of magnified photographs before and after, and comparison of the photographs (Babcock 1955). This was modified further by Sibinga (1959) who increased the photographic magnification from a factor of 6 to 35. This made it possible to conduct studies of nail growth over a period as short as 1 month.

Babcock (1955) understood the problems in the methods involving the lunula and cuticle as reference points, as they both might conceivably change during the study. The method suggested for overcoming this was inventive, but unacceptable these days for ethical reasons. The nail was marked with a deep scratch which was then filled with bismuth amalgam. This made it radio-opaque and allowed comparison with the underlying bony reference points on X-ray. A follow up X-ray, after re-filling the scratch with amalgam, allowed growth estimation. The concern over variation in the non-nail plate reference point can be partly surmounted by using two reference points and possibly halving the error. This can be done by making a scratch at the tip of the lunula and measuring the distance to the distal limit of the nail plate attachment, visible through the nail plate. Subsequent measurements are made from both the lunula and the edge of nail plate attachment. Their sum should always be equal as a way of verifying the method (Fig. 1.38).

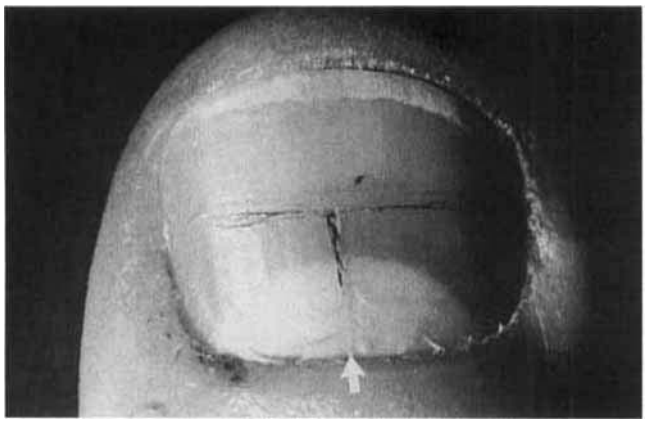


Fig. 1.37 T-shaped mark etched on nail for nail growth measurement (Dawber 1970a). Arrow points to posterior nail fold reference point. Note the absence of a cuticle.

Surface imaging of the nail, exploiting natural irregularities, can be used in lieu of marks placed by the observer. This has been reported by de Doncker and Piérard (1994) in a study of nail growth during itraconazole therapy. The technique was not pushed to its full potential, as only clippings and not the entire *in vivo* nail plate were assessed. It was thought that longitudinal nail growth increased during therapy because surface beading became more spaced apart.

All these methods involve estimation of linear growth. As a measure of total matrix activity this could be misleading. Hamilton sought to measure volume by the following equation (Hamilton *et al.* 1955):

Thickness (mm) × breadth (mm) × length grown per day = volume

Johnson also tried to measure volumetric growth with respect to linear growth, ignoring time (Johnson *et al.* 1991). This entailed the measurement of thickness and mass at different points in the avulsed nail plate. The method presumed that linear measurements in the longitudinal axis of the nail plate were proportional to time and that no element of compaction complicated the issue.

Attempts to measure volume take on particular significance in disease states provoking Beau's lines. In a condition where the bulk of the nail is manifestly affected, measurement of linear growth alone may give misleading results (Fig. 1.39).

Van Noord measured the length and weight of clippings (P.A.H. Van Noord, personal communication).

Physiological factors and nail growth

Most studies concern fingernails. Their rate of growth can vary between 1.9 and 4.4 mm/month (Sibinga 1959). A reasonable guide is 3 mm/month or 0.1 mm/day. Toenails are estimated to grow around 1 mm/month. Population studies on nail growth have given the general findings that there is little marked seasonal change and nails are unaffected by mild intercurrent illnesses (Hillman 1955; Sibinga 1959). The height or weight of the individual made no sigificant difference (Hillman 1955; Hewitt & Hillman 1966). Sex makes a small difference in early adulthood, with men having significantly ($P < 0.001$) faster linear nail growth up to the age of 19 (Hamilton *et al.* 1955). They continue to do so with gradually diminishing significance levels, up to the age of 69, when there is a crossover and women's nails grow faster than males. There is rough agreement from Hillman in an earlier study, although he found that the crossover age was around 40 (Hillman 1955). However, males continued to have a greater rate of nail growth throughout life if volume was measured, and not length (Hamilton *et al.* 1955). Children under 14 have faster growth than adults.

Pregnancy may increase the rate of nail growth (Hewitt & Hillman 1966) and poor nutrition may retard it (Gilchrist & Dudley Buxton 1938).

Temperature is an influence with unclear effects. Bean (1980) kept a slightly idiosyncratic record of his own fingernail growth by making a scratch at the free edge of his cuticle on the first day of each month for 35 years. His record showed a gradual slowing with age. It initially showed a seasonal variation with heightened growth in the warm months. This variation became less marked with age, combining with a move from Iowa to Texas where seasonal contrasts are reduced. Other studies to determine the influence of temperature have compared nail growth rates for people in temperate and polar conditions. An original study in 1958

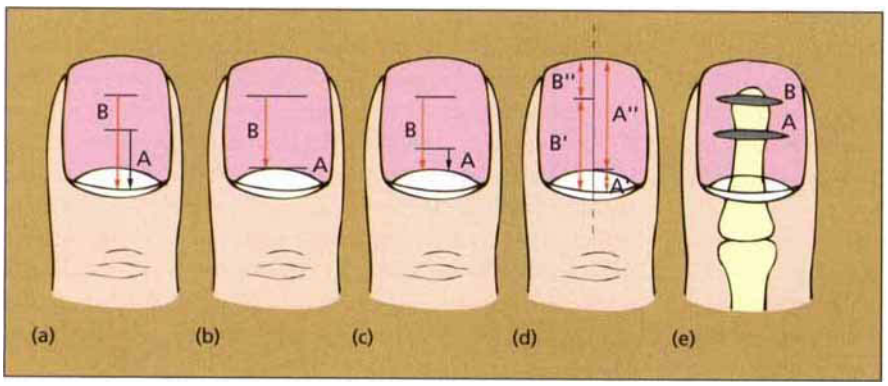


Fig. 1.38 Methods of nail growth measurement. (a) Reference, cuticle; growth B – A; c magnifier, Dawber (1970a). (b) Reference, lunula, growth, B; Hillman (1955); c × 6 reference photo, Babcock (1955), c × 35 reference photo, Sibinga (1959). (c) Reference, lunula; growth, B – A; c magnifier, Gilchrist & Dudley Buxton (1938) (d) Reference, cuticle and nail attachment margin; growth, $(B' - A') + (A'' - B'')/2$; verification by $A' + A'' = B' + B''$. (e) Reference, bone feature on X-ray, bismuth amalgam in nail scratch; growth, depends on landmark.

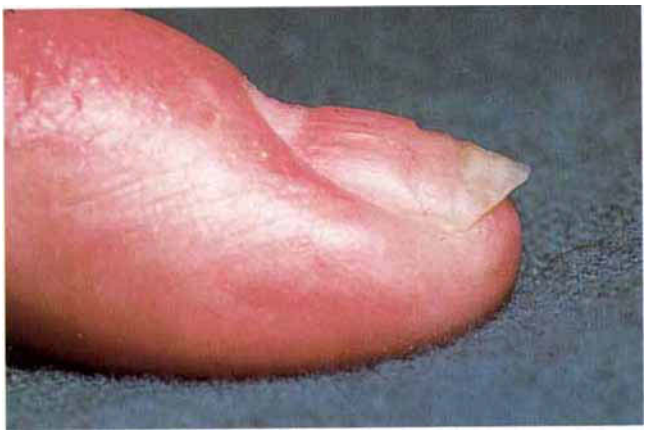


Fig. 1.39 Side view of a nail with Beau's line, indicating the change in nail bulk

(Geoghegan *et al.* 1958) found that nail growth was significantly retarded by living in the Arctic. Subsequent studies from the Antarctic found that there was no change in nail growth (Donovan 1977; Gormly & Ledingham 1983). These studies are not scientific, and it is unclear whether they are commenting on the improvement in thermal insulation since 1958 or nail physiology.

Nail growth in disease

Systemic disease

Insufficient numbers of seriously ill people have been followed as part of a larger study to give good statistical evidence concerning the influence of disease on nail growth. There is plenty of evidence from small numbers that some severe systemic upsets disturb nail formation. The observations of Justin Honoré Simon Beau in 1836 (Weismann 1977) detailed the development of transverse depressions upon the nails of people surviving typhoid. The form of nail growth interference represented by Beau's lines is seen in many conditions (see Beau's lines in Chapter 2). Severe illness in the form of mumps has been noted to bring linear growth to a standstill (Sibinga 1959). Other acute infections are quite variable, with 10 cases of acute febrile tuberculosis failing to have significant effect (Sibinga 1959). In the same study, chronic nephrosis produced exceptionally slow nail growth. Paradoxically, they also found that cadavers appeared to continue the growth of their nails in the 10 days post mortem during which they were assessed. The effect of death was less marked than mumps; something adults with mumps might agree with.

Local disease

Local diseases can influence nail growth. Dawber *et al.* (1971) noted that onycholysis was associated with increased nail growth. This was true whether it was related to psoriasis or

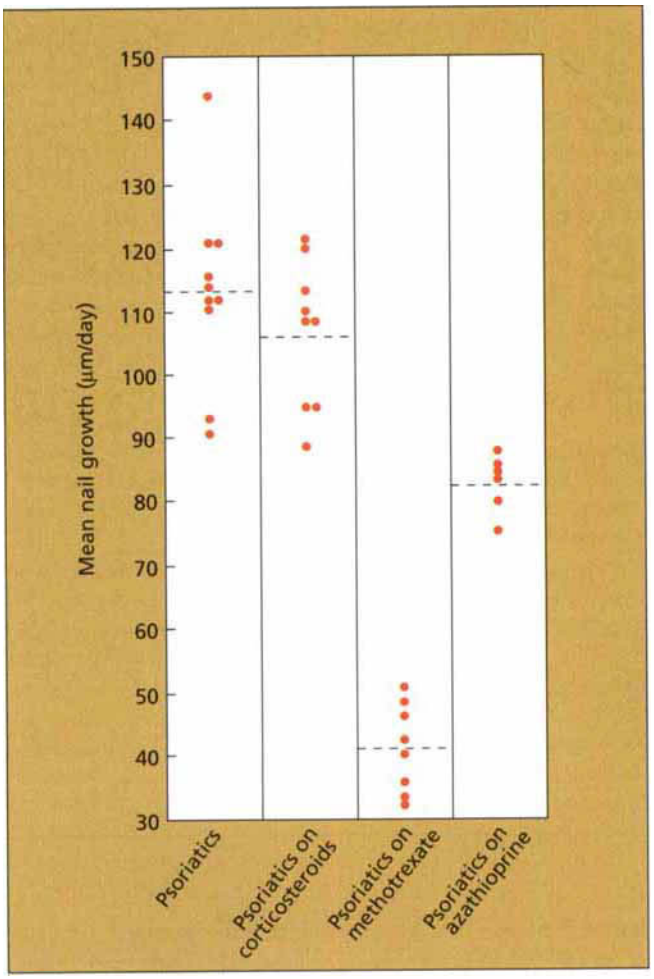


Fig. 1.40 Effect of therapy on nail growth in psoriasis (Dawber 1970b).

idiopathic. It is interesting that psoriasis may also produce Beau's lines and so reduce the bulk of the nail. It is not even clear whether Beau's lines represent a reduction in linear growth. They have been noted after retinoid therapy, and yet this group of drugs has been noted to increase nail growth in psoriasis (Galosi *et al.* 1985). The surface morphology of the nail in a Beau's line reflects a change in rate of nail plate production in different zones in the matrix and a loss of co-ordination with longitudinal growth; it is a product of pathology in space and time. Perhaps a nail that is growing faster is unable to accumulate bulk. Other systemic psoriasis treatments may reduce the rate of nail growth (Dawber 1970b) (Fig. 1.40).

Trauma may also influence nail growth, where wrist fractures are the most common example. Details of nail and local hair growth have been recorded in instances of reflex sympathetic dystrophy where Beau's lines and hypertrichosis on the dorsum of wrist, arm and hand may coincide. It is not clear whether the nail changes represent increased or decreased growth in these circumstances. Hypertrichosis indicates an extension of anagen, such that hairs that might normally fall

Table 1.5 Influences on nail growth.

Faster	Slower
Daytime	Night
Pregnancy (Hewitt & Hillman 1966)	1st day of life (Schnick 1908)
Minor trauma/nail biting (Gilchrist & Dudley Buxton 1938; Hamilton *et al.* 1955)	
Right hand/dominant	Left hand/non-dominant
Youth	Old age (Hamilton *et al.* 1955)
Fingers	Toes (Pfister & Heneka 1969)
Summer (Bean 1980)	Winter/cold (Roberts & Sandford 1958)
Middle finger	Thumb and little finger (LeGros Clark & Buxton 1938; Orentreich *et al.* 1979)
Men	Women (LeGros Clark & Buxton 1938; Hamilton *et al.* 1955)
Psoriasis (Landherr *et al.* 1982)	Finger immobilization (Dawber 1981)
pitting (Dawber 1970a)	Fever (Sibinga 1959)
normal nails (Dawber 1970a)	Beau's lines (Weismann 1977)
onycholysis (Dawber *et al.* 1971)	
Pityriasis rubra pilaris (Dawber 1980)	Methotrexate, azathioprine (Dawber 1970b)
Etretinate (Baran 1982)	Etretinate (Baran 1982)
Idiopathic onycholysis of women (Dawber *et al.* 1971)	Denervation (Head & Sherrin 1905)
	Poor nutrition (Gilchrist & Dudley Buxton 1938)
Hyperthyroidism (Orentreich *et al.* 1979)	
L-DOPA (Miller 1973)	
AV shunts (Orentreich *et al.* 1979)	Yellow nail syndrome (Samman 1978)
Calcium/vitamin D (Hogan *et al.* 1984)	Relapsing polychondritis (Estes 1983)
Benoxaprofen (Fenton & Wilkinson 1983)	

out at 5 mm or less, become longer and may gain a greater diameter. It does not necessarily mean that the hairs are growing *faster* and so in common with Beau's lines it represents a change in the pattern of appendage growth rather than a simple alteration of rate.

Immobility alone may result in a reduction in rate of nail growth and whilst this factor prevails after wrist fracture, reflex sympathetic dystrophy entails significant changes in blood supply that may have their own effects.

Table 1.5 includes influences upon nail growth that are reported, but not always of statistical significance.

References

Babcock, M.J. (1955) Methods for measuring fingernail growth rates in nutritional studies. *Journal of Nutrition* **55**, 323–336.

Baran, R. (1982) Action therapeutique et complications due retinoide aromatique sur l'appareil ungueal. *Annales de Dermatologie et de Vénéréologie* **109**, 367–371.

Bean, W.B. (1980) Nail growth. Thirty five years of observation. *Archives of Internal Medicine* **140**, 73–76.

Dawber, R. (1970a) Fingernail growth in normal and psoriatic subjects. *British Journal of Dermatology* **82**, 454–457.

Dawber, R.P.R. (1970b) The effect of methotrexate, corticosteroids and azathioprine on fingernail growth in psoriasis. *British Journal of Dermatology* **83**, 680–683.

Dawber, R.P.R. (1980) Ultrastructure and growth of human nails. *Archives of Dermatological Research* **269**, 197–204.

Dawber, R.P.R. (1981) Effects of immobilization on nail growth. *Clinical and Experimental Dermatology* **6**, 1–4.

Dawber, R.P.R., Samman, P. & Bottoms, E. (1971) Fingernail growth in idiopathic and psoriatic onycholysis. *British Journal of Dermatology* **85**, 558–560.

de Doncker, P. & Piérard, G.E. (1994) Acquired nail beading in patients receiving itraconazole—an indicator of faster nail growth? A study using optical profilometry. *Clinical and Experimental Dermatology* **19**, 404–406.

Donovan, K.M. (1977) Antarctic environment and nail growth. *British Journal of Dermatology* **96**, 507–510.

Estes, S.A. (1983) Relapsing polychondritis. *Cutis* **32**, 471–476.

Fenton, D.A. & Wilkinson, J.D. (1983) Milia, increased nail growth and hypertrichosis following treatment with Benoxaprofen. *Journal of the Royal Society of Medicine* **76**, 525–527.

Galosi, A., Plewig, G. & Braun-Falco, O. (1985) The effect of aromatic retinoid RO 10–9359 on fingernail growth. *Archives of Dermatological Research* **277**, 138–140.

Geoghegan, B., Roberts, D.F. & Sampford, M.R. (1958) A possible climatic effect on nail growth. *Journal of Applied Physiology* **13**, 135–138.

Gilchrist, M. & Dudley Buxton, L.H. (1938) The relation of fingernail growth to nutritional status. *Journal of Anatomy* **9**, 575–582.

Gormly, P.J. & Ledingham, J.E. (1983) Nail growth in antarctic conditions. *Australian Journal of Dermatology* **24**, 86–89.

Hamilton, J.B., Terada, H. & Mestler, G.E. (1955) Studies of growth throughout the lifespan in Japanese: growth and size of nails and

Table 1.6 Different methods of nail constituent analysis.

Method	Element	Reference
I. *Structural and mineral constituents*		
Raman spectroscopy	Water, proteins and lipid	Gniadecka *et al.* 1998
Immunohistochemistry	Keratin	Heid *et al.* 1988
Polymerase chain reaction	Deoxyribonucleic acid	Kaneshige *et al.* 1992, Tahir & Watson 1995
Electron microscopy	Cystine	Salamon *et al.* 1988
X-ray diffraction	Mg, Cl, Na, Ca, S, Cu	Sirota *et al.* 1988; Forslind 1970
Colorimetry	Fe	Jacobs & Jenkins 1960
II. *Exogenous materials*		
Atomic absorption spectometry	Cd, Pb, Zn, Ca, Cr, Fe, Cu, Mn, Ni, Co, Na, K	Wilhelm *et al.* 1991, Forslind 1970; Nowak 1996
Mass fragmentography	Metamphetamine	Suzuki *et al.* 1989
Gas chromatography	Amphetamine, cocaine	Suzuki *et al.* 1984; Cirimele *et al.* 1995; Miller *et al.* 1994
Flow injection hydride generation atomic absorption spectrometry	Arsenic	Das *et al.* 1995
III. *Biological markers*		
High performance liquid chromatography	Furosine (glycosylated keratin), terbinafine	Sueki *et al.* 1989; Dykes *et al.* 1990
Microscopy	Lipid: triglyceride	Salamon *et al.* 1988
Adsorption differential pulse voltametry	Ni	Gamelgaard & Anderson 1985
Enzymic assay	Steroid sulphatase	Matsumoto *et al.* 1990
Neutron activation analysis	Zinc, selenium	Rogers *et al.* 1991; Van Noord *et al.* 1987, Yoshizawa *et al.* 1998

their relationship to age, sex heredity and other factors. *Journal of Gerontology* **10**, 401–415.

Head, H. & Sherrin, J. (1905) The consequence of injury to peripheral nerves in man. *Brain* **28**, 116.

Hewitt, D. & Hillman, R.W. (1966) Relation between rate of nail growth in pregnant women and estimated previous general growth rate. *American Journal of Clinical Nutrition* **19**, 436–439.

Hillman, R. (1955) Fingernail growth in the human subject. *Human Biology* **27**, 274–283.

Hogan, D.B., McNair, S., Young, J. & Crilly, R. (1984) Nail growth, calcium and vitamin D. *Annals of Internal Medicine* **101**, 283.

Johnson, M., Comaish, J.S. & Shuster, S. (1991) Nail is produced by the normal nail bed: a controversy resolved. *British Journal of Dermatology* **125**, 27–28.

Landherr, G., Braun-Falco, O., Hofmann, C. *et al.* (1982) Fingernagelwaschtum bei psoriatikern unter puva-therapie. *Hautarzt* **33**, 210–213.

LeGros Clark, W.E. & Buxton, L.H.D. (1938) Studies in nail growth. *British Journal of Dermatology* **50**, 221–229.

Miller, E. (1973) Levodopa and nail growth. *New England Journal of Medicine* **288**, 916.

Orentreich, N., Markofsky, J. & Vogelman, J.H. (1979) The effect of ageing on the rate of linear nail growth. *Journal of Investigative Dermatology* **73**, 126–130.

Roberts, D.F. & Sandford, M.R. (1958) A possible climactic effect on nail growth. *Applied Physiology* **13**, 135–137.

Pfister, R. & Heneka, J. (1969) Wachstum und Gestaltung der Zahnnagel bei Gesunden. *Archiv der Klinische und experimentale Dermatologie* **223**, 263–274.

Sibinga, M.S. (1959) Observations on growth of fingernails in health and disease. *Pediatrics* **24**, 225–233.

Weismann, K. (1977) J.H.S. Beau and his descriptions of transverse depressions on nails. *British Journal of Dermatology* **97**, 571–572.

Nail plate biochemical analysis

Methods of analysis

A great range of methods have been used to analyse the organic and inorganic content of nails. Table 1.6 gives a guide, indicating how particular methods are appropriate for different constituents.

Nail proteins

From Table 1.6 on analytical methods it is clear that a considerable number of endogenous and exogenous materials can be sought in the nail plate. The protein mesh into which the elements fit is made primarily of the intracellular protein keratin. The highly ordered structure of the proteins in the nail plate helps explain the degree of chemical and physical resistance in contrast to the characteristics of skin. The proteins of hair and nail alike have extensive folding maintained by extremely stable disulphide bonds. Although these bonds can also be found to a lesser extent in the stratum corneum of normal skin, they have a different geometry in the two sites as demonstrated by Raman spectroscopy. This is expressed as *gauche–gauche–gauche* for hair and nail and *gauche–gauche–trans* for stratum corneum (Gniadecka *et al.* 1998). The latter is less stable. The altered geometry of disulphide bonds and the extreme folding of protein molecules in hair and nail results in a different degree of hydration. The looser structure of skin allows more free water, whereas the structure of hair and nail allows very little. This contrasting degree of hydration means

that skin is capable of sustaining metabolic processes not seen in nail (Gniadecka *et al.* 1998). Keratins and the associated proteins fall into the following categories:

- low sulphur proteins (40–60 kDa);
- high sulphur proteins (10–25 kDa);
- high glycine/tyrosine proteins (6–9 kDa).

It is believed that the low sulphur keratins form 10-nm filaments and the latter two groups of proteins form an interfilamentous matrix. The diversity of keratins within humans and between different species lies in the permutations of these three proteins (Gillespie & Frenkel 1974) and the diversity of the keratins themselves. Over 30 high sulphur proteins have been identified in human nail by polyacrylamide gel electrophoresis (Marshall 1980).

Nail plate keratin fibrils appear orientated in a plane parallel to the surface and in the transverse axis (Forslind 1970). They fall roughly into an 80 : 20 split between 'hard' hair type (trichocyte) keratin and 'soft', epithelial keratin (Lynch *et al.* 1986). These two variants are similar in many respects and share an X-ray diffraction pattern of α-helices in a coiled conformation, also confirmed using Raman spectroscopy (Gniadecka *et al.* 1998). Hard keratins split into the classical association of acidic and basic pairs, with extensive amino acid homologies with the epithelial forms (Hanukoglu & Fuchs 1983). In spite of regions of homology, the 'hard' and 'soft' keratins are distinguishable by immunohistochemistry (Lynch *et al.* 1986; Heid *et al.* 1988; Westgate *et al.* 1997). The relative resilience of the two groups of keratins is also reflected in their solubility in 2-mercaptoethanol. At 50 mmol/L concentrations, only epithelial 'soft' keratins are extracted from nail clippings. The concentration needs to be raised to 200 mmol/L before significant quantities of 'hard' keratin dissolve (Kitahara & Ogawa 1991).

The main lipid of nail is cholesterol. The total fat content is 0.1–1%, contrasting with the 10% found in the stratum corneum. The water content is less than that of skin, being 7–12% compared with 15–25%.

Mineral constituents of nail

X-ray diffraction is one of the best tried methods of elemental nail analysis. Much of the initial work was done by Forslind (1970). He observed that the hardness of the nail plate is unlikely to be due to calcium, which the analogy with bone has suggested. Detailed resumés of normal nail mineral content have been made (Zaias 1990).

Much interest has been demonstrated in the analysis of nails as a source of information concerning health. A significant increase in the nail content of Na, Mg and P was noted in a survey of 50 patients with cirrhosis (Djaldetti *et al.* 1987). In a comparison of term and preterm infants, a decrease in aluminium and sulphur was found in term deliveries. The high aluminium content in preterm infants was considered of possible relevance to the osteopenia observed in this group (Sirota *et al.* 1988). Copper and iron have been observed at higher levels in the nails of male 6–11 year olds in comparison with females (Alexiou *et al.* 1980). Iron levels in the general population were found to be equal in men and women, but higher in children and highest in the neonate (Jacobs & Jenkins 1960).

Biological markers in nail plate

In some respects, nail analysis can be compared with a blood test, but involving the examination of a less labile source of information. Analysis of chloride in nail clippings of a juvenile control population and those suffering cystic fibrosis, revealed a significant increase of chloride, by a factor of 5, in the latter. This has led to the suggestion of 'screening nail by mail' for inaccessible regions, where sending nails would be relatively easy.

The glycosylated globin molecule, used for estimation of long-term diabetic control, has been used as a model in studies measuring nail furosine in diabetes mellitus. The nail fructose-lysine content is raised in this disease and has shown a correlation with the severity of diabetic retinopathy and neuropathy (Oimomi *et al.* 1985). Nail furosine levels have also shown a good correlation with fasting glucose and may even compete with glycosylated haemoglobin as an indicator of long-term diabetic control (Sueki *et al.* 1989).

Steroid sulphatase and its substrate, cholesterol sulphate, have been assayed in the nails of children being screened for X-linked ichthyosis and found to have adequate sensitivity and accuracy to be useful (Djaldetti *et al.* 1987; Matsumoto *et al.* 1990; Serizawa *et al.* 1990). Sudan IV-positive material in nails has been measured as a guide to serum triglycerides (Salamon *et al.* 1988).

Selenium is a trace element critical for the activity of glutathione peroxidase, which may protect DNA and other cellular molecules against oxidative damage. High concentrations are seen to protect against the action of certain carcinogens in some animal models and consequently its role in human cancers has been explored. Analysis of the selenium levels of different rat tissues suggest that blood selenium may be the best indirect measure of liver selenium and nail selenium may best reflect whole body levels and the level in skeletal and heart muscle (Behne *et al.* 1996). Nail selenium levels in those being screened for oral cancer (Rogers *et al.* 1991) and carcinoma of the breast (Djaldetti *et al.* 1987; van den Brandt *et al.* 1994) showed no significant differences between affected and control patients. However, in a prospective study, toenail selenium levels had a weak predictive value for the development of advanced prostate cancer, where low levels of selenium predisposed to this malignancy (Yoshizawa *et al.* 1998). Examination of a wide range of trace elements in the nails of women with breast cancer failed to show any difference from normal controls (Garland *et al.* 1996) and analysis of nail for zinc showed no significant difference between pellagra patients with low serum zinc and normal controls (Vannucchi *et al.* 1995).

Nail clippings can be used as a source of DNA which, after amplification by the polymerase chain reaction, is of forensic use. Early work required 20–30 mg of nail (Kaneshige *et al.* 1992) and this figure has decreased to 9 mg, where the DNA for the HLA-DQa alleles are used to assess homology with blood samples (Tahir & Watson 1995).

Exogenous materials in nail analysis

Exogenous materials can be considered in two groups: environmental and ingested substances. In the first category, cadmium, copper, lead and zinc were examined in the hair and nails of young children (Wilhelm *et al.* 1991). This was done to gauge the exposure to these substances sustained in rural and industrialized areas of Germany. Both hair and nail reflected the different environments, although the multiple correlation coefficient was higher for hair than nails.

Water taken from wells in arsenic-rich rock has resulted in arsenic poisoning on a major scale in West Bengal, India over the last 10 years. About 50% of ingested arsenic is excreted in the urine, smaller amounts in the faeces, hair and nails. Nail analysis has been used in the Bengal population as well as in other populations suffering arsenic poisoning. Levels were estimated using flow injection hydride generation atomic absorption spectroscopy which allows analysis using very small samples and enables comparisons between different tissues. The Bengal experience suggests that there are similar concentrations in hair and nail, with a trend towards higher concentrations in the latter (Das *et al.* 1995). During an episode of arsenic posioning in Alaska, the level of arsenic in nail was four times that found in hair (Harrington *et al.* 1978). A study in New Hampshire, USA, found that in subjects drinking from arsenic-rich wells, there was a doubling of toenail arsenic for a 10-fold rise in water arsenic content (Karagas *et al.* 1996).

The features of arsenic poisoning were different in Alaska and Bengal, with far more cutaneous and systemic signs of toxicity in the Bengal population in spite of similar levels in body tissues. This was attributed to coexistent dietary deficiencies and ill health in the Bengalis.

In addition to hair and nail, teeth can also act as indicators of long-term unwanted substances and, in particular, heavy metals. One account suggests that hair reflects a period of 2–5 months, nails 12–18 months and teeth a far longer period measured in years (Nowak 1996). These figures are likely to be subject to the length of the hair, the site of nail sampling (toe vs. finger) and the age of the subject.

Nickel analysis has been performed to establish occupational exposure (Gamelgaard & Anderson 1985).

The use of forensic nail drug analysis has been reported by the Japanese where over 20 000 people were arrested for the abuse of methamphetamine in 1987 (Suzuki *et al.* 1984, 1989). It was found that the drug entered the nail via both the matrix and nail bed. Chronic drug abusers could be distinguished from those with a single recent ingestion by scraping the undersurface of the nail before analysis. This would remove the nail bed contribution and the drug it contained in the 'one-off' abuser.

Simultaneous hair and nail analysis has been performed to compare the capacity of the tissues to reflect chronic drug abuse in those taking cocaine (Miller *et al.* 1994) and amphetamines (Cirimele *et al.* 1995). Miller *et al.* (1994) found that concentrations of cocaine and its derivatives were higher in hair than in nail, whereas Cirimele *et al.* (1995) found that the concentrations of amphetamines and its metabolites were similar in both tissues. Analysis of nail clippings from the newborn by gas chromatography–mass spectroscopy can provide evidence of exposure to cocaine during embryogenesis. Given the point of nail formation, it is likely that the levels will reflect exposure after the 14th week (Skopp & Pötsch 1997). Inclusion of the antifungal, terbinafine, via the nail bed has also been observed (Dykes *et al.* 1990). Access of the drug to the nail plate via the nail bed may be one of the important factors allowing effective therapy to be delivered in less time than it takes to grow a nail (Matthieu *et al.* 1991; Munro & Shuster 1992). *In vitro* models for the uptake and delivery of terbinafine by nail plate have been employed to examine aspects of this process (Rashid *et al.* 1995).

Following single large doses of methamphetamine, it can be detected by mass fragmentography in saliva up to 2 days later, hair up to 18 days and in nail for the next 45 days (Suzuki *et al.* 1989). Chloroquine (Ofori-Adjei & Ericsson 1985) has also been measured in nail clippings for research purposes up to a year after ingestion.

References

Alexiou, D., Koutselinis, A., Manolidis, C. *et al.* (1980) The content of trace elements in fingernails of children. *Dermatology* **160**, 380–382.

Behne, D., Gessner, H. & Kyriakopoulos, A. (1996) Information on the selenium status of several body compartments of rats from the selenium concentrations in blood fractions, hair and nails. *Journal of Trace Elements in Medicine and Biology* **10**, 174–179.

van den Brandt, P.A., Goldbohm, R.A., vant Veer, P. *et al.* (1994) Toenail selenium levels and the risk of breast cancer. *American Journal of Epidemiology* **140**, 20–26.

Cirimele, V., Kintz, P. & Mangin, P. (1995) Detection of amphetamines in fingernails: an alternative to hair analysis. *Archives of Toxicology* **70**, 68–69.

Das, D., Chatterjee, A., Badal, K. *et al.* (1995) Arsenic in ground water in six districts of West Bengal, India: the biggest arsenic calamity in the world. *Analyst* **120**, 917–924.

Djaldetti, M., Fishman, P., Harpaz, D. & Luric, B. (1987) X-ray microanalysis of the fingernails in cirrhotic patients. *Dermatologica* **174**, 114–116.

Dykes, P.J., Thomas, R. & Finlay, A.Y. (1990) Determination of terbinafine in nail samples during treatment for onychomycoses. *British Journal of Dermatology* **123**, 481–486.

Forslind, B. (1970) Biophysical studies of the normal nail. *Acta Dermato-Venereologica* **50**, 161–168.

Gamelgaard, B. & Anderson, J.R. (1985) Determination of nickel in human nails by adsorption differential-pulse voltametry. *Analyst* **110**, 1197–1199.