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Review

The Ultrastructure and Growth of Human Nails

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Clinicians used to observing the slow rate of clearance of diseased or damaged nail are apt to see the nail apparatus as a rather inert structure. Paticularly during the last 15 years, ultrastructural and physiological research has shown that under a variety of circumstances the nail matric and its product, the nail plate, are in fact the centre of marked biological activity.

Dystrophies specific to nail tissue are rarely diagnosed accurately, largely because dermatologists are loathe to resolve differential diagnostic conflicts by resorting to histology; scarring and deformity due to biopsy are the usual reasons for not undertaking microscopic studies. The longitudinal biopsy technique of Zaias [1] does not lead to significant scarring; if done correctly only a fine inconspicuous line in evident after complete healing (Fig. 1a–c). In general,

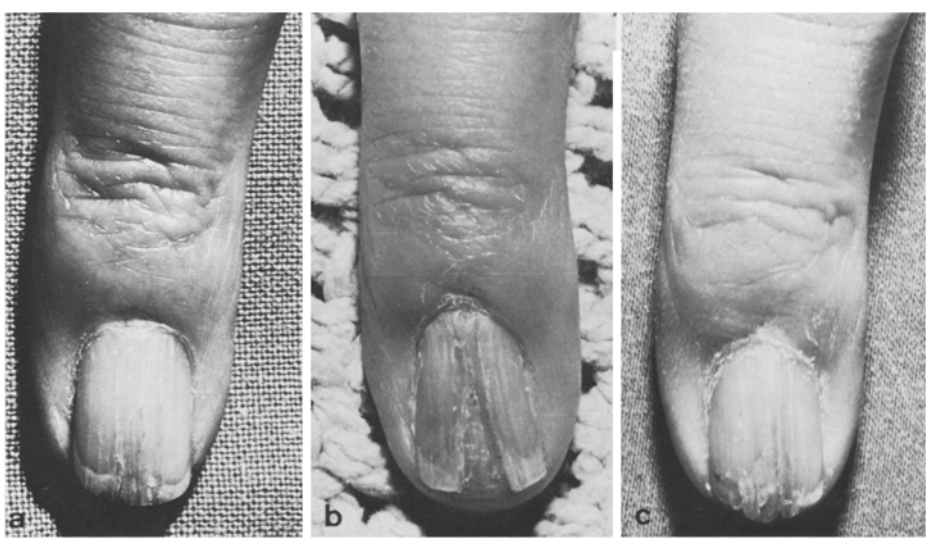

Fig. 1. a Lichen planus of the nail. **b** Same nail as in Fig. 1a, 4 weeks after biopsy. **c** Same nail as in Fig. 1b, 6 months after biopsy

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diseases, such as psoriasis and lichen planus show similar histological changes in the nail apparatus as in the skin [1, 2]. Wilkinson et al. [3, 4], describing a case of idiopathic (20-nail) dystrophy of childhood, showed that a spongiotic process was present and not lichen planus. A patient presenting with longitudinal ridging and fragility of nails was shown to have amyloid deposits in the nail fold and distal nail bed associated with parakeratosis of the nail plate [5]; further investigations revealed systemic amyloidosis. The author has used the Zaias technique consistently during the last 10 years and useful diagnostic and prognostic information has frequently been found. For example, onycholysis due to psoriasis shows the histology of psoriasis and the nail changes seen in Darier's disease are accompanied by acantholytic changes in the nail matrix. The longitudinal pigmented line common in negroes shows the histology of a benign pigmented naevus cell; in white people this is important in excluding malignant melanoma when doubt exists, and little anatomical distortion results. Bowen's disease of the nail apparatus [6] can be confirmed by nail biopsy and squamous carcinoma excluded, thus avoiding radical surgical treatment.

Evolution and Development

In higher primates and man, nails have developed in conjunction with the acquisition of manual dexterity; other mammals do not possess such flattened claws. Close inspection of the evolutionary 'ladder' shows that nails have evolved from claws. The lowest evolutionary level at which claws are present is in amphibia [7]. As will be seen later, in man the intermediate matrix contributes the greatest mass to the nail plate, whereas claws are mainly a product of the dorsal matrix [8].

The nail apparatus is formed from an invagination of the primitive epidermis on the dorsum of the terminal phalanges. In this respect it is similar to the hair follicle. This first appears during the 9th week of gestation. By the 13th week the nail bed and nail fold possess a granular layer with keratohyalin granules which disappear when the hard nail plate is formed [9], though the ventral part of the root, which subsequently gives rise to the intermediate nail matrix, never forms a granular layer. By 24 weeks, the free nail plate is visible to the naked eye and at full-term the free edge of the nail plate extends over the hyponychium [9, 10].

It is presumably this embryological association with the integumentary epidermis which predisposes the nail apparatus to those diseases which primarily affect the epidermis.

Nail Matrix and Plate Structure

The nail plate is formed from the nail matrix. There is still controversy regarding which part of the differentiated epidermis produces the definitive hard nail plate. Figure 2 shows the matrix as defined by Lewis [9]; the fact that he delineated three matrix areas fits the evolutionary facts [8, 11] and the little cell kinetics work carried out thus far in humans. This will be discussed in more detail later. The sohlenhorn ('sole horn') of Boas [12], the most distal part of the nail bed, takes no part in the formation of the hard nail plate; however, in view of its importance in producing horny subungual tissue in claws and hooves, it has been suggested as the site of

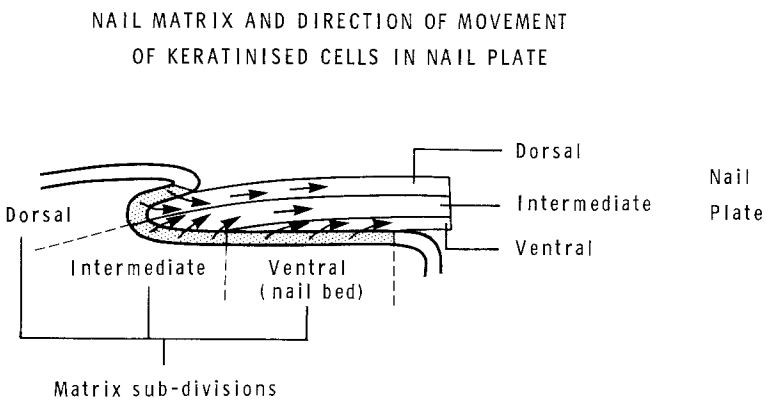

Fig. 2. The nail matrix and nail plate

excess horny subungual tissue in diseases such as pachyonychia congenita and pityriasis rubra pilaris.

The nail matrix epidermis has a distinct basal layer whose cells interdigitate [13]. As with the interfollicular epidermis, adjacent basal cells possess desmosomal contacts whilst the cell surfaces in contact with the basement membrane have hemidesmosomes. Differentiation of keratinocytes in the transitional area takes place over several cell layers [14]. Despite the short distance from the germinal to the fully keratinised layer, transmission electron microscope studies have shown little cytoplasmic endoplastic reticulum and only small Golgi apparatus. However, differentiating cells in the 'prickle cell' area have more RNA, ribosomes and polysomes than equivalent epidermal cells. No keratohyalin granules are formed during nail matrix keratinisation; in this respect it is similar to hair formation [15, 16]. Microfibrils are manufactured during differentiation having a diameter of approximately 75 Å; these become orientated vertically relative to the axis of growth of the hardened nail plate [16] in contrast to the longitudinal orientiation of the equivalent microfibrils in the hair cortex. Lysosomal cytoplasmic organelles, the membrane-coating granules, discharge their contents into the intercellular spaces in the transitional zone [17]. The exact function of this organelle is not known. In the epidermis it is currently thought to play some part in desquamation but evidently this cannot be the function in the nail apparatus in view of the firm adhesion between nail plate cells [18]. Ribosomes containing RNA are still present in transitional cells until the stage of plasma membrane thickening; mitochondria have already become degraded by this stage. Such retention of ribosomes and also nucleoli to a late phase of keratinisation differs from hair development but is similar to feather formation in birds [19]. In general, cells forming the dorsal, and ventral nail plate lose their cytoplasmic and nuclear structures at an earlier stage of keratinisation; nuclear and nucleolar remnants are frequently seen in the mature intermediate nail plate. Since distinct differences are evident in the structure of the dorsal, intermediate, and ventral nail plate and the corresponding transitional areas it is useful to describe these separately [9]:

1. Dorsal Nail Plate

This stains very poorly with routine stains, such as eosin. Individual cells are very flat and are closely apposed by many gap junctions [20]; the shininess and smoothness of the nail surface is probably related to this characteristic. In the transitional area there is a strong reaction for bound cysteine (SH) and phospholipids are present [14]. Cells about to keratinise take up acid phosphatase stains associated with the presence of nucleotides released from RNA and DNA. The mature dorsal nail plate does not contain these nucleic acids.

2. Intermediate Nail Plate

A much broader transitional zone is present in this area. Nuclear remnants are retained and stain yellow with thioflavine T. Membrane thickening and disulphide bonding of keratin occur earlier than in the dorsal zone and there is also less bound phospholipid release. Bound cysteine is found in highest concentration in the upper part of the intermediate region. Acid phosphatase activity can be demonstrated in both the transitional zone and the nail plate. In contrast to the dorsal and ventral matrix, non-specific esterase activity is present in the transitional area (and the earlier stages of keratinisation); this is absent from the fully hardened nail.

Fully keratinised cells are less flat than those of the dorsal plate and contain many vacuoles. The intermediate plate comprises the main bulk of the nail plate, analagous to the cortex of the hair shaft; unlike the latter, however, transmission electron-microscopic studies reveal irregular staining suggestive of uneven keratinisation and retention of non-keratinised structures in the flully formed intermediate plate. The non-fibrillary keratin demonstrates a granular structure similar to hair cuticle cells. The darker staining of the nail plate, non-fibrillary keratin compared to the microfibrils is in general similar to that seen in the hair cortex where the matrix protein is relatively rich in sulphur. The fibrillary protein is arranged in a dorso-ventral orientation.

3. Ventral Nail Plate (from Nail Bed Matrix)

This is the thinnest layer being only one or two cells thick; nuclear remnants are visible in keratinised cells. Bound phospholipids are present throughout the epidermis in this region. Acid phosphatase and non-specific esterase are absent (cf. the dorsal and intermediate zones), whilst bound cysteine can be detected in the transitional region. The histochemical characteristics of this layer suggest the occurrence of pressure keratinisation [21].

Nail Plate Biochemistry

Keratin

Jarrett and Spearman [14] have shown by histochemical methods that cystine, containing stable disulphide bonds, is concentrated particularly in the intermediate nail plate at the periphery of individual cells; the lowest concentration is found in the dorsal plate. The reverse position applies with regard to bound sulphydryl

groups, the highest concentration being present in the dorsal nail plate. Total sulphur concentration is similar in the dorsal and intermediate plates [22]. Nail keratin analysis has revealed essentially the same fractions present in nail as in hair:

(i) α-fibrillar, low sulphur protein.
(ii) Globular high sulphur matrix protein.
(iii) High glycine-tyrosine rich matrix protein.

Amino-acid analytical studies on nail keratin [23] show higher cysteine, glutamic acid, and serine and less tyrosine in nail compared to wool. Most of the cysteine is probably in the intermediate plate [14]. Proline and threonine are in greatest concentrations in the high sulphur proteins of the keratins; moving boundary electrophoresis, used to compare high sulphur fractions of hair and nail has shown that there are marked differences, suggesting that a different mixture of proteins comprises the high sulphur fractions of hair and nail [24].

Calcium

This is found both as the phosphate in hydroxyapatite crystals in the cytoplasm and bound to phospholipids particularly in the dorsal and ventral nail plates [25]. Using alizarin red staining, Cane and Spearman [19] showed calcium to be the chief metal in nail. The concentration of calcium is approximately 0.11 % by weight, i.e., ten times greater than in hair [26]. It has been suggested that nail calcium is not part of the intrinsic structure [22] but absorbed into the nail from extrinsic sources such as soaps; nail is relatively porous and calcium could enter as ionic calcium or bound to fatty acids. In support of this theory is the finding of significantly greater quantities of calcium in the terminal free edge of the ventral plate. Pautard [25, 26] believes that calcium is a constituent part of the nail structure; supporting this is the presence of the same calcium/magnesium ratio (4.5:1) in the nail as in blood [27]. Both ideas are probably relevant. The author agrees with Forslind [20] that calcium content contributes little if anything to the hardness of the nail plate.

Phospholipids

The nail plate contains significant amounts of phospholipids particularly in the dorsal and ventral layers [28]. Free fats and long chain fatty acids are detectable but like calcium it has been suggested that such constituents are of extrinsic origin.

Nail Growth

The nail grows distally as a flat plate largely because of pressure forces from each dividing component of the matrix within the confines of the posterior nail fold and the proximal part of the lateral nail folds; the plate is of course hardened before it becomes exposed and free from the direct influence of the nail folds. Kligman [29] transplanted adult human nail root into interfollicular skin and demonstrated that the nail grows vertically away from the skin surface as a pillar of keratin: presumably because of the influence of the nail root dermis also transplanted, the viable epidermis retained its characteristic appearance. Hashimoto et al. [30] suggested that forward orientation of matrix cells may also contribute to the production of a flat nail plate.

Cell kinetic studies on human nails have been limited by the difficulty of obtaining tissue; many of the techniques used to study DNA synthesis, the mitotic cycle and transit times in skin require serial observations (and biopsies) and are therefore not directly applicable to the nail matrix. Mitotic activity can certainly be seen in the basal area of the dorsal, intermediate, and ventral matrices. Autoradiographic studies in the squirrel monkey [31] showed that labelled glycine was incorporated into the dorsal and intermediate matrix but not into the nail bed. Since glycine is a constituent part of keratin it was therefore suggested that the nail bed plays no part in the formation of the nail plate. However, in primitive monkeys of this type it is known that the nail bed is poorly developed; also Norton et al. [32] showed in human volunteers that the nail bed does incorporate labelled glycine but in lower concentrations than the intermediate nail matrix which produces the bulk of the nail plate. Tritiated thymidine uptake studies suggest the fastest mitotic rate is in the intermediate matrix, the slowest being the nail bed matrix [32].

Nail growth measurements have mostly related to studies on the distal movement of a reference mark etched on the nail plate over a fixed period of time. Such studies do not measure the volume of keratin produced per unit time which therefore limits the interpretation of the results. Attempts have been made to overcome this problem by also measuring terminal nail thickness using graduated calipers [32]. It is false to assume that nail 'growth' as measured in this way is directly related to matrix basal cell mitotic activity though skin epidermis mitotic rates and transit times correlate well under most circumstances.

Linear nail growth has been a popular subject for study in health and many disease states for more than a century. Normal nail growth varies from over 150 μm per day in children to less than 60 μm per day in old age [32, 33]. There is a linear reciprocal relationship between age and nail growth [34]. The middle fingernail grows significantly faster than the other fingers, the thumb and little fingernail showing the slowest growth rate [32, 33, 35]. Most studies have shown no significant difference in nail growth between the sexes or between the right and left hands. Toenails grow at a half to one third of the rate of fingernails [36]. It has been suggested that nails continue to grow for up to 10 days after death [37] but this is likely to be an artefact due to shrinkage of periungual soft tissues [36]. A persistently cold environment may decrease nail growth [38, 39]; daytime growth rates are greater than corresponding night rates [40]. Many women have noted the more frequent need for nail cutting during pregnancy; this observation was confirmed by Halban and Spitzer [41]. A slowing of nail growth has been observed in many disease states, including Beau's lines [42] and other general physical or psychological stresses [43] including the first day of life [44]; immobility due to plaster cast application [45, 46] and denervation [45]; Kwashiorkor and Marasmus [47]; Yellow Nail Syndrome [36], and systemic treatment with methotrexate and azathioprine [48]. Nail growth is markedly increased by regular terminal damage to the nail plate from nail biting and the trauma of manual labour [36, 49]. It is of interest that linear nail growth is increased in certain diseases in which cell kinetic studies have shown epidermal hyperproliferation. In psoriasis both onycholytic and pitted nails grow significantly faster than clinically normal nails in psoriatic subjects; also, the latter demonstrated a faster rate than normal subjects [33]. Similar results have also been found in pityriasis rubra pilaris and congenital bullous ichthyosiform erythro-

derma [36]. It thus appears that the nail matrix become hyperproliferative in those diseases proven to have increased 'turnover' of the whole integumentary epidermis. This differentiates the nail matrix from the hair bulb; the external root sheath and interfollicular epidermis shows a similar increase in cell proliferation rates in psoriasis as the skin generally; however, no increase has yet been demonstrated in hair follicle matrix cells. It seems unlikely that the hair matrix can increase cell turnover any further than the very rapid rate that already exists in the normal anagen bulb. Adult women not infrequently develop onycholysis for which no cause can be found [36]. It has been suggested on clinical grounds that idiopathic onycholysis of this type is a rare manifestation of psoriasis. Affected nails have been shown to grow faster than normal nails but the unaffected fingernails in contrast to clinically normal nails in psoriasis, have a normal growth rate [50].

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