

## BIOPHYSICAL STUDIES OF THE NORMAL NAIL

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**Abstract.** The keratin filament organization in the normal human nail has been investigated by means of micro X-ray diffraction. The X-ray data obtained have been correlated to electron microscope and light microscope findings concerning the structure and histology of the hard nail plate. Analyses of the calcium content in normal human nails have also been performed. These investigations show that the hardness of the nail can be explained on the basis of cell arrangement, cell adhesion, and the ultrastructural arrangement of the keratin fibrils.

A thorough knowledge of normal nail histology and of the structural organization of the cell contacts as well as of the intracellular components is essential in explaining the functional properties of the nail. Such information will also provide a basis for a better understanding of pathological conditions of the nail. In the past, most studies on the human nail have been confined to investigations by means of the light microscope. The recent publication by Hashimoto et al. (7) is one of the first comprehensive electron microscope investigations of the normal nail structure.

In the present report, in order to facilitate the interpretation, the findings are described in relation to a coordinate system superposed on the nail plate. The  $x$ - and  $y$ -axes in this coordinate system lie in the plane of the nail plate, the  $z$ -axis being at right angles to this plane. The  $x$ -axis is chosen to lie in a direction parallel to the growth direction, the  $y$ -axis at right angles to the former axis (Fig. 2).

*The morphology of the nail plate.* Macroscopically, the normal nail plate has double curvatures. The longitudinal curvature lies in the  $(x, z)$ -plane, the transverse curvature lying in the  $(y, z)$ -plane. At the light microscope level most of the details have been exhaustively explored (8, 9, 10, 15).

The nail plate (Fig. 1) was earlier described as

a uniform sheet of keratinized cells originating from a ventral matrix in the proximal nail fold. This matrix was considered to terminate at the distal end of the lunula. The fully developed nail plate is supported by the part of the nail bed distal to the lunula (9, 15).

The modern terminology originates from the work of Barton Lewis (10) who describes the nail as derived from three different epidermal sites. The dorsal nail plate ( $D$ ) is formed by the dorsal matrix constituting the most proximal parts of the roof and the floor in the proximal nail fold ( $DM$ ). The intermediate nail plate ( $I$ ) is formed by the nail matrix of the floor of the proximal nail fold distal to the dorsal nail matrix. The distal border of this matrix coincides with the distal border of the lunula ( $L$ ). The soft ventral nail plate ( $V$ ) is derived from the tissue lying distal to the lunula border. In longitudinal sections the root matrix of the intermediate nail plate is seen to overlap the matrix of the ventral nail plate at the lunula border. Support for Lewis' interpretation has been given by Hashimoto et al. (7), among others.

Dynamic aspects of the nail growth has been provided by an autoradiographic study reported by Zaias & Alvarez (19).

*Organization of the keratin fibrils in nail cells.* Studies on the orientation of the keratin fibrils within the nail cells have been performed by Astbury & Sissons (1) as well as by Derksen, Heringa & Weidinger (3) on nails comprising both dorsal and intermediate nail plates. The main bulk of keratin fibrils were found to be oriented perpendicular to the growth axis of the nail plate and parallel to the nail surface, mainly in the  $y$ -axis direction.

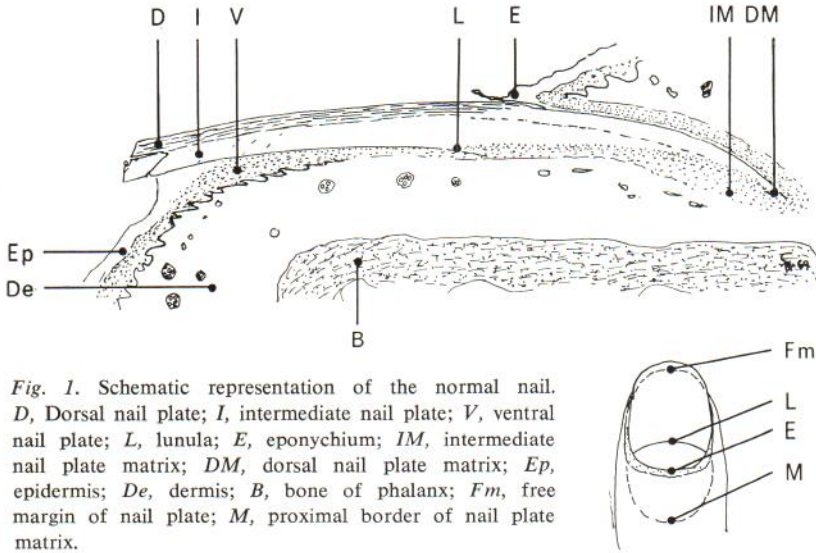


Fig. 1. Schematic representation of the normal nail. D, Dorsal nail plate; I, intermediate nail plate; V, ventral nail plate; L, lunula; E, eponychium; IM, intermediate nail plate matrix; DM, dorsal nail plate matrix; Ep, epidermis; De, dermis; B, bone of phalanx; Fm, free margin of nail plate; M, proximal border of nail plate matrix.

*Investigations of the nail in relation to its hardness.* The hardness of the nail has been attributed to an alleged high content of calcium (8). No quantitative data to support this proposal have been published. Disulphide bridges of cystine are known to stabilize the fibrous keratins. The cystine content of mature nails, 9.4% by weight, is relatively high when compared to a value of 1% in callus and 4.1% by weight in stratum corneum disjunctum (14). Other biophysical studies on the nail are rare, the exceptions being investigations on the indentation hardness (12) and on the strength of nails (18).

So far there has been no attempt to relate results from investigations of the morphological organization of the cells, and/or the structural organization of the keratin fibrils within the cells to the physical properties of the different parts of the nail plate, or to the nail plate as an entity. An X-ray diffraction study of the macromolecular organization of epidermal keratin was published by Swanbeck in 1959 (17).

The present work is concerned with the normal nail structure in an attempt to relate the structural organization of keratinized tissues to their functional properties. The terminology suggested by Lewis (10) is used in the present communication and only the hard nail plate, i. e. the dorsal and the intermediate nail plates are considered in this report.

## METHODS AND MATERIALS

*Electron microscopy.* Human nail clippings and small dissected tissue blocks from monkey nail roots (Rhesus macacus) were fixed in buffered 1% osmium tetroxide (13) for 20 min. In an attempt to improve the contrast some clippings were fixed for 72 hours. Dehydration and embedding in Epon followed the principles given by Luft (11). Sections were obtained with glass knives on an LKB Ultratome set for a section thickness of 600 Å. However, it was not possible to obtain such thin sections of this resistant material which displaces the zone of maximum shear stress away from the knife edge. The sections were transferred by means of a hole grid (6) to a saturated solution of uranyl acetate for 40 to 60 min at 60°C (2) for contrast enhancement. After rinsing in doubly distilled water the sections were mounted on carbon-coated grids and examined in a Zeiss EM 9 electron microscope at 50 kV and at primary magnifications of  $\times 1700$  and  $\times 7000$ . High resolution micrographs ( $\times 20\,000$  and  $\times 40\,000$ ) were obtained in a Siemens Elmiskop I, operated at 60 kV.

*X-ray diffraction.* Human nail clippings from 5 persons of both sexes and of different age (8 weeks to 35 years), with no known skin or generalized disease, as well as nails from 2 monkeys (Rhesus macacus) were used as specimens. A Chesley micro X-ray diffraction camera (Norelco) collimated with a glass capillary having a diameter of 300  $\mu\text{m}$  was used in the experiments. In order to record possible short spacings some specimens were exposed in a wide angle flat film camera with the same collimation which had a specimen to film distance of 50 mm. Nickel-filtered  $\text{CuK}\alpha$ -radiation ( $\lambda = 1.54 \text{ \AA}$ ) was used throughout the experiments. The nail clippings were exposed with the primary X-ray beam parallel to the z-axis (Fig. 2 a) or parallel to the y-axis (Fig. 2 b) or parallel to the x-axis (Fig. 2 c), so as to give X-ray dia-

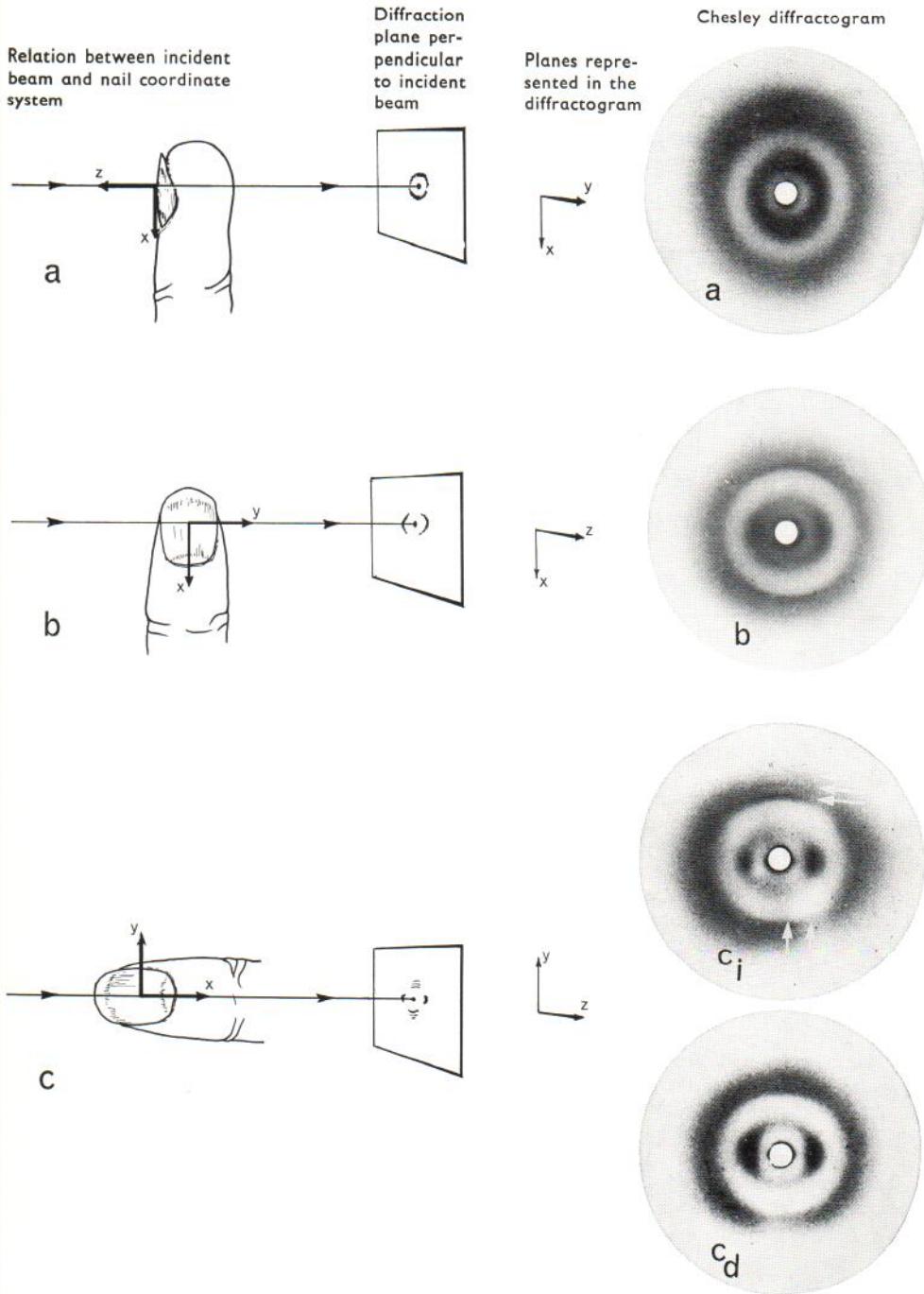


Fig. 2. X-ray diffraction experiments on nail clippings with representation of the relation between the incident X-ray beam and the nail plate and the diffractograms. The latter represent patterns obtained from the intermediate nail plate with the exception of pattern  $c_d$  which

is from the dorsal nail plate. Magnification,  $\times 2$ .

Horizontal arrows in pattern  $c_i$  indicate 3.9 Å, 4.3 Å and 5.2 Å reflexions reading downwards. Vertical arrows indicate 27 Å and 8.8–10.8 Å equatorial reflexions.

Table I. Calcium content of human nails determined by atomic absorption spectroscopy

All specimens consist of finger nail clippings from both hands of the subject except for BF<sup>1</sup> which contains toe nail clippings and GL<sup>(2)</sup> which contains a mixture of finger and toe nails

Specimen	Weight of fat-free specimen	Total volume (ml)	(Ca) weight %
<i>conc. HCl</i>			
BF I	0.5033	25	0.095
BF II	0.0586	3	0.184
BF <sup>1</sup>	0.2506	25	0.084
OI	0.3168	25	0.107
RL	0.1910	25	0.100
LI	0.1361	25	0.081
SI	0.8770	25	0.089
BN I	0.1953	25	0.072
BN II	0.0555	3	0.084
<i>conc. HNO<sub>3</sub></i>			
BF 6-8	0.1070	25	0.079
BF 9-10	0.1789	25	0.112
GL <sup>(2)</sup>	0.4956	25	0.188

grams from the nail in all three dimensions. Diffraction registrations were also obtained from the different morphological entities of the nail clippings, i.e. the dorsal (D) and the intermediate nail plate (I), after dissection in a low-powered light microscope.

X-ray diffraction patterns obtained from porcupine quill tips served as identification standards for  $\alpha$ -keratin.

*Calcium determination.* Human nail clippings were collected from 7 persons of both sexes (age 11-43 years) with no known general or skin diseases. The collection period covered 8 months in order to reduce possible short-term variations. After several washings in diethyl ether to make specimens fat-free, the clippings were immersed in concentrated hydrochloric acid for 7 days or more in order to extract the calcium present. In two cases the clippings were ground into a powder before the acid treatment. Some of the clippings were dissolved in concentrated nitric acid which also dissolved the organic portion. The determination of calcium was performed on a Perkin Elmer Atomic Absorption Spectrophotometer Type 290 with reference solutions containing 40 ppm calcium in the form of calcium chloride in concentrated hydrochloric acid or concentrated nitric acid (Table I).

## RESULTS

*Electron microscopic investigation.* At low magnifications the information obtained confirmed the results from light microscope investigations. During dissection of the nail clippings under the light microscope it was found that the border between the dorsal nail plate and the intermediate nail plate constituted a natural cleaving plane. The in-

termediate nail plate was also less resistant to mechanical stresses (cf. Fig. 3) since large pieces of this structure were easily loosened and separated from the dorsal nail plate at the free margin.

In the electron micrographs little structural detail except for the cell membrane were seen at primary magnifications at and below  $\times 4000$  due to the very dense sections of the mature nail. The cells of the dorsal nail plate appeared very flat with their smallest diameter perpendicular to the nail plate surface in the z-axis direction. The intercellular space between these cells was occupied by a substance that was electron-dense and which filled the intercellular space completely. Sometimes the central dense line was bisected by a faint intermediate line. The total width of this junction measured up to 250 Å (Fig. 4 a). In contrast to the almost straight cell membranes of the dorsal nail plate the borders of the intermediate nail plate cells had a much more meandering outline in the section. The distribution of dense intercellular substance was more or less discontinuous and the intercellular space filled by this substance was  $>200$  Å (Fig. 4 b). In the intermediate nail plate cells, a preferred orientation of the keratin filament in the y-axis direction was recognized at higher magnification.

In the electron micrographs the histochemically demonstrated border between the dorsal and the intermediate nail plates (10) was not obvious but the differentiation characteristics between these two layers were seen to be achieved by an almost continuous change in the cell adhesion pattern. Juvenile and adult mature human nails as well as monkey nails were similar in nature with respect to the organization seen in the electron micrographs. A slightly undulating contact line between the cells of the dorsal nail plate and a dominant filament orientation in the y-axis direction constituted the difference between the transverse sections and the longitudinal sections described above.

*X-ray diffraction experiments.* The X-ray diffraction experiments were performed on untreated nail clippings containing the dorsal and intermediate nail plate as well as on the untreated isolated constituents. In the experiments with the X-ray beam coinciding with the z-axis a pattern of rather extended arcs or almost circular reflexions were obtained (Fig. 2 a). A somewhat higher order of orientation was revealed with the X-ray

beam coinciding with the  $y$ -axis (Fig. 2 *b*). However, with the incident beam in the  $x$ -axis direction a pattern of good order was obtained, indicating a main fiber orientation in the  $(y, z)$ -plane (Fig. 2 *c*). No significant differences between nails from children and from adults or between human and monkey nails were found.

It became apparent that the degree of orientation in the X-ray patterns could be related to the different portions of the nail plate described in recent literature (7,10). The X-ray diffraction patterns from the isolated dorsal nail plate and the isolated intermediate nail plate had the same general features as had the patterns from compound nail clippings. However, in the intermediate nail plate, with the X-ray beam coinciding with the  $x$ -axis, shorter arcs as well as greater detail indicated an increased orientation of the keratin material as compared with a similar registration from the dorsal nail plate. This keratin pattern from the intermediate nail plate much resembled that of human hair fibres in its relative wealth of diffraction maxima (Table II).

The diffraction patterns contained no information of a separate phase (e.g. a calcium mineral) in either the dorsal or the intermediate nail plate.

The atomic absorption spectrophotometric analyses of the calcium content in normal nails show only traces of this element. As can be seen in Table I, no single value exceeds 0.2% by weight.

## DISCUSSION AND CONCLUSIONS

The electron microscope investigation demonstrated that the continuous cell junctions in the dorsal nail plate were similar to the tight junction type of cell contact, zonula occludens, described by Farquhar & Palade (4), whereas the somewhat wider and discontinuous junctions of the intermediate nail plate resembled the intermediate junction type, zonula adhaerence.

The mechanical properties of the nail plate are partly related to the properties of the cells constituting the different nail plate units. In the dorsal plate it is therefore conceivable that the intercellular junctions permit a good interaction between the cells, and thus a highly rigid plate is formed. In the intermediate nail plate the interlocking cell borders denote a large cell surface in comparison with the cell volume. This part of the nail plate has more plastic properties than is ob-

Table II. X-ray diffraction observation on nail clippings

S = Strong, M = Medium, W = Weak

Nail	Intensity	Porcupine quill tip
<i>Observed equatorial reflexions</i>		( <sup>a</sup> )
27 Å	S	27 Å
8.8–10.8 Å	S	9.2–10.5 Å
<i>Observed meridional reflexions</i>		( <sup>b</sup> )
23 Å	M–W	24.5 Å
5.2 Å	S	5.18 Å
4.3 Å	M–S	4.2 Å
3.9 Å	M	3.9 Å <sup>c</sup>

<sup>a</sup> Data obtained from G. Swanbeck in *Aspects of Protein Structure* (ed. G. N. Ramachandran), pp. 93–101. Academic Press, New York, 1963.

<sup>b</sup> Data obtained from A. Lang: *An X-ray Study of  $\alpha$ -keratin. II. Diffractometer measurements of the complete diffraction pattern of Canadian porcupine quill.* *Acta Cryst* 9: 446–451, 1956.

<sup>c</sup> Data obtained from W. T. Astbury–J. W. Haggith: *Pre-Transformation stretching of the so-called 5.1 Å and 1.5 Å spacings in  $\alpha$ -keratin.* *Biochim Biophys Acta* 10: 483–490, 1953.

served in the dorsal nail plate (Fig. 3). Referring to the dorsal nail plate it is interesting to note that the intercellular coupling of the cortex cells in hair fibers is very similar to that of the dorsal nail plate in terms of widths and continuity as seen in electron micrographs.

The details of the X-ray diffraction patterns obtained from nail clippings showed close resemblance to those obtained from porcupine quill tips which represent the preferred fibrous keratin specimen for X-ray diffraction (Table II). The degree of fibrillar orientation is reflected in the X-ray pattern and a low degree of orientation is revealed as arcs or even complete circles. A high degree of orientation gives arcs reduced to dots in cases of perfect alignment of the fibers. Generally, a greater number of reflexions is then seen in comparison with disoriented patterns. In the X-ray diffraction photographs from nail clippings, the specimen possessing the highest order of fibrillar orientation showed a meridional 5.2 Å reflexion characteristic of fibrous  $\alpha$ -proteins. In these cases it was also possible to see the broad equatorial reflexions corresponding to spacings of 25–27 Å and 10 Å, characteristic of  $\alpha$ -keratin.

The equatorial arcs of the X-ray patterns were

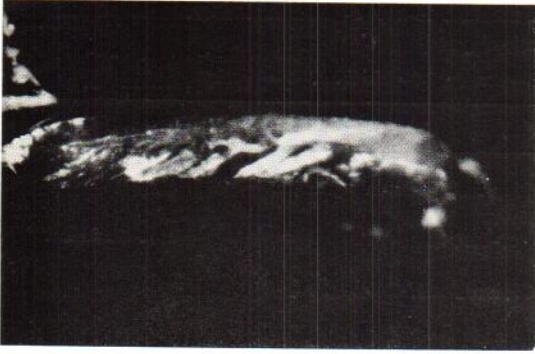


Fig. 3. Photograph of nail clipping sectioned in the  $(x,z)$ -plane. Oblique incident light,  $\times 50$ . The lamellar structure of the dorsal nail plate is suggested in the left hand part of the photograph. The oblique lamellae of the intermediate nail plate are artefacts imposed by the shearing forces of the scissor edge which compressed the plastic material of the intermediate nail plate.

due to the preferred orientation of the keratin fibrils. However, the spread in angular distribution, i.e. wider arcs, was more pronounced in the dorsal nail plate. This supports the concept of a fibril arrangement that provides for the torsional rigidity of the plate and a high breaking strength.

The water content of living cells is known to be about 70% by weight. Under different conditions of relative humidity the water content of stratum corneum varies significantly (16), a fact which is related to its relatively high content of water soluble substances ( $> 20\%$  by weight). This value is high in comparison with that of hard keratin (14). No quantitative data concerning the content of water-soluble substances in the isolated dorsal and/or intermediate nail plate have been published. In the hair follicle a decrease in water content occurs at a stage where the protein synthesis appears to have ceased (5). This favours the concept that the final consolidation of hard keratinized cells includes water deprivation. It is conceivable that such a water deprivation can introduce a disturbance of the fibrillar orientation order. Complementary investigation by means of water substitution in not-fully-consolidated nail root cells as well as drying of such cells in combination with X-ray diffraction methods should provide information on the filament lattice deformation introduced by water deprivation.

The information gained from the electron micrographs and the X-ray diffraction experiments revealed a fibril orientation in the plane of the

nail surface, the  $(x, y)$ -plane, with a pronounced orientation in the  $y$ -axis direction (Fig. 2). Such an arrangement provides a reinforcement preventing cleavage of the nail in the growth direction ( $x$ -axis direction) which would otherwise easily damage the nail root.

If calcium minerals were of importance in producing the hard structure of the nail, as believed by several authors, the mineral should be expected to appear in the form of apatite or calcite crystals as encountered in bone, teeth, and shells. Such crystals ought to show a preferred orientation within the nail plate giving a crystal pattern in the X-ray diffraction experiments even if the mineral content were comparatively low. Using histochemical methods, Jarret & Spearman (8) have demonstrated a calcium content in nails although no quantitative data are given in their report. In the very soft *ventral nail plate* a comparatively high calcium content was reported, only slightly less than that of the dorsal nail plate. This implies that any calcium mineral phase present would hardly be of importance to the hardness of the nail plate. Using calcium-EDTA to chelate and remove related metals in alizarin red stained sections those authors have probably introduced calcium into the tissue and their result should be taken with some caution. The low calcium content reported in the present study makes it unlikely that a calcium mineral salt is responsible for the strength of the nail. If the hardness of the nail plate were due to some other mineral salt we would likewise expect it to appear as a separate crystalline phase in the X-ray diffraction photographs. A possible mode of interaction between the calcium ions and the keratinized tissue is, however, the capacity of these ions to replace protons, to a great extent irreversibly, under physiological conditions. Such an exchange would be expected to influence the physical properties of the fibrous protein and on the tissue.

The present investigation indicates some functional relationships between the cellular arrangements, the cell junction types, and the structure of the nail plate. At the macroscopic level the cutting property can be ascribed to the arrangement of the dorsal and the intermediate nail plates. The dorsal nail plate, per se, is rather brittle and the nail plate as a whole gains in strength by the cooperation of the dorsal and the intermediate nail plates. The method employed for making

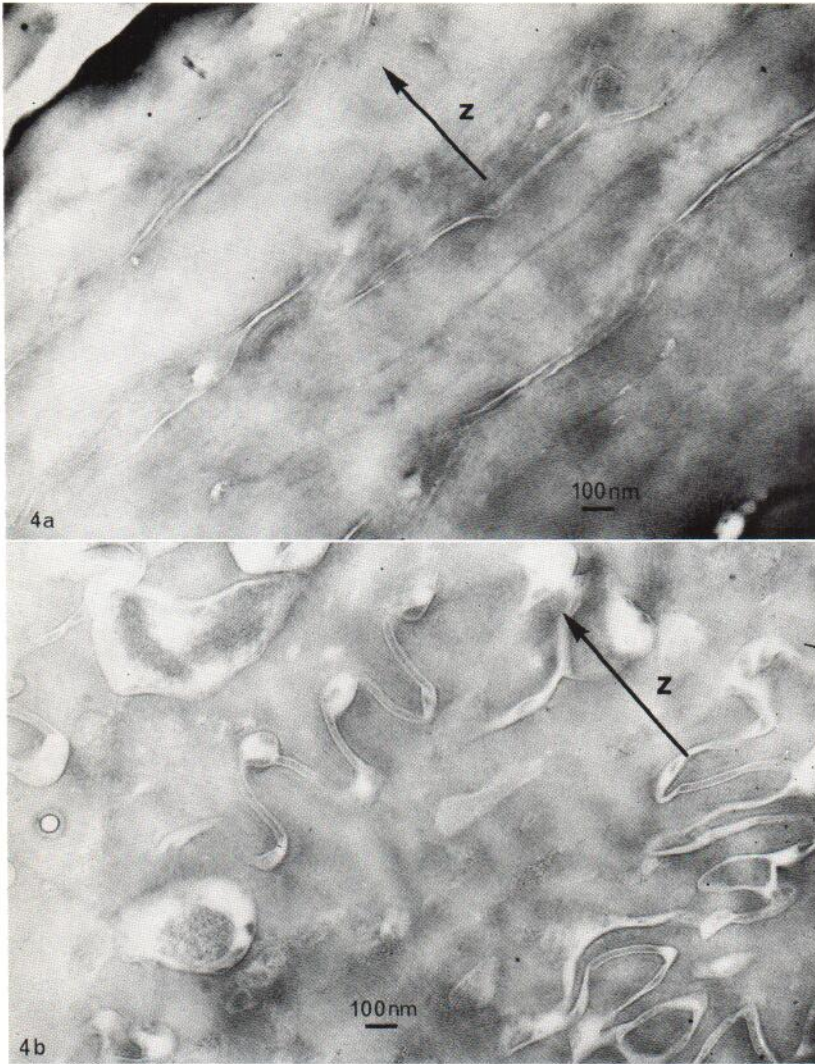


Fig. 4 (a). Section through the dorsal nail plate in the  $(x, z)$ -plane. (b) Section through the intermediate nail plate in the  $(x, z)$ -plane. Primary magnification,  $\times 20\,000$ .

sharp razors by enclosure of a very hard and brittle core between two sheets of comparatively plastic material illustrates the function of the composite design. In the razor the brittleness of the material forming the cutting edge is counteracted by making the central core thin and flexible, permitting bending deformation without undue stresses in the brittle material of high elastic modulus. The necessary rigidity of the razor is attained by enclosure in a non-brittle, easily deformable material which can take strain under stress relaxation due to plastic flow. In the nail the most important force vector has a palmar-dorsal direction, i.e. coinciding with the  $z$ -axis. Consequently, the deformable material is posi-

tioned on the palmar aspect of the brittle dorsal nail plate.

For any plate-like structure a considerable gain in the load-carrying capacity is attained by the introduction of a double curvature to prevent lateral buckling. The longitudinal curvature (in the  $(x, z)$ -plane) in the normal nail plate could be due to a difference in growth rate between the cells in the nail plate and/or to a pressure forming the curvature. Pressure-induced curvature would, in the present case, originate from the pressure of the tissue proximal to the eponychium, which constitutes the roof of the proximal nail fold and the opposed growth pressure. The connective tissue forms what could be called a "dorsal band"

which has ventral connections with the periosteum of the distant phalanx serving as an anchorage for the connective tissue. At the microscopic level the epidermis of the dorsal part of the eponychium is continuous with the epidermis of the roof of the proximal nail fold (Fig. 1). This latter epidermal area has a straight border towards the connective tissue. Such an arrangement is conceivable if it is recognized that the tissue of the roof of the nail fold is exposed to the constant forces of the growing nail plate and the pressure of the connective tissue "dorsal band". Such forces are likely to introduce an orientation of growing cells leading to a lamellar shape of the cell aggregates in vertical sections. The dorsal nail plate originating from the deepest part of the proximal nail fold is the first part of the hard nail plate to become fully keratinized. The more distal the origin of the cells forming the hard nail plate the less is the pressure of the "dorsal band" and the pressure of growth and, consequently, the tendency to a finite lamellar form of the cells. The vertical section through the nail plate as seen in electron micrographs fully agrees with this interpretation. Such a pattern of growth will produce an apparent difference in growth speed of the cells of the dorsal nail plate compared with those of the lower part of the intermediate nail plate. Autoradiographic experiments (19) give ample support to this interpretation. In autoradiographs of longitudinal nail sections obtained at a stage where the labelled areas of dorsal and intermediate nail plate have passed the lunula, the developed silver crystals appear in a rather sharp line in the intermediate nail plate whereas the line is broader and blurred in the dorsal nail plate.

#### ACKNOWLEDGEMENT

The calcium analyses were performed at the laboratory of Svenska BP in Stockholm through the generous courtesy of its director Mr Raoul Niklasson and with the able assistance of Mr Bo Dahl. For assistance in making electron microscopic preparations I thank Mrs Ingrid Jusinski.

Financial support from the Swedish Association for Medical Research and from grant FG-SW-105 of the U.S. Department of Agriculture is gratefully acknowledged.

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Received February 2, 1970

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