

QUANTITATIVE MICRORADIOGRAPHY OF NORMAL HUMAN NAIL

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Abstract. The dry mass and sulphur distribution in human nail was studied by quantitative microradiography. No significant difference in dry mass was observed between the dorsal and the intermediate nail plate. The increase in dry mass per unit volume from a region close to the proximal matrix cells up to the mature dorsal nail plate was roughly fourfold. The dry weight of the mature dorsal nail plate was of the order of 0.7 g/cm². The corresponding relative sulphur content was 3-6% by weight. The results are discussed in relation to a previously proposed explanation of the hardness of the nail and the present knowledge of keratinized tissues.

A recent investigation of the morphology and structural organization of the nail plate at different levels of resolution made possible an explanation of the hardness of nails (5). It was shown that this property may be explained on the basis of cell arrangement, cell adhesion, and the structural arrangement of the fibrous protein in the nail cells. The dorsal and the intermediate nail plate which constitute the hard nail plate form in different regions of the nail root (6). However, the proposed explanation of the hardness is not dependent upon differences in element composition of the cells in these two morphological entities.

One of the characteristic features of keratin is the insolubility in water to a considerable extent depending on stabilizing disulphide bonds (3). The sulphur in keratin is bound to the cystine which amounts to 9.4% by weight in human nails (2, 10). Conventional histochemical techniques do not provide for quantitation of elements and/or dry weight in situ. However, a freeze-sectioning and freeze-drying technique developed for quantitative microradiography facilitates such an analysis (7). This technique therefore provides a means to elucidate the proposed importance of the internal structural organization of the nail cells.

MATERIAL AND METHODS

The distal phalanx of the second toe was collected from four deceased persons of both sexes between 25 and 40 years of age. The nail root was dissected from the bone of the phalanx and was then embedded in a carboxymethyl cellulose-water mixture of satisfactory consistency, adhering to a metal specimen mounting. The mounted specimen was immediately quenched in isopentane cooled by liquid nitrogen and subsequently transferred to a cryostat where freeze-sectioning was performed at -25°C. Each section was mounted over a rectangular hole in a metal disc covered with a thin film of zapon varnish. A reference system, consisting of a stepwedge of Mylar® film was mounted close to the section before exposure to continuous X-rays ($\lambda = 5-20 \text{ \AA}$) for quantitative dry weight determination. Microdensitometry of the X-ray absorption image of the stepwedge and the specimen respectively provide data for dry mass determination (8, 9, 11).

For sulphur determination the same specimen was exposed to strictly monochromatic X-rays so as to produce two micrographs recorded at two different wavelengths, one on each side of the K-absorption edge of sulphur (7). The absorption difference registered by microdensitometry in the two microradiographs recorded at two different wavelengths reflects the content of sulphur per unit area (7). When this analysis is combined with the dry weight determination the percentage by weight of sulphur can be obtained (7). The measured points were located over the section to make possible an analysis of the dry weight and sulphur both in the growth direction and perpendicular to the nail surface (Fig. 1).

For the evaluation of the dry weight per unit volume, the specimen thickness was determined with a measuring device based on a frictionless instrument, the Microcator 4501-B manufactured by C. E. Johansson, Eskilstuna, Sweden. The instrument was calibrated with high precision gauge blocks (C. E. Johansson, Eskilstuna, Sweden) and allowed reading of thickness variations by pointer deflections corresponding to variations less than 1 μm (4). The precision of the measuring device is given as $\pm 0.15 \mu\text{m}$ (4). The flat measuring tip has a diameter of 10 μm and is opposed by a flat tip of equal area on the other side of the section. Each specimen was measured at six different points representing areas where the

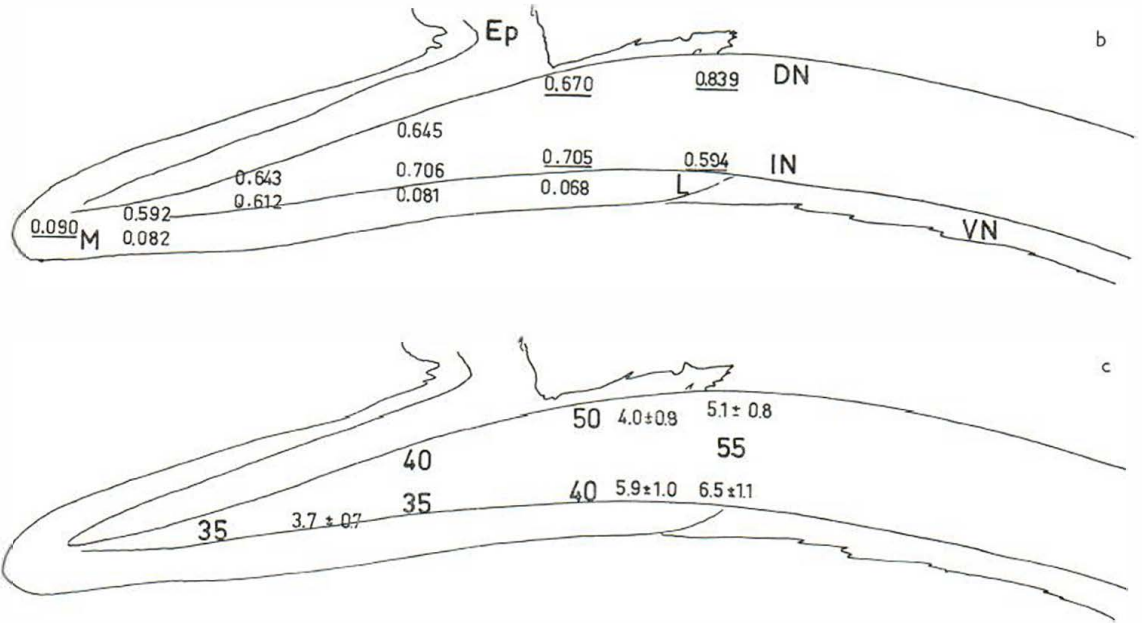
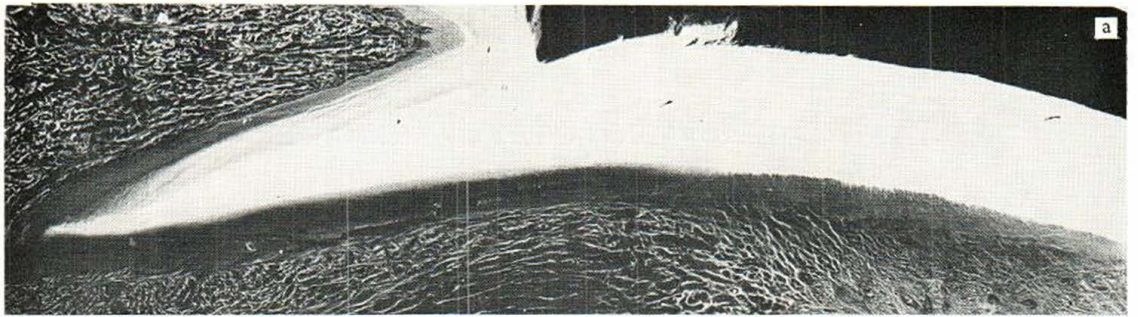


Fig. 1. (a) Microradiograph of human toe nail sectioned at right angles to the nail plate surface and parallel to the growth axis. Light areas indicate high absorption due to high mass per unit area. $\times 32$. (b) Schematic representation of Fig. 1a with some of the mass data corrected for thickness variations and the location of points used for

comparison of mass per unit volume (number with bar). Ep, epidermis; DN, dorsal nail plate; IN, intermediate nail plate; VN, ventral nail plate; M, matrix region; L, lunula. (c) Schematic representation of Fig. 1a with sulphur values at different locations and the thickness measurement locations (bold characters).

densitometry was performed. The thickness evaluation with the modified Microcator gives a high precision with mineralized tissues (4). This is also true for the hard nail plate where no marks of impression could be observed. However, the thickness measuring device used will undoubtedly make minor impressions in the specimen. It was estimated that in the transition zone between the matrix and the nail plate the error produced in this way could amount to values up to $5\mu\text{m}$ as estimated from the first value recorded to a value obtained after 1 min of continuous load. The thickness value in this region was consequently given the same value as that of the neighbouring hard nail plate.

RESULTS

The microradiograph of a nail root constitutes an absorption image of the cellular structures as revealed by their differences in dry mass. Structures containing low amounts of dry mass will thus appear dark whereas structures with a high content of dry mass appear light. With the direction chosen for sectioning, the nail root appears wedge-shaped (Fig. 1). The transition zone of cells is narrow ($\sim 200\mu\text{m}$) and of approximately

the same width up to the lunula. It constitutes a region of keratin synthesis adjacent to the matrix cells. In most sections, the thickness was greater at the lunula end than at the proximal end, though in a few thin sections this tendency was less pronounced. Several thickness measurements in each section were thus necessary to obtain correct values for the dry mass per unit volume (Fig. 1 c).

For the statistical analysis of the measurements, four sections from each of the four subjects were investigated in all analyses of dry mass distribution. The sulphur measurements were obtained from one section from each subject.

The increase in dry mass per unit volume from the root to the mature dorsal nail plate can be demonstrated by taking the ratio between these extremes. An analysis of variance was performed for this ratio in four sections from each of the four subjects (1: chapt. 10.4-5). The mean of the ratios from the sixteen sections was 0.24 with a 95% confidence interval ranging from 0.13 to 0.35 (Table I). During the development of the nail a fourfold increase in dry mass per unit volume was thus observed (Fig. 1 b).

The absorption images of the mature nails did not indicate a substantial difference in mass per unit volume between the intermediate and the dorsal nail plate. Two such ratios were taken from each of the sixteen sections constituting a hierarchical classification of observations (1: chapt. 14.7). The mean for all ratios was 1.13 and by means of analysis of variance the 95% confidence interval was obtained ranging from 0.95 to 1.31. Since the confidence interval includes the value 1, no statistical difference in dry mass per unit volume was obtained between the intermediate and the dorsal nail plate.

Table I. Analysis of variance for the ratio between the dry mass per unit volume in the proximal nail root and the mature dorsal nail plate

Source of variation	Sum of squares	Degrees of freedom	Mean square	Expected mean square
Subjects (A)	0.05707	3	0.01902	$\sigma_B^2 + 4\sigma_A^2$
Sections (B)	0.09113	12	0.00759	σ_B^2

Model: $x_{ij} = \mu + A_i + B_{j(i)}$
 95% confidence limits for $\bar{x} \dots =$
 $= 0.24 \pm t_{0.05} \text{ (d.f. 3)} / (0.01902/16) = 0.24 \pm 0.11.$

Table II. Analysis of variance for the ratio between the dry mass per unit volume in the intermediate and the dorsal nail plate

Source of variation	Sum of squares	Degrees of freedom	Mean square	Expected mean square
Subjects (A)	0.30975	3	0.10325	$\sigma_C^2 + 2\sigma_B^2 + 8\sigma_A^2$
Sections (B)	0.84804	12	0.07067	$\sigma_C^2 + 2\sigma_B^2$
Regions (C)	0.65999	16	0.04125	σ_C^2

Model: $x_{ijk} = \mu + A_i + B_{j(i)} + C_{k(ij)}$
 95% confidence limits for $\bar{x} \dots =$
 $= 1.13 \pm t_{0.05} \text{ (d.f. 3)} / (0.10325/32) = 1.13 \pm 0.18.$

Measurements from the dorsal nail plate in the lunula region and distal to this region were taken for a statistical analysis of the mean dry mass value. The structure of the analysis was identical to that presented in Table II. (1: chapt. 14.7). The mean value of the dry mass per unit volume was thus obtained as 0.68 g/cm³ with a 95% confidence interval ranging from 0.57 to 0.79 g/cm³ (Table III).

The sulphur values obtained were of the order ~3-~6% by weight. No data pattern to indicate a difference in the relative sulphur content of the dorsal and the intermediate nail plate was observed (Fig. 1 c).

DISCUSSION

The microradiographs indicated that there were no conspicuous dry mass differences between the mature dorsal and intermediate nail plate. From analyses of variance of the dry mass data observed in the lunula region, no significant differ-

Table III. Analysis of variance for the total dry mass per unit volume in the dorsal nail plate

Source of variation	Sum of squares	Degrees of freedom	Mean square	Expected mean square
Subjects (A)	0.12326	3	0.04109	$\sigma_C^2 + 2\sigma_B^2 + 8\sigma_A^2$
Sections (B)	0.09194	12	0.00766	$\sigma_C^2 + 2\sigma_B^2$
Regions (C)	0.08303	16	0.00519	σ_C^2

Model: $x_{ijk} = \mu + A_i + B_{j(i)} + C_{k(ij)}$
 95% confidence limits for $\bar{x} \dots =$
 $= 0.68 \pm t_{0.05} \text{ (d.f. 3)} / (0.04109/32) = 0.68 \pm 0.11.$

ence in mass between the two nail plates was obtained. This result does not exclude the possibility of a significant difference had a larger set of specimens been analysed. However, as judged from the present data, such a difference would be of little biological significance. The correspondence of the mass values of the dorsal nail plate and the intermediate nail plate supports the recently proposed explanation of nail hardness. It has thus been suggested that the difference between cells of the dorsal nail plate and the intermediate nail plate is one of organization rather than of different composition (5).

The fourfold increase in dry mass from the level adjacent to the matrix cells to the mature dorsal nail plate is consistent with the electron microscope picture which shows the mature cell to be completely filled with fibrous material (5).

When investigating the matrix cell layer and the transition zone, only the most proximal part of the wedge-shaped nail root was explored. Cracks which were oriented approximately parallel to the nail plate prevented an exhaustive exploration of the transition zone towards the lunula region (Fig. 1a). The dry mass values recorded in the transition zone may consequently be somewhat too low. In the soft regions of the nail the thickness measuring device made shallow impressions. This would give too low thickness values resulting in an overestimation of the dry mass values per unit volume. Consequently, thickness measurements were carried out on the hard nail plate. The systematic and random errors in the thickness measurements of the hard nail plate can be neglected when compared with the biological random variation.

The preliminary sulphur analyses suggest a close relationship between the dry mass of keratin and the relative sulphur content ($\sim 3\text{--}\sim 6\%$ by weight). Amino acid analyses have given a cystine content of 9.4%, which corresponds to roughly 2.4% of sulphur. The value obtained may be too low due to losses of material in the preparation procedures preceding the amino acid analyses (2, 10). Since the present data are based on a small number of measurements, a conclusive statement of the sulphur content in human nail cannot be given. In one subject exceptionally high values of sulphur were obtained ($\sim 6\text{--}\sim 9\%$ by weight) which cannot be explained satisfactorily at present.

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The resolution of the microradiographic technique allows determination of elements at the cellular level, though the resolution does not permit an exact location of sulphur within the keratinized cell. By autoradiography at electron microscopic resolution it should be possible to investigate this problem.

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