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A method for measuring the various constituents of the human hair follicle

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SUMMARY

Hair follicles from scalp biopsies (temporal and parietal regions) were isolated by microdissection. This technique allows preservation of the whole structure of the follicle in its fibrous sheath, or isolation of certain elements: bulb and dermal papilla.

Each follicle is examined by transmission optical microscopy and its image is digitized into sixty-four grey levels by an image analyser. Follicle images are memorized on a hard disc, then recalled individually for measurement.

The image analysis consists of thresholding, interactive selecting, then measurement of the following elements: diameter of the hair follicle, volume of the bulb, height of the keratogenous zone, mean diameter of the hair and size of the dermal papilla. These parameters were related to a clinical classification (terminal, dystrophic, vellus). This morphometric study constitutes an objective approach which is different from, but complementary, to the classic trichogram (telogen/anagen).

INTRODUCTION

The morphology of the hair follicle is influenced by both environmental (nutritional) and systemic factors (hormonal). The cyclic activity of this organ as well as pathological disturbances considerably modify its anatomy. Two techniques of investigation are classically used for the morphological description of the hair follicle: serial microscopic sectioning and plucking. The former is mainly used for biochemical and ultrastructural studies, but is extremely time consuming and expensive for morphometric measurements, as it requires 3-D reconstruction. The latter stretches the hair follicle and largely destroys its original integrity. Because of these limitations, there have been few reports in the literature, concerning measurements of the various constituents of the hair follicle. Van Scott & Ekel (1958) then Van Neste *et al.* (1986) compared the volume ratio of the hair root to its papilla in normal and pathological scalps.

The best technique available for obtaining intact and viable hair follicles from scalp biopsies is microdissection, as proposed by Green *et al.* (1986). This relatively fast method enables one to collect hair follicles surrounded by their fibrous sheaths which contain the dermal papilla, the whole structure of the hair unit being preserved. This technique is coupled here to automatic image analysis in order to perform easy morphometric measurements of the various constituents of the hair unit.

Morphometric parameters are related to a clinical classification and their interrelationships are discussed.

MATERIALS AND METHODS

Human scalp biopsies coming from hair transplantation were used for this study. Punches of 5 mm in diameter were made on the temporal (hairy) and parietal (bald) regions of the scalp of male subjects, 40–50 years of age, showing clinical signs of male pattern baldness.

The biopsies were immersed in phosphate buffer saline solution (PBS) and observed under a stereomicroscope (magnification = 40). Hair follicles were isolated by microdissection. Some were submitted to advanced dissection according to Messenger (1984), in order to remove the dermal papilla. Whole hair units or isolated follicles plus their dermal papilla were individually memorized in the vertical position by using the A/D converter (6-bit) of a Quantimet 900 (Cambridge Instruments Co). Each rectangular image (Fig. 1), of 170 × 400 pixels, was stored on hard disc (20 mB Cynthia). The resolution of the image was about 5·5 μm per pixel. When recalled to the image analyser for measurements, grey tone images were coded by pseudo-colours which provided easy visualization of the different constituents of the hair unit: outer epithelial sheath, hair shaft, bulb and dermal papilla (Fig. 2).

Another way to isolate the dermal papilla consisted of extracting the hair shaft from the epithelial sac. The resultant images appear in Fig. 3. Once the bulb has been removed, the epithelial sac contains the dermal papilla surrounded by the imprint of the bulb, which is similar to the images observed in histological sections.

Each constituent was detected by thresholding and cleaned from background noise by a 2-pixel square opening. Binary images were interactively selected by using the light-pen (Fig. 4). The binary image of the hair shaft was cut into horizontal segments, 10 pixels in height, in order to minimize the bias introduced by the curvature of the hair and/or its oblique direction. The diameter (d) was expressed as the mean value of about ten horizontal Feret diameters (F_h). The diameter of the follicle (D) was determined in the same way as the hair diameter.

The keratogenous region was identified as a lighter segment (higher grey values), above the hair root (Fig. 5). Its approximate length or height (h) was calculated from the area (A) and the horizontal Feret diameter (F_h):

$$h = \frac{A}{F_h}. \qquad (1)$$

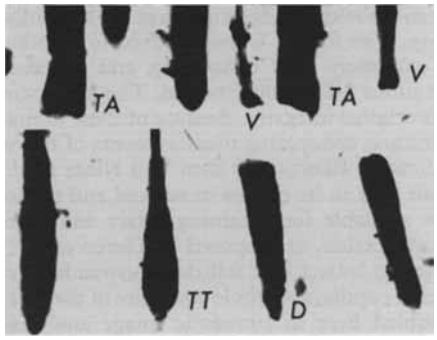

Fig. 1. Digitized grey images of hair follicles, recalled from hard disc and organized into a montage display of eight images. TA = terminal anagen, V = vellus, TT = terminal telogen, D = dystrophic.

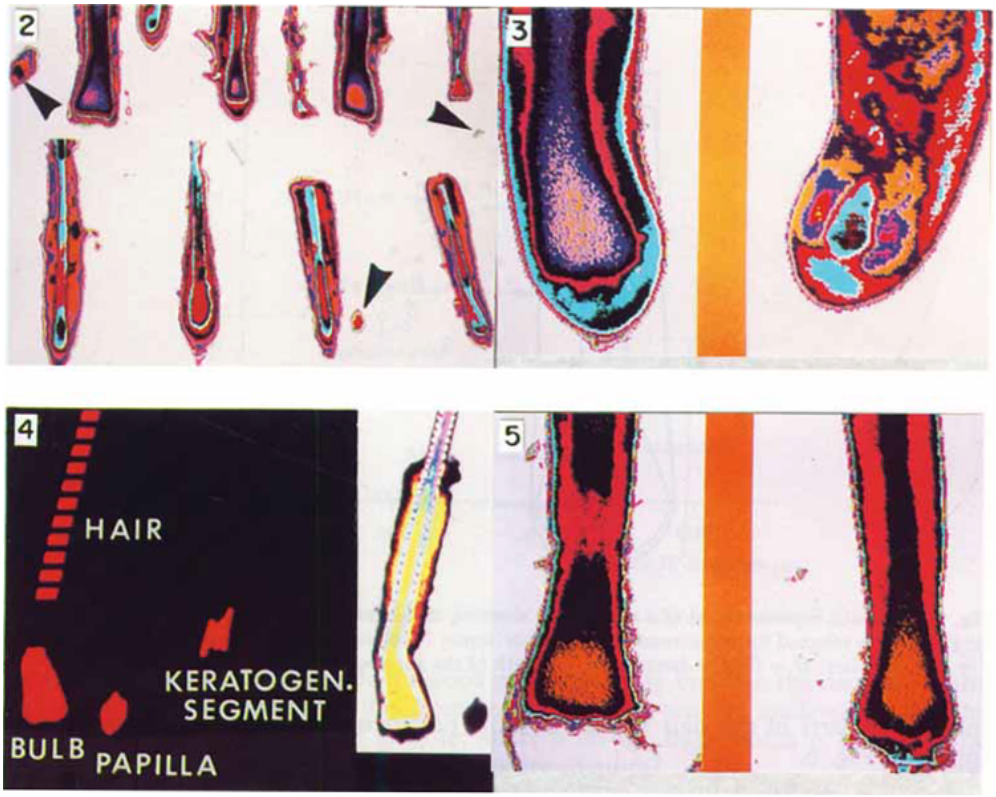

Fig. 3. Two successive images of one hair follicle (electronic zoom × 2) Left: the hair bulb is in its epithelial sac; right: after the bulb has been removed, the epithelial sac contains the dermal papilla (blue), surrounded by the imprint of the hair root.

Fig. 4. The red binary images have been interactively selected from the image on the right hand side after thresholding. Measurements are performed on them.

Fig. 5. Two terminal anagen bulbs (electronic zoom × 2). The left one shows a red segment between the bulb and the hair shaft, which has been attributed to the keratogenous zone.

This formula was preferred to the direct measurement of the vertical Feret diameter, because of the uncertainties in determining the upper and lower boundaries. From the profile area (A) of the hair root and its vertical Feret diameter (F_v), by making a model assumption one can estimate its volume. The bulb having a limited geometrical form: conical, cylindrical, ovoid, its approximate volume (V) was estimated on the basis of the prolate bi-axial spheroid:

$$V = \frac{8A^2}{3\pi F_v} . \qquad (2)$$

The projected area (A) of the papilla was measured either inside the epithelial sheath or outside when dissected. This structure being much less rigid than the bulb, takes various geometrical forms and consequently its volume was not calculated. The

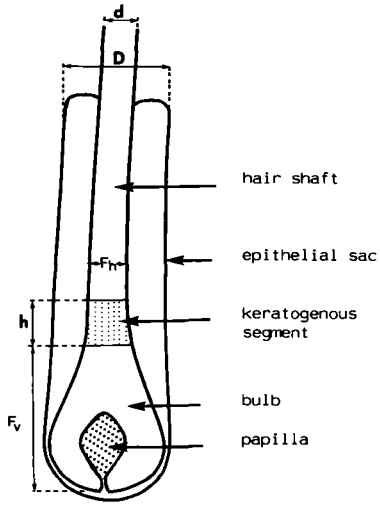

Fig. 6. Schematic representation of a hair follicle showing the anatomical location of the constituents and the parameters selected for measurements, F_h = horizontal Feret diameter; F_v = vertical Feret diameter; d = hair diameter; D = follicle diameter; h = length of the keratogenous segment.

anatomical parts of the hair follicle and the parameters selected for measurements appear in Fig. 6.

RESULTS

The follicles were classed by an expert clinician into terminal, dystrophic or dysplastic (thin, elongated, sometimes hooked hair roots, constrictions of hair shaft and/or bulbs) and vellus. Some remain unclassified, being judged unrepresentative of these three groups. The small number of telogen bulbs did not permit the constitution of sub-groups (anagen, telogen). Moreover, this function distinction was not easy to ascertain among the vellus and dystrophic. The results are summarized in Table 1. While some follicles had no keratogenous region, or no papilla, others had neither.

Compared with the terminal follicles, dystrophic follicles show a reduction of the volume of the bulb (× 5) which is much more pronounced than the reduction of their

Table 1. Quantitative data on each constituent of the hair unit, in each clinical group.

Hair sub-unit	Terminal	Unclassified	Dystrophic	Vellus
Follicle—D (μm)	300 (200–520)	230 (173–274)	230 (170–338)	160 (84–177)
Hair—d (μm)	95 (68–148)	65 (50–70)	75 (70–88)	40 (25–45)
Bulb—V ($mm^3 \times 10^{-3}$)	7 (2–13)	2·5 (0·8–3·4)	1·5 (0·3–3·5)	1 (0·3–2·4)
Papilla—A ($\mu m^2 \times 10^3$)	23 (13–52)	11 (3·5–16)	11 (8–14)	4 (3–6)
K. zone—h (μm)	290 (154–396)	155 (93–248)	150 (82–225)	100 (77–105)
Number of hair units (n)	38	12	7	9

Values given are means with range in parentheses.

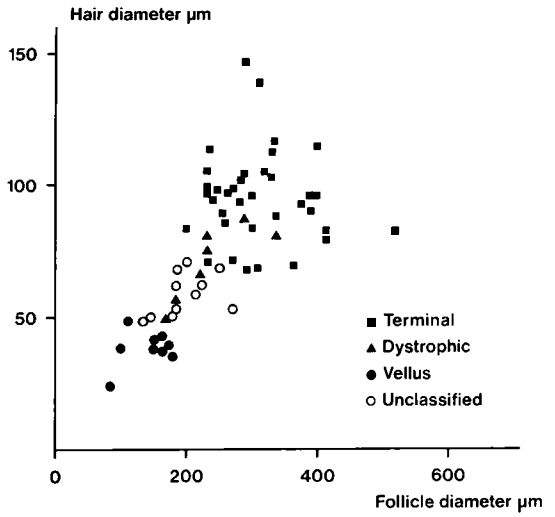

Fig. 7. Individual plots of hair diameter (d) versus follicle diameter (D).

papilla (× 2). In the vellus, there is a good proportionality between the decrease in the size of the bulb and of its papilla. Some analogies between the unclassified and the dystrophic follicles are worthy of note: apart from the bulb which is larger in the unclassified group, all the other parameters are identical.

Some relationships between structural and matrix constituents are presented in the following figures.

Hair and follicle (Fig. 7)

A linear correlation exists between the two diameters when considering all groups, but no correlation seems to exist when considering the terminal group only. The regression line joins the origin of the graph ($y = 3{\cdot}3x$). Plots show that the involution of these structures is continuous and progressive and that three groups: terminal, unclassified and vellus can be separated from one another simply by the measurement of the calibre of the hair. Dystrophic follicles are dispersed among terminal and unclassified.

Hair and bulb (Fig. 8)

The best linear correlation was found between these two parameters ($r = 0{\cdot}82$). Dystrophic follicles can be distinguished from vellus by their hair diameter; with an equivalent bulb volume, dystrophic follicles produce coarser hair. The unclassified group assumes a better junction between the terminal and vellus than between the dystrophic and terminal.

Hair and papilla (Fig. 9)

When the dermal papilla could be isolated from the hair follicle, the diameter of the hair seemed to be closely correlated with the size of the papilla. Vellus constitutes a clear homogeneous group, displaying a very small papilla associated with fine hair ($< 50\,\mu m$).

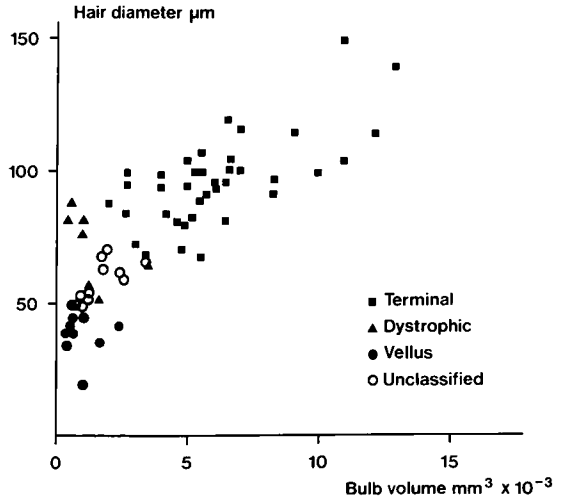

Fig. 8. Individual plots of hair diameter versus bulb volume.

Bulb and papilla (Fig. 10)

The two structures are anatomically interconnected, but their respective sizes seem to be relatively independent. For the same volume of bulb, the size of the papilla can vary up to twofold.

The keratogenous segment

The length of this segment is not correlated with any other parameter. Figure 11 shows a plot of the length of the keratogenous segment versus the volume of the bulb.

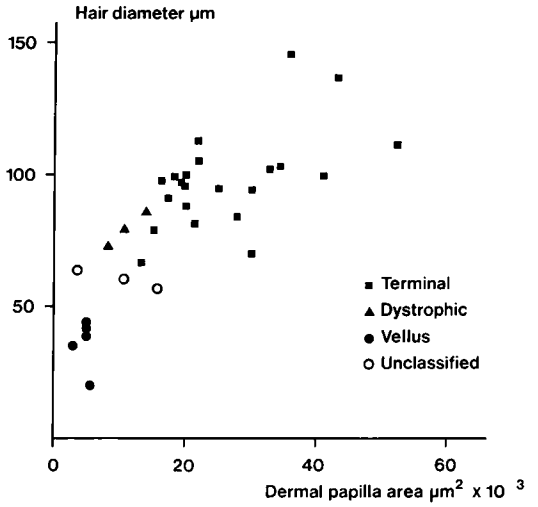

Fig. 9. Individual plots of hair diameter versus dermal papilla area for those follicles which had a papilla.

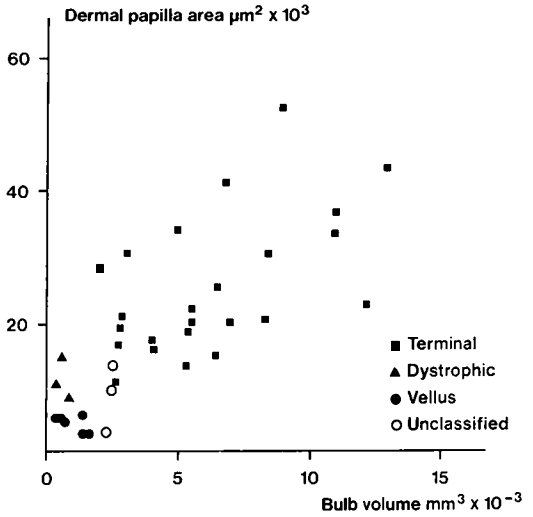

Fig. 10. Dermal papilla area plotted against bulb volume (when the papilla could be isolated).

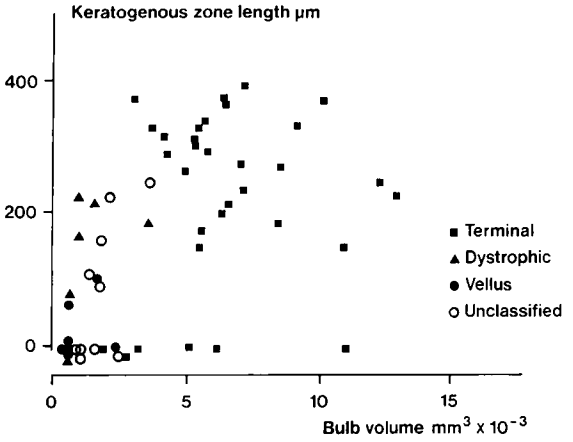

Fig. 11. Length of the keratogenous segment plotted against bulb volume. Many follicles had no detectable keratogenous region and were located near the zero value of the *y*-axis.

Many hair follicles did not have a keratogenous segment but their clinical description corresponded with that of anagen hair (cf. also Fig. 5).

DISCUSSION

The function of an organ can generally be deduced from its anatomical appearance and a certain number of morphometric measurements. Since an organ such as the hair unit depends on tissues from various embryological origins (i.e. mesoderm and ectoderm) for its biological activity or growth, its study should involve its different

parts. Microdissection is a method which preserves the whole structure of the hair follicle

Among the constituents of the hair unit, some have a functional role (papilla, root matrix), while others are products (hair). The knowledge of their respective evolutions can be improved by studying the degenerative process of the hair growth in balding scalps. Our results are in favour of a continuous process in the degeneration of the hair follicle, whereas clinical observation attempts to perform a discontinuous classification. This fact may explain the difficulties encountered by the clinician confronted with the various patterns featured by the hairy system. For example, Porter (1971) has defined the clinical grade of intermediate (or 'indeterminate') hair. This peculiar grade has been observed by Savin (1987), during regrowth of hair on the scalp following topical application of minoxidil. The present study seems to be in favour of the existence of 'indeterminate' hair: our unclassified group showed a good homogeneity of its morphometric parameters and could be distinguished from terminal and vellus groups by the calibre of the hair. In the degeneration of the hair unit, the reduction of the size of the papilla seems to be the first event. Dystrophic and vellus have equal volumes of bulb, although being comparatively reduced with respect to the terminal. Dystrophic produces coarser hair than vellus while the papilla of the dystrophic is double that of the vellus. These comparisons support the idea that the papilla determines the calibre of the hair (Ibrahim & Wright, 1982). Van Scott & Ekel (1958) showed that the mitotic activity of the matrix was correlated with the number of cells in the papilla. The involution of the bulb would then be the second event, as observed in the follicles of dystrophic hairs.

Vellus can be considered as the homothetic image of the terminal, in accordance with the scheme proposed by Uno *et al.* (1967). The 3·3 ratio between follicle and hair diameters, whatever the clinical grade, leads to the conclusion that vellus, 'indeterminate' (unclassified) and terminal could have been identified by the measurement of the calibre of the hair only. This warrants fast and non-invasive investigations by the plucking technique.

Descriptions of dysplastic and dystrophic hair roots in the literature are rather confusing, and sometimes at odds with each other. A. G. Messenger (personal communication) wonders whether 'dystrophic' follicles might in fact be artefacts due to plucking, or follicles in their early catagen phase. We believe that the number of follicles in this transient phase of the hair cycle would have been very small, irrelevant to the 10% of follicles which constitute the dystrophic group in our study.

In any case, the morphometric study of dystrophic hairs needs further developments. The volume of the bulb was not a sufficient parameter to define adequately the dystrophic follicle. An additional form parameter (elongation?) might have been useful in this study.

The presence of a keratogenous segment is considered as a good marker for the growing phase (anagen) during the cyclic activity of the hair follicle. Van Scott *et al.* (1957) and Bartosova (1967) describe it as a darker region in the upper portion of the bulb. The image analyser, even though working on sixty-four grey levels, does not confirm these observations. None of the thirty-eight terminal hair follicles had a darker segment above the bulb, but most had a lighter segment between the end of the bulb and the hair shaft. The anatomical location is superimposed on the upper segment of the keratogenous region as described by de Villez (1986). Nevertheless, the absence of correlation between the length of this segment and all other parameters would justify further investigations including some biological indexes (i.e. radioactive labelling).

This study shows that both microdissection and image analysis represent fast and easy tools for performing morphometric measurements of the hair unit, which could be used in routine work for the constitution of a bank of morphological profiles. Precise

information on the state of the scalp of each patient would improve the efficacy of therapy.

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