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REPORTS

# Morphology of Corneocytes from Human Nail Plates

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**A technique is described which permits the isolation of individual corneocytes from the superficial layers of the human nail plates. Tesa-film D is used to strip off the cells. The tape is mounted on a glass slide, stained with a mixture of methylene blue and rhodamine B. The parameters were size (surface $\mu^2$), shape (regular, irregular), nuclear inclusions and trabeculae.**

**Specimens were obtained from 3 groups of patients (finger- and toe nails): (1) 60 healthy subjects with normal nails, males and females, in 3 age-groups (babies, adults, aged); (2) 10 patients with fast growing nails with psoriasis vulgaris and psoriatic nail involvement; (3) 9 patients with slow growing nails with lichen planus with nail involvement including one patient with Zinser-Engman-Cole-syndrome (dyskeratosis congenita). The nail growth rate was determined with a dissecting microscope-technique.**

**Corneocytes of the dorsal nail plates of normal nails are of irregular polyedrical shape, not nucleated and show distinct but irregular trabecular network. Within each age-group, corneocytes are of rather uniform size but increase significantly ($p \leq .001$) with age (e.g., thumb in males: 597 vs. 920 vs. 1008 $\mu^2$). Accelerated nail plate growth results in smaller corneocytes, and slowed down nail plate growth in larger corneocytes.**

**It is concluded that cell proliferation (and abnormal keratinization) has a measurable effect on the size of corneocytes from the nail plates.**

Exfoliative cytology has been used in the past to characterize the morphology of the human stratum corneum [1,2]. Removal techniques were improved to gain access to individual corneocytes [3]. Surface size, diameter, shape, nuclear inclusion and trabeculae of single horny cells were reported for normal skin [3], regional variations [4], sex and age [5], and effects of increased or decreased epidermopoiesis and abnormal keratinization [6]. The technique was also applied to mucous membrane cells [7], and corneocytes being produced and retained in the follicular infundibula, that are sebaceous filaments and comedones [8]. The only blank spot on the surface of the human body as regards corneocytes are the nail plates and the hair cortex. Lack of information came from the inability to remove individual corneocytes, for instance from the nail plates. There are some preliminary observations concerning the nail plate architecture, but only microscopical and electron microscopical techniques were used [9,10]. The material was processed, underwent artifacts of fixation and was cut vertically.

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With none of the present available removal techniques was it possible to sample individual horny cells so that they could be viewed horizontally and measured. The purpose of this presentation is to describe a new technique for the removal of corneocytes from the dorsal nail plates. Regional variations, effects of age and growth rate of nail plates on the morphology of individual corneocytes was investigated.

## MATERIALS AND METHODS

### *Tapes*

Preliminary trials with over 20 commercially available adhesives, tapes, glues, sticky slides, cyanoacrylate, Scotch Tape and Tesafilm were unsatisfactory. Either no cells or only clumps of cells could be removed. It was found that only the Tesafilm D (Beiersdorf, Germany) yielded good and reproducible results. It is a commercially available translucent, particularly sticky tape, similar to a Scotch tape. D stands for dauerhaft (German = durable).

### *Removal Technique*

A 1–2 cm long piece of Tesafilm D was gently pressed onto the nail plate and immediately thereafter peeled off. The removed corneocytes are recognized on the transparent tape. To avoid the removal of corneocytes from the surrounding nail folds or hyponychium, as is especially the case with the small nail plates in young age-groups, a template with a central window of 5 × 5 mm was stuck onto the nail plate prior to the use of Tesafilm D.

### *Staining*

A drop of a 3:1 mixture of Löffler's methylene blue (Merck AG, Darmstadt, Germany) and rhodamine B (Chroma, Schmidt & Co, Stuttgart, Germany) as previously described [6] was pipetted on the glass slide. Excess of stain was removed after 40–60 sec. The specimens were ready for evaluation within minutes.

### *Morphological Criteria*

*Cell outlines* were classified into regular (hexagonal, pentagonal or round) or irregular.

*Trabeculae* from impression of neighboring corneocytes were classified as regular or irregular.

*Nuclear structures* were assessed as absent, shadows, fragments, fully nucleated or halos.

*Cell surface* was determined by projection microscopy with a projection mirror (Carl Zeiss, Oberkochen, Germany), using a microscope and a 100-oil-immersion lens with a linear magnification of 2.000. Cell outlines were projected on drawing papers and the surface was measured with a planimeter (Ott, Kempten, Germany). The error of this technique is less than 3% [6]. Recently we have used a semi-automatic picture-analyzing apparatus, projecting the corneocytes directly on a MOP-AMO3 (Kontron, 8057 Eching/Munich, Germany).

Fifty cells from each specimen were measured and results expressed in $\mu^2$.

### *Statistics*

Sizes of over 17.000 cells were examined with either Student's *t*-test or paired comparisons.

### *Subjects*

*Healthy subjects with normal nail plates:* Sixty subjects, females and males from 3 age-groups were selected: babies 0–1 yr old; adults 16–36 yr old; aged over 75 yr. From each subject 4 nails were investi-

gated; thumb and fourth finger of the left hand; big toe and 4th toe of the left foot. From the aged subjects measurements were only available from thumb and big toe.

*Subjects with abnormal nail plate growth and keratinization:* It is known that epidermopoiesis and keratinization affect the morphology and size of corneocytes. Increased epidermopoiesis such as in stripped skin, following application of tretinoin [6] or in psoriasis (unpublished results) results in smaller corneocytes. Decreased epidermopoiesis such as following topical application of glucocorticosteroids [6] or in aged subjects (reference 5, and unpublished data) results in large cells. Since stripping off the nail plates or increasing the rate of nail plate growth by tretinoin is unfeasible, natural occuring conditions with abnormal nail plate growth rates were used instead.

*Increased nail plate growth:* Psoriatics with and without nail plate involvement have an accelerated nail plate growth (reference 11, and personal data). Ten psoriatic patients, females and males with pits and/or psoriatic onycholysis, age 38 ± 10 yr were used. Finger nails 1 through 4 from the left hand and the big toe nail from the left foot were sampled.

*Slowed down nail plate growth:* Eight patients with lichen planus and nail involvement and 1 patient with Zinser-Cole-Engman-syndrome (dyskeratosis congenital with nail dystrophy) were chosen. For conveniency, these 9 patients are listed under the term lichen planus. All subjects were males, age 33 ± 15 yr. Fingernails 1 through 4 from the left hand and the big toe nail from the left foot were sampled.

## RESULTS

### *Normal nail plates*

*Morphology.* Corneocytes from the dorsal nail plate have a fairly regular polyedrical shape with rounded-of corners (Fig 1*A*, *B*). Trabecula are somewhat irregularly distributed, but well visible. The cells do not contain a nucleus or nuclear

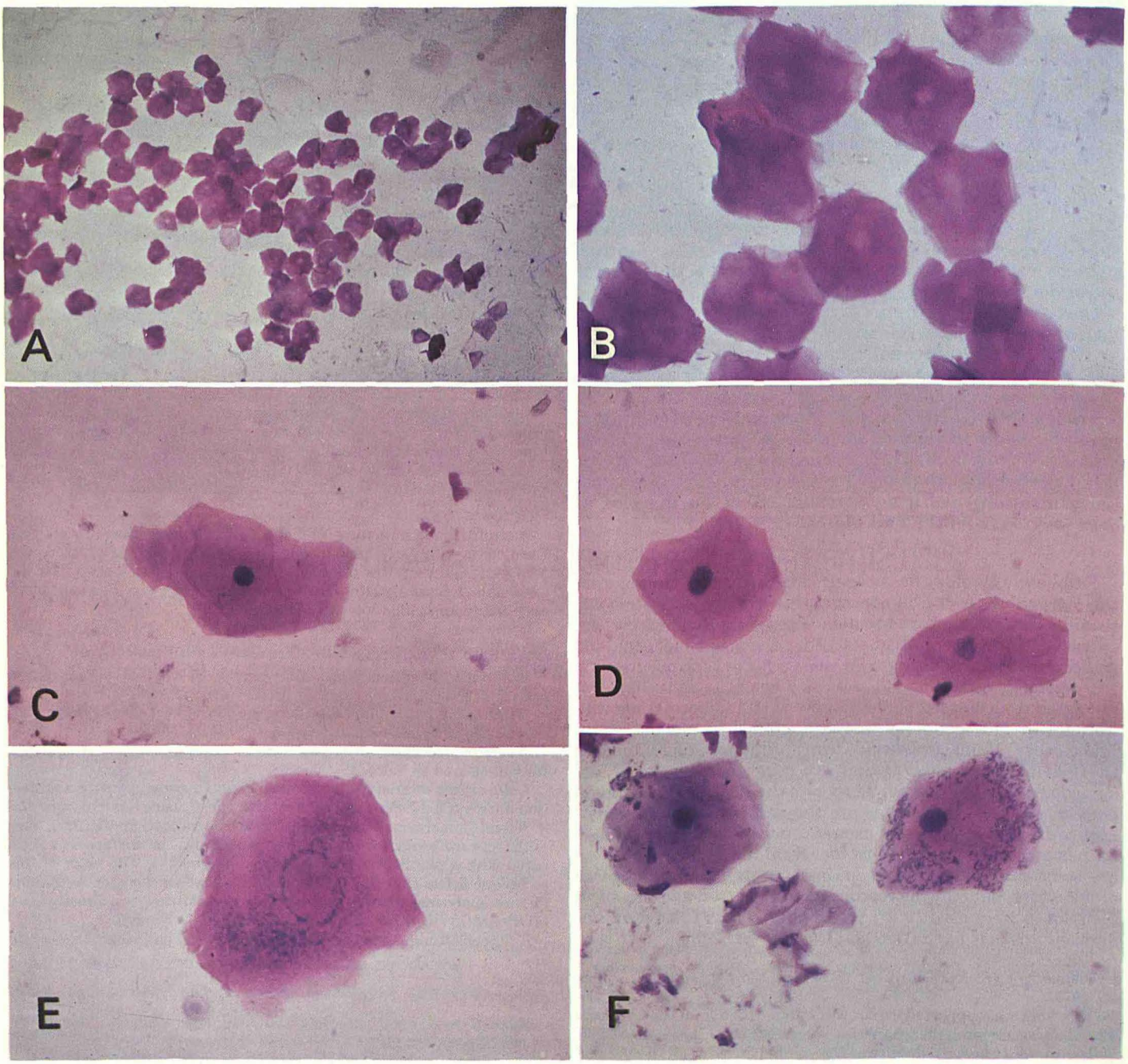


FIG 1. Corneocytes from dorsal nail plates. *A* = normal nail plate. Overview showing many individual cells attached to the Tesafilm D (× 64). *B* = higher magnification of *A*. Rather regular cell shapes; pale staining in former nuclear areas, (× 160). *C* and *D* = Zinser-Cole-Engman-syndrome. Irregular shaped parakeratotic cells (× 640). *E* = Halo-cell from psoriatic nail plate (× 1600). *F* = Zinser-Cole-Engman-Syndrome. Bacterial colonies on parakeratotic cell (× 640).

remnants although the central area of the corneocyte, where the nucleus was once located, does stain less intensively than the remainder of the cell (Fig 1 *B*). Corneocytes from the nail plate differ in morphology from corneocytes from the stratum corneum of all body sites so far investigated [3–8] and can easily be distinguished as such. No difference between finger nails and toe nails could be demonstrated.

*Size*

*Effects of age:* Age has an effect on the size of corneocytes: they increase significantly from the younger age group to adults and again to aged (Fig 2). For instance the corneocytes from the big toe of male babies were 605 ± 41 vs. 920 ± 176 in adults vs. 1008 ± 160 $\mu^2$ in the aged. Similar data for the thumb are given in Fig 3.

*Effects of sex:* Within each age-group there was no difference in cell size between females and males, neither for the finger nor for the toe nails (Fig 4).

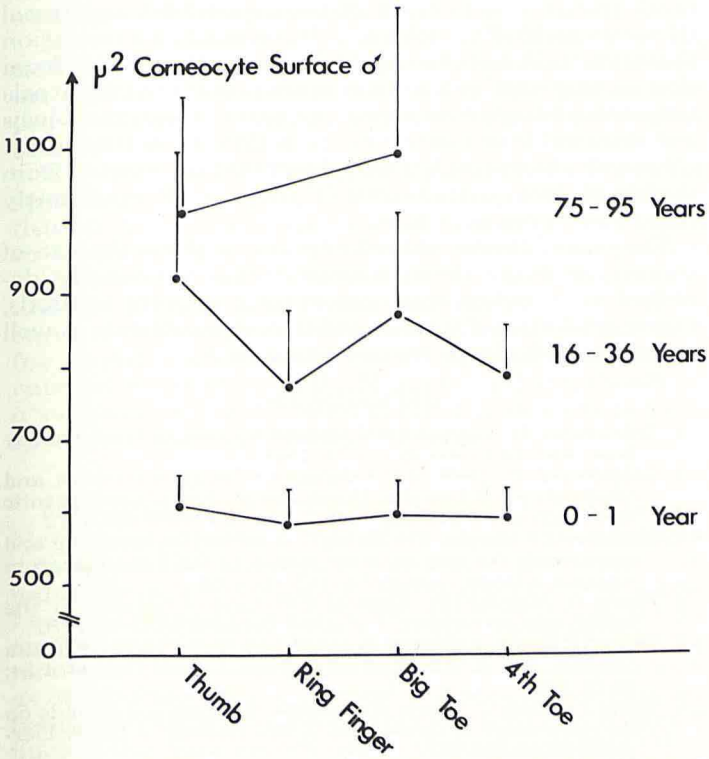


FIG 2. Corneocyte surface in $\mu^2$ from finger and toe nails. Effects of age. Increasing size with age. M ± SD.

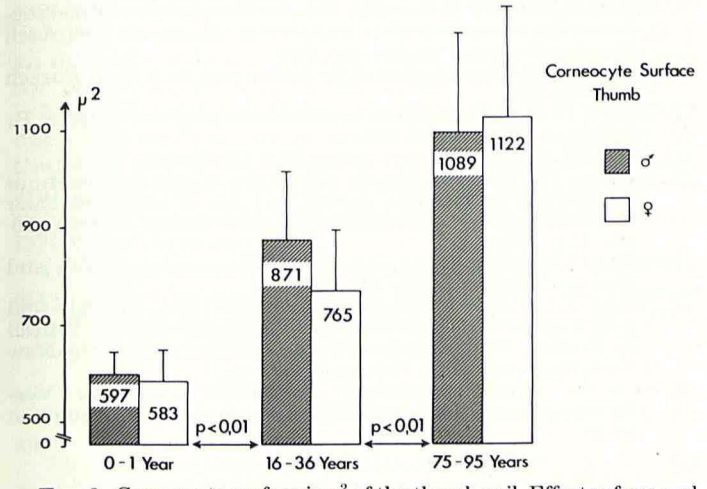


FIG 3. Corneocyte surface in $\mu^2$ of the thumb nail. Effects of age and sex. There is a significant difference between the 3 age-groups, but none between females and males.

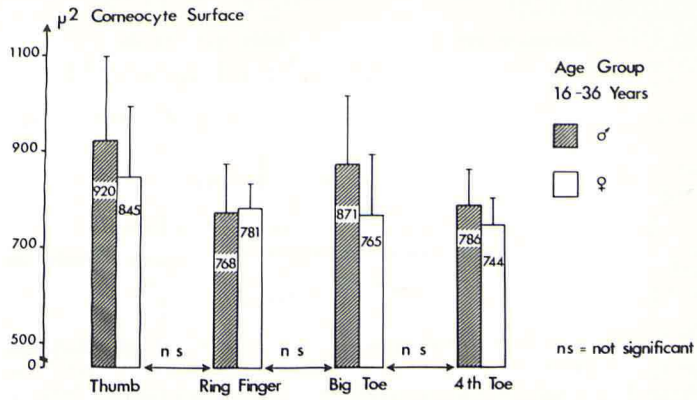


FIG 4. Corneocyte surface in $\mu^2$. Effects of site. Within one age-group (adults) there is no significant difference between finger and toe nails.

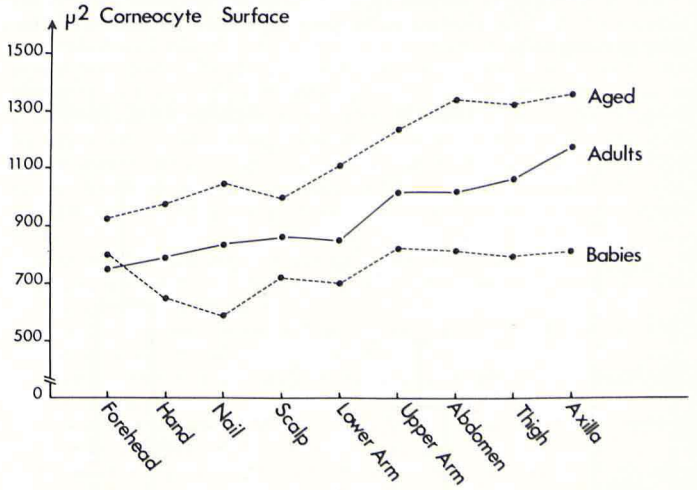


FIG 5. Corneocyte surface in $\mu^2$ from 9 different sites of the body. Effects of age. Increasing size with age. Cells from the nail plates are rather small.

*Effects of site:* Corneocytes from finger- and toe nails of the same subjects are of rather uniform size, and no significant difference was found (Fig 4). Corneocytes from the nail plates are in the lower range when compared to previously studied corneocytes from various parts of the human body (Fig 5), and are much smaller, for instance than corneocytes from glabrous areas such as abdomen, thigh or axilla [5].

*Abnormal nail plate growth and keratinization*

*Morphology:* It was easy to characterize cells from diseased nail plates. They were of irregular, often bizarre shape, with concave and convex border lines. Cells were often elongated or had pointed cell corners. Most prominent was the nuclear area. Solid cell nuclei and nuclear remnants with deeper basophilic staining contrasted with normal corneocytes (Fig 1*C*, *D*). Psoriatic patients often yielded halo-cells [2], which were not seen in any other patients in this study (Fig 1*E*). The nuclear-cytoplasmatic ratio was rather small when compared to the morphology of corneocytes from psoriatic skin lesions (unpublished data). Sometimes corneocytes were covered with bacteria of coccoid shape (Fig 1*F*).

*Size:* Corneocytes from nail plates of patients with psoriasis (increased growth rate) are smaller than those from nail plates of patients with slowed down nail plate growth. There was no overlap of the means and the difference was significant for 3 of the 5 nails studied (Fig 6, 7).

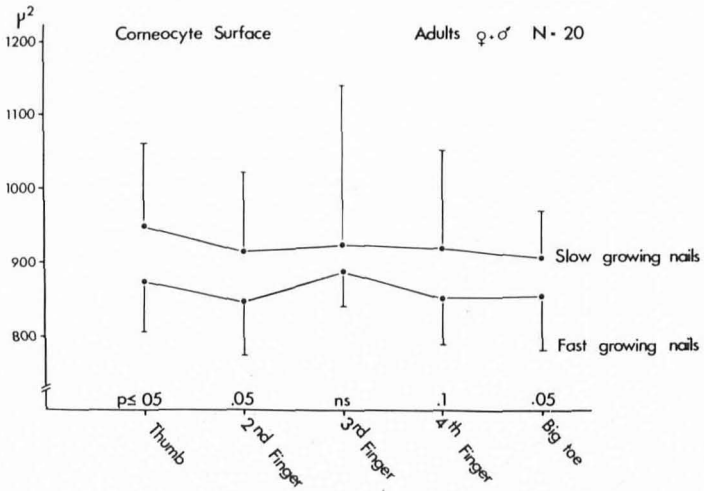


FIG 6. Corneocyte surface in $\mu^2$. Effects of nail plate growth and keratinization. Fast growing nails (psoriasis) show smaller cells than slow growing nails.

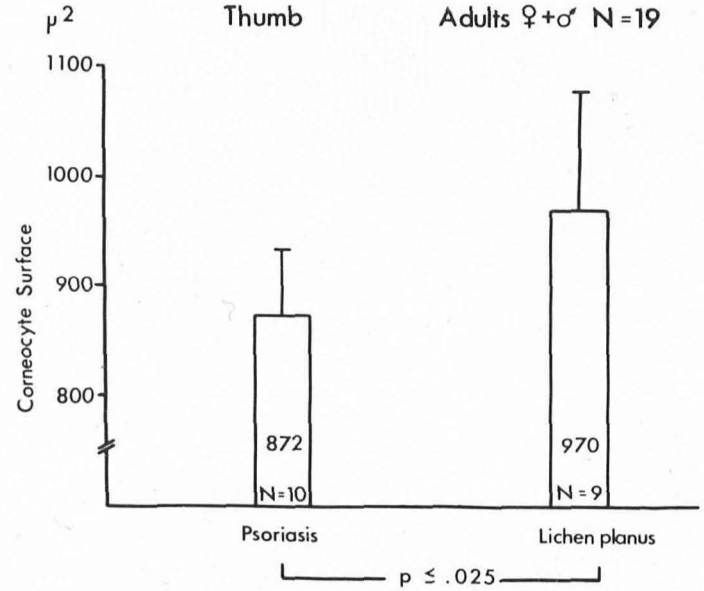


FIG 7. Small corneocytes in psoriasis, large cells in lichen planus. Corneocyte surface in $\mu^2$.

## COMMENT

In order to carry out corneocyte surface measurements from cells of the nail plates it was necessary to obtain individual and intact cells. Earlier studies were hampered because of inadequate sampling. The adhesive slide technique of Goldschmidt and Kligman [1] or the detergent scrub technique of McGinley, Marples, and Plewig [3] do not permit proper isolation of cells. Either none or too many cells, adherent in sheets, come off the nail plate. The transparent tape Tesafilm D, which permits microscopic visualization and photographic demonstration because of its translucency overcomes this handicap. The method is easy to perform and well reproducible. Quantitative cell counts, like with the detergent scrub technique [3,6] are, however, not possible.

The results show constant patterns insofar as horny cells from nail plates increase in size with age. Babies have small, adults significantly larger, and aged subjects again significantly larger cells than the latter group. The increased size of corneocytes with age seems to be a constant biological factor. The question remains why there is a larger cell surface with age. We tried to correlate this with the growth rate (epidermopoiesis) of the keratinizing tissue, interfollicular epidermis or nail plates. Slowed down epidermopoiesis results in larger cells. Bean [12] recorded the growth rate of his own thumb nail after 10, 20 and 25 years of observation. With time his nail plate grew slower. Hamilton, Tereda, and Mestler described reduced nail growth rate with age [13]. Sibinga [14], with a different technique, did not find, however, a difference in nail plate growth between babies and adults. The literature is reviewed by Pinkus [15]. Differences in the disproportional growth of various body sites after birth may also be used to explain our findings. This ratio is 1:2 for the head, but 1:9 for the distal parts of the extremities.

It also fits into our concept that faster than normal growing nail plates yield smaller cells. Corneocytes from psoriatic patients, epidermal or from nail plates, are smaller than normal (H. Goldschmidt, Philadelphia, M.D., personal communication September 1978; and own unpublished data). Corneocytes from slow growing nails such as from lichen planus or dyskeratosis congenita are larger than normal. Admittedly, epidermopoiesis and abnormal keratinization merge in these latter conditions.

Recently, Stein [16] has also shown that corneocytes from the legs of elderly patients (over 75 yr of age) are significantly larger than in younger adults.

The general theme that cell growth rate affects the size of corneocytes in the aforementioned fashion was recently described in an experimental system using stripping methods, topical application of tretinoin and glucocorticosteroids, as well as patients with allergic contact dermatitis [6].

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