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Use of nonintrusive tests to monitor age-associated changes in human skin

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Synopsis

Nonintrusive tests can be used to objectively characterize and quantitatively evaluate *in vivo* those changes in the physiological properties of skin due to aging. Such a testing procedure presents no untowards risks and has proven to be quite palatable to normal healthy volunteers. A number of age-associated changes in skin structure and function can be monitored in this manner. These include changes in SKIN SURFACE ANATOMY such as loss of DERMATOGLYPHICS and altered patterns of CORNEOCYTE DESQUAMATION. Physiological decrements such as diminished eccrine sweating, epidermal CELL RENEWAL and HEALING of superficial skin wounds can also be evaluated. Moreover, by using a variety of excitants which when placed on the skin induce specific reactions, viz. erythema, wheals, stinging, etc., an age associated loss to express these reactions has been demonstrated.

INTRODUCTION

It seems likely that changes which occur in human skin with advancing age may be of some value in monitoring the senescent process not only for that organ but perhaps for the entire individual as well (1). Such an ability to measure aging biologically on the basis of structural and functional alterations in skin rather than just chronologically on the basis of birth certificate information would be of great benefit to the investigative gerontologist. Unfortunately, most testing procedures which are used to study skin structure and function require biopsies or some other surgical manipulation to be performed. There is no doubt that most human subjects, especially those with no dermatological problems, find these invasive testing procedures objectionable. What is really needed is a testing strategy that is palatable to human volunteers. Such procedures should be conveniently administered, cause little or no discomfort, present no untoward risks and leave no permanent scars or pigmentary changes.

The object of this paper is to summarize some preliminary results of a pilot study of aging human skin designed primarily to test the feasibility of this nonintrusive approach. This is a cross-sectional investigation and involves a comparison of two

age-cohorts: young adults 20–35 years of age and older adults 60–75 years of age. Both groups are comprised of normal healthy white volunteers (6 males and 6 females in each) who had given informed consent. To avoid differences due to changes in environmental conditions or protocols, both cohorts were tested concurrently and all assessments were made independently by three graders.

REPLICAS AND SKIN SURFACE BIOPSIES

The skin surface is organized into complex patterns of ridges and furrows which presumably enable the stratum corneum to undergo deformation in a variety of directions without subsequent loss of integrity (2). With the exception of palmar surfaces ("finger prints") information on the dermatoglyphics of the remaining body areas is minimal, especially regarding age changes.

With this in mind a special technique has been developed which, for the purpose of studying dermatoglyphics, is superior to the usual replicas using silicone or dental wax impressions. This entails applying a thin layer of polyvinylidine chloride emulsion (Duran, Merck) which is actually a liquid form of Saran Warp®. When dry, a thin sheet can be peeled off which is an exact negative replica of the underlying skin surface. This translucent specimen serves as a permanent record of the dermatoglyphics of that subject. Recent studies indicate that the dermatoglyphics of older adults are highly irregular and lack the orderly arrangement of geometric patterns typical of the young (3). Exposed areas seem to exhibit the greatest alteration in dermatoglyphics with patterns in some areas of the dorsal hand being almost totally obliterated (Figure 1).

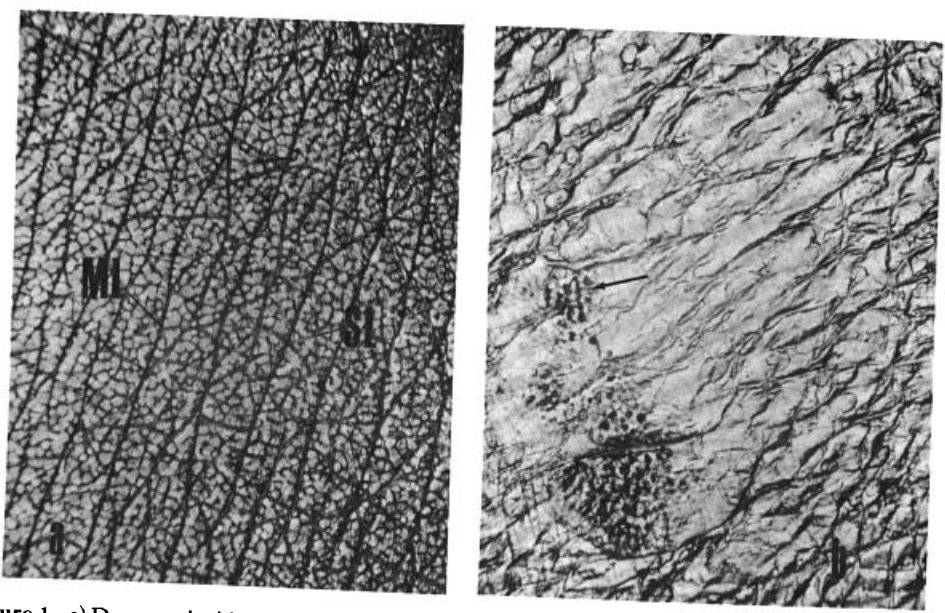

Figure 1. a) Dermatoglyphic pattern of the dorsum of the hand of the young. Major (ML) and secondary (SL) lines traverse to form a highly ordered pattern of triangles. Within the triangles the corneocytes are arranged in a honeycomb fashion (arrow) (×15). b) Dermatoglyphic pattern of the dorsum of the hand of the old. Note the lack of an ordered geometric pattern due to absence of secondary lines. Remnants of the honeycombed corneocytes are occasionally observed (arrow) (×15).

Such a loss of dermatoglyphics may be correlated with a decrease in skin elasticity and an increased tendency to crack and fissure. The underlying events for this change are not completely understood but may be related to atrophic changes in the dermis (4).

By changing the nature of the replica material one can obtain information regarding other structural and functional changes in aged skin. For example, the silicone technique as originally devised by Sarkany and Gaylarde (5) and later improved by Harris, Polk and Willis (6) can be used to evaluate sweating. To take an imprint a mixture of silicone base and catalyst (Syringe Elasticon, Kerr) is applied as a thin film immediately after drying the skin surface. Because sweat is immiscible with silicone, each sweat droplet forms a globular hole in the silicone layer. This rubbery sheet can then be peeled off and forms a permanent record for which the density and output of active sweat glands can be estimated.

Preliminary results using this procedure suggest that sweating capacity is diminished in older adults (Figure 2). Previous studies by Silver, et al. (7) have indicated that the number of digtal sweat glands visualized by a starch-iodine film technique decreases with advancing age. By using these nonintrusive approaches in conjunction with pharmacological agents known to promote (Pilocarpine) or block (Scopalomine) sweating, additional insight on the nature of the apparent age-related differences in eccrine gland function should be obtained.

The skin surface biopsy method of Marks and Dawber (8) results in the removal of a sheet of horny cells from the superficial stratum corneum, usually five to six cell layers. It should be emphasized that this is not a replica technique but actually removes the outermost portion of the horny layer and thus provides biological material which can be subsequently analyzed in a variety of ways. To obtain this sample, one drop of

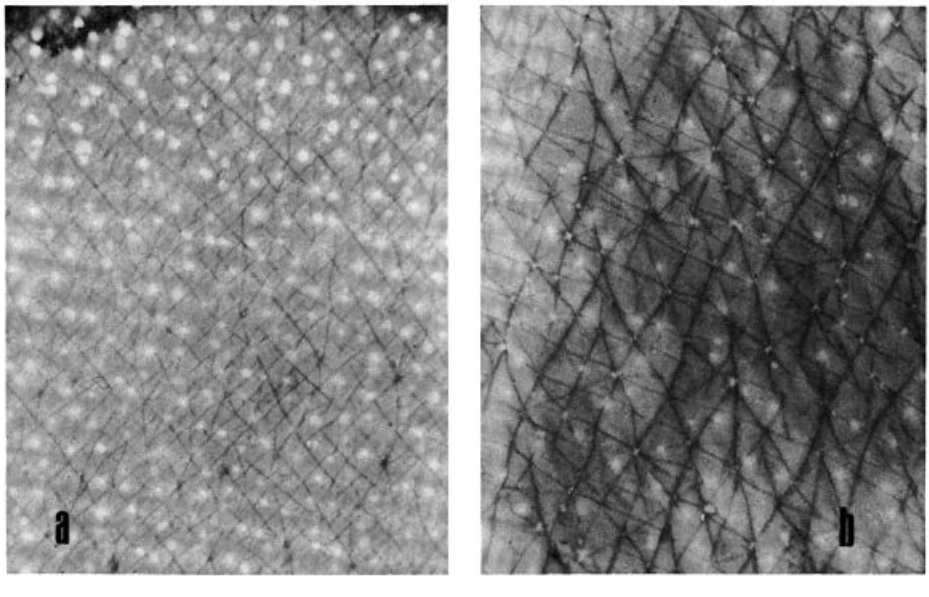

Figure 2. a) Sweat gland imprint from the volar forearm of the young. Light circles represent perforations in the film of silicone material formed by sweat droplets from functioning sweat glands (×15). b) Sweat gland imprint from the volar forearm of the old. Note diminished amount of functioning sweat glands (×15).

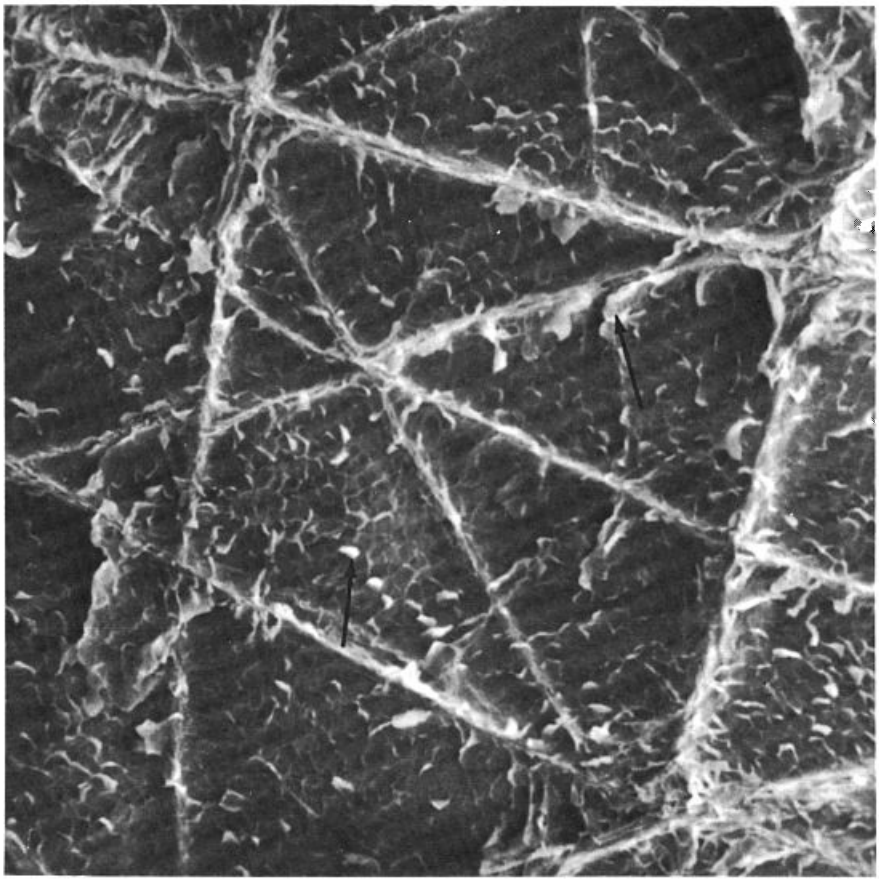

Figure 3. Scanning electron micrograph of a skin surface biopsy from the calf of the young. In addition to the easily recognized ridges which constitute the dermatoglyphic pattern, the desquamation of corneocytes in single and small groups can also be resolved (arrows) (×250).

cyanoacrylate adhesive (Aron-alpha, Vigor Co.) is placed on a 3 × 1 glass slide which is then pressed against the area to be sampled. After approximately 30 s, the glass slide is removed with its adherent horny layer. This specimen can then be used to study the dermatoglyphic patterns as well as the configuration and patterns of desquamation with the scanning electron microscope (Figure 3).

INDIRECT ASSESSMENT OF EPIDERMAL PROLIFERATIVE ACTIVITY

Horny cells are continually being lost into the environment due to exfoliation. Normally, the renewal system of the epidermis operates under steady-state kinetics; thus for every cell lost, a new cell must be produced in the basal layer. Since all the intervening layers are of a simple transit type, cytokinetic analysis of the stratum corneum provides indirect assessment of epidermal cell proliferation (9,10).

One parameter which can be measured in such a renewal system is transit time, i.e., the time required for a cell to travel through a compartment. Since horny cells are tightly

Table I
Non-Intrusive Assessments of Epidermal Proliferative Activity[1]

Site	Transit Time (days)	Number of Cell Layers	Turnover Rate (h/layer)
Volar Forearm			
<35	19.8 ± 1.39	17.0 ± 0.83	28.3 ± 1.2
>60	28.1 ± 2.66	16.8 ± 0.66	$0.8 ± 3.8
Upper Inner Arm			
<35	17.7 ± 2.02	14.3 ± 0.61	30.0 ± 2.6
>60	25.5 ± 2.63	13.9 ± 0.81	46.6 ± 6.5

[1]Results are means ± S.E.

bound, they move in unison through the stratum corneum. Thus, in this special case, transit time is equivalent to turnover time, i.e., the time required for a compartment to renew or replace itself. This value can be measured nonintrusively by determining with the aid of a Wood's lamp, the time required for a fluorescent marker (Dansyl Chloride) to disappear from fully stained horny layer (10). This dye binds avidly to the horny layer only and its rate of disappearance is not influenced by washing or protecting the surface. Our preliminary results with Dansyl Chloride Disappearing Method (Table I) indicate that for both the volar forearm and upper inner arm, stratum corneum transit times of young adult subjects are approximately 18 to 20 days. In older subjects, this was lengthened about eight to nine days at both sites indicating that epidermal proliferation decreases with age.

It should be emphasized that transit time values can be misleading unless they are related to the number of cell layers (11). If cell proliferation is the same, the time for a cell to reach the skin surface will be longer if there are more cell layers. Thus, transit times should be corrected for differences in number of cell layers and converted to turnover rates. Estimating cell layers entails raising up small blisters with ammonium hydroxide, cryostat sectioning the blister roof and treating it with alkali (12). The horny cells swell up and the number of cell layers can easily be counted. Our preliminary results (Table I) reveal a site difference but no age differences with regard to the number of cell layers in the stratum corneum. Thus, the increased transit time values observed in older subjects is truly a reflection of diminished proliferative activity. In fact, the calculated turnover values indicate that on the average it takes about 30 hours to replace each horny cell layer in younger subjects and about 45 hours for older subjects.

In addition to providing a stratum corneum sample for cell layer counts, the blister roofs can be subjected to additional types of analyses, such as moisture avidity or physiochemical properties (13). It is apparent that much will be learned about the structural and functional properties of the stratum corneum from such a comprehensive examination of blister roof samples.

WOUND HEALING

The unroofed blister site represents a reasonable standard superficial wound at which the dermatoglyphics have been completely obliterated. Thus, by observing the restoration of the original markings we can objectively evaluate the rate of wound

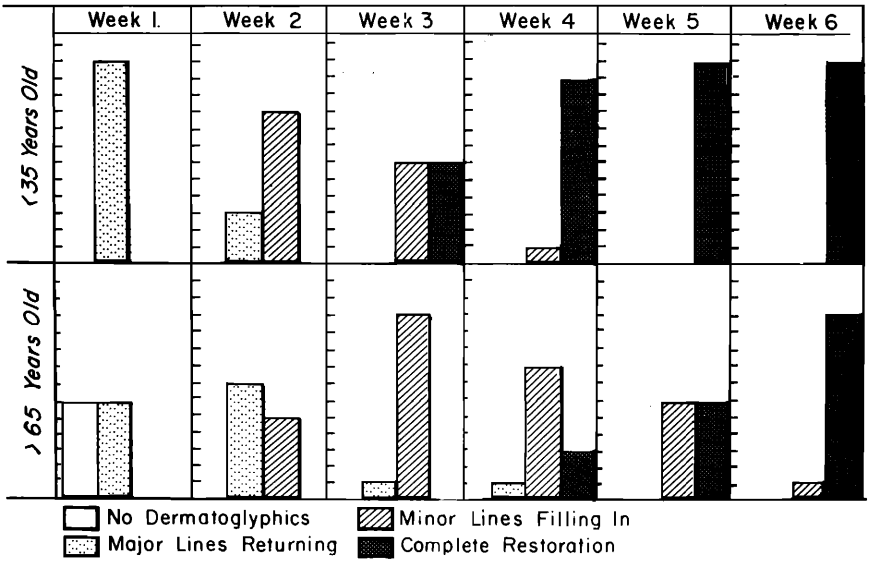

Figure 4. Wound healing following unroofing of NH_4OH blisters on volar forearms of human subjects.

healing. Figure 4 shows some preliminary results with regard to the volar forearm site. Note that by the end of the first week, all young subjects had begun to reestablish major lines, while half of the older ones still show a complete absence of lines. By the end of the second week, most of the young and many of the old had reestablished all major lines and were beginning to fill in the minor ones. The differences in rate of wound healing become even more pronounced in subsequent weeks. By the fourth week, all young subjects had completely restored their dermatoglyphic pattern. In contrast, only 25% of the older group had achieved this stage, and in fact, one individual had not even completed reestablishment of the major lines. By the end of the sixth week, all but one of the older subjects had completely restored their dermatoglyphics. A similar progression of events was observed in the upper inner arm site.

BLISTER FORMATION

The ability to raise up blisters has proved useful in a variety of ways in this investigation. Frosch and Kligman (14) originally described the induction of blisters by ammonium hydroxide (NH_4OH). In this procedure, a 1:1 aqueous solution of NH_4OH is placed in a 14-mm well drilled in an acrylic plastic block which is held snugly to the skin surface. During the exposure, the site is examined under good lighting at 30-s intervals. The time required for the appearance of tiny vesicles around the follicles is termed minimal blistering time (MBT). The exposure is continued and the additional time required for a fully tense blister to form, blister filling time (BFT), is also measured.

Table II compares blister formation of the two age groups on the volar forearm and upper inner arm. At both sites, the young achieved an MBT response after 13 to 14 min of exposure, while in striking contrast, the older subjects as a group took only half as long. At the present time, it is not clear what accounts for these differences.

Table II
Blister Formation Induced by NH_4-OH Exposure

Site	M.B.T.[1]	B.F.T.[2]
Volar Forearm		
<35	12.5 ± 1.68	11.7 ± 1.96
>60	6.6 ± 0.87	24.8 ± 5.66
Upper Inner Arm		
<35	14.0 ± 2.11	17.3 ± 3.00
>60	6.1 ± 0.87	31.5 ± 6.79

[1]Minimal Blistering Time, mean ± S.E. in min.
[2]Blister filling Time, mean ± S.E. in min.

Table II also shows that in younger subjects, within 10 to 15 min after achieving an MBT, a tense blister appears. By contrast, Blister Filling Time is over 25 min for older subjects. Indeed, in two cases, the experiment was terminated at 90 minutes without obtaining a blister even though a follicular (MBT) pattern was evident. This suggests that integumental reactivity may be impaired in older subjects.

To gain additional insight into this problem, a brief survey of integumental reactivity was conducted using a variety of excitants which when placed on the skin induce specific reactions, viz. erythema, wheals, vasodilation, stinging, etc. Preliminary findings reveal that in general older subjects as a group are less reactive (Figure 5). Such an age-related loss in the capacity to express certain inflammatory reactions has serious implications. For one thing, it means that certain clinical signs may be muted or missing entirely, making diagnosis of even common dermatological problems extremely difficult in older patients. Moreover, in various studies, e.g., allergic contact sensitization, the results may be meaningless unless it is reasonably certain that older panel members possess an adequate effector system for expressing the response.

EXFOLIATIVE CYTOLOGY

Since cells are continually being shed from the stratum corneum it becomes a simple matter to collect them for subsequent analysis (15). One method of collecting these corneocytes is by the sticky slide technique (16). In this procedure, a glass 3 × 1 slide coated with either tacky adhesive or double-sided scotch tape is pressed firmly on the skin surface to be examined. Upon removal, the specimen which retains the topographical relationships of the horny cells (corneocytes) can be stained for visualization. This method seems to be especially useful in studying the pattern of desquamation. Figure 6 shows sticky tape slides taken from the legs of a normal subject and one with dry skin. Note the appearance of large clumps in the dry skin preparation indicating that the corneocytes are shed as large aggregates rather than small clusters of individuals as is normal.

Another method for examining the cytology of exfoliated cells is the detergent scrub technique (17). In this procedure, a glass well covering 3.8 cm^2 in area is held firmly to the skin. One ml of buffered 0.1% Triton X-100® is added and the skin surface gently rubbed with a teflon scrubber for one minute. The resulting wash fluid which contains individual horny cells can then be processed in a variety of ways.

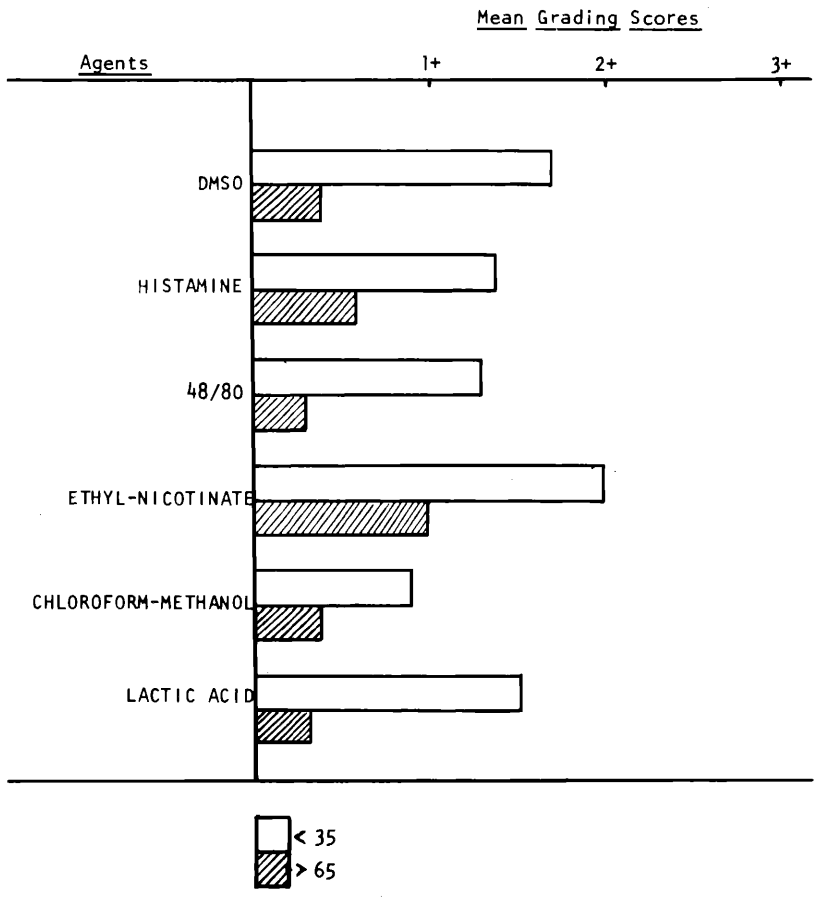

Figure 5. Integumental reactivity tests. Values represent mean grading from three independent observers.

One extremely important parameter is the corneocyte count, the number of desquamation cells per square centimeter of skin surface. As such, it provides an indirect measure of epidermal proliferative activity since it is these cells which eventually migrate to the skin surface and are desquamated. In a previous study (18), age-related differences were determined in two groups: a) individuals with no history or signs of scalp disease and b) individuals with dandruff, a clinically noninflammatory hyperproliferative scaling disorder of the scalp. The results indicated that dandruff subjects had significantly higher corneocyte counts from the scalp than their corresponding age-matched controls. In fact, this elevated corneocyte count can be a very reliable way to assess the efficacy of various anti-dandruff medications (19). However, what is important to the investigative gerontologist is that in both groups, the older subjects had significantly lower corneocyte counts than the corresponding younger cohort. These results support the conclusion that with advancing age, the proliferative activity of the human epidermis is significantly reduced not only in normal individuals but also in those with a hyperproliferative disease such as dandruff (18).

The detergent scrub technique also provides a simple method for the visualization of

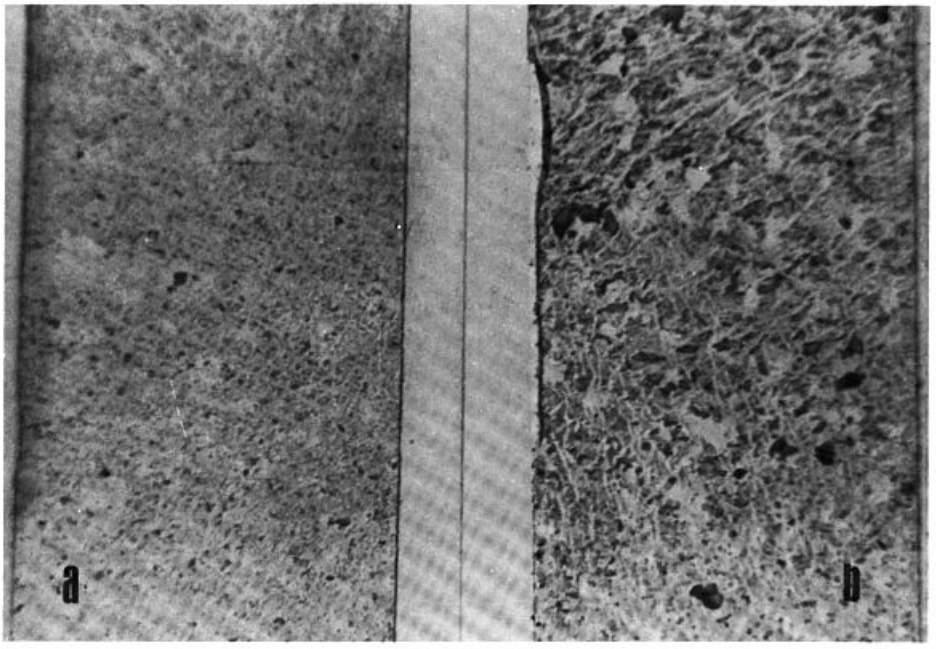

Figure 6. a) Sticky slide from shin area of young. b) Sticky slide from shin area of older subject with a dry skin problem.

the corneocytes with the light microscope. The appearance of corneocytes from a healthy young subject is shown in Figure 7. Note that these cells lack nuclei, one of the results of the terminal differentiation of epidermal cells (20). These cells also tend to be polygonal in shape with relatively smooth borders. They show lines at the periphery corresponding to regions of overlap with adjacent cells indicating a highly structured epidermis. With advancing age, increased frequency of atypical cells (Figure 8) which lack these general features can be observed. Although clinical significance of these unusual cell types is still uncertain, there can be no doubt that these cytomorphological alterations have diagnostic importance. Indeed, these specimens are akin to those used for Pap Smear cytodiagnosis and exfoliative examination of the skin should be equally as rewarding.

Our studies also indicate that changes in corneocyte size may permit a sensitive evaluation of altered skin physiology, especially epidermopoiesis. By using the projected area feature of the Vickers M-85 microcpectrophotometer, changes in corneocyte size can be rapidly and precisely measured (21). Table III gives the results obtained for the volar forearm and upper inner arm sites. Note that for both age cohorts, the volar forearm cells tend to be smaller than those of the upper inner arm. In fact, a size gradient of increasing cell size exists as one goes from the wrist to the axilla. Thus, extreme caution should be exercised to sample specific sites if corneocyte size is to be a meaningful measurement. In this manner an age-related increase in cell size at these two sites has been demonstrated (15). Previous studies by Plewig, et al. (22) have also shown similar age-associated changes in corneocyte size; however, we have been unable to confirm any sex differences in this parameter.

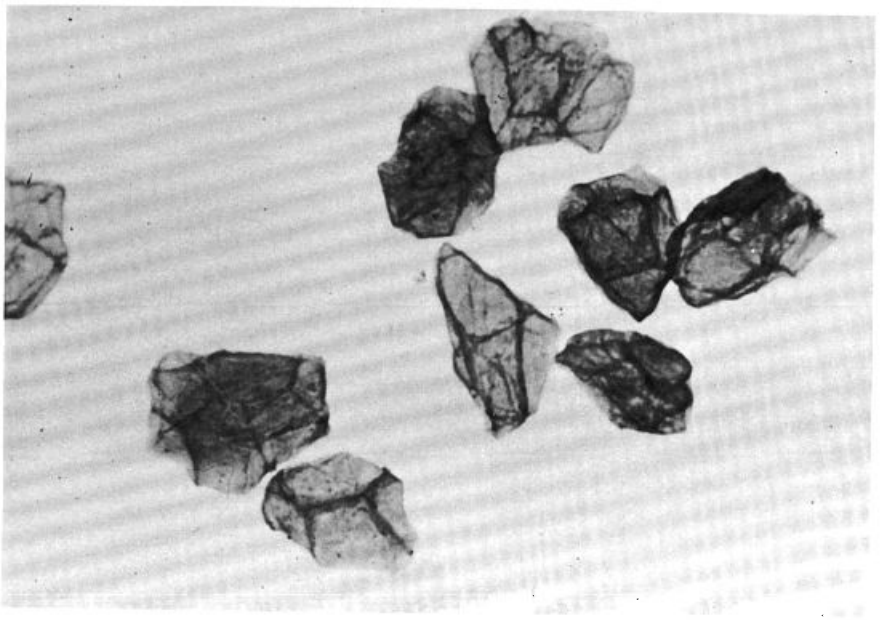

Figure 7. Light micrograph showing the morphology of a normal exfoliated corneocyte.

It should be noted that with advancing age, there is an increased variance of cell size measurements among the subjects. Figure 9 shows projected area measurements of volar forearm cells from another panel of subjects of various ages. Note that the values are fairly tightly clustered in the early years and exhibit a much wider range later. What

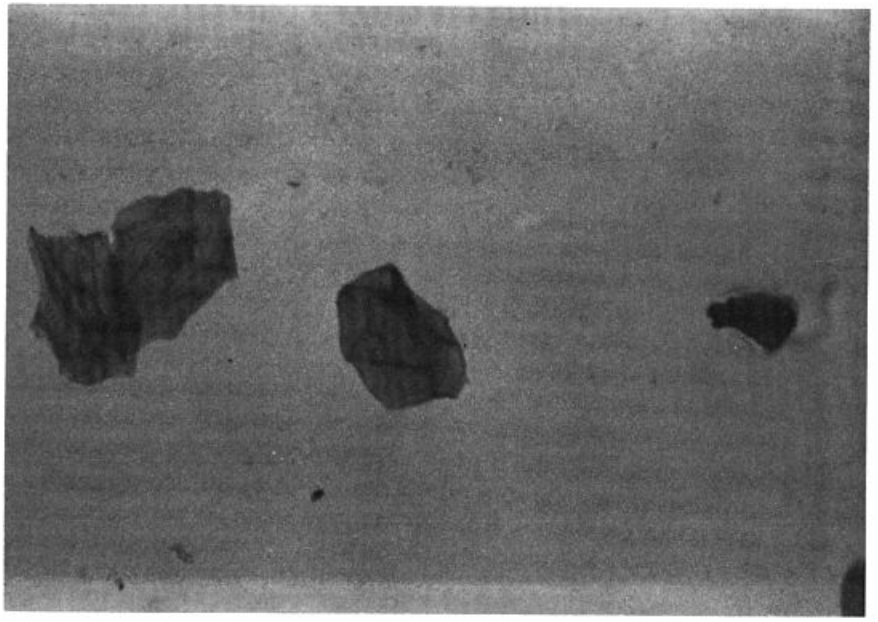

Figure 8. Light micrograph showing the morphology of some atypical exfoliated corneocytes.

Table III
Projected Areas of Corneocytes[1]

Cohort	Volar Forearm	Upper Inner Arm
<35	838.7 ± 17.4	984.4 ± 29.6
>60	936.4 ± 46.1	1,076.3 ± 36.3

[1]Values are means ± S.E. in μ^2.

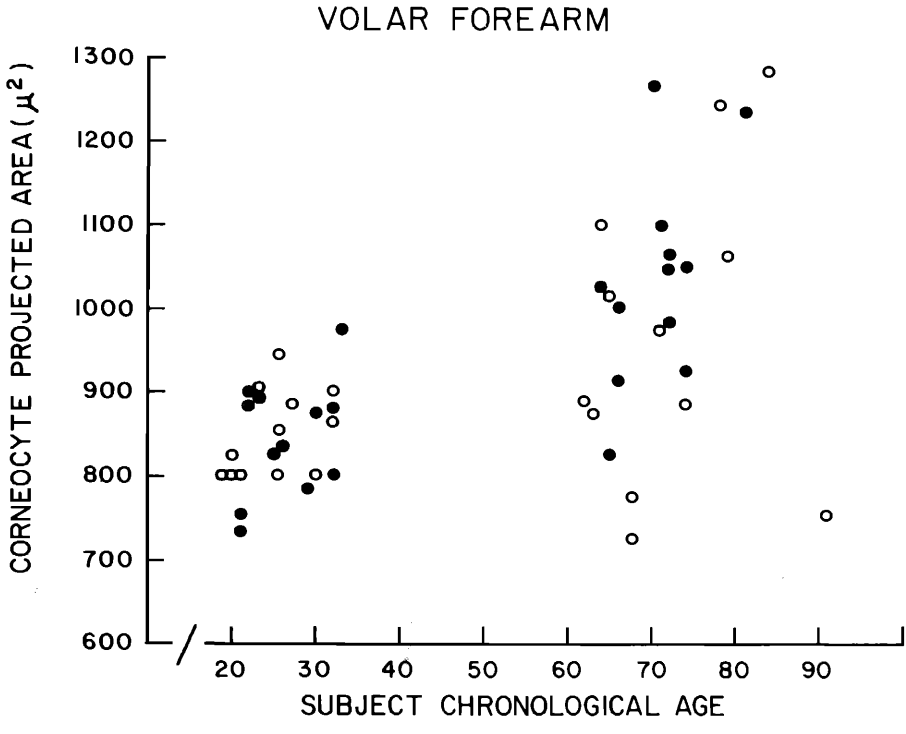

Figure 9. Scatter plot showing the projected areas of exfoliated corneocytes from the volar forearm of human subjects of different ages.

accounts for this extraordinary spread of values among aged individuals is perhaps one of the most important questions we can ask. By correlating ethnic origins, occupational histories, life styles, etc. with age-associated differences revealed by our nonintrusive testing procedure, we hope to estimate the relative importance of genetics, environment and other factors which clearly contribute to "aging." Thus we anticipate that the knowledge gained will serve as the foundation for the development of effective therapeutic and prophylactic treatment for easing dermatological problems associated with "aging."

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