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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

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).

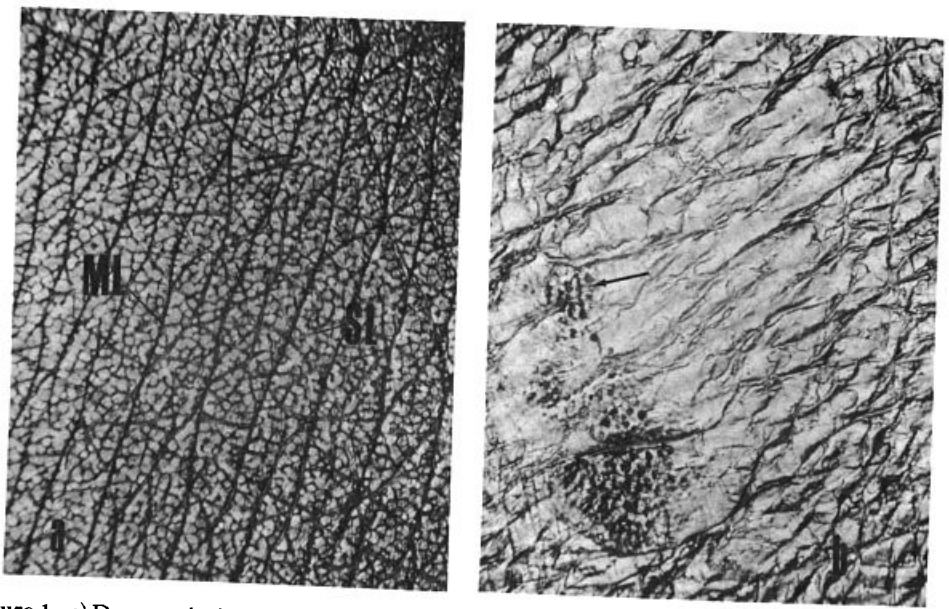

Figure 1. a) Dermatoglyphic pattern of the dorsum of the hand of the young. Major (ML) and secondary (SL) lines traverse to form a highly ordered pattern of triangles. Within the triangles the corneocytes are arranged in a honeycomb fashion (arrow) (×15). b) Dermatoglyphic pattern of the dorsum of the 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

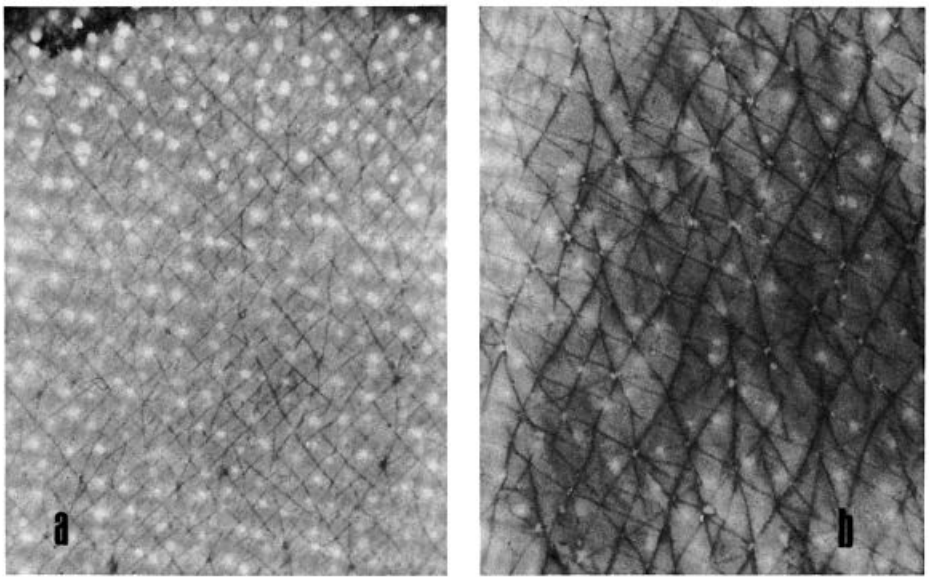

Figure 2. a) Sweat gland imprint from the volar forearm of the young. Light circles represent perforations in the film of silicone material formed by sweat droplets from functioning sweat glands (×15). b) Sweat gland imprint from the volar forearm of the old. Note diminished amount of functioning sweat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

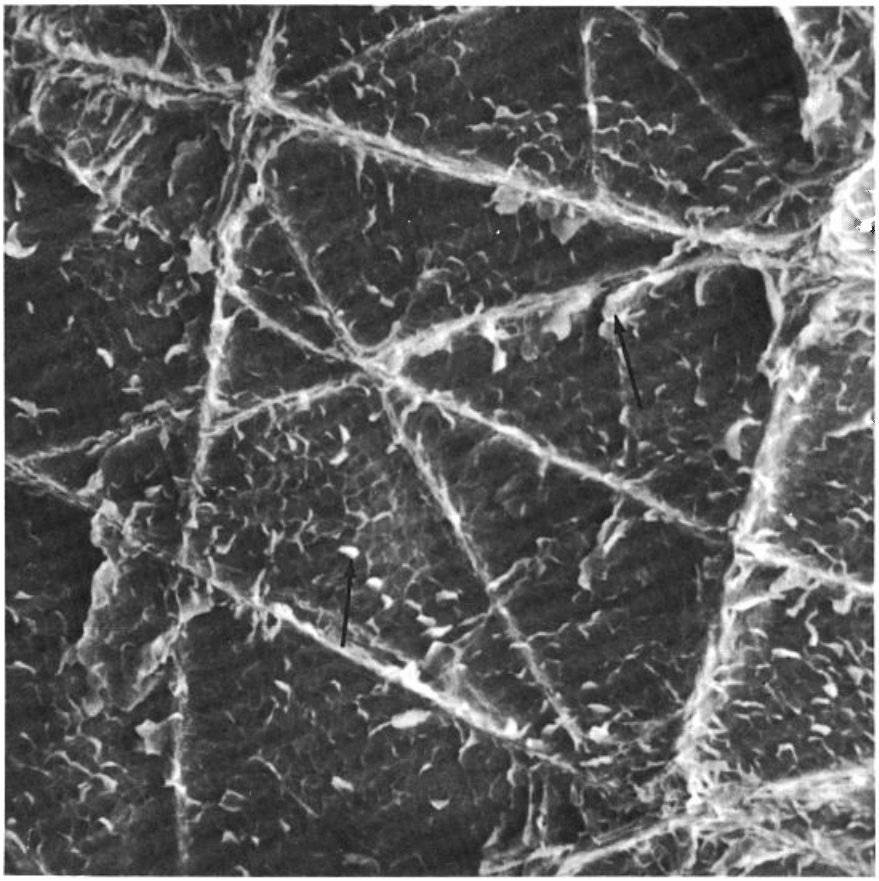

Figure 3. Scanning electron micrograph of a skin surface biopsy from the calf of the young. In addition to the easily recognized ridges which constitute the dermatoglyphic pattern, the desquamation of corneocytes in single and small groups can also be resolved (arrows) (×250).

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

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