Table 1. *Analysis for Hyp and Hyl of skin biopsies from patients with generalized scleroderma (acrosclerosis) and normal controls*

Subjects	No.	Hyp (μg/10 mg DDS)	Hyl (μg/10 mg DDS)	Molar ratio Hyp/Hyl
Controls	56	849.3±71.37	56.33±7.30	19.49±1.883
Scleroderma	82	590.5±100.29	44.97±7.27	16.86±2.024
Student's *t*-test		$P<0.001$	$P<0.001$	$P<0.001$

with those of Harris & Sjoerdsma (6), Neldner et al. (10) and our own earlier values (3). We calculated the molar ratio Hyp/Hyl on the basis of the findings published by Neldner et al. (10) and found a similar low ratio.

The possibility exists that an underhydroxylated proline (Pro) as compared with lysine (Lys) would result in an abnormal collagen. The low molar ratio Hyp/Hyl found in this study indicates the occurrence of a pathological collagen in scleroderma. A change from one collagen type to another may well have taken place, a transition which is known to occur in other pathological conditions (11, 12).

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Measurement of the Horny Layer Turnover after Staining with Dansyl Chloride: Description of a New Method

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Abstract. A simple non-radioactive technique is described. The corneal layers were stained with 5% dansyl chloride ointment during 24–48 h of occlusion. The number of newly formed corneal layers were counted on sections from punch biopsies obtained at various intervals after the staining. The rate of formation is given as layers formed per 24 hours. The technique makes it possible to detect an increase in the turnover rate when it is more than 15–32% above normal. The biological material can also be utilized for comparative studies utilizing biochemical and histological methods. The average rate of formation was

1.15±0.09 (S.E.M.) layers per 24 hours in a group of 10 subjects.

Key words: Epidermis; Proliferation, epidermal; Corneal; Dansyl chloride; Method

In most biochemical studies on the epidermis, observations on its histology and the cell proliferation have not been included in the evaluation of the findings. To combine such investigations it is necessary to collect all such observations from the same material without unduly damaging or alterating the biological specimens during processing by the methods used. This paper describes a method for measuring the turnover of the horny layer with these precautions in mind.

The rate of proliferation in the basal layer of the epidermis is the major regulatory factor for the transit of cells through the stratum Malpigii and for the formation of the newly formed corneal cell layers. The cellular replacement of the horny layer in human epidermis has been estimated by utilizing tetrachlorosalicylanilide as a corneal marker (1, 2). The interval between the time of staining and complete disappearance of the marker some weeks later can be used to calculate the daily replacement rate of the horny layer, when the number of the corneal layers is known. This is valid provided that all the corneal layers are stained initially and that the proliferation is constant throughout the period. The technique was modified by Jansen & Kligman (4), by exchanging the previous marker with dansyl chloride.

In order to establish whether the turnover rate changes during the period when the horny layer is marked with the fluorochrom, several biopsies may be taken. The appearance of non-marked corneal cell layers could then be counted by studying sections from several punch biopsies, instead of measuring the decay of the superficial fluorescence. Preliminary observations indicated that the number of corneal cell layers in the horny layer could vary conspicuously in neighbouring biopsies obtained from the same patient. It was also evident that the fluorochrom did not stain all of the corneal layers down to stratum granulosum reproducibly. Thus it is probable that the formation of corneal layers constitutes a more reliable way of estimating the proliferation rate of the basal layer than does the exfoliation of horny layers from the surface of the skin. A method based on this principle is presented in this paper.

MATERIAL AND METHODS

The staining of the normal horny layer with 5% dansyl chloride (4) was evaluated in 4 volunteers. The formation of the corneal layers was then studied in 10 further non-psoriatic subjects, 5 males and 5 females, age 41–77 years. The material was obtained by punch biopsy from non-involved skin on the extensor area of the upper arm. Five of the subjects had chronic leg ulcers, one had lichen simplex chronicus, one had a tinea infection of the feet and the other 3 subjects were healthy. The area of the skin studied was not affected by the clinical condition of the patients during the course of the study. Up to three punch biopsies were taken from the same person at various intervals from 0–10 days after completion of the staining procedure.

Identification of the marker. 5 μm cryostat sections were treated with 0.4 M glycine buffer, pH 10, which resulted in a uniform swelling of the horny layer yet without quenching the fluorescence of the corneal marker. No fluorescence could be detected when 0.4 M NaOH was used according to MacKenzie (6). A Zeiss universal microscope was used. The UV-light was applied as reflectant light for fluorescence. Identification was done by phase contrast microscopy utilizing transmitted illumination.

Staining of the horny layer. The occlusion of the dansyl chloride ointment was tested on two volunteers in the following ways: (*a*) cotton wool covered by a sheet of polythene and adhesive tape (4), (*b*) the Al-test (Imeco, Sweden) and (*c*) the Finn Chamber aluminium disc (Epitest Ltd Oy, Finland). The intensity of the superficial fluorescence was brightest after occlusion with the Finn Chamber discs. These were therefore selected for the study.

The duration of occlusion was 24 h for the first 4 subjects. In one of them the fluorescence was displayed down to the granular layer, as judged from biopsy sections obtained immediately after the occlusion period. However, in the other 3 subjects the corneal layers was unevenly stained. 48 h was required to give complete corneal staining in all 7 persons studied. In these cases a change of the Finn Chamber and the dansyl chloride ointment was made after 24 h.

Spacing of biopsy sites. The staining sites (ca 1 cm Ø) were spaced 2–3 cm apart according to the preliminary observation that the wound healing after the punch biopsy resulted in a more rapid decay of fluorescence 2–3 mm around the wound site.

Measurements. At least five different view fields in a section were observed. In each field an area was selected with intact stratum corneum using the transmitted phase contrast illumination. The selected area was marked by a scale of an ocular micrometer placed perpendicular to the corneal surface. The reflected UV-light was then turned on while the transmitted light was turned off. The margin of the corneal fluorescence was registered on the micrometer. The corneal layers were then counted starting from the observed micrometer setting down to stratum granulosum. In each biopsy specimen 5 measurements in each of 5 sections were counted.

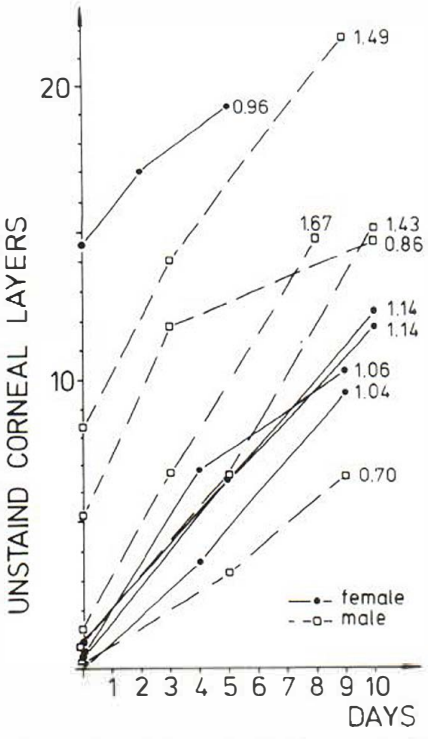

Fig. 1. Corneal formation in 10 subjects. Number of unstained corneal layers formed in a period after the staining with 5% Dansyl chloride in white petrolatum by occlusion for 24 h (upper 4 lines) and for 48 h (lower 6 lines) are shown. The rate of formation expressed as layers formed per 24 h is given for each subject.

The total number of corneal layers varied from one view field to the next by up to 2–3 fold. Therefore, this measure was not suitable as a basis for any calculation. The best measure was the non-fluorescent number of corneal layers lying just above the stratum granulosum.

In the 10 subjects followed up to 10 days after the staining period, three different times were selected for biopsies. The mean and the standard deviation of the 25 observations of the non-stained corneal layers from each biopsy were calculated. The coefficient of regression of these means with respect to time was calculated and given as layers formed per 24 h.

The minimum increase in the turnover rate that could be detected was calculated in the following manner for each subject for the observation points made at 3–5 days and 8–10 days. A new point equal to an estimated mean different from the observed mean by t-test, ($P=0.05$) assuming the same variance, was computed. The slope of the line through this point and that found at the observation on zero day was calculated. Its percentage increase from the calculated coefficient of regression was averaged. This value was used as an estimate of the minimum detectable change.

RESULTS

Only the corneal layers were fluorescent after staining with dansyl chloride, even when the occlusion was prolonged to 48 h.

Variation was found in the depth of staining attained in the stratum corneum after 24 and 48 h of occlusion (Fig. 1). The horny layer was most thoroughly stained after 48 h, and the standard deviations calculated showed less magnitude than at corresponding periods of time after the 24 h occlusion (Table I).

Corneal formation in non-involved skin in 10 subjects is given in Table II. The mean of the layers formed per 24 h was 1.15. The 95% confidence interval of the distribution was ±47% and that of the mean ±14%.

The mean of the increase in turnover rate which could be detected with the present method was 32% for the time interval 3–5 days, and 15% for the interval 8–10 days.

DISCUSSION

The method described is simple and the biopsy material can be used for other purposes, e.g. as freeze dried sections for biochemical analysis according to Lowry (5). It was shown in this material that dansyl chloride did not stain any part of stratum Malpigii. The viable part of the epidermis seems therefore to be accessible without any interferance from the stain.

To yield a good staining of the complete horny layer, occlusion for 48 h was necessary, as shown in Fig. 1. When the occlusion was for 24 h, a variable number of corneal layers were not stained. The variation in each biopsy was also greater when the

Table I. *Means and standard variation of non-stained corneal layers after a period of occlusion with dansyl chloride*

Values are given for both parameters as arithmetic means

Time of occlusion	Immediately after the staining	After 3–5 days	After 8–10 days
24 h			
Mean	5.0	13.7	17.2
S.D.	4.3	4.1	4.0
48 h			
Mean	0.6	5.5	11.0
S.D.	0.8	2.6	3.4

Table II. *Corneal formation given as layers formed per 24 hours measured after corneal staining with 5 % dansyl chloride ointment under occlusion*

Sex	Age	Corneal formation	Duration of occlusion (h)
♀	54	1.14	48
♀	63	0.96	24
♀	63	1.06	48
♀	67	1.04	48
♀	69	1.14	48
♂	41	1.49	24
♂	62	0.70	48
♂	65	1.67	24
♂	66	1.43	48
♂	77	0.86	24
Mean		1.15	

material obtained after occlusion for 24 h was compared with that at 48 h (Table I).

It may be noted that the occlusion for 48 h gave almost straight lines in the material studied up to 10 days after staining (Fig. 1). This indicates that the occlusion did not affect the proliferation rate during this interval. The incomplete staining obtained after occlusion for 24 h might be a reason for the non-linearity found in one of the male subjects (Fig. 1).

The present results which indicate a formation of about 1.15 corneal cell layers per 24 h was in the upper range of three earlier studies, 0.89, 0.72–1.08 and 1.20, utilizing the surface disappearance of the fluorescent corneal marker as the base of measurement (1, 2, 3). The range in the present study varied by a factor of 2. In future studies relating observations on cell proliferation and histology with biochemical studies, it will be necessary to include the normal formation of the horny layer in the skin region used. From the data presented it is feasible to detect at the 5% level an increase in the formation of the horny layer when it is more than 15–32% above the normal rate.

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Membrane-associated Actin in Psoriatic Epidermis

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Abstract. With immunofluorescence technique by using specific antibodies, polymerized actin (F-actin) was found to be present in the peripheral parts of the cells in all layers of epidermis from lesional skin taken from twelve psoriatics, whereas normal epidermis from the same individuals showed no such reactivity. This finding might have some bearing on the induction of these lesions.

Key words: Microfilaments; Plasma membrane

Actin is a contractile protein present in almost all eukaryotic cells. Actin constitutes part of a microfilament system which appears to be associated with the plasma membrane (13). In non-muscle cells these actin-containing filaments have been implicated in a number of cellular functions including cytokinesis, endocytosis, exocytosis, cell adhesion to substratum, cell locomotion, membrane ruffling, maintenance of cell shape and cell division. There is also evidence that the distribution and mobility of plasma membrane components is controlled by submembranous microfilaments and microtubules (1, 11). Actin may be present in a cell in at least two interconvertible states: as unpolymerized actin (G-