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THE EFFECT OF OCCLUSIVE TREATMENT ON HUMAN SKIN: AN ELECTRON MICROSCOPIC STUDY ON EPIDERMAL MORPHOLOGY AS AFFECTED BY OCCLUSION AND DANSYL CHLORIDE

M. Lindberg,[1,3] A. Johannesson[2] and B. Forslind[1]

[1]*Department of Medical Biophysics, Karolinska Institutet, Stockholm,*
[2]*Department of Dermatology, Karolinska sjukhuset, Stockholm,*
and [3]*Department of Dermatology, Södersjukhuset, Stockholm, Sweden*

Abstract. The effect of occlusion on epidermal morphology was studied at electron microscopic resolution. The morphological changes recorded were relatively mild, though conspicuous, and consisted in a dilatation of the intercellular space, appearance of cytoplasmic vacuoles and an oedematous change in the dermis. Stereological analysis showed that the dilatation of the intercellular space had a biphasic time-dependent course. Dansyl chloride applied under occlusion was shown to cause the same type of changes as did simple occlusion, but the changes appeared earlier.

Key words: Epidermis; Patch testing; Occlusion; Dansyl chloride; Electron microscopy; Stereology

Occlusion is an effective way of enhancing the penetration of many substances through the skin barrier. It is generally assumed that this effect is due at least in part to an increased hydration of the epidermis, more specifically of the stratum corneum (11). Both the diagnostic use of occlusion, as exemplified by patch testing, and the therapeutic use are extensively documented in the literature (cf 2).

Previous studies on the effect of chromium solutions on human skin by patch testing suggested that occlusion as such produces morphological changes in the living epidermis (3, 4). Further study of this problem has recently been made in an investigation of the Langerhans' cell in skin under occlusion (9).

Organic stains have been used as markers for epidermal horny layer turnover (1), while in a modified technique (5) dansyl chloride was utilized.

In order to achieve optimal marking of the horny layer cells, dansyl chloride was applied under occlusion (6) for 24–48 hours. It was found that in order to obtain a complete staining of the horny layer, 48 hours of occlusion was necessary. The report does not include a description of the cellular morphology of subcorneal layers. We therefore report here the electron microscopic findings in simple occlusion and in exposure to dansyl chloride under occlusion in normal subjects.

In order to ascertain how well ocular estimation of changes in the volume ratio of the extracellular and intracellular compartments corresponds to objective data, a stereological analysis of the size of these compartments was performed.

MATERIAL AND METHODS

The skin. Three male adults (age 30–45) with no known systemic or skin diseases were exposed to occlusive treatment of the skin of the extensor aspect of the forearm. Dansyl chloride was applied under occlusion on another area of the forearm on 2 of the persons.

The occlusion. Plain aluminium cups (Finn Chambers) were fixed to the skin with Scanpore tape.

Dansyl chloride. 5% dansyl chloride in petrolatum was introduced into the Finn chambers under occlusion.

Time intervals. Biopsies were taken after 1, 3, 6, 24 and 48 hours of occlusion. Control skin was obtained from well outside the occluded area on the forearm.

Specimen processing. Biopsies from 2 persons were fixed in 2.5% glutaraldehyde in a phosphate buffer isotonic to blood (KS-buffer: 300 mOsm). The biopsies from the third person were fixed in 2.5% glutaraldehyde in a Veronal-acetate buffer (Palade ref. 10). All specimens were post-fixed in 1% osmium tetroxide (OsO_4) in the buffer used for the primary fixation. Dehydration was obtained in a graded series of ethanol solutions and Epon was used for embedding. Sections 50–70 nm thick were obtained with an LKB Ultrotome. Contrast staining with uranyl acetate or uranyl acetate in 50% methanol.

Electron microscopy. Electron microscopy was performed with a Philips EM 301 G at 60 and 80 kV.

Stereology. To obtain an objective evaluation of the changes in the relative volume of the intercellular space after different intervals of occlusive treatment, a stereological analysis was performed. Ten fields from the sec-

Table I. *Diagrammatic representation of the most conspicuous findings at occlusive treatment of human skin*

+ indicates positive finding in several sections, (+) indicates positive finding in some section, 0 indicates that no positive findings were obtained

Compartment	Time interval 1 h	3 h	6 h	24 h	48
(a) Simple occlusion					
Intercellular spaces widened Basal layer	0	+	+	+	+
Intercellular spaces widened Str spinosum	0	0	+	+	+
Vacuoles in perinuclear zone	0	0	0	+	+
Vacuoles in cytoplasm	0	0	0	+	+
Intercellular debris	0	0	(+)	(+)	+
Edema in dermis	0	0	+	+	+
Duplication of basal lamina	0	0	0	0	0
Invading mononuclear cells	0	+	+	+	+
(b) Occlusion with dansyl chloride					
Intercellular spaces widened Basal layer	0	+	+	+	+
Intercellular spaces widened Str spinosum	0	0	+	+	+
Vacuoles in perinuclear zone	0	0	0	+	+
Vacuoles in cytoplasm	0	+	0	+	+
Intercellular debris	0	0	(+)	(+)	+
Edema in dermis	0	+	+	+	+
Duplication of basal lamina	0	0	0	0	+
Invading mononuclear cells	0	+	+	+	+

tions used for the descriptive analysis of the cellular changes under occlusion were recorded anew at a primary magnification of ×2 800. These fields were from the two cellular layers above the basal lamina and were randomly chosen within the section in question. At printing, a secondary magnification of ×6.5 was used. A grid of 225 points was superimposed on the electron micrograph in the printing process (cf. Fig. 1). The grid was a square lattice (d=10 mm) which allowed for systematic point counting (12).

In order to minimize the subjective influence on the stereological procedure the photocopies were randomly mixed after an identification number had been allotted to each micrograph. The morphometry was thus performed with as little bias as possible. We have used the "progressive mean technique" (13) to calculate the minimum sample size required for estimation of the relative volume of the intercellular space. It was found that applying 225 points to ten different micrographs from normal skin at the given magnification gave an estimate constantly within ±10% of the final value for the relative volume.

Table II. *Mean values for the relative volume of the intercellular space obtained by stereological analysis at different times of occlusion*

Subjects 1 and 2 KS buffer and subject 3 Palade buffer

	0 h	1 h	3 h	6 h	24 h	48 h
Subject 1						
Mean	7.5	15.2	23.1	20.5	10.8	13.4
SD	1.1	3.2	6.0	6.1	2.1	3.9
n	10	10	10	10	10	10
Subject 2						
Mean	5.9	–	14.5	9.2	9.1	18.0
SD	2.5	–	7.0	3.6	1.8	4.1
n	10	–	10	10	10	10
Subject 3						
Mean	9.0	8.5	16.7	11.6	13.5	16.1
SD	2.7	2.6	6.2	2.7	6.0	4.8
n	10	9	10	10	10	10

RESULTS

Initially the cellular and subcellular changes in human skin under occlusion were studied qualitatively. However, we found it necessary to obtain an objective evaluation of the changes in relative compartment size for a more precise understanding of the cellular changes as a function of time.

Ocular analysis

In the following we refer to Table I and II, where the most characteristic findings are tabulated. The markings of the tables record findings in several sections from all specimens at each time interval.

After 1 hour of occlusion the skin appeared normal in all cell layers. At 3 hours a widening of the basal intercellular space was recorded, a morphological change which persisted throughout all time intervals. The intercellular space of the spinous layer also appeared widened at 6 hours of occlusion and this condition then remained unchanged—if not more accentuated—at subsequent intervals. At 6 hours an oedematous change in the dermis appeared which subsequently persisted. At 24 hours perinuclear vacuoles appeared in the keratino-

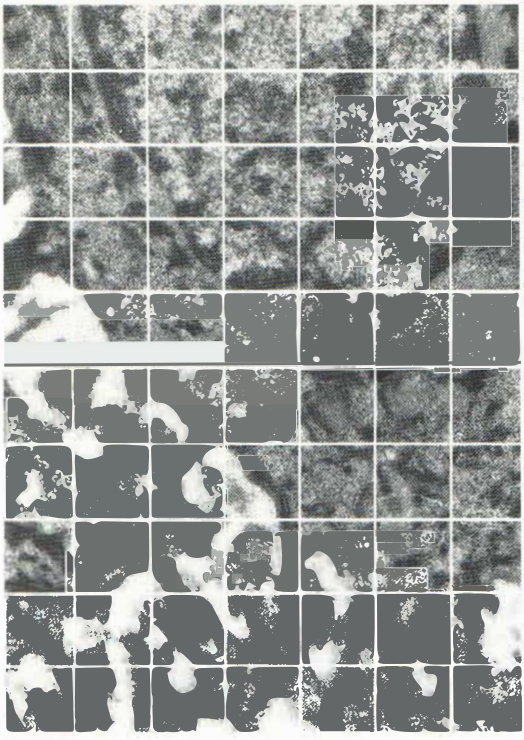

Fig. 1. The basal part of epidermis in normal, non-occluded skin. The square lattice grid used for morphometry is superimposed on the micrograph. ×18 000.

cytes—except in the stratum granulosum. Vacuoles and vesicles in the cytoplasm of the cellular periphery (Fig. 2) were also observed.

The morphological changes induced by dansyl chloride under occlusion were comparable to those of simple occlusion. However, at 3 hours we observed cytoplasmic vacuoles and vesicles in the keratinocytes of the basal and the spinous layers. A dermal oedema was also observed at this time. At 48 hours we recorded a case of duplication of the basal lamina.

The stratum corneum appeared to be more affected by the dansyl chloride at 24 and 48 hours, compared with the morphology under simple occlusion. The change consisted in a loose arrangement of cells and their contents.

Mononuclear cells infrequently seen in normal material were observed in sections from each time interval, except at 1 hour, under simple occlusion as well as in dansyl chloride exposure. In some instances such cells were seen to penetrate the basal lamina and appeared to be propagating in the intercellular space of the basal layer.

Stereological analysis

The stereological analysis showed that the relative intercellular volume of the basal layer changed with the duration of occlusion. Thus a maximum intercellular volume was attained at 3 hours of occlusion. Subsequently a return to smaller but still expanded intercellular spaces occurred in the interval 6–24 hours. At 48 hours of occlusion the intercellular volume again increased (Table II and Fig. 3). The choice of buffer did not seem to influence the course of events.

To test that the difference in the intercellular volume at zero and 3 hours is significant, Student's t-test with pooled variances was applied to all three persons involved (cf. Table II). This revealed a significant increase in size ($p<0.05$) foreach person.

DISCUSSION

Occlusion is thought to increase epidermal hydration and to facilitate penetration of substances applied on the skin. From a clinical point of view occlusion has been regarded as an inert method of introducing various pharmacological and other substances into skin. However, some doubt on this topic was expressed in a study of patch testing in chromium allergy (3).

Recently an experimental study on the effect of water in occlusion in rats has given morphological results indicating pronounced effects on the epidermis (7).

It is interesting to note that the CO_2 emission of the skin increases during the first 3 hours of occlusion, subsequently to remain at a relatively constant level for at least 24 hours (8). In the present study, gross reactive changes, such as a widening of the intercellular spaces, were recorded under occlusion within the first 3 hours (Fig. 3). However, it must be noted that it is virtually impossible to obtain a complete knowledge of what substances are resident in the stratum corneum. It is possible that such substances might influence the reactions of the skin only under special conditions, such as occlusion. It has also been suggested that a local temperature rise is one of the factors important to the effectiveness of occlusive treatment. In any experiment involving the introduction of topically applied pharmacological substances it is therefore pertinent to include simple occlusion, especially if morphology is to be studied.

The overall morphological reactions we observed

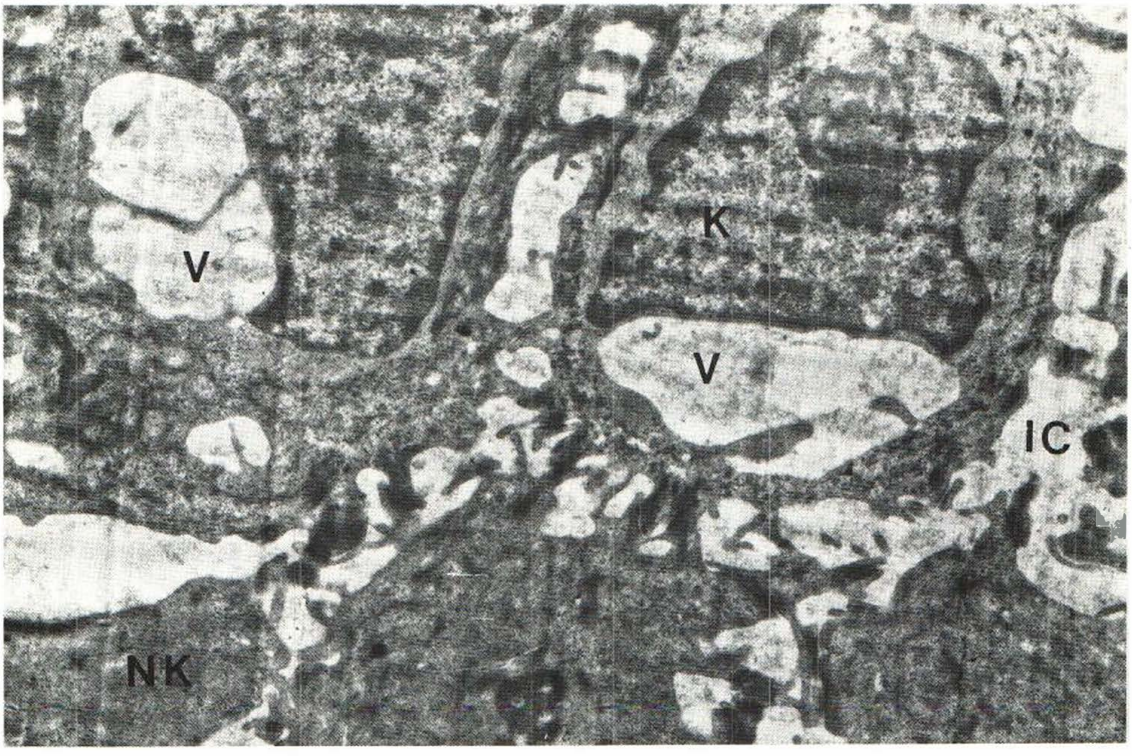

Fig. 2. Basal part of epidermis after 48 hours of occlusion. V = perinuclear vacuole, IC = widened intercellular space with debris, K = keratinocyte, NK = non-keratinocyte. ×14000.

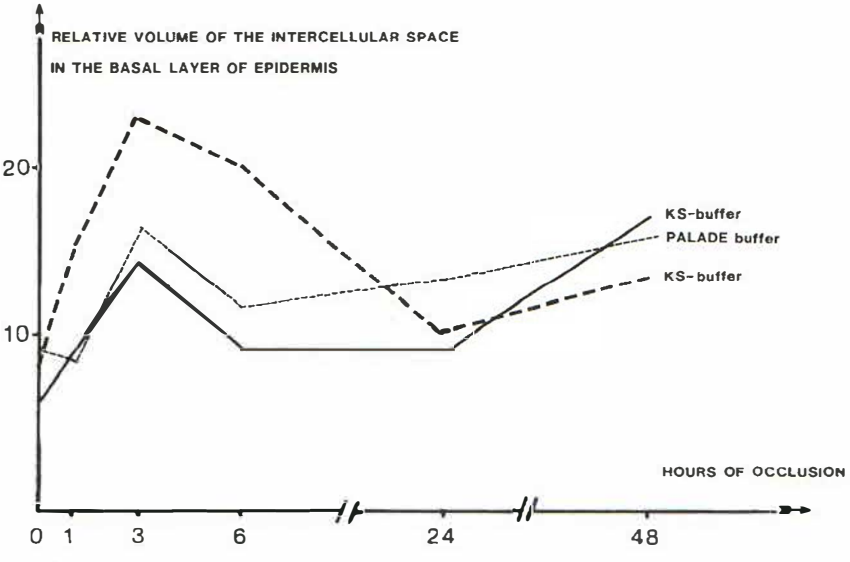

Fig. 3. The diagram shows the relative volume (%) of the intercellular space as a function of time of occlusion in each volunteer (cf. Table II). All specimens were fixed in 2.5% glutaraldehyde. Two different buffer systems were used (cf. Methods). Thick dashed line: subject no. 1, KS buffer. Solid line: subject no. 2, KS buffer. Thin dashed line: subject no. 3, Palade buffer.

in epidermis under occlusive treatment of the skin developed as a function of time. At least in the first hour no clearcut changes were recorded by ocular evaluation in our test subjects. After longer periods of occlusion a widening of the intercellular spaces, notably in the two lowest layers, was observed.

The morphometry performed on the limited number of subjects available to us confirms our subjective evaluation of the effects of occlusion. However, details of the intercellular widening process such as the marked maximum size at 3 hours was not easily resolved by ocular evaluation. The statistical analysis further confirmed that this increase was significant.

It can be seen from Fig. 3 that a steep transient volume change occurs within the first 3 hours. The subsequent decrease in size of the intercellular space coincides with the development of intracellular vacuoles. This could be the result of an adaptation of the epidermis to the conditions imposed on the skin under the occlusive treatment.

It is interesting to note that an increase in the intercellular space occurs in the interval 24–48 hours.

The detailed morphological changes of the epidermis include subcellular as well as cellular changes. The widening of the intercellular space is a first sign of influence on the epidermis which is accompanied by intracellular vacuolization in the keratinocytes. We also note the appearance of intercellular debris and dermal oedema (Fig. 2). Furthermore, the number of invading mononuclear cells appears to increase. However, in electron microscopy, quantitation of the number of invading mononuclear cells could not be satisfactorily achieved due to the small volume of tissue under observation.

Addition of dansyl chloride in the occlusion tests causes minute effects, as seen in the present investigation. The differences appear to be mainly in the time sequence of appearance rather than in changes specific for the agent (dansyl chloride) (cf. Table I).

The present investigation thus confirms that simple occlusion gives rise to morphological alterations in the epidermis detectable at electron microscopic resolution. The most probable cause of the changes observed is the increased hydration caused by occlusion, but several other (unknown) factors are probably at work.

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M. Lindberg, M.D.
Department of Medical Biophysics
Karolinska Institutet
S-10401 Stockholm 60, Sweden