

THE EFFECTS OF OCCLUSION OF THE SKIN ON THE LANGERHANS' CELL AND THE EPIDERMAL MONONUCLEAR CELLS

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Abstract. Clinically normal skin was occluded for 3, 6, 24, and 48 hours with aluminium cups used for patch testing. Electron microscope sections from occluded skin showed morphological alterations in the Langerhans' cells and apposition of mononuclear cells. After occlusion an increased number of mononuclear cells were found in the epidermis, compared with normal skin.

Key words: Patch-test; Occlusion; Langerhans' cell; Electron microscopy

The morphology of the epicutaneous patch test reaction as revealed by electron microscopy has been documented in the literature (2, 10). Structural alterations arising in conjunction with positive chromium patch tests were described recently. It was then emphasized that both occlusion and the vehicles used must be considered when evaluating the morphological results of a patch test (7).

Occlusion allows a high level of penetration for substances applied to the skin, due to prevention of water loss from the skin surface and increased hydration of the stratum corneum and probably even other factors. Occlusion has been reported to increase mitotic activity, to cause epidermal thickening and to induce the Langerhans' cells to move to a more central position in the epidermis (6, 17). In occluded guinea pig skin, as revealed with histofluorescence, the Langerhans' cells had a more dendritic appearance than is normally seen (17).

The fact that occlusion as such produces changes in normal epidermis prompted us to undertake the present electron microscopic study, with special attention to the non-keratinocytic cell population in the epidermis.

MATERIAL AND METHODS

Test patches of empty aluminium cups (Finn chambers attached with Micropore tape) were applied to the skin of the forearm of 3 healthy volunteer male adults free from

skin problems 48, 24, 6, and 3 hours before biopsies were taken. A 2 mm punch biopsy was taken from each patch test area.

A control sample was taken from a non-occluded area of the same forearm. Clinically normal skin was also obtained from the gluteal region of 6 adult males to serve as reference material. The specimens were fixed in 2.5% glutaraldehyde in either a phosphate buffer made isotonic to blood or a Veronal acetate buffer, post-fixed in 1% osmium tetroxide in the same buffer. Dehydration and staining *en bloc* with uranyl acetate was obtained in a graded ethanol series followed by routine embedding in Epon. Sections were cut on an LKB Ultratome, set to a section thickness of 60 nm. Sections were contrasted with uranyl acetate in water or in 50% methanol and were viewed in a Philips EM 301 at 60 kV and 80 kV.

RESULTS

Epidermis

Biopsies from skin areas under occlusion showed distinct changes when compared with normal skin. After 3 and 6 hours of occlusion the intercellular spaces in the basal parts of the epidermis were distended. At 24 and 48 hours a perinuclear vacuolization of keratinocytes had occurred (Fig. 1).

Langerhans' cells

The Langerhans' cells were identified by the presence of so-called Langerhans' cell granules (or Birbeck granules) in the cytoplasm (1) and by the absence of desmosomes and tonofilaments. Langerhans' cell granules had a normal structure in both normal and occluded skin. The racket form of the granule was rarely encountered in our specimens.

In the control specimens, Langerhans' cells were found in the basal layer and up to the upper stratum spinosum. Most of the cells contained mitochondria, a Golgi apparatus, small clear vesicles, lyso-

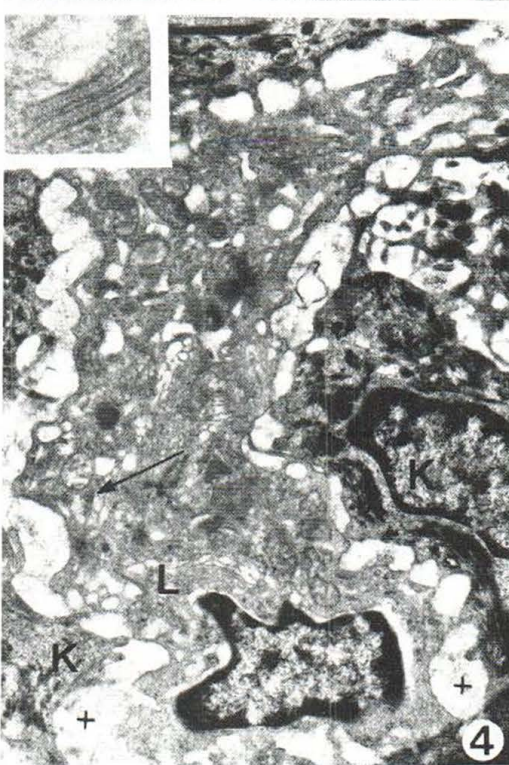
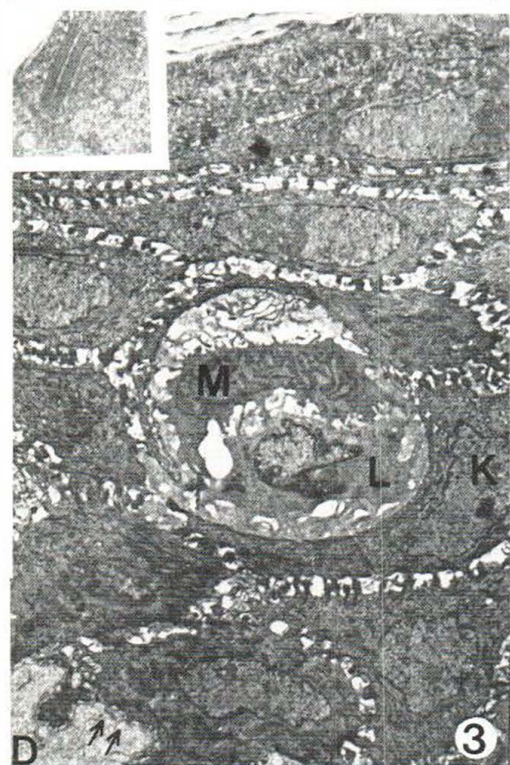
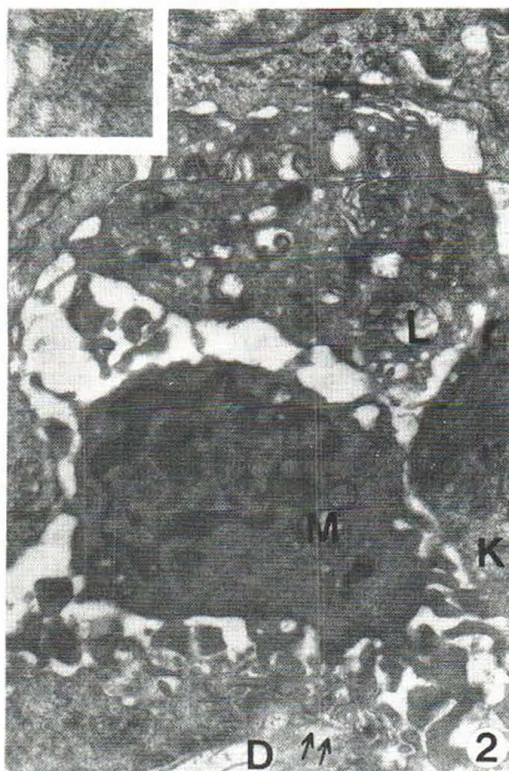
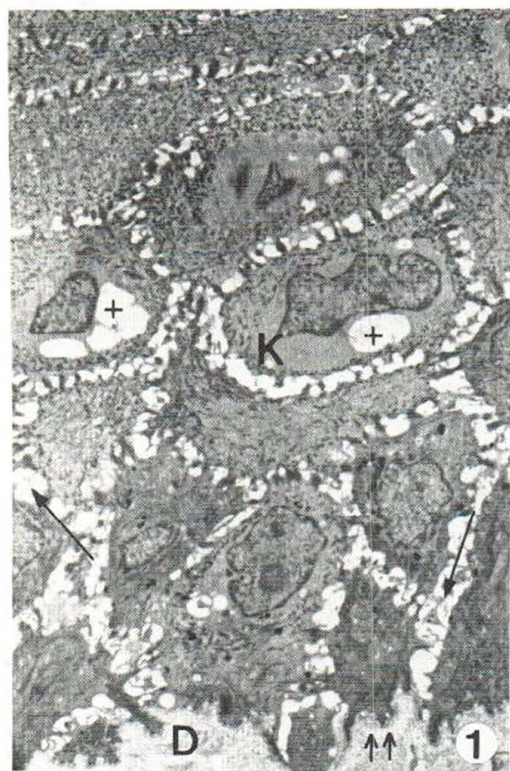




Fig. 5. Mononuclear cell moving in stratum basale at 24 hours of occlusion. Langerhans' cell granulae or melanosomes are not found in this cell. $\times 11\,000$.

somes and a clear cytoplasm. Langerhans' cells having a different appearance, viz. a more electron-dense cytoplasm, fewer cell organelles and few typical granules, were also present in the material. These cells were often rich in filaments and most often found in the basal layers. Apposition between

a Langerhans' cell and a mononuclear cell was found in the basal layer of the epidermis in two of the control specimens from the gluteal region (Fig. 2), but was not seen in non-occluded skin from the forearm.

A conspicuous feature in occluded skin was the apposition of Langerhans' cells to mononuclear cells in *st. spinosum* seen at 3 and 6 hours (Fig. 3). The mononuclear cells showed an indented nucleus with peripherally aggregated chromatin, a dense cytoplasm with filaments, but few other cell organelles. The cells in apposition were clearly separated from surrounding keratinocytes (Fig. 4). Apposition was not found in the specimens taken at 24 hours, but had appeared in the basal layer by 48 hours. After 6, 24, and 48 hours of occlusion, structural alterations were seen in some cells belonging to the Langerhans' cell population. Such cells had larger vacuoles than normal, dilated endoplasmatic reticulum and a more villous cell membrane (Fig. 5). These alterations were most pronounced after 24 hours of occlusion. However, intact, normal-looking Langerhans' cells were also observed in all biopsies.

Abbreviations: K=keratinocyte, L=Langerhans' cell, M=mononuclear cell, D=dermis, $\rightarrow$ =basal lamina.

Fig. 1. Epidermis at 48 hours of occlusion. Widened intercellular space ($\rightarrow$) and perinuclear vacuoles (+) are seen. $\times 3\,500$.

Fig. 2. Apposition of a Langerhans' cell and a mononuclear cell in stratum basale in non-occluded gluteal skin. A distortion of the basal lamina is seen. $\times 11\,200$. *Inset:* A typical granula found in the Langerhans' cell. $\times 90\,000$.

Fig. 3. Apposition of a Langerhans' cell and a mononuclear cell in stratum spinosum at 6 hours of occlusion $\times 3\,100$. *Inset:* Two typical granulae found in the Langerhans' cell. $\times 40\,000$.

Fig. 4. A Langerhans' cell in the lower part of epidermis at 6 hours of occlusion. More vacuoles and a more villous cell membrane than normal are seen. Intercellular debris (+) is also present. $\times 11\,200$. *Inset:* Langerhans' cell granulae seen at the arrow in a higher magnification. $\times 65\,000$.

Mononuclear cells

Mononuclear cells were found in the basal layer of the epidermis at all time intervals of occlusion. These cells had a kidney-shaped nucleus which in some cases was slightly indented. The cytoplasm contained mitochondria, endoplasmic reticulum, a Golgi apparatus, filaments, and small vesicles. Langerhans' cell granules or melanosomes were not found in these cells. In the control specimens, cells with this appearance were not found. Mononuclear cells apparently moving within the basal parts of the epidermis were observed at 24 and 48 hours. These cells exhibited a polarization of cell organelles, filaments in the cytoplasm and a conspicuous nucleus (Fig. 6).

DISCUSSION

One of the most important functions of the skin is to act as a barrier against harmful agents in the environment. Recently much interest has been paid to the Langerhans' cell and its role in immunological defence. This cell type has been shown to have a macrophage-like function in the immunological response (18) and it probably plays a central role in the contact allergic reaction (16). Stimuli which do not produce an allergic reaction on primary contact with skin (e.g. mercuric chloride (15), UV-light (19), PUVA (9), DNCB (8), podophyllum resin (12), tape stripping (11)) have been shown to induce changes in the Langerhans' cell morphology and/or function. Furthermore, it has been proposed that the Langerhans' cell acts as a "reticulo-epithelial trap" (13) and it has been shown (14) that several antigens bind specifically to Langerhans' cells *in vitro*. Our present knowledge of the Langerhans' cell and its function has recently been reviewed (5).

Occlusion may be regarded as a very mild physical stimulus to the epidermis, the most conspicuous effect being to hinder water transport through the skin and, in consequence, increased hydration of the epidermis. On prolonged occlusion, additional chemical effects on the epidermis cannot be excluded at present. In this investigation it was found that simple occlusion affects the Langerhans' cells and that mononuclear cells, normally infrequently seen in the epidermis, appear during occlusion. The epidermis as such is also affected, as revealed by widened intercellular spaces and perinuclear vacuolization of the keratinocytes.

In normal skin the Langerhans' cells are found to

have the characteristics previously described in the literature (3, 4, 20). The fact that occlusion produces detectable changes in these cells, as recorded at electron microscopy, further stresses the fact that occlusion as such is not an inert method of introducing substances into the skin (cf. Forslind & Wahlberg (7)). The alterations seen in the epidermis due to occlusion might be interpreted as a result of the physical stimuli and a subsequent adaptation to the new environment by the viable epidermis.

The function of the Langerhans' cell is probably manifold. A central role is obviously that of initiating the cell-related immunological reactions in the delayed type of hypersensitivity. The Langerhans' cell also seems to be affected by physical and chemical stimuli. However, the wide range of reactions involving the Langerhans' cell makes it possible that the cell acts as an epidermal target cell in a wider sense. The morphological changes recorded in the present study of epidermal occlusion agree with the latter idea.

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