EARLY CHANGES IN HUMAN EPIDERMIS FOLLOWING THERMAL BURN: AN ELECTRON MICROSCOPIC STUDY

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Abstract. Early alterations in the epidermis of human volunteers following 60° and 95°C burns were studied by means of electron microscopy. The blister was induced at 30 seconds following a 60°C burn for 1 and 3 minutes by disintegration of the basal cells just above the basement membrane, and, in addition, for 3 minutes by disruption of the intercellular bridges adjacent to the desmosomes in the suprabasal cells. The keratinocytes of the separated epidermis revealed intracytoplasmic vacuoles, widening of the intercellular spaces and, in some cells, vacuolization of the mitochondria. At 30 seconds following a 95°C burn for 3 seconds, the blister was induced by complete breakage of the basal and suprabasal cells which demonstrated karyorrhexis, aggregated tonofibrils and breaks in the plasma membrane. The keratinocytes of the blister roof showed aggregation of the tonofibrils at the periphery, and vacuolization of mitochondria and endoplasmic reticulum. The desmosomes and basement membrane revealed no alterations with 60°C and 95°C burns.

Key words: Thermal burn; Epidermal blister; Basement membrane

Many studies have been made on the cutaneous thermal burn, though published ultrastructural observations on the burned epidermis are few. Pearson (10) carried out morphologic studies of the mechanism of blister formation in response to graded thermal stress. Cuppage et al. (3) described briefly the ultrastructural alterations of the epidermis in monkey skin following severe thermal burn. Brizio-Molteni et al. (1) examined the change in the basement membrane during development of the eschar in deep second- and third-degree burns. Neither of these papers, however, included details of the initial cellular damage in the burned epidermis.

The present electron microscopic study was designed to elucidate the initial pathologic events in the human epidermis induced by experimental thermal burn at high (95°C) and low (60°C) temperatures for very short periods.

MATERIALS AND METHODS

Heat was applied by placing a flat-bottomed glass tube containing hot water on the skin of volunteer subjects. A 95°C burn for 3 seconds was induced in two volunteers, and a 60°C burn for 1 and 3 minutes in one volunteer. Biopsy specimens were obtained at 30 seconds post-heating.

All tissue was cut into two. One half was processed for routine microscopy and sections were stained with hematoxylin-eosin. The other half was divided into small pieces for electron microscopy. They were fixed in cold 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 2 hr, rinsed in several changes of the same buffer containing 10% sucrose overnight at 4°C, post-fixed in cold 1% osmium tetroxide for 2 hr, dehydrated in a graded alcohol, and embedded in Epon 812. Thin sections were stained with methanolic uranyl acetate and lead citrate, and examined in a Hitachi 11-D or HS-9 electron microscope. One-µm thick sections were stained with toluidine blue to verify the orientation of the skin.

RESULTS

Burn at 60°C

The intercellular spaces were slightly widened and the intercellular bridges were distended in the basal and spinous layers after a burn for 1 minute. In some areas, breakage of the cell membrane next to the desmosomes occurred and left remnants of structurally normal desmosomes attached to one of the two cells from which they were derived (Fig. 1). The local formation of suprabasal vesicles was caused by disintegration of the basal cells just above the basement membrane, to which the remnants of basal cells remained attached (Fig. 2).
In a 3 minute burn, the epidermis was separated from the dermis at the level of basal or suprabasal cells (Fig. 3, inset). The separation occurred ultrastructurally due to disintegration of the basal cells just above the basement membrane, as observed in a 1 minute burn, or due to breakage of the intercellular bridges adjacent to the desmosomes between the suprabasal cells and the cells of the higher layers (Fig. 3). In the latter case, the cell borders were rough in areas where the desmosomes protruded, or smooth in areas where the desmosomes were pinched off. The separated epidermis revealed a widening of the intercellular spaces, with frequent disruption of the intercellular bridges. In a 60°C burn for both 1 and 3 minutes, the epidermal cells contained intracytoplasmic vacuoles (Figs. 1, 2, 3). The mitochondria were intact at 1 minute but some were vacuolated at 3 minutes (Fig. 3). The cell membrane in some keratinocytes (K2 and K3 in Fig. 3) was indiscernible in a burn for 3 minutes. The basement membrane was intact in a 60°C burn.

**Burn at 95°C**

Even at 3 seconds following a burn, dermal-epidermal separation was evident, as there was complete disorganization of the basal and suprabasal cells (Fig. 4). At the dermal-epidermal junction, the remnants of disrupted cells remained attached to the basement membrane by half-desmosomes, or when separated from the basement membrane, the half-desmosomes were present on the disrupted cells (Figs. 4, 5). The tonofibrils were attached to the desmosomes or aggregated in small masses, yet the individual filaments were quite discernible (Fig. 5). In the separated epidermis, the intercellular spaces were widened, while disruption of the intercellular bridges was rarely observed (Fig. 6). The tonofibrils were aggregated at the cell periphery and...
showed a homogenization in which the individual filaments were indiscernible. The mitochondria, in addition to the endoplasmic reticulum, were vacuolated, and showed a loss of cristae. The nuclear membrane was dilated and disintegrated in some areas.

**COMMENT**

Blister formation occurred at the level of basal and suprabasal layers in both 60° and 95°C burns. In a 60°C burn for 1 and 3 minutes, the blister was caused by disintegration of the basal cells just above the basement membrane. In addition, disruption of the intercellular bridges adjacent to the desmosomes at the suprabasal cells also contributed to the blister formation in a 60°C burn for 3 minutes. It should be stressed that the cells in the basal and suprabasal layers usually showed little or no morphological alterations of the nuclei, tonofibrils and plasma membrane in a 60°C burn for 1 minute. On the other hand, the blister of a 95°C burn was caused by complete breakage of the basal and suprabasal cells, in which karyorrhexis, aggregation of the tonofibrils and breaks in the plasma membrane were observed. It is worth noting that the separation in an early burn at 95°C occurred not only just above the basement membrane but also at

*Fig. 2. Blister in a 1 min 60°C burn. Keratinocytes from blister roof are relatively well preserved. MK, mitotic keratinocyte. ×8 100. Inset: HE-stained section of a 1 min 60°C burn, showing focal blister formation. ×200.*
Fig. 3. Separation of keratinocytes (K) at suprabasal levels in a 3 min 60°C burn. Cell border of K1 is rough, while that of K2 and K3 is smooth. D, disrupted desmosomes. ×7,500. Inset: HE-stained section of a 3 min 60°C burn, showing suprabasal blister. ×200.

Fig. 4. Complete disorganization of basal cells in a 95°C burn. Nuclei (N) are fragmented. Tonofilbrils (T) are aggregated or attached to desmosomes. Plasma membrane is broken. BM, basement membrane. ×7,500. Inset: Toluidine blue stained section of a 95°C burn. ×86.

Fig. 5. Dermal-epidermal junction in a 95°C burn. Half-desmosomes (HD) are attached to the basement membrane (BM) or to part of disrupted basal cells. T, tonofilbrils. ×18,000.
lowing a 45°C heat application for 5 minutes, and irregularly swollen mitochondria with pale matrix spaces and disintegration of endoplasmic reticulum were seen. Therefore, there appears to be a fairly good parallel between heat tolerance of cellular organelles of human skin and cultured cells.

On the other hand, some organelles were relatively heat resistant in early burns. The tonofibrils were mostly intact in a 60°C burn, while they were aggregated and homogenized in a 95°C burn. Structures of most desmosomes were well preserved in a 60°C burn. The basement membrane was also intact as reported by Brizio-Molteni et al. (1) in early deep second- and third-degree burns.

ACKNOWLEDGEMENT
Thanks are due to M. Ohara, Kyoto University, for assistance with the manuscript.

REFERENCES

Received May 26, 1976
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