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The Ultrastructure of the Electric Burn in Man: A Transmission Electron Microscopy-Scanning Electron Microscopy Study

REFERENCE: Torre, C. and Varetto, L., **"The Ultrastructure of the Electric Burn in Man: A Transmission Electron Microscopy-Scanning Electron Microscopy Study,"** *Journal of Forensic Sciences*, JFSCA, Vol. 30, No. 2, April 1985, pp. 448–455.

ABSTRACT: The authors describe the ultrastructural aspects of the electric burn observed with the transmission electron microscope (TEM) and the scanning electron microscope (SEM). With the TEM the most apparent feature is the homogenization and arrangement of the filamentous cytoplasmic material in elongated and parallel bundles together with elongation of the nuclei and junctional structures. SEM studies present a three-dimensional vision of the filamentous material that confirms and clarifies the cellular deformation as a whole. An hypothesis is advanced suggesting that the phenomenon is due to the effects produced by the extremely rapid dehydration of the tissue or that the electric current and Joule effect can act upon the filaments and contractile proteins, causing the cell deformation.

KEYWORDS: pathology and biology, skin, burns (injuries), microscopy, human epidermis, transmission electron microscopy, scanning electron microscopy, ultrastructure

The morphology of cutaneous lesions caused by electricity has been vastly studied by means of the light microscope. But, few data are available regarding the ultrastructural study using the transmission electron microscope (TEM) and the scanning electron microscope (SEM), and almost all refer to research on laboratory animals [*1–3*].

In a preceeding preliminary study [*4*] in which we examined electrical injuries using the TEM, we observed an increasing degree of damage from the periphery to the center of the lesion. The most characteristic finding was thought to be that concerning the cytoskeleton which presented coagulation and organization in parallel bundles of the filaments.

In this current paper, we have continued our study using the TEM and complemented it with the SEM.

Materials and Methods

Fragments of skin from the palm of the hand, palmar surface of the fingers, and from the plantar surface of the foot, bearing electrical injuries, were removed from nine corpses 24 h after death and fixed in 2.5% glutaraldehyde in 0.1*M* phosphate buffer (pH 7.4) for 4 h. After fixing, sections perpendicular to the surface of the skin and 2 mm thick were prepared and immersed for washing in 0.1*M* phosphate buffer at pH 7.4 for 48 h.

The TEM samples were postfixed in osmium, dehydrated, and finally included in Araldite®.

Received for publication 12 July 1984; accepted for publication 20 Sept. 1984.

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The skin sections were obtained with the LKB ultramicrotome, stained with uranyl acetate and lead citrate, after which observation was carried out using a Siemens 102 TEM.

The samples to be examined with the SEM were dehydrated by passage through a series of alcohols, then in amyl acetate, then critical point-dried, gold-coated, and observed with a Cambridge Stereoscan 250.

Undamaged skin samples from areas in the vicinity of the lesions were obtained from the same corpses 24 h after death and subjected to identical treatment.

Results

Control Samples with TEM

All the structural and ultrastructural morphologic characteristics of the epidermal cells are easily appreciated (Fig. 1). The nucleus and its envelope, the cytoplasm and its organelles, and the cell membrane and its junctional devices are evident and do not present important alterations. At the level of the dermo-epidermal junction the basal lamina is always clearly appreciable.

SEM Control Samples

The appearance of the section surfaces is homogeneous. The dermis is made up of bundles of collagen fibers which are variably oriented and entwined. The transition from dermis to epidermis is sharp; no structure that could be identified as the basal lamina was observed. The cells of the stratum germinativum appear as elongated, aligned elements. The cells of the stratum granulosum and spinosum appear round or polygonal. All the elements seem to consist of a dense interlacement of cytoskeletric elements with amorphous material (most likely cytoplasmic matrix) deposited upon it. In between cells, there are thin filamentous "bridges" (likely to be junctional structures). Inside, there is frequently a bowl-shaped structure caused by the lodging of the nucleus. In fact, in the center of these spaces we often find round masses presumably caused by nuclei, and often anchored to the surrounding structures by filamentous connections (Fig. 2).

Electric Burn with the TEM

The gravity of the lesions increases from the periphery towards the center. In Fig. 3 note the absence of recognizable structures with the exception of homogeneous bands that are finely granular and vacuolized, located approximately at the level of the malpighian layer, within which we can observe less electron-dense "bands" of constant thickness and comparable with that of desmosomes, and also intensely electron-dense clumps caused by nuclear material. We are evidently in the presence of homogenized filaments and residues of desmosomes. The latter are mostly elongated and substantially parallel to one another; at times they are brief and bizarre in shape. Analogous cytological damage is found in the stratum germinativum; here again, we have the common finding of elongated, parallel bundles of filamentous origin. We never came across detachment of the dermis from the epidermis, but there is tearing of cells belonging to the stratum germinativum with persistence of elongated and distorted cellular residues adherent to the basal lamina. The last-mentioned structure appears discontinuous and not always clearly distinguishable from the peripheral cytoplasmic portions of the basal epithelial processes.

Shifting the field of observation more towards the periphery (Fig. 4), some cellular elements, although bearing alterations, become more recognizable. In particular, the filaments gain individuality as do the junctional structures which continue to have an elongated and basically parallel appearance.

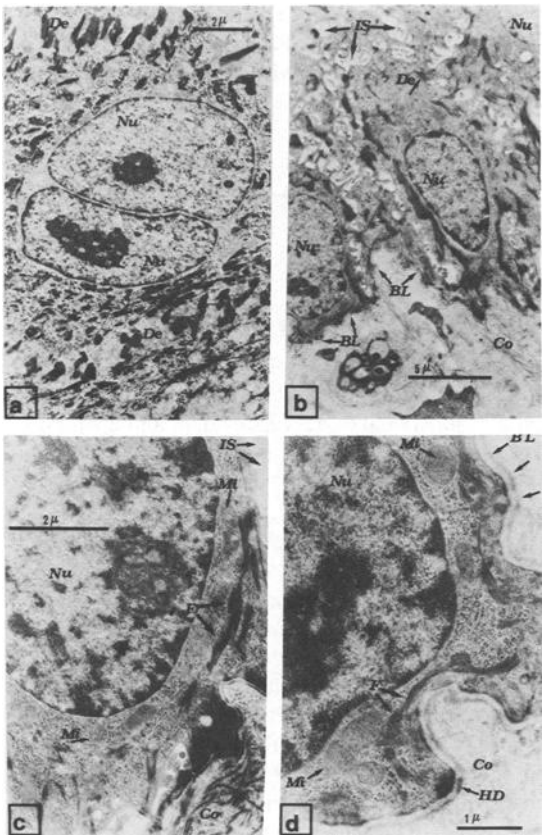

FIG. 1—*Ultrastructural aspects (TEM) of undamaged skin obtained from a corpse 24 h after death, removed from the palm of the hand or sole of the foot contralaterally with respect to the site of the lesion.* (a) *Binucleated cell of the malpighian layer of the epidermis. The nuclei* (Nu) *(or perhaps just a single multilobed nucleus) possess an evident nucleolus and intact nuclear envelope. Short bundles of filaments and mitochondria are within the cytoplasm. Numerous desmosomes are in the periphery.* (b) *Cells of the stratum germinativum of the epidermis. Again, the nuclei* (Nu) *have an intact envelope. The intercellular space* (IS) *is ample and fine cellular processes are recognizable. Desmosomes* (De) *are clearly identifiable and morphologically unaltered. At the dermo-epidermal junction the continuous basal lamina is recognizable* (BL). *In the dermis, collagen fibrils* (Co) *are sectioned either transversally or longitudinally.* (c) *Part of a cell of the stratum germinativum of the epidermis in which the majority of the cytoplasmic organelles are recognizable. The mitochondria* (Mi) *are scarcely affected by the autolytic process and present a double membrane and easily recognizable cristae. At this magnification, the bundles of filaments* (F) *can be separated into their constituent units. There are numerous free ribosomes.* IS = *intercellular space and* Co = *collagen fibrils of the dermis.* (d) *At higher magnification, part of a cell of the stratum germinativum at the dermo-epidermal junction. Note the electron-transparent lamina immediately under the epithelial cell and also one that is electron-dense (basal lamina* = BL). *Hemi-desmosomes* (HD) *are well recognizable.* Nu = *nucleus,* Mi = *mitochondria,* F = *filaments, and* Co = *collagen fibrils.*

The malpighian layer frequently presents round, intracytoplasmic electron-transparent cavities, particularly where cytologic damage is minor. The nuclear envelope is easily recognized and often deformed by the aforementioned cavities. Groups of free ribosomes are recognizable in the cytoplasm and mitochondria are seen. The bundles of filaments and desmosomes have more or less normal appearance. The cells are reciprocally well separated by a continuous cell membrane. The cytoplasmic organelles and cellular boundaries can be clearly observed at the level of the stratum germinativum. The filaments are persistently organized in

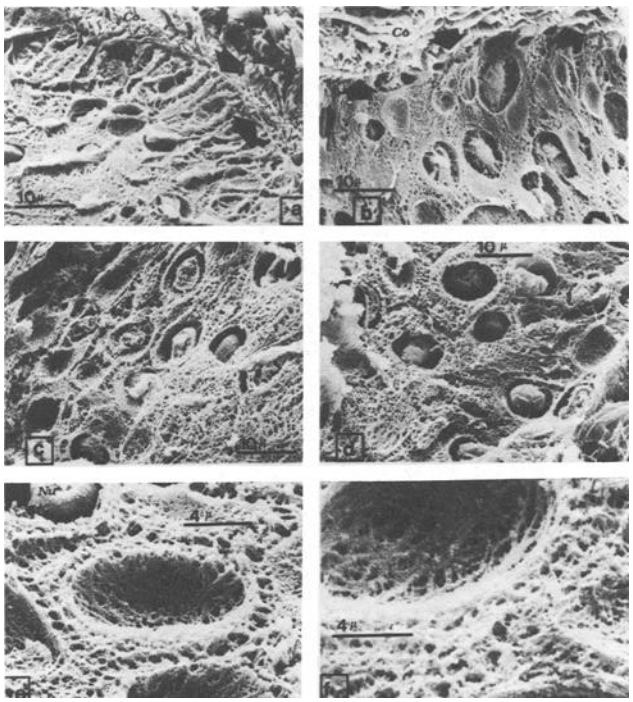

FIG. 2—*Ultrastructural aspects (SEM) of undamaged skin obtained from a corpse 24 h after death, removed from the palm of the hand or sole of the foot contralaterally with respect to the sites of lesion. The technique involves removal of part of the cytoplasm which makes the cytoskeleton stand out.* (a, b) *Basal part of the epidermis in contact with the dermis (the arrows indicate the dermo-epidermal junction). The epithelial tissue appears as a dense net in which it is difficult to distinguish the cell boundaries. Often, one can see nuclei separated from the peripheral part of the cytoplasm by an empty space, but joined to the former by means of thin filaments. At times the nucleus is lost during sectioning leaving a bowl-shaped cavity that contained it.* (c, d) *At the level of the malpighian layer the cytoskeletal net is more evident and the cell boundaries are easier to distinguish, as can be seen with ease at higher resolution* (e, f), *where the "bowl-shaped" cavity that contained the nucleus is surrounded by cytoplasm with a grossly filamentous net-like structure arranged peripherally in rays. These structures appear to continue with identical structures in adjacent cells which can be identified because of the presence of "bowls" with or without nuclei.*

elongated bundles. The relationship between dermis and epidermis is correct; the basal lamina is continuous.

Electric burn in SEM

The most characteristic feature of the lesion is represented by modification of the appearance and orientation of the filaments with respect to the control. Instead of being organized to form an interlacement, these filaments are arranged in rather gross conglutinated bundles, made up mostly from parallel subunits. Recognition of nuclear residues is exceptional but on the surface one may observe circular or elongated depressions, most likely negative impressions of the nuclei (Fig. 5).

Discussion

It seems appropriate to discuss briefly the quality of the control skin samples. We are dealing with skin obtained from corpses 24 h after death. At all levels, note (Figs. 1 and 2) the ex-

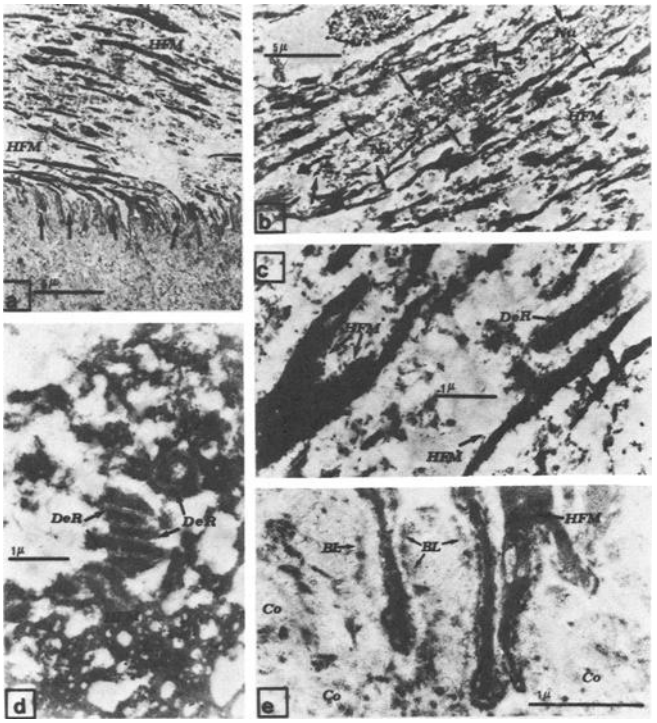

FIG. 3—*Ultrastructural appearance (TEM) of human skin removed 24 h after death from the palm of the hand and sole of the foot, seat of the lesions (subjects died from electrocution). Central part of the lesion.* (a) *At low magnification, there is complete change of the structure of the epidermis in which it is possible to identify electron-dense material consisting of homogenized filaments* (HFM = *homogenized filamentous material). This material is arranged in lengthy clumps that are parallel to one another. Remnants of cells belonging to the stratum germinativum remain anchored to the dermis. The arrows point to the dermo-epidermal junction.* (b) *At a higher resolution, one can see that the homogenized filamentous material, while maintaining its arrangement in elongated and parallel bands, surrounds nuclear residues* (Nu) *which can be recognized by the accumulation of highly electron-dense material. The arrows indicate a very elongated nucleus whose major axis is parallel to the bands of homogenized filamentous material.* (c, d) *With further magnification it is possible to recognize, within the context of the homogenized filamentous material, less electron-dense bands of regular thickness (30 to 40 nm) unmistakably derived from residues of desmosomes* (DeR). *These are generally elongated (in c), at times short and bizarre in shape (in d).* (e) *Detail of the dermo-epidermal junction. The remnants of the cells of the stratum germinativum (consisting essentially of homogenized filamentous material) are gathered as usual in the invaginations of the dermis. The basal lamina* (BL) *is damaged, irregularly electron-dense, and often interrupted. The collagen fibrils of the dermis* (Co) *are still recognizable.*

cellent preservation of the ultrastructural characteristics of the cell membrane, of most of the cytoplasmic organelles, and of the nucleus and its envelope. These findings allow us, on the one hand, to establish the validity of the ultrastructural study of the skin in the corpse in general, and on the other hand, permit us to consider as surely pathological and not thanatological the alterations found in the lesion.

Our observations confirm much of what other authors [*2,3*] have described in laboratory animals and what one of us preliminarly observed in man [*4*]. In particular, our observations in TEM were conglutination and homogenization of the filaments whereas the cytoplasmic organelles were unrecognizable in the zones nearest the center of the lesion. The nuclear rem-

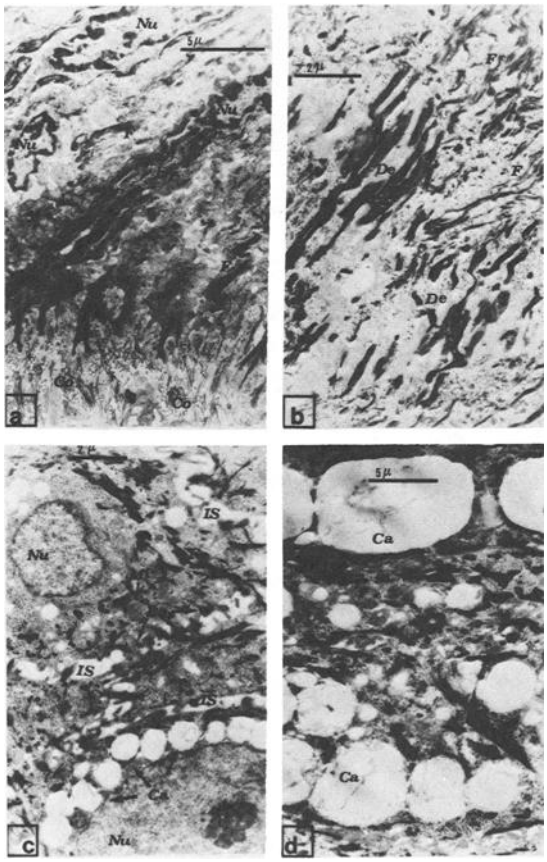

FIG. 4—*Ultrastructural appearance (TEM) of human skin removed 24 h after death from the palm of the hand and sole of the foot, location of the electric burn (subjects died from electrocution). Peripheral portion of the lesion.* (a) *Once again, the nuclei are recognizable* (Nu) *as are the cytoplasmic organelles and the cell boundaries. The filaments are collected in large, long bundles that are parallel to one another. The structure of the dermo-epidermal junction and basal lamina is similar to that found in the controls.* Co = *collagen fibrils of the dermis.* (b) *A typical feature of this portion of the burn is the long desmosomes* (De) *parallel to one another with adjacent filaments* (F) *bearing the same direction.* (c, d) *In numerous cells there are cavitations* (Ca) *that are electron-transparent and not surrounded by a membrane. They are located near the nucleus and often deform its profile without interrupting the nuclear envelope. Bundles of filaments may insert themselves between these structures.* Mi = *mitochondria.* De = *desmosomes.* F = *filaments, and* IS = *intercellular space.*

nants were elongated and arranged in parallel with the filamentous material and the residues of the desmosomes present, even if altered.

As regards the relationship between dermis and epidermis, our results are not in agreement with those of Somogyi et al [*2*]. We have never observed true detachment of the dermis from the epidermis, but there is tearing of the fine processes of the cells of the stratum germinativum; the optically empty space observed in light microscopy is, in our opinion, within the context of the stratum germinativum.

Towards the periphery, the picture fades out with the gradual appearance of the cytoplasmic organelles. In these areas bordered with undamaged skin there are other characteristic alterations which can be observed in transmission, particularly in the stratum germinativum

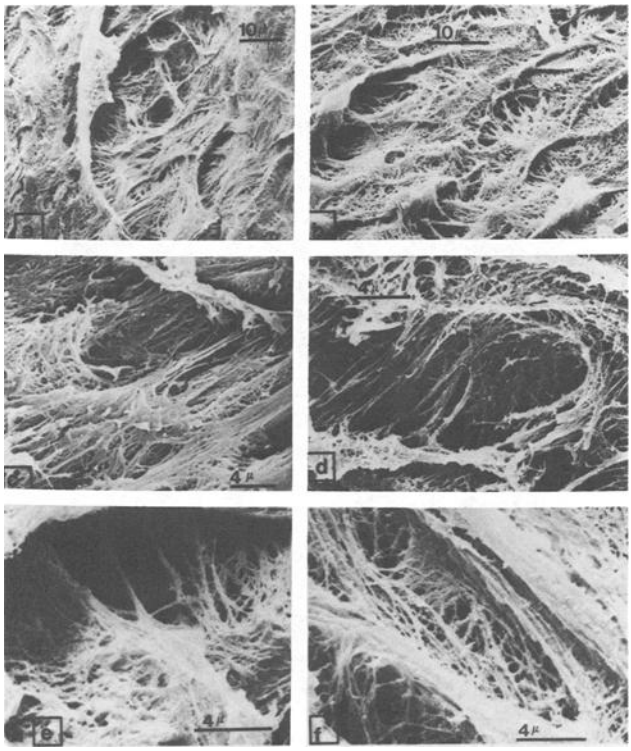

FIG. 5—*Ultrastructural aspects (SEM) of human skin obtained from the corpse 24 h after death from the palm of the hand and sole of the foot, seat of the electric burn (subjects died from electrocution).* (a, b) *The general appearance is profoundly changed because the cytoskeletal material loses its three-dimensional net-like architecture. On the contrary, one may observe long filamentous bundles that are often parallel to one another or are arranged in a vortex. Finding nuclei is exceptional: the cavities apt to contain them are deformed.* (c, d, e, f) *At higher resolution, note how the filamentous material is often homogenized into long thick bundles conferring a "tempest at sea" aspect to the field of observation. Along with these, there are thin filaments that conserve their individuality* (e, f).

and spinosum. Whereas filaments and junctional structures return to normality, electron-transparent cavities appear in the cytoplasm, often in close contact with the nucleus to the point of deforming it at times.

SEM observation of normal skin allows optimal identification of the cytoskeletal structures and nucleus, above all. The technique is therefore most suitable for examining material in which, as we have seen, the most important alterations regard the filaments. In fact, if we compare the undamaged epithelium with the epithelium found in the lesion, we note a net change of the cytoskeleton as a whole. The cytoskeletal material loses its three-dimensional net-like architecture; on the contrary the bundles of filaments appear parallel, elongated, flexuous, or vortical.

Conclusion

On the whole, the results obtained with the SEM and TEM confirm each other reciprocally and are demonstrative of the complementarity of the two techniques, the first being suitable for certain recognition of the finer structures and their damage, the second being more suit-

able for gathering the image as a whole, which permits a more general vision of the cytoskeletal rearrangements.

As regards attempts at a pathogenetic hypothesis, the heat produced by the Joule effect through passage of current undoubtedly explains the destruction of the membranous systems in the areas proximal to the center of the lesions and the cytoplasmic cavities in the peripheral zones. It seems possible that the latter may be due to a "cooking" phenomenon with liberation of vapor and coagulation of the cytoplasmic matrix.

It is more difficult to interpret the alterations of the cytoskeleton; the entire state may be due to conglutination of the elements with apposition of coagulated proteinaceous material. However, the arrangement of the filaments in parallel lines suggests some other mechanism of production: perhaps it is a purely mechanical phenomenon and consequence of the elongation of the cell as a whole. The most likely cause of their deformation seems to us to be found in a mechanical traction of all the structures surrounding the point of contact of the conductor, as a result of the retraction of the tissue caused by the probably considerable and certainly rapid evaporation of water. Nonetheless, there is also suggestion with the hypothesis that direct action of the electric current and heat may be important through their effect upon the filaments and contractile proteins of the cytoskeleton which causes modification of the general architecture with consequential deformation of the cell in its entirety.

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