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A histological study on the mechanism of epidermal nuclear elongation in electrical and burn injuries

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Abstract Epidermal nuclear elongation is one of the most important signs for the diagnosis of electrical injury. In this study, we investigated the mechanism responsible for this phenomenon by comparing the findings from burn injuries and those from contusions. Electrical and burn injuries were made in the dorsal skin of rats using energy ranging from 100 to 790 joules for electrical injury, and 170–690 joules for burn injury. Contusions were also made by compressing the skin with a vice. In electrical and burn injuries, the dermis under the epidermal elongated nuclei was homogeneous and without empty spaces between collagen bundles and the number of dermal fibroblasts per 0.01 mm^2 below the damaged epidermis decreased significantly ($P < 0.05$). The incidence of this change correlated with the depth of denatured dermal collagen fibres and in both types of injuries, dermal cells had no nuclear antigenicity for ubiquitin. The width of the injured epidermis with nuclear elongation decreased significantly ($P < 0.05$) and the elongated nuclei were parallel to the basal membrane. In electrical injury however, nuclear elongation occurred more frequently near the external root sheath. Nuclear elongation of fibroblasts and external root sheath cells was also found, but those of sebaceous gland cells were not detected. Epidermal elongated nuclei were also found in contusions. The evidence strongly suggests that epidermal nuclear elongation in electrical and burn injuries is due to dermal expansion by heat.

Keywords Epidermal changes · Heat injury · Electrical injury · Morphology · Nuclear elongation

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Introduction

Several morphological changes of the skin were thought to be useful for the diagnosis of electrical injury (Winer and Levin 1958; Rozsa et al. 1967; Hunt et al. 1976; Danielsen et al. 1978; Thomsen et al. 1981 a, 1981 b, 1982, 1983 a, 1983 b; Nielsen et al. 1981; Danielsen et al. 1991; Weedon 1992; Xiaohu et al. 1995). Histologically, vacuolation and metallisation in the epidermis are known as those changes with diagnostic value. Recently, DNA typing of material on current conductors has been reported to be of forensic significance, which could supplement conventional scene reconstruction in cases of electrical injury (Ortmann et al. 1998). On the other hand, evaluation of nuclear elongation of the epidermis has been conflicting (Knight 1996; Janssen 1984). Formerly, it was demonstrated that nuclei were deformed by an ion flow in electrical injuries (Heinlein 1962). Recent studies, however, suggested that this elongation was also seen in fire victims and therefore, must be regarded as an expression of thermal injury (Knight 1996; Janssen 1984). An explanation for the detailed mechanism related to this phenomena has not been provided either way. In this study therefore, we examined histological changes in these types of injuries to investigate the mechanism of the nuclear elongation.

Materials and methods

A total of 15 male Wistar rats weighing between 290 and 320 g were used. After being anaesthetised with pentobarbital, the hairs of the dorsal skin were shaved and the skin was wiped with cotton wool soaked in 70% ethanol.

Electrical and burn injuries

Electrical transfer

The electrical energy transfer device consisted of a pair of circular brass electrodes mounted in an acrylic plate. The diameter of each electrode was 8 mm and the distance between the centre of each electrode was 30 mm. The two electrodes were kept in contact with

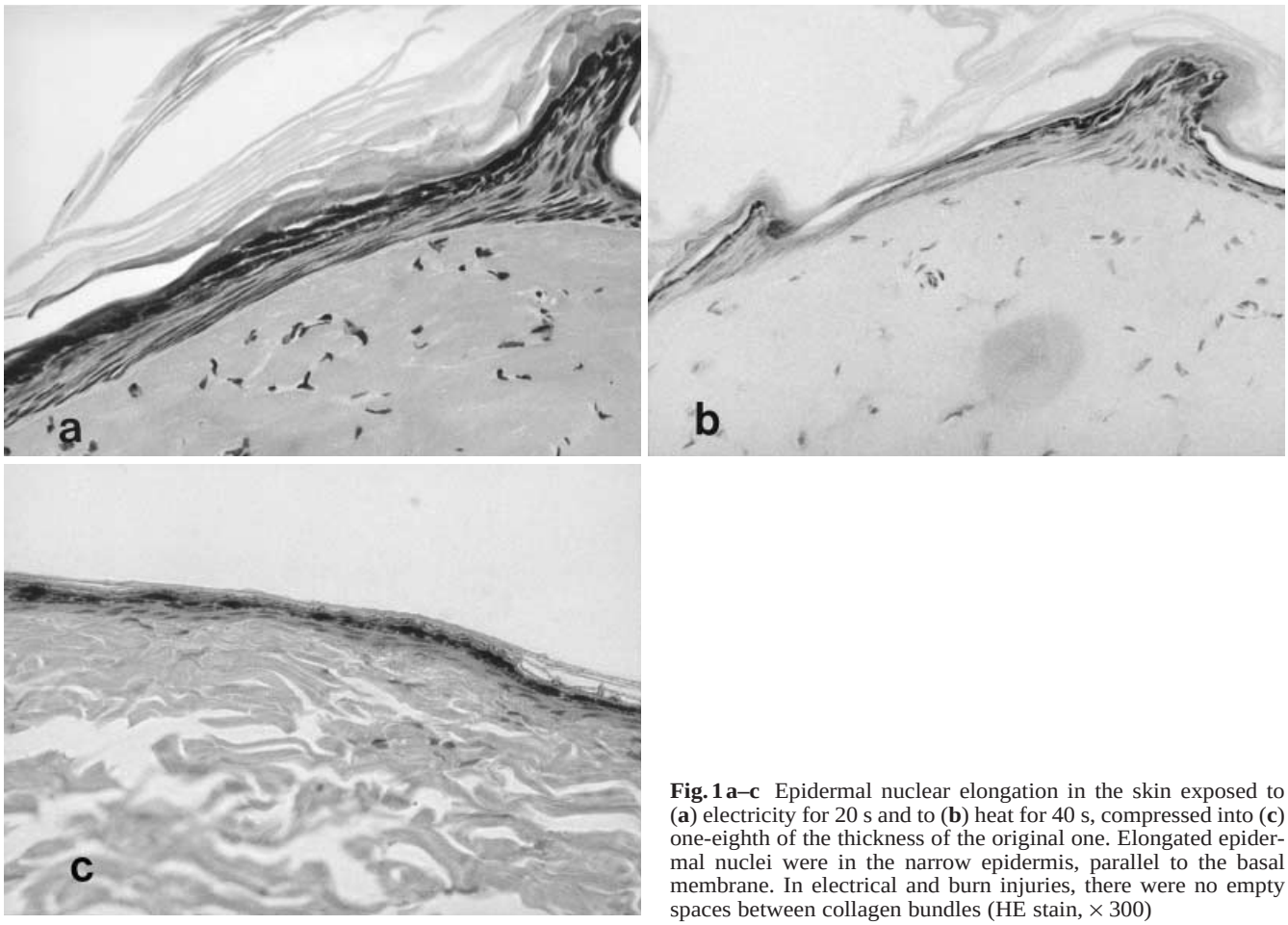

Fig. 1 a–c Epidermal nuclear elongation in the skin exposed to (**a**) electricity for 20 s and to (**b**) heat for 40 s, compressed into (**c**) one-eighth of the thickness of the original one. Elongated epidermal nuclei were in the narrow epidermis, parallel to the basal membrane. In electrical and burn injuries, there were no empty spaces between collagen bundles (HE stain, × 300)

the dorsal skin of the rats by firm manual pressure. Electrical energy (100 V, 50 Hz A/C) was from the mains supply. To calculate the energy generated in the skin, the amount of current was monitored by a galvanometer (Yokogawa Electric Works, type 2014, class 0.5, Tokyo, Japan). The current was applied to each rat (n = 5) at 3 areas for 5, 10 and 20 s, whereby the transferred energy ranged from 100 to 200 joules, 320–400 joules and 400–790 joules, respectively.

Heat transfer

This experiment was carried out using an electrically regulated heater. The resistant wire (Nichrome wire), 0.35 mm in diameter, was heated by applying an electric current (3.5 V, 50 Hz A/C). Heat was applied to the dorsal skin with the wire mounted on an acrylic plate kept in contact with the skin by firm manual pressure. Each rat (n = 5) was exposed to heat for 10, 20 and 40 s, whereby the transferred energy was 170 joules, 340–350 joules, and 670–690 joules, respectively. The rats were killed by intraperitoneal injection of pentobarbital immediately after the experiment.

Histology

In both experiments, 35 specimens of the skin (15 × 5 mm area), 5 of each exposure time and 5 as controls, were taken and fixed in 4% buffered formalin, embedded in paraffin and sectioned at a thickness of 2.5 μm. The sections were separately stained with haematoxylin-eosin and elastica-Masson. Immunostaining for ubiquitin was performed using polyclonal rabbit antibody against rat ubiquitin (DAKO, Denmark) as the primary antibody. The antibody was placed in a humid chamber for 2 h. Thereafter, the streptavidin-biotinylated peroxidase complex method was conventionally employed (Histofine SAB-PO, Nichirei, Japan). Colour development was performed with 3,3'-diamino-benzidine and slides were counterstained with haematoxylin.

Table 1 The number of specimens with epidermal nuclear elongation and dermal changes with epidermal nuclear elongation

Exposure time (total specimens)	Specimens with nuclear elongation of epidermis	Dermal changes with epidermal nuclear elongation	
		Homogeneous dermis without empty space	Number of fibroblasts per 0.01 mm^2
Control (5)	0	0	8.0 ± 2.04
Electrical injury			
5 s (5)	2	2	3.4 ± 1.10*
10 s (5)	5	5	4.5 ± 2.18*
20 s (5)	5	5	4.0 ± 1.36*
Burn injury			
10 s (5)	4	4	4.0 ± 1.35*
20 s (5)	5	4	3.9 ± 1.50*
40 s (5)	5	5	4.5 ± 2.01*

* $P < 0.05$

Table 2 Variation in the depth of the epidermis depending on the type and duration of injury

Exposure time	Depth of epidermis	
	Area with elongated nuclei (μm)	Area without elongated nuclei (μm)
Control	–	28 ± 5.98
Electrical injury		
5 s	9.6 ± 2.47**†	24 ± 8.32*
10 s	16 ± 6.48**	25 ± 4.49*
20 s	19 ± 6.12**	31 ± 8.63
Burn injury		
10 s	12 ± 4.49**††	27 ± 8.91
20 s	12 ± 5.95**	31 ± 7.07
40 s	12 ± 2.26**	32 ± 7.48

* $P < 0.05$
** $P < 0.01$ comparing to the control
The number of samples examined were 5 of each, except for †: 2 and ††: 4

Morphometrical and statistical analyses

In the dermis, the number of fibroblasts under the injured epidermis was counted in 10 randomly selected microscope fields of 0.01 mm^2. The thickness of the epidermis was monitored in 10 randomly selected high power fields from each section. The individual data in each exposure time were analysed first using the one way ANOVA and the Kruskal-Wallis tests. The data between control and each exposure time were analysed using the Dunnett-test. In addition, the data for dermal fibroblasts under epidermal nuclear elongation and epidermal depth were analysed using the Kruskal-Wallis test. P values of 0.05 or less were considered as statistically significant. The ratio of the number of elongated nuclei to total number of nuclei was monitored in 10 randomly selected high power fields on each section. The correlation between the ratio of elongation and the depth of the denatured collagen fibres was also analysed.

Contusions

To evaluate the morphological effect of the pressure applied to the skin on the epidermal cells, two skin flaps were prepared for compression on the back of the rats ($n = 5$). One flap was compressed into a quarter of the thickness of the original one, the other one-eighth of the thickness, for 1 min with a table vice (Matsuo Seisakusho, type 53, Omiya, Japan). The 10 skin specimens (15 × 5 mm area) were fixed and sectioned as described above. The ratio of the number of elongated nuclei to total number of nuclei and the epidermal depth was monitored in 10 randomly selected high power fields on each section. The correlation between the ratio of elongation and epidermal depth was analysed. Immunostaining for ubiquitin was also performed.

The authors carried out the research described in this report in accordance with the Guide for Animal Experimentation, Iwate Medical University.

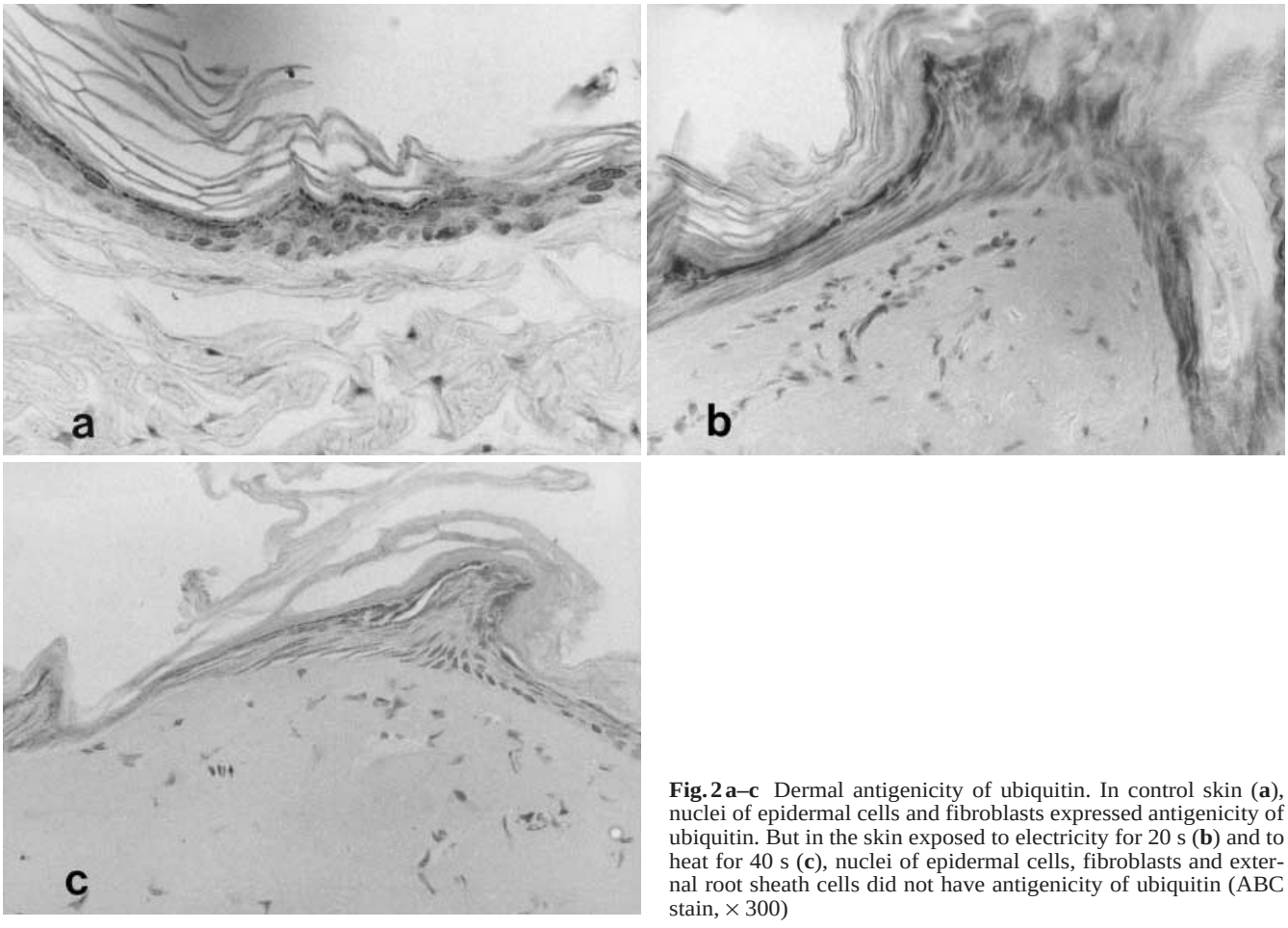

Fig. 2 a–c Dermal antigenicity of ubiquitin. In control skin (**a**), nuclei of epidermal cells and fibroblasts expressed antigenicity of ubiquitin. But in the skin exposed to electricity for 20 s (**b**) and to heat for 40 s (**c**), nuclei of epidermal cells, fibroblasts and external root sheath cells did not have antigenicity of ubiquitin (ABC stain, × 300)

Results

In electrical injury, epidermal nuclear elongation accompanied homogeneous dermis without empty spaces between collagen bundles which were found in normal dermis with routine staining methods (Fig. 1 a, Table 1). Elongated nuclei were parallel to the basal membrane and accompanying thinning of the epidermis (Table 2). The number of dermal fibroblasts decreased significantly in these specimens. In three sections exposed for 5 s, however, neither elongated nuclei nor severe injuries to the dermis were found and the fibroblasts showed no statistically significant decrease in number (7.7 ± 2.26, P = 0.54, Table 1). In the other two sections, elongated nuclei were noted only in the area with significantly thinner epidermis. In burn injuries, epidermal nuclear elongation and the decrease in the number of dermal fibroblasts was found in almost all cases (Fig. 1 b). In both types of injury, epidermal cells, fibroblasts, external root sheath cells and sebaceous gland cells had no nuclear antigenicity for ubiquitin (Fig. 2 a–c). Injured dermis showed reddish discolouration with elastica-Masson stain. The incidence of epidermal nuclear elongation correlated with the depth of denatured dermal collagen fibres (Fig. 3). Unlike burn injuries, the epidermal nuclear elongation tended to accumulate near the external root sheath in electrical injuries (Table 3). Nuclei of fibroblasts and external root sheath cells also lengthened in both types of injury, whereas sebaceous gland cells remained intact (Table 4).

Table 3 The number of specimens with epidermal elongated nuclei classified by their location

Exposure time (The number of total specimens)	Near the external root sheath	Distant from the external root sheath
Electrical injury		
5 s (2)	2	2
10 s (5)	5	2
20 s (5)	5	3
Burn injury		
10 s (4)	4	4
20 s (5)	5	5
40 s (5)	2	5

Epidermal elongated nuclei were also found in contusions (Fig. 1 c), showing a negative correlation between the incidence and the epidermal depth (Fig. 4). In dermal cells, the antigenicity for ubiquitin was preserved.

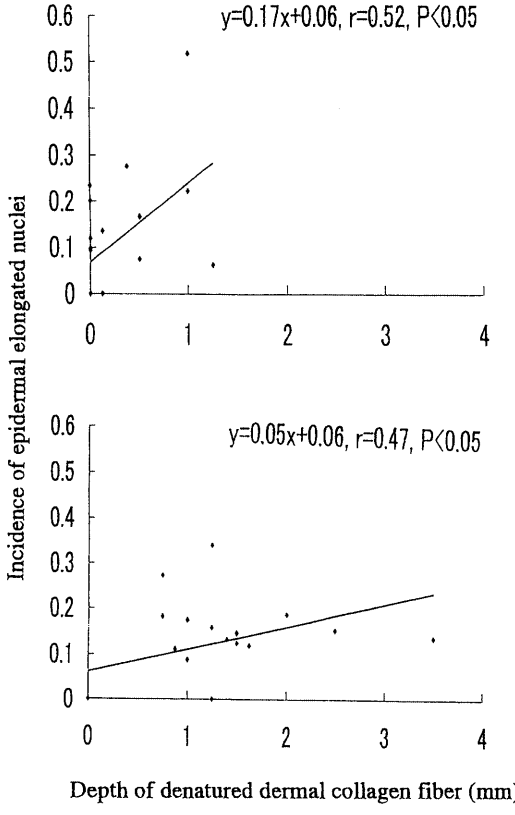

Fig. 3 Relationship between the depth of dermal denatured collagen fibers and the incidence of epidermal nuclear elongation in an electrical injury (*top*) and in a burn injury (*bottom*). In both injuries, a good correlation was found between these two factors

Discussion

Several findings are used for the diagnosis of electrical injury and among them, epidermal nuclear elongation was once claimed to be an electromagnetic effect. However, it has been reported that the same appearance could be observed in burn injuries and blunt force injuries (Knight 1996; Janssen 1984). Electricity generates Joule's heat, thus, this phenomenon in electrical injuries was thought to be caused by the effect of heat.

In the present study, lesions observed in association with epidermal nuclear elongation were essentially identical in both electrical and burn injuries. The epidermal nuclear elongation accompanied homogeneous dermis without empty spaces between collagen bundles and was related to the decrease in the number of underlying dermal fibroblasts which indicated swelling of the dermis. The injured dermis was stained reddish in elastica-Masson stain presumably because dermal collagen fibres affected by heat were swollen and intermixed with electron dense materials which had a high affinity for acid fuchsin (Cuppage and Leape 1973; Karlsmark et al. 1986; 1988 a, 1988 b). The loss of nuclear antigenicity for ubiquitin in epidermal and dermal cells indicated that the proteins were denatured by heat. It was demonstrated that heating could change the immunoreaction of epitopes (Lombardero et al 1990). The incidence of the epidermal nuclear elongation correlated with the depth of the reddish dermal collagen fibres, i.e. the degree of dermal injury. As discussed in previous papers (Knight 1996; Janssen 1984), the elongated nuclei were parallel to the basal membrane and coincided with a decrease in depth of the epidermis. These findings suggest that the epidermis was pressed upward by the dermis

Table 4 The number of specimens with nuclear elongation of dermal cells with and without epidermal elongated nuclei (*EEN*)

Exposure time	Fibroblast		External root sheath cell		Sebaceous gland cell	
	with EEN	without EEN	with EEN	without EEN	with EEN	without EEN
Electrical injury						
5 sec2	1†	2	1†	2	1†	1
10 sec	4	3	3	3	1	1
20 sec	5	5	3	3	1	1
Burn injury						
10 sec	4††	5	4††	1	0††	1
20 sec	5	5	5	0	1	1
40 sec	5	5	5	3	1	1

The number of samples examined were 5 of each except for † 2 and †† 4

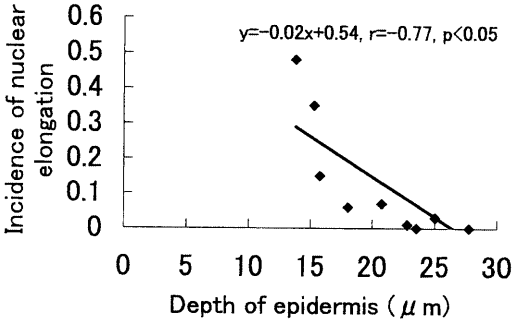

Fig. 4 Relationship between the epidermal depth and the incidence of epidermal nuclear elongation in contusion. There existed a definitive negative correlation between these two factors

swollen by heat and following horizontal stretching produced epidermal nuclear elongation.

Except for the specimens injured electrically for 5 s, no significant correlation was found between the period affected and the number of dermal fibroblasts, or the epidermal depth. On the other hand, the incidence of epidermal nuclear elongation negatively correlated with the epidermal depth of the skin contused with mechanical pressure. Available evidence suggests that dermal pressure would cause epidermal nuclear elongation by making the epidermis narrower. As the degree of the dermal swelling seemed to reach a peak after heat or electrical treatment for 10 s, no significantly different findings would have been observed on the epidermis.

Nuclear elongation was not specific to epidermal cells. Nuclei of fibroblasts and external root sheath cells were also elongated even where the overlying epidermal nuclei remained apparently intact. These nuclei could be elongated more easily than those of epidermal cells, because the dermis pressed the nuclei from both sides. In contrast, the nuclei of sebaceous gland cells hardly lengthened. As these cells which are rich in cytoplasm are larger than epidermal cells and fibroblasts, it was considered that these cells could maintain their shape despite the dermal pressure.

In both types of injury, the epidermal nuclear elongation occurred regardless of the distance from the external root sheath. In electrical injury, however, it took place more frequently near the external root sheath. Thomsen et al. (1981 a) also reported that its low electrical resistance affected dermal changes in electrical injury. It was considered that the abundance of current which passed through the external root sheath would facilitate heat generation in this area.

It was suggested that the dermis expanded by heat compressed the epidermis and horizontal stretching power eventually elongated the nuclei in electrical and burn injuries. Space-occupying lesions, such as dermal vacuolation generated by heat, could cause additional compression and produce elongated nuclei. In other words, epidermal nuclear elongation expresses the condition in which considerable thermal energy was transferred to the skin. Therefore, certain periods of electrification should be required to produce this finding.

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References

Cuppage FE, Leape LL (1973) Morphologic changes in Rhesus monkey skin after acute burn. Arch Pathol 95:402–406

Danielsen L, Thomsen HK, Nielsen O, Aalund O, Nielsen KG, Karlsmark T, Genefke IK (1978) Electrical and thermal injuries in pig skin – evaluated and compared by light microscopy. Forensic Sci Int 12:211–225

Danielsen L, Karlsmark T, Thomsen HK, Thomsen JK, Balding LE (1991) Diagnosis of electrical injury. Am J Forensic Med Pathol 12:222–226

Heinlein G (1962) Modellversuche zur Deutung der morphologischen Veranderungen im Bereich der Strommarke. Elektromedizin 7:174–176

Hunt JL, Mason AD Jr, Masterson TS, Pruitt BA, Colonel MC (1976) The pathophysiology of acute electric injuries. J Trauma 16:335–340

Janssen W (1984) Injuries caused by electricity. Forensic histopathology. Springer, Berlin Heidelberg, pp 261–274

Karlsmark T, Thomsen HK, Danielsen L, Aalund O, Nielsen O, Nielsen KG, Eng M, Genefke IK (1986) Immediate dermal changes in pig skin after exposure to moderate amounts of heat and electrical energy. J Invest Dermatol 87:528–532

Karlsmark T, Thomsen HK, Danielsen L, Aalund O, Nielsen O, Nielsen KG, Genefke IK (1988 a) The morphogenesis of electrically and heat- induced dermal changes in pig skin. Forensic Sci Int 39:175–188

Karlsmark T, Danielsen L, Thomsen HK, Johnson E, Aalund O, Nielsen KG, Nielsen O, Genefke IK (1988 b) Ultrastructual changes in dermal pig skin after exposure to heat and electric energy and acid and basic solutions. Forensic Sci Int 39:235–243

Knight B (1996) Electrical fatalities. Forensic pathology, 2nd edn. Arnold, London, pp 319–331

Lombardero M, Heymann PW, Platt-Mill TA, Fox JW, Chapman MD (1990) Conformational stability of B cell epitopes on Group I and Group II *Dermatophagoides spp*. Allegens effect of thermal and chemical denaturation on the binding of murine IgG and human IgE antibodies. J Immunol 144:1353–1360

Nielsen KG, Nielsen O, Thomsen HK (1981) Device and methods for the measurement of energy transfer in experiments involving thermal and electrical injuries of pig skin. Forensic Sci Int 17:203–209

Ortmann C, Rolf B, Fechner G (1998) DNA typing of cellular material on current conductors. Int J Legal Med 111:177–179

Rozsa Gy, Somogyi E, Nevelos A, Varga T (1967) Nuclear changes in response to electric shock. Acta Med Legal Soc (Liege) 20: 199–202

Thomsen HK, Danielsen L, Nielsen O, Aalund O, Nielsen KG, Karlsmark T, Genefke IK (1981a) Early epidermal changes in heat- and electrically injured pig skin. I. A light microscopic study. Forensic Sci Int 17:133–143

Thomsen HK, Danielsen L, Nielsen O, Aalund O, Nielsen KG, Karlsmark T, Genefke IK (1981b) Early epidermal changes in heat- and electrically injured pig skin. I. A electron microscopic study. Forensic Sci Int 17:145–152

Thomsen HK, Danielsen L, Nielsen O, Aalund O, Nielsen KG, Karlsmark T, Genefke IK (1982) Epidermal changes in heat and electrically injured pig skin. A light microscopic study of sequencies in morphology. APMIS 90:327–332

Thomsen HK, Danielsen L, Nielsen O, Aalund O, Nielsen KG, Karlsmark T, Genefke K, Christoffersen P (1983a) Epidermal changes in heat and electrically injured pig skin. A light microscopic study of the influence of heat energy intensity and electrical current frequency. APMIS 91:297–306

Thomsen HK, Danielsen L, Nielsen O, Aalund O, NielsenKG, Karlsmark T, Genefke IK, Christoffersen P (1983b) Effect of direct current, sodium hydroxide and hydrochloric acid on pig epidermis. APMIS 91:307–316

Weedon D (ed) (1992) Reactions to physical agents. In: Systemic pathology: the skin. Churchill Livingstone, Edinburgh, pp 571–589

Winer LH, Levin GH (1958) Changes in the skin as a result of electric current. AMA. (American Medical Association) Arch-Dermatol 78:386–390

Xiaohu X, Hongquan X, Jiazhen Z, Ping D (1995) A preliminary study of skin electrical injury with computerized image analysis. Forensic Sci Int 73:197–202