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The histological and ultrastructural picture of the paraepidermoidal epithelium subjected to low temperatures. II

Obraz histologiczny i ultrastrukturalny nabłonka paraepidermoidalnego poddanego działaniu niskich temperatur. II

Long-term world research on the mechanism of the action of low temperatures on the living organisms' tissues has not brought any explicit explanations of this complicated process so far. Attempts have been made to explain it during the experimental investigations on tissue models and also in the clinical investigations (1, 3-5, 6-9). The experience of the Clinic of Dental and Maxillo-Facial Surgery of Medical University of Lublin reveals that after freezing of the lesions within oral mucosa or skin with closed applicator with the temperature of –196 C for the time of 1, 2, and 5 seconds, within 24 hours, in the frozen site a necrosis source with simultaneous transudate of tissue fluid appears and a blister is formed.

The blister cracks after several days and erosion occurs which covers with crust. The healing goes on without any complications and after about 14 days in the site of the operation there appears a thin, smooth elastic and, whitish in colour scar which is esthetically and functionally satisfactory.

In the present paper histological and ultrastructural changes were evaluated in individual epithelium layers in the frozen region adjacent to the cryoprobe.

Material and methods were presented in the previous paper.

RESULTS

Group III included epithelium segments of the clinically unchanged oral vestibule mucosa, frozen with the temperature of –196 C in the time of 2 seconds, using the applicator 10mm in diameter collected immediately after the cryoapplication from the same 21 patients.

In the light microscope, under the immersion, with magnification of about 1,000x, the widening of intracellular spaces in the whole epithelium cross-section was observed (Fig. 1). It is much bigger and more regular than in the case of freezing with the same probe in the time of 1 second. The epithelium cells preserve their characteristic shape. The nuclei do not reveal so big changes of shape as in the freezing time of 1 second. The formation of halo around the nuclei is observed.

On the basis of the observations in the electron microscope it seems that in the cryoapplication time of 2 seconds the largest amount of water crystallizes in the intracellular spaces, which leads to the disruption of intracellular desmosomal junctions (Fig. 2, 3). The basement membrane preserves its continuity (Fig. 4), though the widening of intracellular spaces is observed between the basement membrane and the cells of the epithelium basal layer. Occasionally, the damage of hemidesmosomes was seen. After the 2-second cryoapplication, the ultrastructural changes in the keratinocytes in group II appeared and also more distinct thickening of cytoplasm in the cells, thicker filament bundles with blurred structure, fewer glycogen granules, endoplasmatic rete both smooth and rough. Even in the keratinocytes in which the presence of perinuclear clearing was not revealed, the irregular shape of the nuclei was stated, the predominance of heterochromatin over euchromatin, marginal chromatin condensation and vague, condensed nucleolus with blurred structure.

DISCUSSION

As our own research demonstrated, after freezing with the temperature of –196 C within the time of 1 second, neither disruption of keratinocyte cell membrane nor desmosomes injury was stated. However, after the 2-second freezing, desmosomes injury was observed. Variable grade of changes in the cell itself conditioned by cryoapplication time was also noticed. As it is anticipated, changes in the keratinocytes can be twofold in their nature. The first type of changes clearly observed after 1 second of freezing and occurring in the epithelium after 2 seconds of freezing, is characterized by the formation of various size vacuoles in the cytoplasm, usually in the area surrounding the nucleus.

As our own ultrastructural research shows, these vacuoles occur in the hyaloplasm, they are not limited by the membrane, they do not reveal connection with other cell organelle. The second type of changes was characterized by the marked shrinkage of the nucleus, the nuclei hyperchromia, thickening of the cytoplasm and change in shape of cells.

Sometimes, presence of intraplasmatic or intranuclear vacuoles was observed in the keratinocytes with thickened cytoplasm and hyperchromatic nucleus.

After freezing for 2 seconds, the injury of the perinuclear sheath was not stated. After the cryoapplication for 1 and 2 seconds the ultrastructural pictures of paraepidermoidal epithelium under the cryoprobe reveal the crucial injury of intracellular spaces.

The observations made by Ernst and Knoch (2) are also the confirmation of the above observations. The quoted researchers prove that at gradual cooling to the temperature of –20 C ice crystals are formed, usually extracellularly. Although these crystals deform intracellular spaces still, morphologically and physiologically, any irreversible injury of the majority of cells is not observed after defreezing, though occasional injuries of cell membrane can be observed. At gradual cooling to the temperature of –70 C the injury of cell cytoplasm may not take place, either. This phenomenon is used for lyophilization, which serves for the conservation of blood cells, sperm and tissues. Only when fast freezing with the decrease of temperature below –100 C, small ice crystals are formed outside and inside the cell. Simultaneously, the cell structure is subject to irreversible changes. The forming ice crystals generate the deformation and shrinkage of the cell with an increasing electrolyte concentration in it. The referred authors explain various cryobiological effects in different tissues and organs, different water content in these tissues and changeable chances of its diffusion through the cell membranes.

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STRESZCZENIE

Oceniano histologicznie i ultrastrukturalnie wycinki niezmienionej klinicznie błony śluzowej jamy ustnej, zamrażane temp. –196 C przez 2 sek. przy użyciu aplikatora o średnicy 10mm. Wycinki pobierano bezpośrednio po krioaplikacji od 21 pacjentów - w tym 10 mężczyzn w wieku 28 do 76 lat i 11 kobiet w wieku od 17 do 67 lat.

Po 2 sek. zamrażania oprócz znacznego poszerzenia przestrzeni międzykomórkowych we wszystkich warstwach nabłonka stwierdza się wyraźne uszkodzenie desmosomów, a niekiedy także hemidesmosomów. Zmiany w keratynocytach mają dwojaki charakter. Pierwszy typ zmian to zwyrodnienie drobnowodniczkowe cytoplazmy z częstą lokalizacją okołojądrową wodniczek. Drugi typ zmian to znaczne obkurczenie jądra komórkowego z jego nadbarwliwością, zagęszczenie cytoplazmy i zmiana kształtu komórek. Oba typy zmian w komórkach można wiązać z tworzeniem się pozakomórkowo kryształów lodu, a obkurczenie komórki jest już zjawiskiem nieodwracalnym.

EXPLANATION TO FIGURES

Fig. 1. The epithelium of the oral mucosa in the site of application of the cryoprobe (temp. –196 C, time 2 sec.). Visible widening of intracellular spaces in the whole epithelium, most intensely marked in the basal layer. In the basal, prickle and intermediate

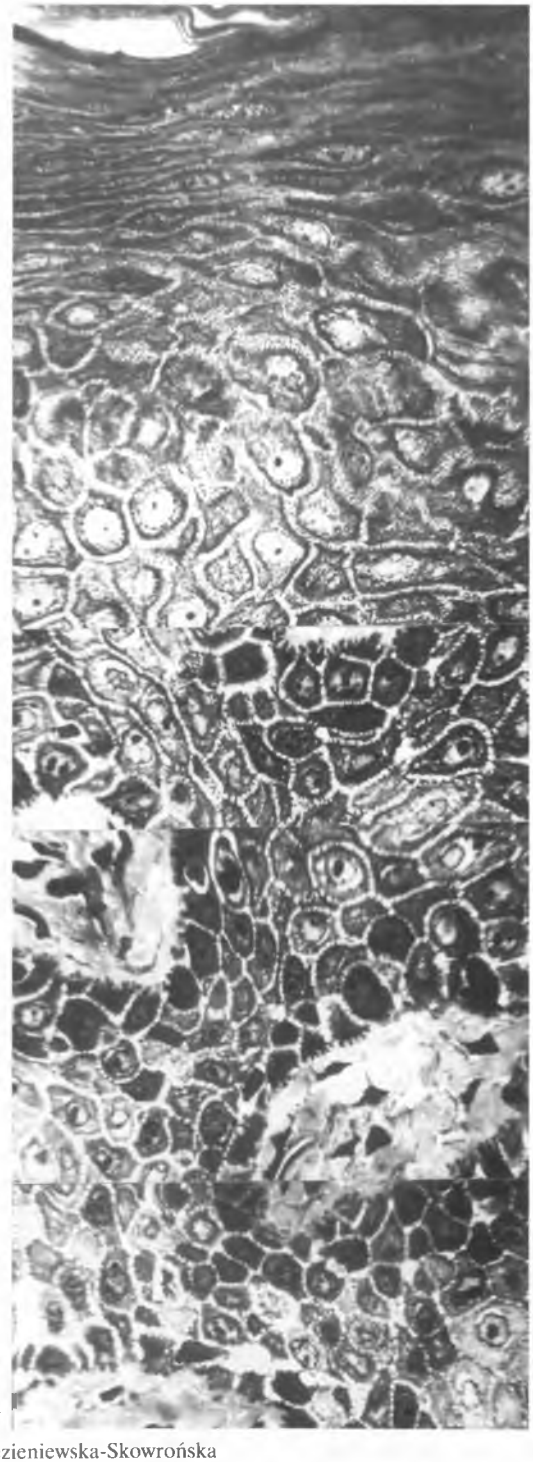


Fig. 1

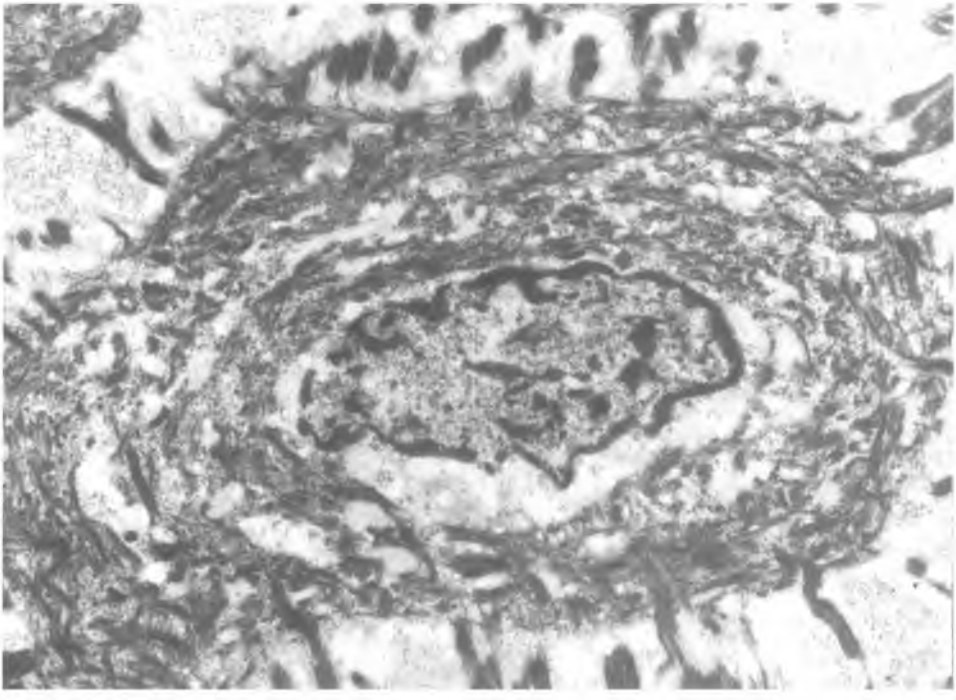
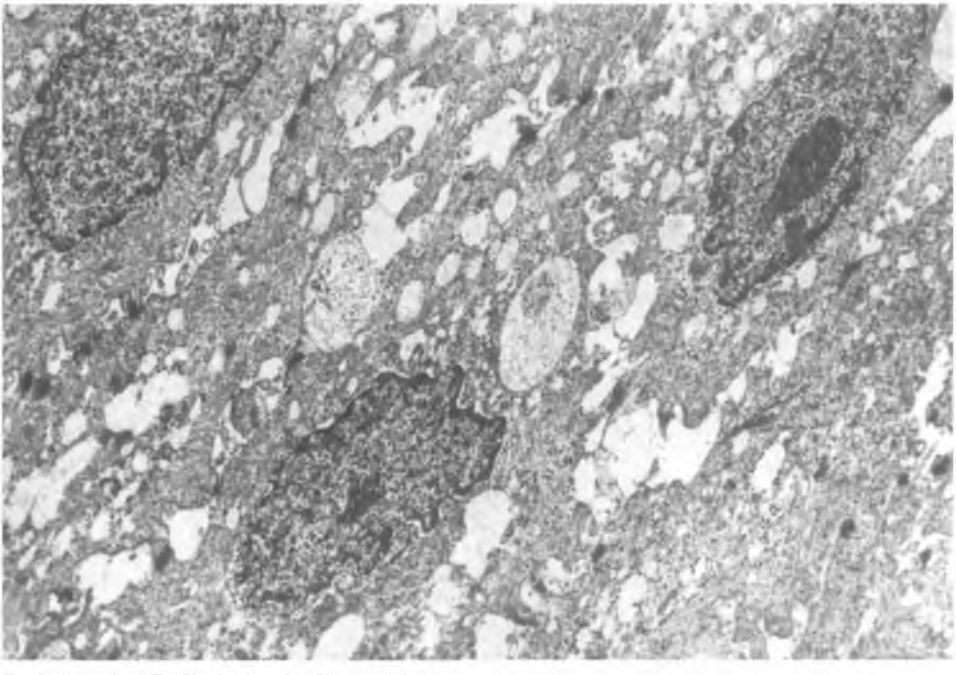


Fig. 2

Fig. 3



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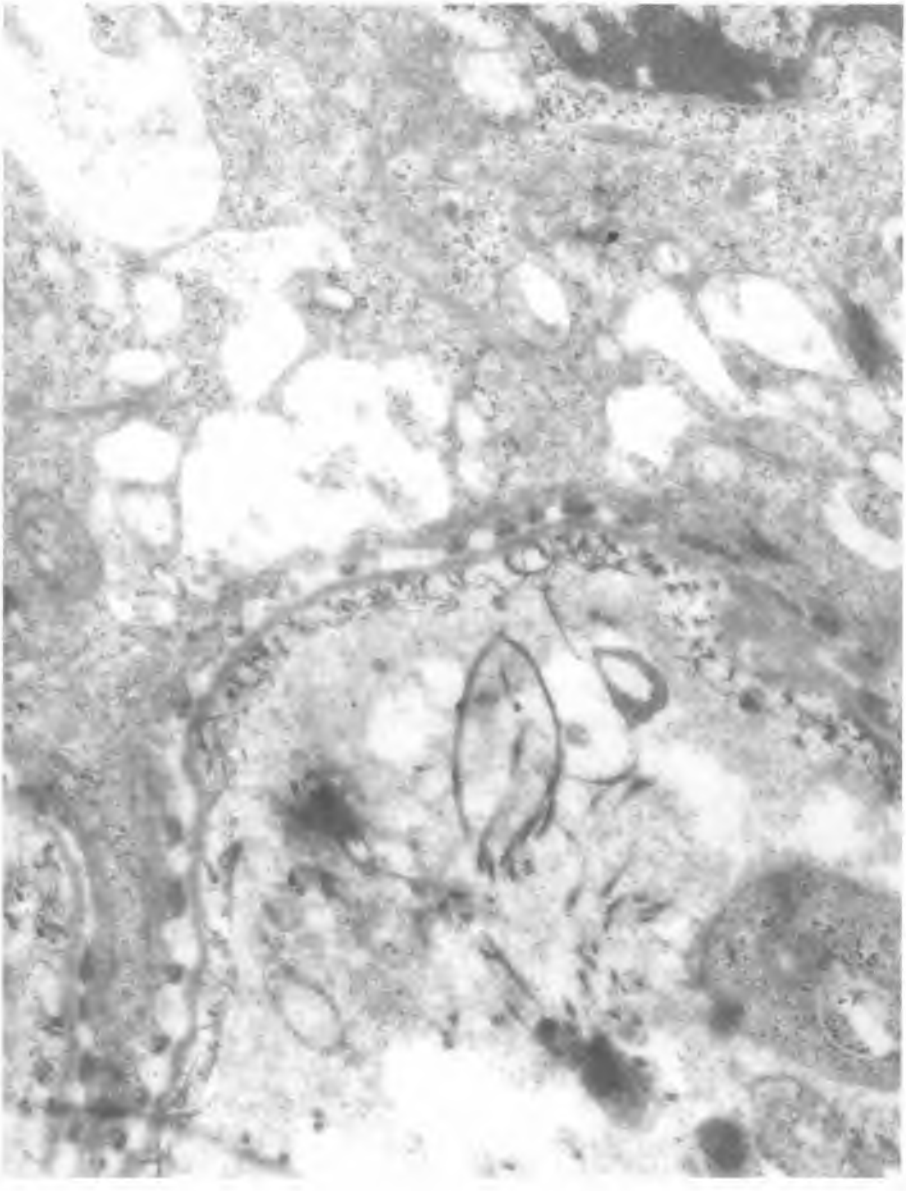

Fig. 4

layers present perinuclear vacuoles and small vacuoles in the cytoplasm. In the superficial layer blurred cell borders, thickened cytoplasm and the lack of the nuclei. Mag. 14,000x.

Fig. 2. The widening of intracellular spaces in the non-desmosomal zones, abruption of desmosomal plates from the cell membrane. In the cytoplasm of the keratinocytes small vacuoles, bundles of disintegrated filaments and visible perinuclear vacuoles. The keratinocyte nucleus contains condensed heterochromatin. Keratinocytes of the upper stratum of the prickle layer. Mag. 10,000x.

Fig. 3. The widening of intracellular spaces with abruption of non-desmosomal junctions. In the cytoplasm of the keratinocytes irregular vacuoles with little density, scattered particles of alpha and beta glycogen and mitochondria with the traits of matrix clearing and the crystolysis. The mitochondrial external membrane preserved. The nuclei of the keratinocytes slightly shrinked with predominance of heterochromatin. Keratinocytes of the lower stratum of the prickle layer. Mag. 4,000x.

Fig. 4. The continuity of the basement membrane preserved. The majority of hemidesmosomal junctions with normal picture. Occasionally seen hemidesmosomes injury and moderate widening of intracellular spaces, between the basement membrane and cells of the epithelium basal layer, considerable widening of intracellular spaces in non-desmosomal zones with the abruption of the continuity of the cell membrane and complete damage of desmosomes. In the keratinocytes visible bundles of intermediary filaments, concentrations of alpha and beta glycogen particles and scarce mitochondria with oedema traits. Border of the epithelium and oral mucosa proper. Mag. 10,000x.