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

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

Up to the present moment the recognized mechanism of cryodestruction of tissue was characterized by complex physical, chemical and biological phenomena. It created several controversial hypotheses explaining this phenomenon:

1. The theory of cell compression which assumes that not all cells are subject to irreversible damage after defreezing, the substantial part of them can resume their former shape (14).

2. The theory of electrolyte concentration which suggests that cell injury is generated by the diffusion of intercellular fluids into the intracellular spaces which causes the increase in electrolyte concentration in the cell up to the toxic level (10).

3. The theory of minimal cell volume which assumes that in the process of freezing of the tissue fluid the concentration of extracellular salt solutions leads to the reduction of cell volume. The difference in the osmotic pressure, being the consequence of it, leads to the permeation of fluids through the cell membrane evoking the osmotic shock, which causes irreversible changes in the cell membrane permeability in the defreezing stage (14).

4. The theory of the destruction of cell structural proteins which suggests that in the tissue freezing process defects form in the semipermeable cell membrane, which makes it lose its permeability. It affects the decrease in fluid concentration in the cell and finally its death (8).

5. The theory of low temperature acting on tissue enzymes which suggests that during freezing of the tissue, not only cell membrane is injured but also the nucleus and membrane structures. Moreover, the mitochondrial and lysosomal enzymes can reveal the rise of activity if the membrane of their structures was not broken during the freezing (14).

6. The theory of breaking of cell membrane continuity caused by sudden water deficiency which suggests that during abrupt cooling of tissues, water leaves the cell faster than the cell membrane allows it in the physiological circumstances (9). The use of cryoapplication time of 5 seconds in the conditions described in previous papers makes it possible to anticipate that with the intensification of injury some of the perceived changes can be irreversible.

The development of cryobiology, especially in recent years, informs that biological membranes are the least resistant to freezing structures (7, 12). The constituents of phospholipids, the physicochemical composition of which in large extent depends on temperature, are subjected to structural and biochemical modification. They mainly regulate the function of plasma and intercellular membranes of the organelle whereas the membranes' constituents such as lipids and various albumin complexes react to the reduction of temperature differently. Low temperatures most quickly destroy lipids, then glycolipids and glycoproteins and finally albumins and their complexes (1-6, 13).

The majority of biochemical reactions in the organism take place in the aqueous environment. Because of different water contents in various tissues (e.g. the liver tissue contains 70% water and bone tissue about 22% water), and also the biochemical tissue content – the cryobiological effects are varied. Similar differences also appear according to the structure and function of cell junctions. The experimental studies concerning the action of low temperatures in the range of temperature from -20^0 C to -190^0 C revealed that the breakdown and devitalization of tissues depends on (11): 1) obtaining appropriately low temperature; 2) the rate of freezing in Celsius degrees per minute; 3) the type of tissue; 4) the rate of tissue defreezing in Celsius degrees per minute; 5) the number of freezing and defreezing cycles following one after another.

The material and methods were shown in the first part of the paper.

RESULTS

Group IV included tissue segments of the clinically unchanged oral vestibule mucosa frozen with the temperature of –196 C within 5 seconds, using the applicator 10 mm in diameter, collected immediately after the cryoapplication from the same 21 patients. On the semithin sections observed in the light microscope it was noticed that there was a widening of intracellular spaces in the whole epithelium cross-section, however, it was less intense than in the freezing time of 2 seconds. Intercellular changes are non-homogeneous (Fig. 1). Apart from cells with considerably deformed nucleus and large perinuclear halo, cells with normal nucleus shape but without the formation of perinuclear halo

are observed. The cells with normal nucleus shape, however, reveal considerable areas of cytoplasm clearings probably caused by the crystallization of intercellular water. In some cells, especially in the cells of epithelium superficial layer, distinct and extensive cytoplasm clearings are visible. Some keratinocytes localized in different layers have evidently condensed cytoplasm and dark nucleus with condensed structure. Both, in the basal and prickle layers slits are visible. They are surrounded by mechanically damaged cells generated as a consequence of the formation of crystals.

As ultrastructural research revealed, the process of crystallization of intracellular water can lead either to slight widening of intracellular spaces preserving the continuity of cell membranes or to the total destruction of intracellular junctions with simultaneous destruction of cell membranes (Fig. 2, 3). It seems that as a result of freezing, the mitochondria are most quickly destroyed (Fig. 4). Usually, the mitochondrial matrix is subject to clearing, crests are considerably shortened or completely blurred but the external mitochondrial membrane is preserved.

In the keratinocytes of all layers, especially of the prickle layer, the disruption of the nucleus sheath is often observed (Fig. 2), especially when the nucleus is surrounded by the electron translucent halo. Sometimes in the nuclei changed in such a way intracellular vacuoles are visible with the density approximate to the density of vacuoles surrounding the nucleus. In the studied group the injury of hemidesmosomes and desmosomes was more often observed as well as considerable widening of intracellular spaces in the non-desmosomal zone with the total destruction of desmosomes and keratinocytes cell membrane (Fig. 3). The cytoplasm of the keratinocytes revealed either numerous, irregular vacuoles being most likely the effect of crystallization of intercellular water or evident density. Within these densities it was difficult to identify cell organelle but sometimes it was possible to differentiate bundles of compact filaments or small clusters of alpha and beta glycogen particles (Fig. 3).

DISCUSSION

The activity of low temperature on the cells of the epithelium of the oral mucosa causes changes in the basement membrane towards its surface. It can be connected with the temperature gradient which enables, through the basement membrane itself, the crystallization of water in the intracellular spaces leading to the widening of these spaces. Simultaneously, the cryoapplication time of 1 second turns out to be too short to freeze all layers of the epithelium cells excluding the process of crystallization of intracellular water. It further leads to the formation of perinuclear halo and the change in the shape of the nuclei. The freezing time of 2 seconds appears to be long enough to decrease evidently the crystalliza-

tion of intercellular water, decreasing the intensification of the nuclei deformation.

As opposed to freezing in the time of 1 second, the process of crystallization of water in the intracellular spaces envelops evenly all epithelium layers. However, after the cryoapplication time of 5 seconds it is observed, that there appears an even widening of intracellular spaces in all layers of the epithelium but with the smaller intensity than after freezing in the time of 2 seconds. The picture of intercellular changes is not explicit. Cells are observed both with considerably deformed nucleus and large perinuclear halo and with regular nucleus shape, however with considerable clearing regions in the cytoplasm without the formation of perinuclear halo.

CONCLUSIONS

1. In the region of application of the cryoprobe, changes in the cells of the epithelium of the oral mucosa are most intense in the area of the basement membrane.

2. The widening of intracellular spaces and the formation of vacuoles in the cytoplasm as well as in the nuclei of the keratinocytes is the effect of the crystallization of intra- and extracellular water.

3. Observed changes of cytoskeleton and mitochondrial keratinocytes in the process of cryoapplication should be considered as damaging with the possibility of their reversible character in favourable conditions.

4. In the site of cryoprobe application, cell membrane and nuclear membrane are interrupted.

5. The results of the conducted research, considering tissue peculiarity, can be the basis for elaboration of the technique of cryosurgical operation and defining the range of cryodestruction according to the temperature, the size of applicator used and the freezing time.

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STRESZCZENIE

Złożony mechanizm kriodestrukcji tkanek pozostaje nadal nieznany, pomimo licznych prowadzonych badań i postulowanych hipotez tłumaczących to zjawisko. W obecnej pracy analizowano zmiany histologiczne i ultrastrukturalne nabłonka niezmienionej klinicznie błony śluzowej jamy ustnej, zamrażanego temp. –196 C w czasie 5 sek., przy użyciu aplikatora o średnicy 10 mm.

Wycinki pobierano bezpośrednio po krioaplikacji u 21 pacjentów – w tym 10 mężczyzn w wieku od 28 do 76 lat i 11 kobiet w wieku od 17 do 67 lat. Po 5 sek. okresie zamrażania opisywane zmiany po 1 i 2 sek. zamrażania nasilają się, a ponadto występuje ogniskowe przerwanie błony komórkowej i otoczki jądra, znacznie nasilony obrzęk mitochondriów oraz ogniskowe zagęszczenie cytoplazmy z zatarciem struktury organelli.

Wyniki naszych badań potwierdzają wcześniejsze spostrzeżenia, że najmniej odporne na zamrażanie są struktury błonowe komórki. Wyniki przeprowadzonych badań mogą stanowić podstawę do opracowania techniki zabiegu kriochirurgicznego i określenia zasięgu kriodestrukcji w zależności od temperatury, czasu zamrażania i wielkości użytego aplikatora.

EXPLANATION TO FIGURES

Fig. 1. The cross-section of the epithelium of the oral mucosa in the site of the cryoprobe application (temp. –190 C, time 5 sec.). Little, meagre or considerable widening of intracellular spaces in the whole epithelium density together with the formation of slits in the epithelium. Basement membrane focally, not clearly visible. Keratinocytes shrinked with hyperchromatic nuclei, sometimes with perinuclear vacuoles. In the upper strata of the prickle layer and in the intermediary layer the cytoplasm of some keratinocytes is thickened, while the cytoplasm of other keratinocytes is cleared with the presence of various size vacuoles. Mag. 1000 x

Fig. 2. The widening of intracellular spaces is accompanied by breaking off numerous desmosomal plates which lie loosely in these spaces. Cell membrane considerably injured, not clearly seen. Organelle of cytoplasmatic keratinocytes difficult to identify, only bundles of compact filaments can be seen. Perinuclear vacuoles around one of the nuclei. The nuclei also contain intranuclear vacuoles with the average electron density and the nuclei sheath is visibly interrupted in several places. Keratinocytes of the upper stratum of the prickle layer. Mag. 6,000 x.

Fig. 3. Considerable widening of intracellular spaces with the injury of desmosomal and non-desmosomal zones and focal continuity disturbances of the keratinocytes' cell membranes. The cytoplasm of the keratinocytes thickened with the presence of scarce, irregular in shape clearings. Identification of the cytoplasmatic organelle impeded. Only accumulation of alpha and beta glycogen can be revealed and also bundles of disintegrated filament. The nucleus of one of the keratinocytes is evidently shrinked with the predominance of heterochromatin and the presence of caryoplasm clearings. Keratinocytes of the prickle layer. Mag. 3,000 x.

Fig. 4. In the cytoplasm of the keratinocytes present quite numerous glycogen granules, scarce filament bundles, tiny or larger vacuoles not clearly separated from the remaining cytoplasm. In the mitochondria, crystolysis features and evident matrix clearings.

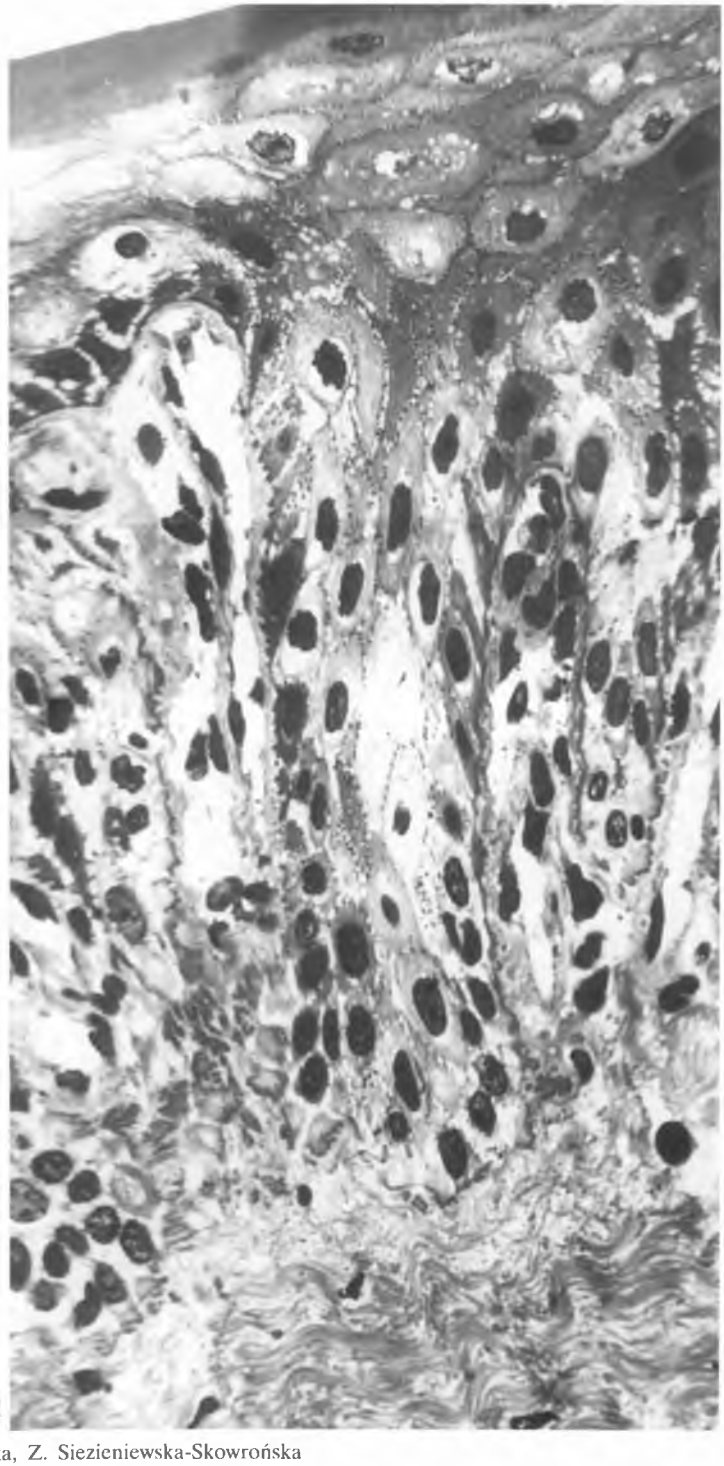

Fig. 1

E. Gałkowska, Z. Siezieniewska-Skowrońska

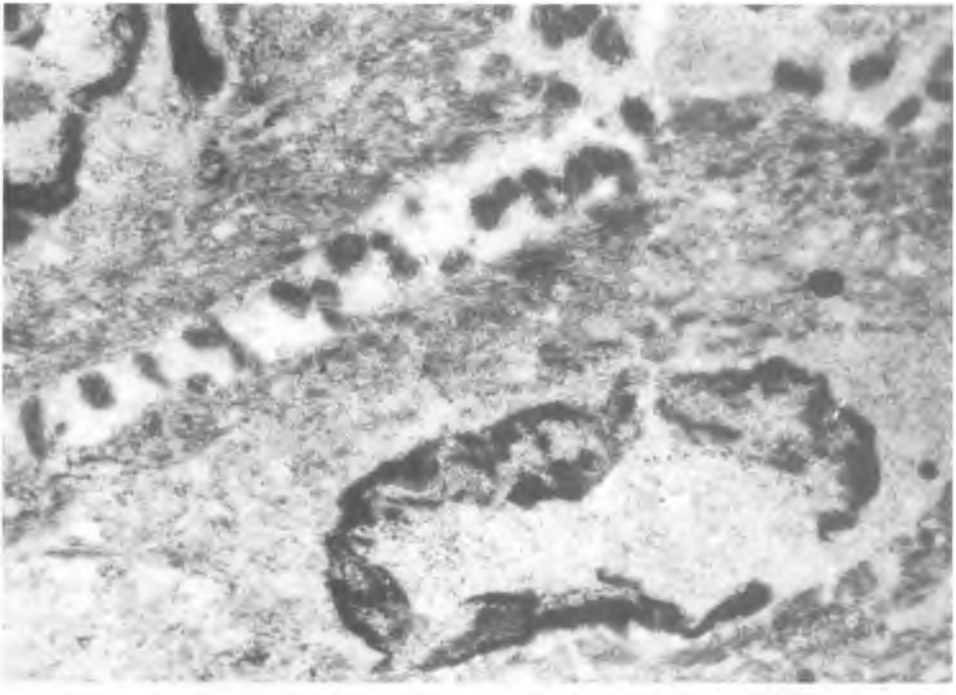

Fig. 2

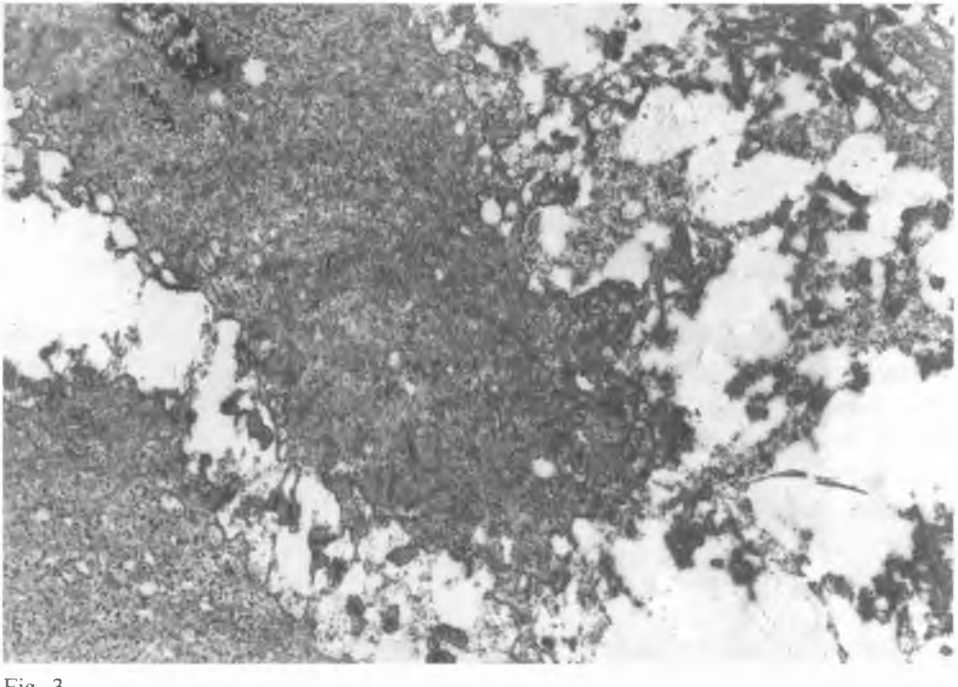

Fig. 3

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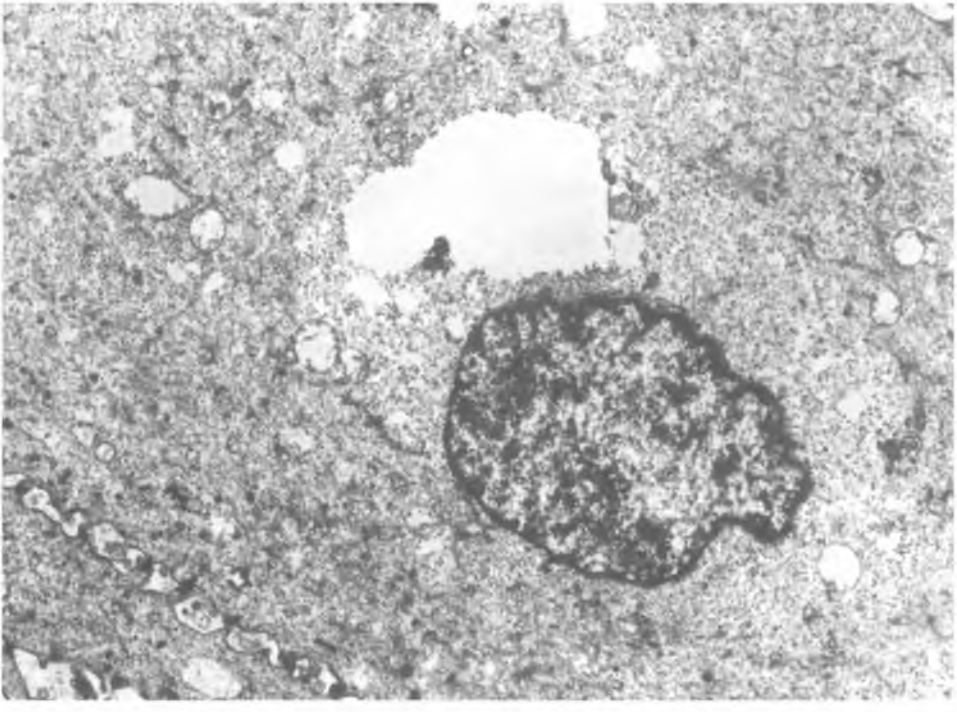

Fig. 4

External mitochondrial membranes with preserved continuity. The nucleus of the keratinocyte scarcely shrinked with predominance of heterochromatin. Small widening of intracellular spaces in non-desmosomal zones. Keratinocytes of the lower stratum of the prickle layer. Mag. 4,000 x.