CHANGES IN THE ULTRASTRUCTURE OF THE HUMAN EPIDERMIS ON FREEZING

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Samples of skin from middle-aged and old persons, taken at operation, were either frozen to −35°C for 24 h in a deep-freezer or repeatedly frozen and thawed on a freezing microtome at −70°C. Electron-microscopic examination of the samples showed dilatation of the intercellular spaces, destruction of the cell membranes, homogenization of the tonofibrils, a decrease in volume of the mitochondria, and partial destruction of their internal structure. The cell nuclei were particularly visibly injured chiefly in the upper layers of the epidermis, for the nuclear membrane was ruptured and masses of structureless nucleoproteins were liberated into the cytoplasm. Destruction and fragmentation of the cytoplasm and nuclear membrane were accompanied by the formation of myelin-like structures. Freezing to −70°C caused more severe changes in the fine structure of the epidermal cells with the formation of multiple empty spaces, evidently caused by ice crystals.

KEY WORDS: *epidermis; freezing of tissues; cell ultrastructure.*

Insufficient attention has been paid to the study of the effect of subzero temperatures on tissue structure and such data as has been obtained are largely contradictory. Most indicate that rapid freezing to temperatures close to 0°C causes destruction of the tissues through the formation of ice crystals [2, 4]. However, there is virtually no factual evidence of the effect of low temperatures on the ultrastructure of the epidermis.

The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

Skin samples from middle-aged and old persons taken at operation were kept for 24 h in a deep-freezer (−35°C) or repeatedly frozen and thawed on a freezing microtome at −70°C. After fixation in 2% OsO_4 and embedding in Araldite, the material was examined in the IEM-7A electron microscope.

EXPERIMENTAL RESULTS

Freezing to −35°C was accompanied by compaction of most of the keratin scales, weakening of the bond between them, and the appearance of finely granular high-contrast inclusions in the spaces between the scales. The cytoplasm of the granule cells was condensed, the degree of contrast of the tonofibrillary-keratohyalin complexes was slightly reduced, and the cell membrane remained intact. Structureless empty spaces of different sizes were often found in the prickle cells close to the nucleus. Some mitochondria were reduced in volume and contained dense, granular or honeycombed contents without visible cristae, whereas others were swollen and contained a translucent matrix and residual fragmented cristae. The tonofibrils in the bundles were fused together to form almost homogeneous bands. The structure of the free ribosomes and their number were not appreciably altered.

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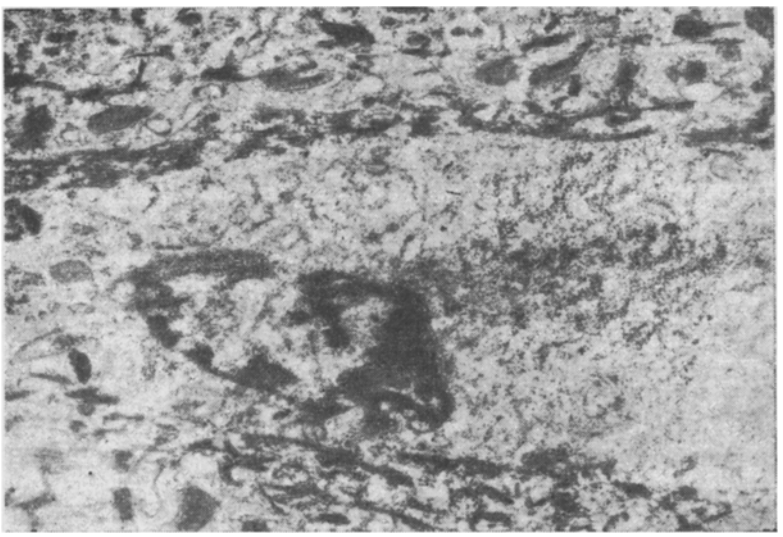

Fig. 1. Residue of nucleus of prickle cell with multiple ruptures of nuclear membrane and massive liberation of nucleoproteins into cytoplasm. Freezing to −35°C, 57,600×.

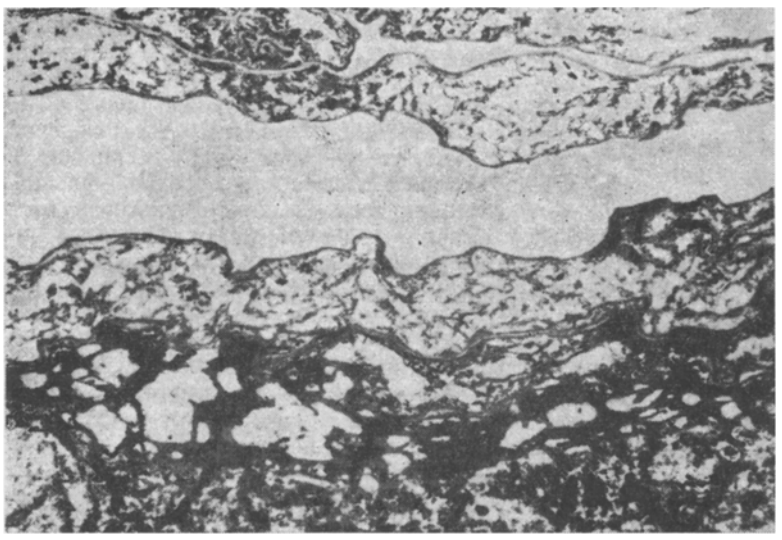

Fig. 2. Multiple empty spaces in keratin scales and granule cell as the result of ice crystal formation. Freezing to −70°C, 57,600×.

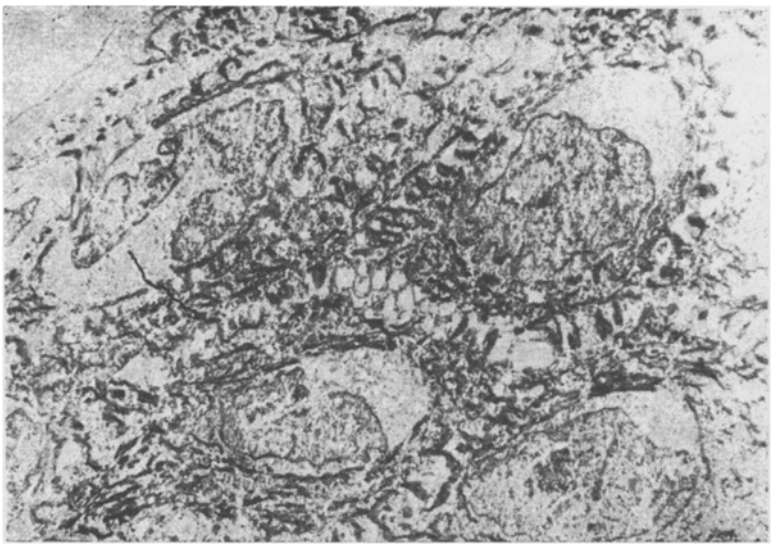

Fig. 3. Dilatation of intercellular space with their destruction and changes in structure of nuclei and cells of stratum granulosum and stratum spinosum. Freezing to −70°C, 16,800 ×.

Over a wide area in the cells of the stratum spinosum and stratum basale the cell membrane could not be defined and it was clearly distinguishable only in the region of the desmosomes, the internal lamellar structure of which was well preserved. In the zone of the intercellular spaces at the site of the previous cell membrane, round and apparently twisted membranous formations, known as myelin-like bodies, were sometimes visible. The most noticeable and substantial changes during freezing to −35°C occurred in the nuclei of cells of the stratum granulare and, in particular, the stratum spinosum of the epidermis. They were characterized by ruptures of the nuclear membrane and the liberation of finely scattered nucleoprotein granules of the nucleus into the cytoplasm, where they were distributed over the area occupied previously by the intact nucleus (Fig. 1). Inside the remains of the nucleus could be seen a clearly defined heterochromatin region close to the nuclear membrane, and fibrillary and granular components in the central zones of the nucleoplasm. The structure of the nucleolus could not be made out. Remains of the much smaller nucleus were frequently distributed among the mass of nucleoproteins liberated into the cytoplasm. In the zone of the ruptured nuclear membrane myelin-like figures could sometimes be seen. Some nuclei, especially in the cells of the basal layer, retained their shape and volume, but their internal structure, including the nucleolus, was damaged to some degree. The thickness and degree of contrast of the basement membrane were slightly reduced. The cell membrane of the basal cells could not be identified. The interstitial spaces separating the basement membrane from the hemidesmosomes were dilated.

Freezing to −70°C caused much greater damage to the ultrastructure of the epidermis. Numerous small and large empty spaces appeared in the horn cells and granule cells (Fig. 2). Changes in the structure of the cytoplasm and nuclei of cells of the stratum spinosum were similar to those of the type described above, but more severe (Fig. 3). The number of myelin-like structures also was increased, especially in the zone of the intercellular spaces.

The results show that freezing the epidermis to −35 and −70°C causes considerable damage to the ultrastructure of the cytoplasm, cell membranes and, in particular, the nucleus. The partly pulverized contents of the nucleus escape through tears in the nuclear membrane into the cytoplasm, filling the space previously occupied by the nucleus. Under the light microscope, this may give the impression that the nucleus is intact. The structure of the ribosomes was virtually unchanged. Simpler changes in the ultrastructure of the nuclei have been observed after freezing of polymorphonuclear granulocytes in liquid nitrogen, even after the addition of cryoprotective agents [3], and after brief (5 sec to 1 min) freezing of rabbit areolar tissue at temperatures of −35, −60, and −80°C [5]. According to data in the literature [5], repeated freezing and thawing of isolated cells causes swelling of most mitochondria, with clearing of their matrix and destruction of the cristae. The

writer's own observations showed that only a very few mitochondria responded in this way and most became condensed and developed a fine honeycombing of their internal structure, possibly on account of the formation of small ice crystals. Freezing to −35°C was virtually not accompanied by the appearance of intracellular empty spaces or zones of reduced density, i.e., by crystal formation. These phenomena were observed following exposure to a temperature of −70°C and they were most severe in the surface layers of the epidermis, where they may perhaps reflect the rate of freezing of the tissue and the effect of multiple freezing and thawing. The present experiments showed that the source of formation of the myelin-like structures was evidently fragments of the cell membranes and, in particular, of the nuclear membrane.

Homogenization of the tonofibrillary system is evidently linked with phenomena of irreversible dehydration and drying and it is also observed during postmortem destruction of the epidermis [1].

LITERATURE CITED

1. I. N. Mikhailov, Izvest. Akad. Nauk SSSR, Serial Biol., No. 5, 657 (1973).
2. E. O'Flaherty and W. T. Roddy, J. Am. Leather Chem. Ass., 26, 172 (1931).
3. T. I. Malinin, Cryobiology, 9, 123 (1972).
4. R. Plank, Z. Allg. Physiol., 17, 221 (1918).
5. J. K. Sherman, Cryobiology, 9, 112 (1972).