EFFECT OF FREEZING THE SKIN ON FORMATION OF MULTINUCLEAR CELLS IN ITS EPITHELIUM

V. V. Kadakas

UDC 612.79.014.3-088.6

The number of binuclear and multinuclear epithelial cells formed in the skin of the rat's ear after transient and slight freezing, like the degree of general degenerative changes in the tissues, depends on the time during which the tissue is in a frozen state at a temperature close to the temperature of freezing.

* * *

It was shown that after transient freezing, binuclear and multinuclear cells are formed in the stratum germinativum of the cutaneous epithelium, in the sebaceous glands, and in the outer epithelial sheath of the hair follicle in mammals, including man [2, 3, 5, 11]. Earlier experiments revealed that in the epidermis of the ear of albino rats these cells develop in the greatest number in the most distal part of the ear, which under the experimental conditions used was in a frozen state for longer than the rest of the ear. In addition, variability of the number of binuclear and multinuclear cells formed was observed in these experiments.

The object of the present investigation was to examine whether these differences are related to the conditions of freezing and to study the general character of changes in temperature in the course of freezing and thawing under the experimental conditions.

EXPERIMENTAL METHOD

Experiments were carried out on 18 male albino rats weighing 200-250 g. Under ether anesthesia the distal part of the ear was frozen by spraying ethyl chloride on to its posterior surface for 3-5 sec. The intensity of spraying was varied slightly depending on the intensity of the release of ethyl chloride from the ampules. The ear temperature was measured during freezing and thawing by means of a needle thermocouple introduced into the connective tissue between the epithelium and cartilaginous plate. Galvanometer readings were recorded every 5 sec. The lowest temperature attained was also recorded. Thawing took place at room temperature. The rats were killed two days after the experiment, always at the same time of day (from 12 noon to 1 P.M.). The ears were amputated and fixed in Zenker-formol and Carnoy's fluid. Paraffin sections 7 μ in thickness were stained with Mayer's hematoxylin-eosin. In every case the number of binuclear and multinuclear cells was counted in the stratum basale in 1000 cells of this layer.

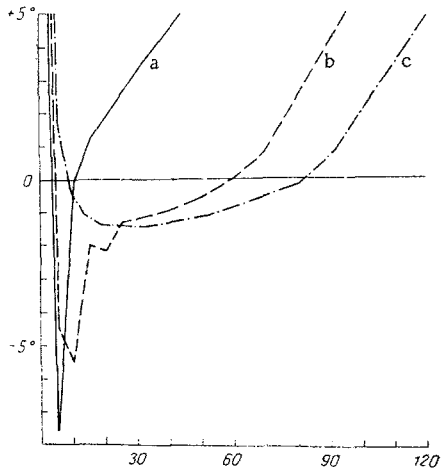

Fig. 1. Different types (a, b, c) of temperature curves recorded during freezing the ears of albino rats. Abscissa, time from beginning of freezing (in sec); ordinate, temperature (in °C).

EXPERIMENTAL RESULTS

A few seconds after the beginning of freezing, as a rule the ear suddenly turned white at its edge and became hard. Transillumination showed that the frozen area was opaque. The freezing front advanced rapidly toward the base of the ear. Thawing began from this part and usually ended at the edge. During thawing the intensity of the white color gradually diminished.

Laboratory of Experimental Histology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad (Presented by Active Member of the Academy of Medical Sciences of the USSR D. A. Biryukov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 1, pp. 80-83, January, 1969. Original article submitted July 1, 1967.

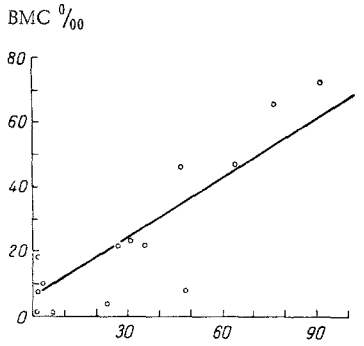

Fig. 2. Relationship between number of binuclear and multinuclear cells (BMC) in stratum basale of cutaneous epithelium and time during which tissue was in a frozen state. Abscissa, time (in sec); ordinate, number of BMC (in ‰). Equation of line of regression $y = 6.25 + 0.622x$; $P < 0.001$.

Analysis of the temperature curves obtained shows that in different cases freezing and thawing followed different courses (Fig. 1). In most cases the temperature of the ears fell on the average during the first 4 sec from +28 to -7.8° (with variation from -2.6 to -15.5°), after which it rose rapidly (Fig. 1, curves a, b). This shows that overcooling of the tissues took place, followed by the sudden formation of ice and liberation of the latent heat of crystallization.

If the freezing ended quickly, thawing was complete in 10 sec (Fig. 1, curve a; mean of 5 cases). If, on the other hand, the duration of freezing was longer and continued even after freezing of the tissues, as shown by the occurrence of a new, although slight, decrease in temperature after the step on the temperature curve, subsequent thawing in some cases took 1 min or longer (Fig. 1, curve b; mean of 5 cases). These differences were perhaps attributable to the formation of different quantities of ice in the tissues. Between these two types there were also curves of intermediate character. In two cases the fall in temperature was slower, and no overcooling was recorded (Fig. 1, curve c). This slowing of the fall in temperature before the freezing point was reached was due in all probability to liberation of the latent heat of crystallization after overcooling in neighboring areas. This interpretation follows from experimental work [6] to study the principles governing freezing in different parts of a frozen object. Very probably in some other cases the end of the thermocouple did not lie in the region of maximal overcooling. There is therefore reason to suppose that overcooling in some cases was even deeper than was recorded.

A relationship was discovered between the degree of necrobiotic changes in the ear tissues and the duration of freezing and thawing. Severe injury with death of all tissues took place in cases when the ear was left in a frozen state more than 1-1.5 min (3 cases). With a shorter duration of thawing, necrosis did not develop in the tissues. Inflammatory changes developed in the subcutaneous connective tissue, and the appearance of binuclear and multinuclear cells and other changes described by the author previously were observed in the epidermis and sebaceous glands. If the temperature rose above 0° immediately after crystallization of the water, these processes were less marked or absent altogether.

The number of binuclear and multinuclear cells formed in the epidermis thus also depended on the conditions of freezing. This relationship is illustrated graphically in Fig. 2, showing that a definite correlation is present between the time during which the tissue was in a frozen state and the number of binuclear and multinuclear cells formed ($P < 0.001$, coefficient of regression $b = 0.622$, number of degrees of freedom $f = 13$, $t = 4.90$, standard deviation $\sigma = 14.0$). As mentioned above, the number of cells examined was particularly large in the most distal part of the ear, which was in a frozen state for a longer time.

Freezing of water in the tissues in the case of slow freezing takes place extracellularly, but if freezing is rapid and there is appreciable overcooling, it also takes place intracellularly [4, 8, 10, 14]. Since freezing of the ears in the present experiment took place after appreciable overcooling, it can be assumed that intracellular freezing took place. This assumption is also confirmed by death of the cells in cases when the ear remained in a frozen state for longer than 1-1.5 min. In the case of slow freezing producing only extracellular crystallization of water, the skin can remain in a frozen state close to freezing point without appreciable damage for a much longer time [8, 14].

The possibility of survival of the cells of some tissues after intracellular ice formation during rapid freezing is now accepted by many investigators [1, 7, 9, 12, 12]. This possibility was emphasized, in particular, at a symposium of the American Society of Cryobiologists held in Madison in 1965. The view is held that it is not the presence of ice in the cells itself but the size of its crystals which is the deciding factor [15]. At very low temperatures the crystals grow rapidly, and this explains the need for rapid thawing [8].

The results of these experiments thus confirm that cells of the cutaneous epithelium and its derivation, and possibly other tissues of the rat's ear can tolerate transient and slight freezing with, in all probability, intracellular ice formation. The degree of reactive changes in the cells of the epidermis during freezing, including the number of binuclear and multinuclear cells formed, depends on the time during which the tissue is in a frozen state at a temperature close to the freezing point.

LITERATURE CITED

1. L. K. Lozina-Lozinskii, in: Collected Transactions of the Institute of Cytology [in Russian], No. 12, Moscow-Leningrad (1966), p. 33.
2. V. V. Podvysotskii and R. G. Pironé, Arkh. Biol. Nauk, 12, No. 3, 214 (1906).
3. F. M. Khaletskaya, Arkh. Biol. Nauk, 39, No. 1, 127 (1935).
4. E. Asahina, Contr. Inst. Low Temp. Sci. Hokkaido Univ., No. 10, 83 (1956).
5. E. Fuerst, Beitr. Path. Anat., 24, 415 (1898).
6. B. J. Luyet, Cryobiology, 2, 198 (1966).
7. P. Mazur, Cryobiology, 2, 181 (1966).
8. H. T. Meryman, Proc. Roy. Soc. B.,147, 452 (1957).
9. H. T. Meryman, Physiol. Rev., 37, 233 (1957).
10. L. Rey, Conservation of Life by Cold [Russian translation], Moscow (1962).
11. A. Rischpler, Beitr. Path. Anat., 28, 541 (1900).
12. R. W. Salt, Nature, 184, 1426 (1959).
13. J. K. Sherman, Anat. Rec., 144, 171 (1962).
14. O. Smith, Biological Action of Freezing and Overcooling [Russian translation], Moscow (1963).
15. Summary of the Panel Discussion, Cryobiology, 2, 330 (1966).