THE INFLUENCE OF PRELIMINARY GLYCEROL TREATMENT ON SUCCINIC DEHYDROGENASE ACTIVITY OF SKIN CONSERVED THROUGH FREEZING

S. A. Vankova

The use of freezing for the conservation of tissues and organs is considered as the most reliable method, providing for their preservation for unlimited length of time. However, the investigators are confronted with a number of questions of theoretical significance. Reference is made to issues related to the influence exerted by low temperature upon the structure and function of cells, to the importance of rate of freezing and thawing, action of various protective substances and, more particularly, glycerol in obviating intracellular crystallization and the like.

The perusal of the pertinent literature available disclosed the great paucity of systematic histochemical research into tissues and organs, conserved through freezing. So far as conserved skin is concerned in particular, no investigations whatsoever were discovered in the literature reviewed.

Object of the present work is the cytochemical study of succinic dehydrogenase activity changes in skin conserved through freezing, following preliminary treatment with glycerol.

Materials and Methods

The study was carried out on skin obtained from the abdominal region of four male albino rats, weighing between 180 and 200 grams. One part of the skin obtained from each rat underwent treatment in 15% glycerol, at room temperature, for one hour, and thereafter, together with the second part of the material, underwent freezing in a mixture of dry ice and acetone for 40 min (temperature —79° C) and transfer in a refrigerator at —20° C over terms of 3, 7, 14, 30, and 60 days. The third part of the skin from each animal was not subjected to preliminary treatment whatsoever and served as control. After elapsing of the fixed time, the freezed material was thawed in physiological solution at temperature +40° C. Histochemical investigation of the succinic dehydrogenase activity on the whole fresh and conserved material without fixation was conducted after the method of Nachlas and co-workers (1957), with incubation in a medium containing sodium succinate as a substrate, and Nitro BT as electron acceptor. Specificity control of the reaction was performed by means oelimination of the substrate from the incubation medium.

Results

A. Fresh, non-conserved skin. The reaction is positive in all cellular elements of the skin. It is localized in tiny, dark-blue tinged granules, filling out the cytoplasm of the cells. In the basal layer of the epidermis, these granules are numerous and densely aggregated in the perinuclear cytoplasm. An intense reaction is observed in the spinous layer also. A weaker reaction is noted in the granulous layer, whilst in the horny layer, it is completely negative. At dermal level, strong succinic dehydrogenase activity is established in all the cellular elements except for the mastocytes in which a red instead of a dark blue staining occurs. In the fibrous elements of the connective tissue, the reaction is negative.

B. Skin conserved through freezing without preliminary glycerol treatment. In comparison with non-conserved skin, the changes observed here involve, on the one hand, the intensity of the reaction and, on the other, the changes in mitochondria. As early as at the third day of conservation, a rise is noted of the ferment activity in all cellular elements of the epidermis, dermis and subcutaneous striated musculature. It is associated to clearly outlined bloating of mitochondria. Parallel to lengthening of the conservation term, the changes become intenser in both directions referred to above, reaching their maximum in the material preserved for a duration of 14 days. At the latter stage, a particularly strongly manifested and intense reaction is observed in the cells of the epidermis and connective tissue, smooth muscles and transversely striated muscular fibers. In all the elements listed above, the mitochondria are strongly bloated. At conservation term lasting for two months, the intensity of reaction is maintained at the same level (Diagram 1-a).

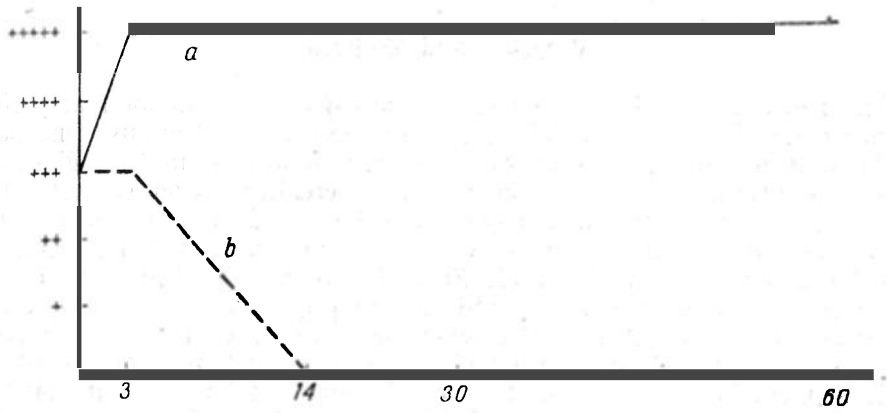

Diagram 1

C. Skin, conserved through freezing and preliminary treatment with 15% glycerol. At 3 days from the conservation no changes in the reaction intensity are established in the skin treated in advance with glycerol, as

compared to the control of non-conserved skin. In instances of rather prolonged conservation of the material, the succinic dehydrogenase activity is strongly reduced, and at 14 days, it is fully negative in all the cells of the epidermis and dermis (Diagram 1-b). The positive reaction persists only at places in the transversely striated musculature.

Discussion

The localization of succinic dehydrogenase activity in the cells of the epidermis and dermis complies with the data reported by other authors (4, 8, 9, 10, 14). Our results, obtained in the course of histological demonstration of this particular enzyme in the fresh skin, are in accordance with Walker's concept (15) stating that the intensity of the histochemical reaction depends largely upon the concentration of mitochondria. Bearing in mind the biochemical, cytochemical and electron microscopic data, published hitherto by a number of investigators (2, 3, 5, 6, 11, 12, 13), it is assumed that the pattern of distribution and localization of succinic dehydrogenase activity in the skin, outlined in the present study, corresponds fully to the picture of mitochondria in its cells. The pink coloration of mastocytes recorded, in our opinion, is due to the fall of enzymic activity. According to some authors (7, 8, 10), the pink staining of the cells during work with Nitro BT is due to the reduced enzyme activity and, moreover, instead of blue, red tinged monoformasan is obtained because of the reduction of one end of the ditetrasol molecule only.

The rise in intensity of the cytochemical reaction for the demonstration of succinic dehydrogenase and the bloating of mitochondria, observed in skin conservation through freezing without previous treatment with glycerol, might be explained by the deleterious effect of freezing and thawing on the mitochondrial membranes. This gives us sufficient reason to state that conservation through freezing leads to structural disorders in the mitochondria. The steadily rising intensity of the reaction up to the 14th day from the beginning of conservation shows that the changes taking place bear a progressive character. In all likelihood, this is due to the preservation of the material at moderately low temperatures (—20° C), which is equivalent to a slow-rate thawing. It is a well known fact that freezing proper does not constitute a critical moment for the cell so much, as slow thawing which leads to the so-called migrating precrystallization, occurring at temperature about —50° C. In addition, attention should be called to other factors, exerting harmful effect on the cells in the process of freezing. On the first place, the possibility should be mentioned of increasing the concentration of electrolytes in the cells as a result of crystallization of the water and its differentiation into a separate phase — a process leading to the osmotic shock (1). The osmotic shock, in turn, causes changes in the pH of the medium (6) to which mitochondria are particulary sensitive (3, 6).

As regards the action of glycerol, our observations show that in its presence, the succinic dehydrogenase activity of skin does not rise, and simi-

larly, no increase of mitochondria is established. Nevertheless, the lowered intensity of the histochemical reaction in the later stages demonstrates that preliminary treatment of the material with glycerol fails to protect the skin from changes resulting in loss of succinic dehydrogenase activity. Most probably, glycerol exerts a dehydrating effect on the cells which is deleterious also to the mitochondria and their ferment system.

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ВЛИЯНИЕ ПРЕДВАРИТЕЛЬНОЙ ОБРАБОТКИ ГЛИЦЕРИНОМ НА СУКЦИНДЕГИДРОГЕНАЗНУЮ АКТИВНОСТЬ КОЖИ КОНСЕРВИРОВАННОЙ ПУТЕМ ЗАМОРАЖИВАНИЯ

С. А. Ванкова

РЕЗЮМЕ

Исследовали гистохимическим путем сукциндегидрогеназную активность кожи крысы, консервированной в течение различных периодов времени при помощи замораживания после обработки материала 15%-ным глицерином. Полученные результаты сравнили с контролем — свежей кожей и кожей, замороженной без предварительной обработки глицерином. Установили понижение интенсивности реакции и полное ее прекращение в последних сроках консервирования.