# AUTORADIOGRAPHIC INVESTIGATION OF METHIONINE $S^{35}$ INCLUSION IN VARIOUS METHODS OF SKIN CONSERVATION

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In the past several decades the interest in problems related to organ transplantation has been steadily increasing. In close relationship with the latter problem, also various issues of organ conservation and tissue bank creation have been further developed under the guidance of research studies on the possibilities of preserving their biological structures and metabolism under different conditions of conservation. Autoradiography is a method enabling the investigation of the various aspects of metabolic processes in the cells and tissues and their significance for the respective structures. In the latter studies, both inorganic and organic substances are employed, marked with radioactive isotopes. The incorporation of radioactive sulfur labelled methionine in the cells and tissues is considered by the investigators in this field as an indicator of protein synthesis and, more particularly, as indicator of the exchange of sulfur-containing proteins (2, 6, 8).

The purpose of the present work is to investigate through autoradiography the inclusion of methionine $S^{35}$ in skin, conserved according to a variety of methods.

A number of authors have studied the inclusion of this isotope in the normal skin of various animals (1, 3, 4, 6). In the literature surveyed no reference was found to studies on skin and other organs, conserved for transplantation purposes.

## Material and Methods

Conservation was performed on material comprising 20 male albino rats, weighing between 140 and 280 g and distributed into five groups of 4 animals each. The abdominal skin of the animals from the first four groups was conserved according to one of three methods: in physiological solution with 200 000 U penicillin per liter at temperature 0—4° C; in ready made hydrolysate conserving solution (13), similarly at temperature 0—4° C; through freezing at —79° C and subsequent preservation at —20° C. The skin of the animals from group I was conserved for a two-month interval, group II — for one month, group III — for fifteen days and group IV — four days; the material from the fifth group was not subjected to conservation — it served as control. Conservation was carried out in such a manner that it terminated for all the objects in one and the same day. Pieces measuring about 2 $mm^2$ were obtained from all conserved objects as well as

from the 4 control animals, and were incubated «in vitro» in Krebs—Ringer's phosphate solution, supplemented by methionine $S^{35}$ at total activity 1 μ c per milliliter. Each piece underwent separate incubation in 2 ml of the solution at temperature 37° C and constant stirring over a period of one hour. After incubation, the pieces were washed out for 2 minutes in 5 ml Krebs—Ringer's phosphate solution without isotope and fixed for two hours in alcohol/acetic acid at 3:1 ratio, and thereafter embedded in paraffin and cut with microtome at thickness of the sections 8 microns. To provide for absolute equality and comparability in further processing, on one and the same object glass samples of the three types of conservation were fixed for equal periods of time. After removal from the paraffin, gelatin sublayer was applied on the sections, and after drying, the specimens were covered with liquid photographic emulsion, type P. The preparations thus treated were exposed to 0° temperature for ten days and following development and fixation, they were stained with hematoxylin and eosin.

## Results

A. Fresh, non-conserved skin. In the control autoradiograms from non-conserved skin of rat, methionine $S^{35}$ incorporation is established mainly in the epidermis and epithelial vaginae of the hairs. In the subcutaneous connective tissue and in the transversely striated muscles the inclusion is much less pronounced (Fig. 1).

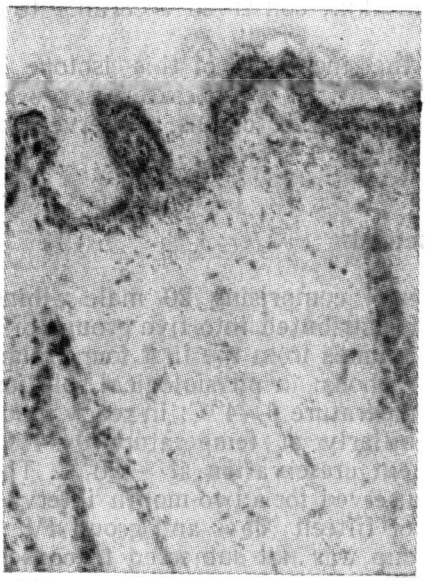

Fig. 1

Fig. 2

Fig. 3

Fig. 4

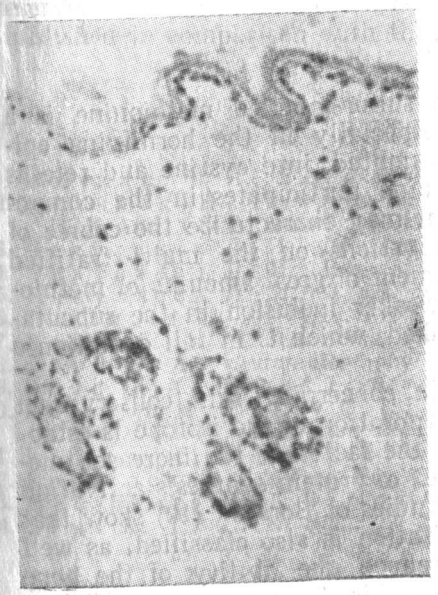

Fig. 5

B. Skin conserved through freezing at —79° C (Fig. 2). The autoradiograms obtained show that the blackening of the emulsion overneath the epidermis and hair follicles in the skin preserved in this manner is much more intensive than in the fresh, non-conserved skin. The incorporation of the isotope in the connective tissue and in the subcutaneous transversely striated musculature does not differ from that in the fresh skin. The autoradiograms obtained from skin conserved for various periods of time show no difference between each other in terms of degree of blackening of the emulsion.

C. Skin conserved in physiological solution at 0 — 4° C. (Fig. 3) With this particular method of skin conservation, incorporation of the isotope is noted at four days, showing no difference whatsoever from that in fresh skin. In 15-day long conservation at identical conditions, the blackening of the emulsion overlying the epidermis and hair follicles, as well as overneath the dermis is much weaker. (Fig. 4) In the autoradiograms from skin preserved for 1 and 2 months, no inclusion of the isotope whatsoever is established in the cellular and tissue elements of the skin.

D. Skin conserved in ready prepared hydrolysate solution at 0 — 4° C. The results regarding methionine $S^{35}$ incorporation in the skin, conserved in this manner, do not differ from those obtained during conservation in physiological solution. At 4 days, the blackening of the emulsion over any of the skin elements is the same as in the control; at 15 days it is much weaker and following 1 and 2 months preservation, it is completely absent. (Fig.5).

## Discussion

According to Zsinkin (2), radioactive sulfur-labelled methionine is a convenient protein synthesis indicator, especially in the hornifying epithelium, owing to the fact that it is transmuted into cystine and retains the sulfuric atom. Furthermore, methionine participates in the composition of most of the proteins and thus, it may characterize the course of the total protein metabolism. In investigations on the multi-stratified flat hornifying epithelium (3, 11), ammassment of great amounts of methionine in the epidermis and considerably weaker inclusion in the subcutaneous connective tissue have been established, which is in full compliance with the results obtained by us.

In the interpretation of personal data concerning skin conservation through freezing, in which intensified incorporation of the isotope is established, one should take into consideration the fact that the increased methionine $S^{35}$ uptake in the tissues is an index of protein synthesis activation (2, 4, 6, 11, 14). On the other hand, the studies of Borsook (10) show that the cytolytic agents among which lyophilization is also classified, as well as freezing and thawing, considerably reduce the ability of the bone marrow cells to incorporate tagged amino acids. This gives us sufficient ground to assume that the more intensive incorporation of radioactive sulfur tagged methionine in the skin, conserved through freezing, is not due to intensification of the metabolite processes in the same. Hence attention should be called to the works of Boyd and Board (9), Board (7)

demonstrating that in many tissues in contact with photographic emulsion, a latent image is obtained at the absence of radioactive elements. Board (7) is in the opinion that such images are to be attributed to the presence of glutathione and cysteine and, more particularly, to their sulfhydril radicals, which are strongly reducing agents. Bearing in mind that freezing might readily lead to protein splitting (12), the assumption is warranted that during skin conservation through freezing, a non-specific blackening of the emulsion occurs, due to the rise of the sulfhydril radicals' level. The latter is corroborated also by our histochemical investigations of sulfhydril groups in skin conserved through freezing, where a much stronger reaction occurs than in fresh skin (studies yet unpublished).

As regards methionine $S^{35}$ inclusion during conservation in solutions, our results show that the rather prolonged preservation under similar conditions leads to a weakening to full loss of the ability of cells to incorporate the isotope. The latter fact might be interpreted as a reduction of their metabolite capacities. Here too, it might be accepted that the level of sulfhydril groups rises subsequent to the occurrence of autolytic processes, resulting in splitting of sulfur containing proteins, which, however, does not account for a stronger blackening of the emulsion, owing to the transfer of the products obtained into the surrounding preserving solution. The latter phenomenon is confirmed by the results obtained by the authors of the paper during histochemical study of the sulfhydril groups in skin preserved in solutions, where a reduction of the intensity of the reaction is established in comparison with that in fresh, non-conserved skin.

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## АВТОРАДИОГРАФИЧЕСКОЕ ИССЛЕДОВАНИЕ ВКЛЮЧЕНИЯ МЕТИОНИНА $S^{35}$ ПРИ КОНСЕРВИРОВАНИИ КОЖИ РАЗНЫМИ СПОСОБАМИ

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РЕЗЮМЕ

При помощи авторадиографии исследовали включение метионина, меченного радиоактивной серой, в кожу крысы, консервированную в течение различных периодов времени путем замораживания и в растворах. При консервировании путем замораживания установили усиленное почернение фотографической эмульсии по сравнению с неконсервированной кожей, что толкуют как неспецифический образ, обусловленный присутствием сульфгидрильных радикалов. В коже, консервированной в растворах, наблюдалсь сниженное включение изотопа, обусловленное нарушением обменных процесоов в клеточных элементах кожи во время консервирования.