HOMOPLASTIC TRANSPLANTATION OF SKIN TREATED WITH WEAK SOLUTIONS OF FORMALIN

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UDC 612.79:612.6.02.017.1].014.46:
615.28:547.281.1

Treatment of skin with weak solutions of formalin reduces its content of water-soluble proteins. Rejection of the formalinized homograft in dogs occurs later than after transplantation of skin not treated with formalin. After repeated grafting of formalinized skin on guinea pigs and rabbits, quickening of its rejection was not observed.

True survival of skin has not yet been obtained clinically after homoplastic transplantation.

However, for clinical requirements and, in particular, for the treatment of patients with burns, not only the true survival of a skin graft, but even the temporary covering of skin defects is of considerable importance. For this reason the prolongation of times of temporary survival of skin following homografting is currently important.

An acceptable method which can be used to prolong the survival of skin following homoplastic transplantation onto patients is treatment of the grafts by substances reducing its antigenic activity, and thus minimizing the rejection response. Billingham and co-workers [10], Hasek [9], and Kahan [11] isolated water-soluble protein fractions, possessing the properties of transplantation antigens, from splenic tissue. Since the rejection reaction arises as a result of immunization of the recipient with substances originating from the graft, it can be assumed that, if the liberation of water-soluble proteins from a skin graft is prevented, the activity of the transplantation-immune response will be reduced, and the period of survival of the graft will be correspondingly prolonged.

One method which can be used to reduce the liberation of water-soluble proteins from a graft is by increasing the size of the protein molecules. As Walker [12] and Fel'dman [7] have shown, formaldehyde has the property of reacting with certain groups of the protein molecule and of forming methylene bridges between them. This leads to an increase in the molecular weight and a decrease in the solubility of the protein.

Grafts of formalinized skin have been used by several workers in experiments on rabbits [1, 3, 4, 5]. To treat the skin they used formalin solutions of high concentrations (2-10%). No appreciable increase in the survival periods of the formalinized skin homografts were obtained.

Rozvadovskii [6], Dmitrienko, [2], and Eingorn and co-workers [8] carried out homoplastic transplantation of bone treated with weak solutions (0.5-1%) of formalin and obtained much better results than after homografting bone conserved by other methods. As these workers showed, morphological evidence of a reaction of incompatibility to the formalinized bone homograft was absent. The reason for the weak rejection reaction to the formalinized bone homografting may have been that formalin converts water-soluble transplantation antigens into a water-insoluble state, thus reducing the possibility of their liberation into the recipient's body.

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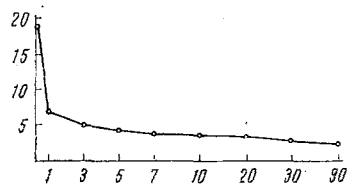

Fig. 1. Changes in protein content in skin in relation to period of keeping in 0.5% formalin solution. Abscissa, duration of treatment of skin with formalin (in days); ordinate, protein content (in mg/g).

For the reasons described above it was decided to determine the effect of treating skin with weak solutions of formalin on the survival period following homografting and on the quantitative content of water-soluble proteins in the skin grafts.

EXPERIMENTAL METHOD

To determine the content of water-soluble proteins, the skin was cut into small pieces with scissors, ground in a mortar with quartz sand, and treated with physiological saline in the ratio 1:2. The homogenate was allowed to stand for 24 h and then centrifuged for 30 min at 6000 rpm.

Protein in the supernatant was determined by the micro-Kjeldahl method. The results showed that after 24 h the content of water-soluble proteins in the skin, when preserved in 0.5% formalin solution, was reduced to one-third of its initial level. Later the content of water-soluble proteins in the skin continued to decrease, but much more slowly, reaching 17% on the 10th day, 11% on the 30th day, and only 7.8% of its initial level on the 90th day (Fig. 1).

To determine the times of rejection of the formalinized skin, experiments were carried out on 15 dogs which were homografted with skin preserved in 0.5% formalin solution for periods of between 20 days and 3 months.

The animals were anesthetized, a full-thickness skin graft was removed from the lateral surface of the chest, measuring 10 × 10 cm, and the resulting defect was replaced with formalinized skin. As a control, a fresh skin homograft of the same size, not treated with formalin, was transplanted to the other side of the chest. Before transplantation, the formalinized skin was washed 3 times for 2 h in several changes of physiological saline in order to remove the formalin.

EXPERIMENTAL RESULTS

The first signs of rejection of the formalinized homograft were observed after 11.8 ± 3.8 days. Individual areas of condensation and changes in the color of the grafted skin appeared. Complete rejection of the graft occurred after 19 ± 4.5 days. In the control the first signs of rejection were observed after 6.8 ± 1.3 days, and complete rejection took place after 10 ± 1.9 days.

The period of survival of the formalinized skin homograft was thus on the average twice as long as the survival period of skin not treated with formalin. Statistical analysis by Student's method showed that differences between the times of survival of fresh and formalinized skin grafts were significant ($P < 0.01$).

In all cases, the fresh homograft characteristically disturbed the animals much more than the formalinized graft. As a rule, on the 6th-7th day after transplantation, the dog bit off the dressing over the graft of fresh skin. Active destruction of the graft could then be seen, in the form of infiltration and suppurative liquefaction of the grafted skin.

Rejection of the formalinized graft took place with much less disturbance. In these experiments, the animal apparently withstood the grafting procedure better. The dog made no attempt to bite off the dressing, and after its removal it did not lick off the graft as was observed in the animals with a fresh skin graft. Usually the individual areas of condensation which appeared gradually spread throughout the graft, after which it was rejected as a continuous scab, under which a clean granulating surface was exposed.

In the next series of experiments the rate of rejection of retransplanted skin homografts treated with formalin (the second set reaction) was investigated. Three series of experiments were performed on guinea pigs (35 animals) and two series of experiments on rabbits (12 animals). Complete rejection of the fresh primary homograft by the guinea pigs occurred on the average after 10.6 days, and by the rabbits after 14 days. Rejection of the formalinized homograft by the guinea pigs occurred after 13.6 days, and by the rabbits after 14.6 days. In the case of regrafting, fresh homografts were rejected by guinea pigs on the average after 6 days, and by rabbits after 6.6 days. Formalinized skin regrafted on guinea pigs was rejected after 13.4 days, and on rabbits after 14 days.

These results show that following transplantation of a formalinized homograft the second set reaction does not develop, and they suggest that formalinized skin can be used for homoplastic transplantation under clinical conditions.

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