SURVIVAL OF SKIN PRESERVED IN WEAK FORMALIN SOLUTIONS

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It has recently been reported [2, 4] that if an amputated ear of a rabbit is immersed for 48 h in a 2-20% solution of neutral formalin at 2-4°, the skin of the concha auriculi remains viable.

In a paper on the implantation of formalin-preserved skin [1], the authors described experiments on preservation of rabbit's skin in 2-3-8% formalin in Ringer's solution at 2-3° for 48 h. Judging by the results of the experiments, skin flaps treated in this way and then washed with cold Ringer's solution to remove formalin were dead in every case. The attempts made by P. M. Medvedev, L. S. Priezzhaeva, and V. P. Teodorovich [3] to transplant skin preserved in 2-5-10% formalin solutions likewise were unsuccessful.

In the present study the authors attempted to find a concentration of the formalin solution which would act, not as a fixing agent, but as a preservative.

EXPERIMENTAL METHOD

Two series of experiments were conducted. The technique of the authors cited above was used, but the formalin solution was weaker. In one series of experiments the amputated ears of the rabbits were kept for 48 h at 2-4° in a 1% formalin solution, and in the other series, in a 0.5% solution. For the next 4 days the ears were washed in physiological saline, cooled to the same temperature and changed daily. Next, pieces measuring 6 × 8 mm were cut from the skin on the outer side of the concha auriculae, and implanted beneath the skin of the contralateral ear of the same or different rabbits. The pieces of implanted skin were introduced under the skin of the outer side of the concha auriculae, not far from its root. Grafts from 20 rabbits were studied. Half the grafts were investigated 8 days after implantation, the remainder 16 days after. Sections were stained with hematoxylin-eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

The results of the histological study of the grafts showed that three of those treated with 0.5% formalin solution and one of those treated with 1% formalin were alive. It can definitely be accepted that all these grafts were alive when implanted, bearing in mind their condition on the day of fixation.

The picture of the implant preliminarily kept in a 1% formalin solution was especially demonstrative (Fig. 1). It was fixed 16 days after implantation, by which time the epidermis of the graft, having grown outside its borders, had formed a closed cyst. For most of its thickness the implant was densely infiltrated by the host's wandering cells and only the top quarter of the dermis was relatively free from them. The epidermis showed reactive thickening in response to inflammatory changes in its underlying dermis. Whereas the thickness of the epidermis in the rabbit's skin varied around 16 μ and it consisted of only two or three layers of cells, the thickness of the epidermis of this particular implant was on the average 40 μ and it consisted of five or six layers of cells. Irritation of the epidermis was also demonstrated by the uneveness of its border with the underlying connective tissue; small areas of downward penetration into the connective tissue could be seen. The epithelial root sheaths were thickened to the same degree. The epithelium of the cyst varied in thickness from 48 to 120 μ. Its thick layer was well differentiated into strata: basale, spinosum, granulare, and corneum. Beneath it lay the young, newly-formed connective tissue of the host, the apparently thickened continuation of the capsule formed around the implant. Among the numerous fibroblasts lying parallel to the epidermal lining of the cyst, equally numerous wandering cells were visible. Here and there the epithelium of the cyst invaded the underlying connective tissue. The cyst wall resembled young regenerating skin. It is interesting that in this case the developing skin had a dual origin: its epidermis was obtained from the donor and its connective tissue from the recipient.

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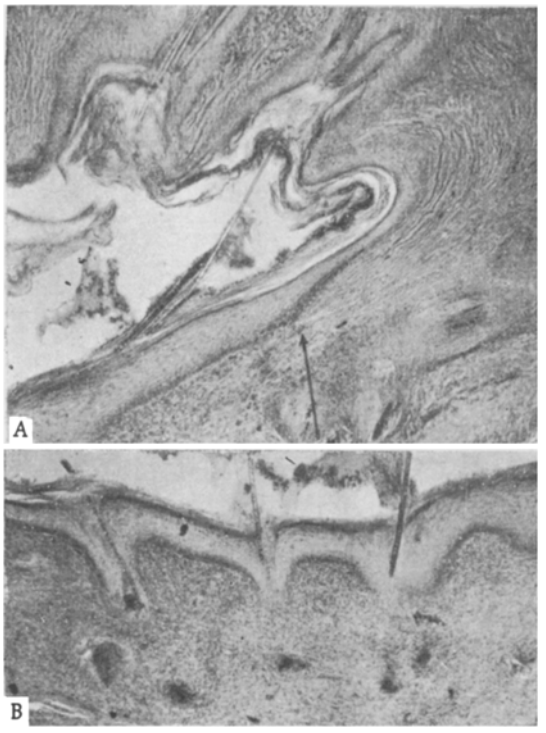

Fig. 1. Section through 16-day implant. Skin before grafting was preserved in 1% formalin solution. A) Implant and epidermis growing beyond its borders (indicated by an arrow); B) wall of a cyst formed by the proliferating epidermis of the implant and the newly formed connective tissue of the host. Photomicrograph. Objective 10×, Gamal P.

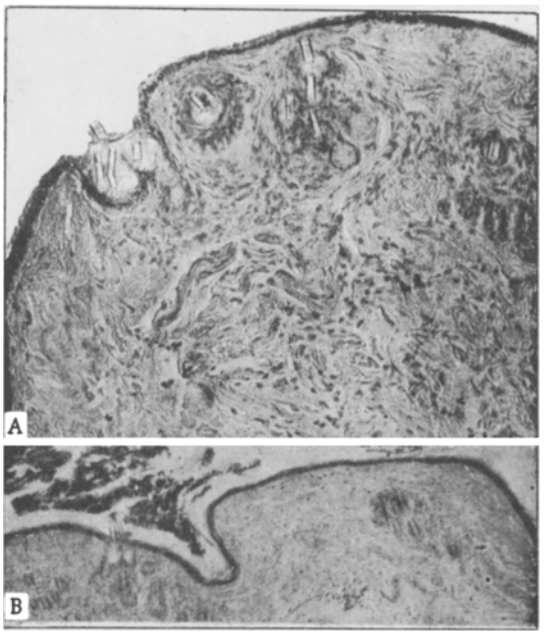

Fig. 2. Section through a 16-day implant. Skin before grafting was preserved in 1% formalin solution. Photomicrograph. Objective 20× (A), 10× (B). Gamal P.

One of the implants treated preliminarily with 0.5% formalin solution was alive at the time of taking the biopsy specimen. In this implant, fixed 8 days after grafting, the epidermis also was thickened, in some places to 32 μ, and it consisted of four or five rows of cells. The epithelial root sheaths also were thickened. On both sides of the graft small tongues grew out from the cut ends of the epidermis. One of these tongues was 320 μ long and it consisted of a uniform sheet of epithelium composed of three or four layers of cells, 20 μ thick. This sheet of cells was differentiated into stratum germinativum and stratum corneum. On the opposite side of the implant the tongue was less well developed and its length was no more than 100 μ. This implant was only comparatively slightly infiltrated by the recipient's wandering cells.

The histological picture of two other implants, previously kept in 0.5% formalin solution, showed that they were alive at the moment of grafting. They were fixed 16 days after grafting, and by this time they were more or less dead. The tongues formed by the implants were also dead. The epithelium of the implants, mostly desquamated and infiltrated by wandering cells, in places was considerably thickened (to 60 μ).

The remaining implants had the appearance of well fixed skin (Fig. 2) and, an interesting point, they were hardly infiltrated at all by the host's leukocytes. A dense mass of wandering cells was seen only around the implant. The absence of infiltration in the thickness of these implants must evidently be attributed to the fact that the tissues fixed with formalin had not yet begun to be destroyed during the period of the experiment, and their disintegration products, capable of attracting the recipient's leukocytes, had not begun to enter the surrounding zone.

It is difficult to explain the fact that only a very small proportion of the implanted pieces of skin, preserved in weak formalin solutions, was viable. However, the authors are convinced that the tissues of the skin, preserved for 48 h in a cold 0.5-1% solution of formalin, can survive. Survival is more frequent in the case of pieces treated with the 0.5% solution.

SUMMARY

Amputated ears of a rabbit were preserved in 0.5 and 1% formaldehyde solutions for two 24-hour days at +2-+4°C. Then they were kept for washing in cool physiological saline for 4 days. Pieces of skin 6-8 mm in size taken from the outer ear surface were implanted subcutaneously to the contralateral ear of the same or other rabbits.

The implants were fixed in 8 or 16 days. Histologic study showed that the greater part of the implants looked like fixated skin. They were but little infiltrated by the host's migratory cells. However, 3 pieces preserved in 0.5% formaldehyde solution and 1 piece in 1% solution were alive. Reactive thickening of the epidermis was clearly observed in these pieces as well as its growth beyond the limits of the implants.

The implants were infiltrated by the recipient's leukocytes. Thus, the experiments showed that the tissues of the skin preserved in 0.5-1% cool formaldehyde solution for 2 days may remain alive.

LITERATURE CITED

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