# THE USE OF FLUID MEDIA FOR THE PRESERVATION OF TISSUES

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In recent years, grafting of preserved tissues has been widely used in plastic surgery.

Transplantation of fresh or preserved tissues is also used in many biological problems [4, 8]. At present, in homografts, a number of tissues which have been kept in various ways are used [2, 3, 5, 9, 10, 11, 12, 14, 15].

The most widespread of the present day methods of preservation is to freeze at low temperatures, and to dry while the tissue is frozen [1, 6, 7, 16]. However, these methods by no means always allow tissue to be preserved in a viable condition. It is known that at low temperatures many tissues lose their viability as a result of physicochemical changes within the cells [13]. When bone or cartilage is freeze-dried, the cells cease to be viable soon after they have been removed. When such tissues are grafted, they merely play the part of a framework on which the new living bone or cartilage is laid down, and their conservation therefore does not present any great difficulty. The preservation of soft tissues is a more complex problem.

It is known that skin whose viability has been lost in preservation plays merely the part of a biological bandage when used as a homograft. It is quite evident that one of the principal requirements to be fulfilled in preservation is to preserve the life of the tissues.

Also, the method of freeze-drying requires a special quite complex apparatus, which is not always available.

The use of liquid media for conservation requires that the tissue shall remain alive only for a short time. Reliable, effective, and simple methods of conservation must then be sought.

A necessary condition for conservation of bone marrow used in the treatment of blood diseases and radiation sickness is that it should retain its vitality. It is important to observe this condition also when skin is preserved, because the most striking therapeutic effect makes use of the vital properties of the transplant.

In the work reported here we have made a comparative evaluation of the vitality of skin fragments preserved in various fluid media.

As media for preservation we have used Hanks complete nutritive medium 199, Belyakov's cold-resistant solutions, physiological saline, and Tyrode's solution with added antibiotics.

The cutaneous fragments were preserved in a refrigerator at 3-5° in the solutions mentioned above. Initial experiments showed that the most reliable preservative was nutritive medium 199, consisting of a number of principal amino acids vitamins, hormones, salts, and sugar. It therefore contains substances necessary for the preservation of the vital activity of the cells under conditions of reduced metabolic activity. On this account we have used medium 199 for the preservation of skin and bone marrow.

The preservation was carried out as follows. Cutaneous fragments from the outer surface of the ear, and bone marrow were taken from rabbits under sterile conditions, and the samples were placed in glass flasks containing medium 199 to which 10% donor serum and 150-200 units per ml of medium had been added. The amount of nutritive medium for skin was adjusted to represent 1 ml per $cm^2$ of skin, or nine times the volume of the bone marrow. The flasks were hermetically sealed, and kept in a refrigerator at 3-5°.

The viability of the cutaneous fragments preserved under these conditions was determined by the extent of healing which took place when they were used as autografts, and from the growth of the cutaneous explants into a tissue culture. We also studied the morphological changes occurring in the skin during its preservation.

A supravital stain with a 1% aqueous solution of eosin, luminescence microscopy, and tissue cultures were used to determine the viability of the bone marrow.

Altogether 54 rabbits were kept under observation, and they received full thickness skin grafts preserved in medium 199 for 2-6 weeks. At the end of these times, the preserved skin fragments measuring 2.5 × 3 cm were transplanted onto the ear of the same rabbit. Fragments preserved in medium 199 retained their normal color and elasticity. As a rule the epidermis was firmly connected to the underlying tissues. When the tissues were kept for a long

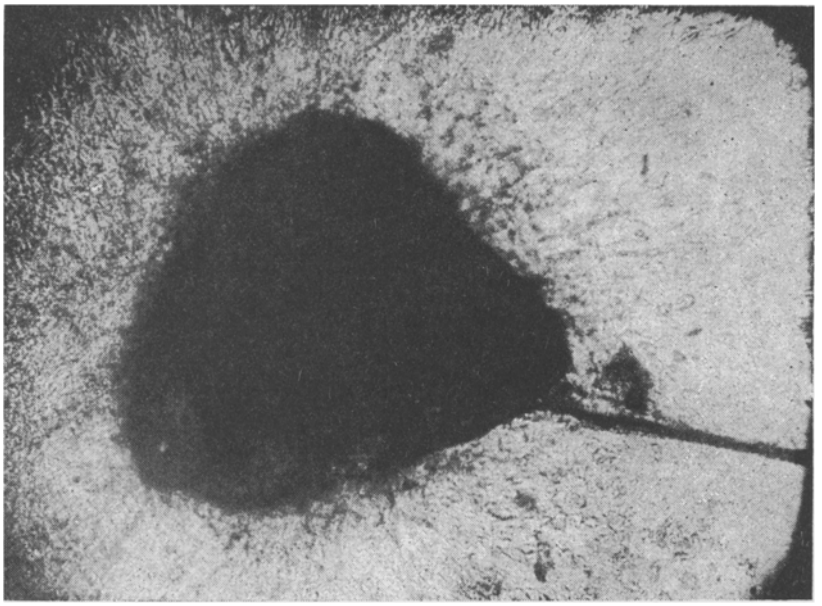


Fig. 1. Three-day tissue culture (rabbit skin), which had previously been preserved in medium 199 for 30 days.

time, the hair in the fragments showed growth. In no single case was there any swelling or desquamation of the epidermis, such as takes place when skin fragments are preserved in other fluids. In the great majority of cases, the cutaneous fragments preserved in medium 199 for times up to six weeks became firmly established. After they had been grafted, the fragments were mobile and elastic, they had a good blood supply, and fur grew from them. The grafts remained alive during the whole six-month observation period.

The vitality of different kinds of preserved skin, taken either from rabbits, from human corpses, or from patients suffering from burns, and kept for times up to 45 days, was tested in tissue culture. In most cases, zones of epithelial and connective-tissue growth were observed.

Figure 1 shows a typical growth of newly-formed epithelial cells of an explant taken from a fragment of rabbit skin which had been preserved for 30 days.

It was established histologically that the structure of the skin had been maintained completely during preservation of from 2 to 6 weeks; the cell nuclei and all the layers of the epidermis stained well with hematoxylin, and the cutaneous structures had been preserved. The dermis showed a moderate but unimportant swelling and some loss of collagenous fibers, but the connective tissue cells were not changed. In no single case was there any desquamation of the epidermic. After the 35th day of preservation, in certain cells there was some pyknosis of the true dermis.

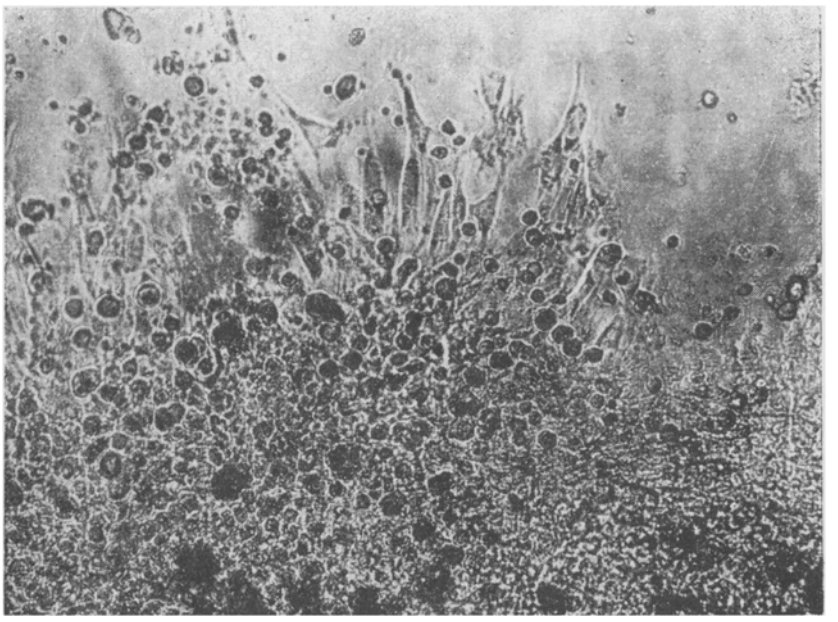
Phenol was used in medium 199 as an indicator, which gave the fluid a pink color. During preservation, the color of the solution changes. If the tissue is viable, the medium becomes more acid with time, and takes a yellow color.

With this method of preservation, by the end of the 6th week, the medium becomes impoverished and acidified. The appearance and properties of the graft do not alter, even when kept for more than two months. Although the skin fragments take when grafted, some degenerative changes in the epidermal layer may be observed. Therefore,

in our view, six weeks of preservation is the limit, without a change of medium. At the moment we are experimenting on prolonging the times of preservation in medium 199 by renewing it.

The viability and state of preservation of the stored bone marrow was listed by staining with 1% aqueous eosin and 1:1000 acridine orange, daily from the 1st to the 15th day, and by tissue culture.

It has been shown that when preserved in medium 199 at 3-5° for five days, up to 80-85% of bone marrow fragments remained viable, when preserved for 10 days the figure was 60-65%, and for 15 days it was 43%. When a tissue culture was made from preserved bone marrow, after these times extensive growth around the explants was observed (Fig. 2).



Three-day tissue culture (rabbit bone marrow) previously preserved in medium 199 for 15 days.

During preservation morphological changes of the bone marrow take place, and are associated with the degeneration and necrosis of the less robust cellular elements. The most resistant portions are the nucleated cells of the red-cell series, and lymphocytes. Under certain cases, as was demonstrated by supravital staining with a 1% aqueous solution of eosin, or by luminescence microscopy, the tissues remain viable for 4-5 months.

Therefore, the method of preservation in medium 199 which we have studied has many advantages over other methods of preservation in fluid media. It does not require any special expensive apparatus, it enables the vitality of the tissues to be maintained for a comparately long time, so that it may be used in regional and district hospitals when collecting skin or bone marrow from cadavers.

This method holds out good prospects for the further determination of the optimal conditions for the preservation of the vitality of bone marrow cells.

## SUMMARY

The viability of skin and bone marrow was determined during preservation in various fluid media at temperatures ranging from 3 to 5°. Physiological saline, Tyrode's solution, Belyakov's solution at 31$^{E}$, Hanks' solution, and medium 199 were used for the storage of skin and bone marrow. Tests of the viability of skin and bone marrow fragments preserved by these methods proved medium 199 to be the best preservative. Storage of rabbit and human skin graft in medium 199 to which 10% donor serum and antibiotics had been added enabled viability to be preserved for six weeks, as was confirmed by the prolonged taking of the autografts, as well as by the growth of skin explants in tissue culture. Preliminary observations have shown that the bone marrow cells preserved in medium 199 retained their viability for up to two weeks.

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**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.**

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