fluids should be made horizontally, *i.e.*, without restricting the availability of atmospheric oxygen.

A study by the surgical staff of the Children's Hospital of the preservation of aortic segments for surgical repair of large blood vessels has produced results in accordance with these principles. Their publication[17] describes the conditions under which blood vessels may be maintained in 10% homologous serum for as long as 50 days.

In conclusion, 10% serum is superior to mineral oil as a refrigeration menstruum because it provides nutrient and dilutes or buffers the acids which result from metabolism. At 6-8° oxygen is required to support metabolism, while at 0° viability is extended by completely filling the storage tubes with liquid or by covering the tissue with a blood coagulum.

[17] Peirce, E. C., Gross, R. E., Bill, A. H., and Merrill, K., *Ann. Surg.*, 1949, **129**, 333.

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17132. Sterilization of Skin.

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It is frequently stated that in the strict bacteriological sense it is impossible to sterilize skin. This concept apparently arises from the use of methods which do not insure that an appropriate disinfectant is applied in active form for an adequate interval of time. Since the rich and complex nutrients used for cell cultivation provide an excellent pabulum for a variety of microorganisms, long experience in sterilization of leprous and normal skin prior to biopsy for tissue culture induces us to present evidence that skin sterilization is not a difficult problem.

Early attempts to sterilize well scrubbed skin by painting with tincture of iodine were at times successful and at times failures. The inconstancy of the results suggested the desirability of controlling the action of iodine with respect to concentration and time of action. By covering the area to be biopsied with a heavy patch of fabric, dripping iodine solution (tincture of iodine 1:2 in 70% alcohol by weight) on this patch until it was saturated, and then removing the patch and recleaning with alcohol after an interval of 5 minutes, the occurrence of scattered contamination was terminated. During the next several years it was noted that epithelial cells rarely if ever migrated from fragments explanted from the papillary or uppermost layer of the skin, while abundant epithelial cells appeared around fragments explanted from the deeper or reticular layer of the skin.

Upon the first occasion when a biopsy was taken after painting the skin with 2% Mercurochrome in Duponol as a wetting agent, good epithelial growth around papillary explants appeared for the first time in our experience. Nevertheless, one after another of the culture tubes gave evidence of contamination by staphylococci until 40% of the cultures had been lost. The period of applying the iodine (1:2) patch was next shortened to 2 minutes, without trouble from contamination and with the result that the superficial layer of skin produced moderate to excellent epithelial migration.

In order to inquire whether other disinfectants might be as effective as dilute iodine under these conditions and possibly less inhibitory to epithelial migration *in vitro*, three strips of skin were removed from the same individual simultaneously, one having been prepared by a two minute dressing with tincture of iodine 1:2, the second with Zephiran 1:1000, and the third with 2% aqueous Mer-

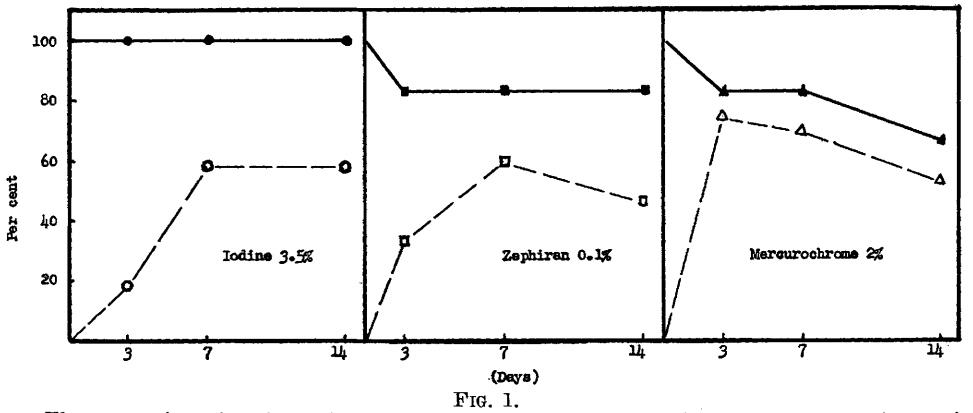

FIG. 1.
The proportion of explants free of contamination (solid symbols) and the proportion positive for epithelial cells (open symbols). Thirty-three cultures in each group.

curochrome. The results (illustrated in Fig. 1) were the usual ones after iodine patching, namely, no contamination, but appreciable inhibition of epithelial migration. With Zephiran 17% of the cultures were lost by contamination within the first 3 days, while migration of epithelium was not significantly improved. The Mercurochrome treatment was definitely less inhibitory to epithelial cells, but did not afford an increase in the number of useful cultures, due to a 33% loss by contamination. Since fibroblasts grew from the skin with equal readiness irrespective of the disinfecting agent, the dilute iodine produced the greatest proportion of positive and usable cell cultures.

Lest the aqueous solution of Mercurochrome might have given results poorer than those obtainable in the presence of a wetting agent, the effectiveness of Mercurochrome 2% in Duponol was compared against tincture of iodine diluted 1:4 in 70% alcohol, each being applied as a moist pack for 2 minutes prior to the biopsy of rat skin. During the first two weeks of cell cultivation *in vitro*, the incidence of contamination proved to be 20% from the Mercurochrome biopsy and 4% following the use of the 1:4 dilution of iodine.

Subsequent work with tincture of iodine 1:4 in 70% alcohol, with application for five minutes, indicates complete reliability, but that the same care must be exercised in its removal as in the case of the 1:2 solution. Further experience is required to evaluate the routine effectiveness of the 1:4 dilution during application for only two minutes.

Discussion. In Price's excellent report on disinfection of skin,[1] it is emphasized that the germicidal action of 7% iodine tincture practically ceases upon drying. It is shown that 2% of iodine in alcohol 70% by weight evaporates more slowly and avoids the objectionable irritating effects of the 7% tincture. This 2% solution was more effective following a single surface application than any other class of disinfectant studied. In other sections of the report the time factor is repeatedly emphasized. The results reported represent an application of the principles elucidated by Price.

It is not improbable that a mixture of penicillin, streptomycin and sulfadiazine for skin treatment, and in the cell culture media would provide a more appropriate measure of optimal epithelial migration than any of the disinfectants used. In view of the long period of cell cultivation required in leprosy research, this comparison has been impractical for two reasons: (a) the danger of inhibiting the infectious agent, and (b) the inability of such a mixture to inhibit the development of fungi from the skin explants.

[1] Price, P. B., *J.A.M.A.*, 1938, **111**, 1939.

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