Effect of Glutaraldehyde on Skin Grafts[1] (36918)

E. A. SANTIAGO-DELPIN,[2] E. J. YUNIS, C. O. CALLENDER,[2] AND J. S. NAJARIAN[3]

Departments of Surgery and Laboratory Medicine, University of Minnesota Hospitals, Minneapolis, Minnesota 55455

The ultimate goal of the transplantation biologist is to encounter methods and substances which will allow acceptance of the transplanted organ without crippling the host's general immune response. Glutaraldehyde, a commonly used tissue fixative, has been reported to prolong skin homografts if previously incubated in a dilute solution of this substance (1). It was postulated that the surface characteristics of the skin had been modified, rendering the graft less antigenic. The present report investigates the nature of this prolongation, as well as the viability of the graft.

Materials and Methods. Twenty-five percent aqueous glutaraldehyde was obtained from Curtin Company, Minneapolis. Phosphate-buffer saline (PBS) was utilized for all the dilutions of glutaraldehyde as well as for washing the grafts after incubation. A standard solution of glutaraldehyde in PBS (pH 7.38, 2.8 mg/ml) was prepared before each experiment. This is the optimal concentration recommended by Schecter (1).

Adult CBA mice were used as donors, and adult C3H or CBA mice as the recipients. Eight-millimeter grafts were fashioned from the donor tail skin. These were submerged for 20 min in a glass beaker holding 25 cc of either PBS alone, or the glutaraldehyde-PBS solution at room temperature. After incubation, the grafts were washed five times with PBS solution, and then implanted on the posterolateral chest wall of the recipient mice. Control grafts were incubated with PBS only. The technique of Billingham and Silvers was followed (2). After 10 days the plaster jackets were removed and the grafts observed daily. Rejection time was defined as the loss of 75–85% of the graft.

Two to three weeks after destruction of the isografts a second skin graft was performed on the opposite side, using the technique described above. After removal of the plaster, observations were again made until "rejection" of the second graft occurred. Both allogeneic and syngeneic grafts were done, with and without glutaraldehyde incubation. There were 16–20 mice in each first-set experiment, and 8–10 mice in each second-set group. Statistical analysis was done using a chi-square method for medians as described by Dixon (3). A 2×2 contingency table was used.

Results. Gross and microscopic patterns. Untreated allogeneic grafts looked normal upon removal of the plaster jacket. Grossly, they were minimally swollen, were fixed to their base, and had normal color and texture. In the allogeneic model, acute rejection was heralded by swelling of the graft, followed shortly by a dark patchy discoloration, dryness and necrosis of the graft. An elevated dry scab was the final lesion, and this eventually healed by leaving a small, shiny contracted scar in its place.

In contrast, all grafts pre-treated by incubation in glutaraldehyde looked abnormal from the start. For the first few days after removal of the plaster jacket, the graft looked swollen and moist, but shortly thereafter it became dry, scaly and hard, with the edges slightly curled upwards. All grafts were firmly adherent to their base. The exact rejection time was very hard to determine since changes were gradual. The color became lighter, the surface markings faded, and the graft started to contract, apparently by losing substance from the edges. Both allo-

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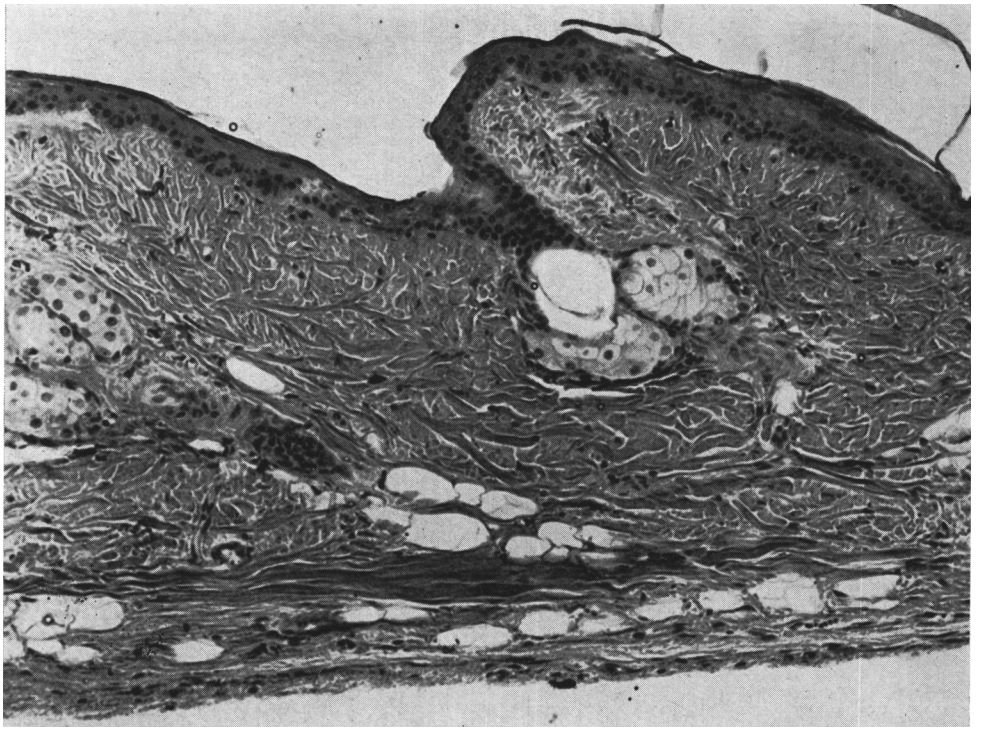

FIG. 1. Untreated isograft. Histology appears normal.

geneic and syngeneic glutaraldehyde-treated grafts looked grossly identical.

Untreated syngeneic grafts took permanently and looked normal from the start. Follow-up from 3–8 months failed to show any change in size or texture. Histological section through a normal untreated syngeneic graft at 2½ months is shown in Fig. 1. All the layers are well defined, skin appendages are present, round-cell infiltration is absent, and only the collagen fibers seem to be increased. In comparison, Fig. 2 shows a histological section through a typical glutaraldehyde-treated syngeneic graft at 2½ months, which did not show gross evidence of "rejection". In spite of this, the graft appeared thickened, with poor attachment to the base, with minimal cellularity, and no definable fibers or dermal appendages. The base shows moderate round-cell infiltration and mild fibroblastic invasion of the graft is seen, but no vascular invasion. Regenerating epithelium could be observed under the edges of the graft.

Graft survival data. Median survival time (MST) for the untreated allogeneic first-set reaction was 15.5 days. Glutaraldehyde incubation significantly prolonged the survival to 35 days ($X^2 = 24.06778$, $p < 0.001$). Untreated syngeneic grafts took permanently. Untreated syngeneic second grafts also took permanently. Glutaraldehyde-treated syngeneic grafts "rejected" at 95 days, and glu-

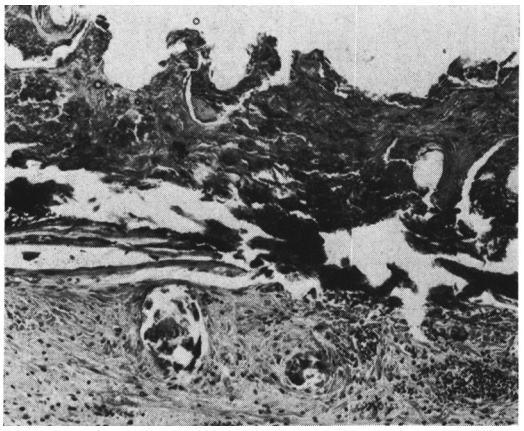

FIG. 2. Glutaraldehyde-treated isograft. Destroyed, bizarre skin histology.

TABLE I. Syngeneic Graft Survivals.

Syngeneic (CBA to CBA)	No glutaraldehyde (∞)[b]	—
	Glutaraldehyde incubation 95 (77–112)[a]	No glutaraldehyde (∞)[b]
		Glutaraldehyde incubation 90 (77–98)

[a] Median survival time (range in parentheses).
[b] ∞ Permanent takes.

taraldehyde-treated second grafts for this group also "rejected" at 3 months. (Table I) ($X^2 = 0.20168$, p = N.S.)

Discussion. These results confirm the fact that glutaraldehyde allows skin grafts to stay on longer. Our median survival time of 35 days corresponds to the average survival time of 39.2 days reported by Schecter (1). It should be emphasized though, that "rejection time" is read by loss of substance and diminution in the size of the graft, and that this is not a completely accurate way of determining graft death. Since hardening, loss of pliability, and slight pallor occur early in the course, those parameters are lost for determining rejection time. Caution is suggested in the interpretation of results involving glutaraldehyde-treated grafts.

The first-set syngeneic, glutaraldehyde-treated grafts are destroyed in 90 days, as are the secondary glutaraldehyde-treated grafts. No accelerated destruction was noted. This suggests that the destruction of the first graft is not immunologic. Further weight is given by the histologic findings. Hypocellularity, histologic disorganization, and specifically, absence of vascularity, in spite of a grossly "unrejected" graft suggest mummification rather than a living graft. In view of these results we question the role of glutaraldehyde in the prevention of rejection under the experimental conditions described.

Summary. Experiments were performed in order to investigate the mode of action of glutaraldehyde in the prolongation of skin grafts. 1. Glutaraldehyde-incubated grafts had prolonged survival (35 days) when compared to untreated controls (15.5 days). 2. Glutaraldehyde-treated isografts were destroyed at 95 days. 3. Glutaraldehyde-treated second isografts were also destroyed at 3 months. 4. Histologic examination of glutaraldehyde-treated grafts showed a bizarre, poorly vascularized, poorly defined histology, probably incompatible with viability.

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