TABLE II. Rejection of Skin Homografts in Normal Adult Guinea Pigs Possessing or Lacking Delayed Type Iso-hypersensitivity (IH) and Serum Factor (SF).

| Donor | | Recipient | | | |
|---|---|---|---|---|---|
| IH | SF | IH | SF | No. grafts | Graft survival (days) |
| + | — | — | + | 4 | 13 |
| + | — | + | — | 11 | 13–15 |
| — | + | — | + | 22 | 13–17 |
| — | + | + | — | 4 | 9–14 |

This mechanism might conceivably account for the high incidence of the disease in recipients with serum factor (Exp. A); however, it could not account for the equally high incidence of the disease observed in recipients lacking serum factor and possessing iso-hypersensitivity (Exp. B; Fig. 1). An alternative basis for a "graft-versus-host" mechanism would be provided if the effective lymphoid cells (which are from animals genetically lacking serum factor) were for some reason selectively tolerated by the recipient hosts. However, in an experiment in which full thickness (1 $cm^2$) skin grafts were exchanged among guinea pigs of Rockefeller Institute stock, matched for possession or lack of serum factor and iso-hypersensitivity, no immunological tolerance to homologous tissue was detected (Table II). Thus, all skin homografts were rejected within a time range (9-17 days) not far removed from that which others have noted for first-set homografts (12); whereas, a majority of autologous (control) grafts appeared well seated and healthy at 22 days and beyond.

The possibility that some mechanism other than graft-versus-host reaction may be responsible for erythematous disease is being explored.

*Summary.* Adult, immunologically competent, non-inbred guinea pigs injected with selected homologous lymphoid cells develop a wasting, sometimes fatal illness. Termed "erythematous disease," it is induced by spleen and lymph node cells from guinea pigs lacking heritable serum factor toward which they possess naturally occurring delayed type iso-hypersensitivity.

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## A Simple, Rapid Skin-Graft Technic in Rats. (26455)

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A modification of the punch technic for skin grafting as performed on mice(1,2) has been successfully adapted to rats. Improvements developed during studies on 242 rats have resulted in a regularly obtained healthy skin graft. Sutures are unnecessary in this technic. In mice a tape vest can be used(3), but because of the greater strength of rats, we have found it necessary to use a plaster jacket. The graft is well immobilized in a

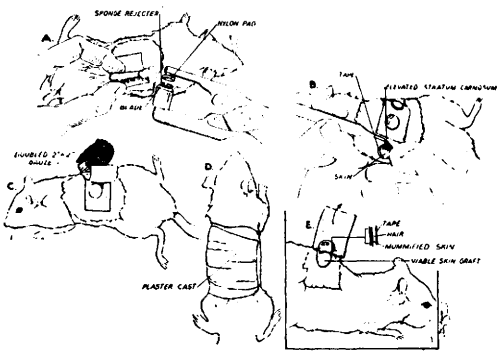


FIG. 1. Punch skin graft technic in rats. A. A double layer of skin is elevated in preparation for the punch graft. B. Stratum carnosum is removed from each of the 2 skin discs. C. Skin is placed in graft bed and immobilized with a second strip of tape and covered with a gauze roll. D. Position of the plaster cast at completion of graft. E. Case and dressing are removed in 8 days. A superficial layer of skin is removed with the tape, leaving viable skin graft.

perfectly fitted graft bed by using light vertical pressure.

*Method.* Young adult male Long-Evans rats, weighing 100 to 150 g, were anesthetized by ether inhalation. "Scotch" Brand pressure-sensitive tape (2.5 cm wide) was used in all animals. A leather punch, with a blade 1.25 cm in diameter, was made to cut against a nylon pad, to avoid dulling the blade's cutting edge (Fig. 1-A). Sponge rubber in the lumen of the blade eliminated the need to remove the graft manually, which often disturbs the graft's adherence to the tape. The graft site was on the posterior chest wall, 2.5 cm caudad to the shoulder girdle. This area could be well covered by the plaster cast without interfering with the shoulder girdle. Grafts placed cephalad to this site were often inadequately immobilized because of neck movement.

The hair of the graft area is shaved and the area is prepared with tincture of iodine. After 3 minutes the area is washed with ether to remove moisture and skin oils. A 5 cm strip of "Scotch" tape is pressed onto the skin. The tape and adherent skin are elevated carefully by pinching the tape in the center (Fig. 1-A). The elevated tape with 2 layers of skin, panniculus adiposus and panniculus carnosis are punched as one. The 2 discs that are obtained are connected by a fibrous band, which is then divided. The panniculus carnosus is then excised with scissors; care is taken not to separate the skin from the adherent tape (Fig. 1-B). The muscle layer is excised most easily if the scissor blades are held parallel to the direction of the muscle fibers.

The graft bed requires no preparation, and bleeding is negligible. The circular disc is inserted into the same-sized defect, with the surrounding tape in place (Fig. 1-C). The graft must be well immobilized to insure that a "take" will occur. A second 5 cm strip of tape is placed over the discs and the first strip of tape. The double thickness of tape is then covered by a doubled 5 × 5 cm layer of gauze and is ready for casting. A single sheet of plaster splint, measuring 7.6 × 38 cm, is folded at one-third its width and is wetted. Warm water is used for more rapid setting of the plaster. The cephalad and caudad edges of the plaster are made slightly constrictive by winding the splint obliquely (Fig. 1-D). If the fit is too loose, the rat can wriggle out of the plaster. Picric acid can be used to prevent the rats from chewing the case, but this is seldom necessary.

The ventral aspect of the cast is cut with heavy scissors on the eighth day. The plane between the 5 × 5 cm gauze and the tape is found so that the tape can be removed separately from the plaster. The end of the tape is slowly lifted up, with gentle separation of the tape from the skin. A scalpel blade can be used to apply counter-traction and prevent pulling off the graft. A plane of cleavage appears in the disc of rat skin graft (Fig. 1-E) between the stratum corneum and the stratum lucidum. The implanted graft disc of skin without hair or outer dessicated squamous cells is smooth and glistening.

*Results.* The early detection of onset of the homograft reaction, with the graft denuded of hair and dessicated cells, is remarkably easy. Very minor amounts of edema and changes in the color or size of the graft can be readily identified. The later stages of the homograft reaction are obvious.

*Summary and conclusions.* A modified graft technic is described which has been found to be rapid and reliable in rats. Two

people can complete this graft technic in 15 to 25 rats in one hour. The adherence of the skin, hair and outer dessicated layers of the skin to the tape allows an unobstructed view of the viable graft and permits detection of minor changes, as compared with the control site. When the graft disc was placed without an overlying tape, or when the cast and tape were removed by the animal, the superficial dessication and hair prevented the early changes of slight edema, faint brownness in color, and reduction of graft diameter, from being seen or recognized as the early onset of the homograft reaction.

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## Fluorescent Treponemal Antibody Test Using the Reiter Treponeme.* (26456)

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The fluorescent treponemal antibody (FTA) test(1,2) for syphilis requires *Treponema pallidum* of the virulent Nichols strain as antigen, and as a result is expensive and limited in availability. This suggested an attempt to substitute the Reiter strain of treponeme, which is antigenically related and easily cultivated *in vitro*.

*Materials and methods.* Sera were from a collection of the *T. pallidum* immobilization (TPI) testing unit of the Division of Laboratories and Research; they were from persons verified as syphilitic or non-syphilitic on the basis of clinical or anamnestic evidence, supplemented by a critical test(3,4) for TPI antibody; many of the non-syphilitic individuals presented diagnostic problems in that they reacted serologically with cardiolipin antigens. Normal sera were from healthy members of both staffs and from blood donors. All sera were stored at –25°C. *T. pallidum* of the Reiter strain was cultivated at 35°C in screw-capped tubes containing 25 ml NIH thioglycollate broth† to which heated (2 hours at 56°C) rabbit serum had been added to give a final concentration of 10%. Growth was satisfactory at 2 days, the cultures remaining acceptable for antigen preparation from the 2nd to 5th day. Two ml of culture were removed aseptically from the undisturbed upper stratum and delivered into a 15× 100 mm pyrex tube containing 2 ml 0.002% sodium hypochlorite (NaClO)‡ in 0.85% NaCl solution. The NaClO was essential, the treponemes otherwise clumping, becoming atypical in morphology, and failing to adhere to slides. They were mixed well by gentle swirling, then sedimented by spinning 15 minutes at 1,000 g. The supernate was decanted, and the organisms were washed once by resuspending in 4 ml 0.85% NaCl solution, centrifuging, and decanting as before. They were suspended finally in enough 0.85% NaCl solution to make the count 10-15/430 × microscopic field, *i.e.*, about 2 × $10^6$/ml. *T. pallidum* of the Nichols strain was available as suspensions eluted from fresh or frozen(5) syphilomatous rab-

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† No. 0257-01, Difco Labs., Inc., Detroit, Mich.

‡ B.K chlorine-bearing liquid bactericide; active ingredient sodium hypochlorite 5.25%. Pensalt Chemicals Corp., Phila. 2, Pa. A 1:2,625 dilution was made in 0.85% NaCl solution.