Control of Surface Wound Infection: Skin Versus Synthetic Grafts

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Received for publication 6 February 1973

Auto-, iso-, or xenografts of skin and synthetics placed on surface wounds freshly contaminated with Pseudomonas aeruginosa stabilizes the wound bacterial population in rats over a 24-h period. When these wounds contained a bacterial contamination established for 24 h prior to grafting, only skin and the synthetic polyhydroxyethylmethacrylate were effective in lowering the initial bacterial concentration. Polyurethane foam and nylon velour were not effective in the established infection model. Skin placed on a contaminated wound for 2 h or longer appeared to equilibrate with the underlying muscle so that the bacterial count per milligram of skin was similar to that of the muscle. It was suggested that this preparation would be useful to obtain an estimate of surface contamination without biopsy of the infected muscle. Skin grafts in place for 2 h significantly lowered the bacterial count in a wound with an established infection. A second decrease occurred between 4 and 24 h after grafting. Histological studies of contaminated and exposed panniculus muscle showed that leukocytes tend to migrate from the muscle surface to its base. Skin grafts and polyhydroxyethylmethacrylate appear to reverse the white cell migration so that the cells move toward the surface of the muscle with preservation of normal staining characteristics in the muscle. It is suggested that this alteration in cell movement after graft application might modify the white cell function and result in a greater bactericidal activity. Apparently, grafts lower bacterial levels in an established infection by modifying the host response to the surface contamination.

Brown et al. (6) observed clinical benefits in patients with burns treated with skin grafts from living or cadaver donors. Temporary allografts placed on a wound are currently being used to reduce bacterial growth (5, 13; C. R. Baxter, presented at First Annual Congress of the Society of German Plastic Surgeons, Munich, Germany, 28 September 1970) and clean-up (16, 26) granulation tissue. How skin grafts reduce bacterial numbers in infected surface wounds has not been resolved. Shuck et al. (23) suggested that an antibacterial factor is present within the graft. Alternatively, wound closure may enable host defenses to deal with surface infection while the graft material remains viable (15).

Eade (8) studied the initial number of bacteria present on granulating, infected burn wounds and the number remaining after the application of skin grafts. Within 2 h after either viable or nonviable graft application, there was a decrease in bacteria extracted from the wound into a wash fluid. Sewell et al. (22) reported the use of a dressing formed of lyophilized skin for burned sites but found it satisfactory as a temporary wound cover only in areas free of bacterial contamination. Blocker et al. (3, 4) noted that the covered wound contains less surface exudate for bacterial multiplication than the open wound. Others (1; F. F. Pindax, Ph.D. thesis, Univ. of Texas, Austin, 1958), suggested that phagocytic cells of granulation tissue combined with the bactericidal activity of serum could rapidly eliminate residual organisms not previously removed by mechanical cleansing.

The limited availability of human skin stimulated investigations of other suitable sources of biological dressings such as (i) animal skins (19, 24, 27; F. F. Pindax, Ph.D. thesis, Univ. of
Texas, Austin, 1958); (ii) amniotic membranes (18); (iii) bovine collagen, reconstituted on a dacron mesh for covering uncontaminated surface wounds in mice (10, 25); and (iv) human fetal skin (2). Investigators searching for synthetic substitutes for skin have tested polyamino acid films cast upon either fine mesh gauze or nylon velour (28). Dressler et al. (7) reported the successful control of experimental burn wound sepsis with nylon velour as a prosthetic skin substitute. Etheron, diisocyanate polyether (9), methyl 2-cyanoacrylate, a tissue adhesive (17), ivalon, a polyvinyl alcohol sponge (12), polyurethane foam (14), and polymethacrylate foam (11) are examples of other materials evaluated as temporary synthetic skin prostheses.

The present research attempts to verify quantitatively in a standardized laboratory model the clinical impressions that skin grafts are helpful in the control of surface wound infection. Also, several synthetics were evaluated as possible wound dressings by comparing them with skin for their ability to reduce bacterial contamination of surface wounds. Finally, some insight is reported on the mechanism of action of grafts on contaminated bacterial wounds based on histologic examination of the infected muscle tissue in both grafted and exposed wounds.

MATERIALS AND METHODS

Animals. The animals used were adult, female rats of the Lewis or Sprague-Dawley strains weighing 200 to 300 g. The experimental model and the assay procedures are described elsewhere (21). A brief summary follows.

Bacterial culture and septic model. A culture derived from an isolate of P. aeruginosa was maintained as a frozen suspension in sterile skimmed milk. A 1-ml sample of a rapidly growing suspension of this culture was inoculated into 100 ml of a basal salt medium and incubated on a shaker for 5 to 6 h at 35 C. A 0.1-ml amount of a supernatant of this culture containing approximately 10^7 viable Pseudomonas aeruginosa was seeded onto a 2.5-cm square of exposed panniculus carnosus. Aseptic techniques were used to prepare and remove the skin, exposing the clean panniculus muscle.

The graft materials evaluated were applied to the contaminated panniculus surface either immediately after, or 24 h after, seeding with P. aeruginosa. In the latter condition the panniculus muscle contained an established infection. Grafts were covered with Telfa pads held in place with adhesive tape. When the contaminated panniculus remained ungrafted, as a control, it was protected from abrasion by a perforated, polystyrene weighing tare glued in place with collodion. The tare was inverted so that it did not touch the wound surface.

Graft materials. The following viable skin grafts were applied to either freshly contaminated wounds or wounds with established infections. (i) Rat skin: the full-thickness wound was covered with autograft, isograft (Lewis strain), or allograft (Sprague Dawley) skin. Since grafts were in place for less than 48 h, no significant rejection response was anticipated. (ii) Human skin: split-thickness human cadaver skin (0.033 cm in thickness) obtained under aseptic conditions served as a xenograft. These grafts were harvested within 6 to 8 h of the donor's death. The tissue was wrapped in sterile gauze saturated with a saline solution containing penicillin (500 U/ml) and streptomycin (5 mg/ml). The skin was stored at 4 C. (iii) Porcine skin: split-thickness porcine skin grafts 0.033 cm thick obtained under sterile conditions were obtained from fresh hides, placed in sterile gauze soaked with isotonic saline, and stored at 4 C. Since no antibiotics were added to these grafts, they were utilized in the experimental system as soon as possible.

The following synthetics were used as graft materials serving as nonviable controls for skin. (i) Hydron (Hydron Laboratories): this material is a polycroxyethylmethacrylate. Hydron is heat stable at 120 C and was sterilized by autoclaving in 0.9% sodium chloride prior to use. (ii) Nylon velour: silastic laminate was supplied by D. P. Dressler. An impermeable layer of polyvinyl fluoride serves as a water barrier between a double layer of the velour. The preparation was gas-sterilized prior to use. (iii) Epigard: a polyurethane foam (Parke-Davis Company) is supplied as a sterile product. The effect of polyurethane foam on the bacterial flora of the wounds with established infections was investigated. Polyurethane foam was not evaluated on freshly contaminated wound surfaces.

Bacterial tests at frequent intervals showed our graft materials to be free of P. aeruginosa. This species of bacteria was not present as a normal resident on the skin of our rats.

Quantitation of tissue bacterial content. In this investigation a mechanical method of tissue surface washing and homogenization which provided an objective and reproducible method of sample preparation for bacterial quantitation was adopted (21).

(i) Surface washing was carried out by using sterile plastic cylinders of uniform bore, which were pressed tightly against the surface of the contaminated panniculus muscle to be sampled. A 10-ml sample of sterile, iced saline was pipetted into the cylinder. The fluid was agitated by using a glass T-bar driven by a Tri-R motor at a speed (approximately 1,500 rpm) sufficient to produce a vortex in the fluid. After 5 min, the fluid was drawn off for quantitation of bacteria present in the wash fluid. The inside diameter of the cylinder was 1.5 cm, and in all of the wash experiments the total bacteria extracted was determined for an area of muscle, skin, or synthetic equal to that of the inside of the cylinder.

(ii) Tissue homogenization was carried out as follows. By aseptic technique, graft materials and underlying panniculus muscle tissues were removed, in specified experiments, from 1 to 24 h postgraft
application. The tissues were individually weighed, diced, and placed in sterile 50-ml capacity glass grinders to which were added 10.0 ml of sterile iced saline. Each grinder was placed in an ice bath, and the sample was homogenized for 5 min at 720 rpm, utilizing the constant-torque power of a 0.5-horsepower motor on a 39-cm drill press. All samples were kept chilled after processing until they could be quantitated for bacterial content.

(iii) Bacterial quantitation was carried out as follows. The bacteria were estimated by a standard plate count method, substituting a selective growth medium, acetamide agar (21), for the usual medium. Colonies were counted after 48 h of incubation at 35 C. The bacterial count was expressed as the number of cells per unit surface area of tissue washed or, in the homogenization tests, as bacteria per milligram of tissue. The bacterial count of the biopsy sample divided by the tissue weight provided the bacterial density or concentration. These concentration values were transformed to the log10 scale to normalize the data and reduce inequality of variances. The data were expressed as the mean log10 (log10) ± the standard error of the mean. Significance of individual treatment modes was determined by the Student's t test. Data were considered significant when the means differed at a probability less than or equal to 0.05.

Histology. Light microscopy was carried out by using biopsy samples of graft materials and underlying muscle which had been obtained for histological evaluation. The sections were stained with hematoxylin and eosin.

RESULTS

Application of grafts to freshly contaminated surfaces. Figures 1 and 2 present data on the application of grafts to freshly contaminated wound surfaces. Graft materials evaluated include rat skin autografts, human and porcine skin xenografts, nylon velour-silastic laminate and Hydon. The grafts were removed from 1 to 24 h after application and contamination of the wound surface. Control animals were contaminated but remained ungrafted for the duration of the experimental period. Figure 1 indicates the number of P. aeruginosa extracted per milligram of tissue at 1, 2, and 24 h after the application of full-thickness rat skin autografts. The ungrafted controls are presented at the left of each experimental group. At 1 and 2 h after contamination with approximately 107 cells, the P. aeruginosa concentration of the ungrafted panniculus, expressed as (log10) bacteria per milligram of tissue (wt weight), was 4.1 and 3.9. By 24 h the ungrafted panniculus had increased significantly (P < 0.001) to a mean of 5.6.

The (log10) P. aeruginosa contents of the panniculus carnosus under the grafts at 1, 2, or 24 h were similar, ranging from 3.7 to 3.4. The most striking observation was the significantly increased bacterial concentration of the ungrafted panniculus at 24 h, compared to its grafted control (P < 0.005). Also of interest was the relatively stable viable bacterial content of the skin as well as the panniculus under the grafts over the 24-h period. It is worth noting that at 24 h the P. aeruginosa concentration of the skin autografts and their respective underlying tissues were similar, indicating an almost one-to-one relationship between the bacterial concentration of the skin grafts and the underlying muscle.

Split-thickness human and porcine skin xenografts were compared with the full-thickness rat skin autografts in their ability to modify bacterial levels 24 h after the grafting of
freshly contaminated wound surfaces (Fig. 2). The control data show that at 24 h the ungrafted panniculus contained a (log₁₀) of 5.6 P. aeruginosa per mg. Twenty-four hours after the application of full-thickness rat skin autografts, the underlying panniculus contained a mean of 3.5. In addition, 24 h after application of either split-thickness human or porcine skin xenografts to freshly contaminated surfaces, the muscle contained (log₁₀) of 2.7 under human skin and 2.8 P. aeruginosa per mg when covered with pig skin. There was no significant difference in the P. aeruginosa concentration of the panniculus when grafted with any of the three types of skin; however, the bacterial levels were significantly lower than the ungrafted controls. The skin grafts themselves contained a (log₁₀) between 3.6 and 4.

Figure 2 also presents data obtained on P. aeruginosa concentrations 24 h after application of the synthetics Hydron and nylon velour to freshly contaminated panniculus surfaces. At 24 h the (log₁₀) bacterial concentrations of the panniculus carnosus grafted with the synthetics were 3.1 and 2.8. These values were significantly lower than the ungrafted controls (P < 0.001). Of importance was the relatively high P. aeruginosa content of the nylon velour compared with the bacterial contamination of the Hydron grafts. The difference in means of 5.6 and 2.8 was significant (P < 0.001). A statistical comparison between the P. aeruginosa content of the panniculus 24 h after grafting with the viable skins and Hydron showed no significant differences. For muscle tissue beneath the nylon velour, however, the bacterial concentration was significantly less than that observed for muscle underlying rat skin autografts (P < 0.005).

Application of grafts to panniculus wounds containing an established P. aeruginosa infection. An infected surface wound was established by contamination of the exposed and ungrafted panniculus carnosus with 10⁷ P. aeruginosa. Twenty-four hours later the tissue presented a moist surface with patchy areas of greenish purulence. Operationally, this preparation is defined as an established infection. At this time graft materials were applied for periods of 1 to 24 h to evaluate their potential effect on the bacteria in the muscle. Control animals remained ungrafted for the duration of the experiment. Data for the ungrafted panniculus 24, 25, 26, and 48 h after contamination is shown in Figure 3. The (log₁₀) P. aeruginosa concentration at 24 h of 5.6 represents the level of bacteria present in the muscle tissue at the time of graft application. From 25 to 48 h postcontamination the bacterial concentration of the panniculus did not change significantly. The muscle underlying isografts which were in place for 1 or 2 h contained means of 5.5 and 5.3, similar to the 25- and 26-h ungrafted controls. At 24 h, however, the grafted panniculus demonstrated a significant one-log reduction to a mean of 4.2 compared with the 48-h ungrafted controls (P < 0.001) or animals having skin grafts in place for 1 (P < 0.001) or 2 (P < 0.01) h. The skin grafts, themselves, contained a (log₁₀) of between 3.7 and 4.7 P. aeruginosa per mg.

Figure 4 presents data comparing full-thickness rat skin isografts with split-thickness human and porcine skin xenografts and several synthetics in their ability to modify the viable bacterial content in established wound infections. The control data, at the left of the figure show that the panniculus contained a (log₁₀) of 5.3 P. aeruginosa per mg if ungrafted for 48 h. Twenty-four hours after the application of rat skin isografts to established infection, the bacterial concentration of the underlying muscle decreased to a mean of 4.2. In addition, when either the human or porcine skin xenografts
were in place for 24 h, the underlying tissue contained means of 4.0 and 3.9, respectively. Statistical comparisons demonstrated no significant difference in the mean bacterial concentration of the panniculus carnosus when grafted with any of the three types of skin. These grafts significantly reduced the P. aeruginosa concentration of the panniculus by a factor of one-log compared with the ungrafted controls (P < 0.001). The skin grafts themselves contained a \( \log_{10} \) P. aeruginosa concentration of from 3.6 to 3.9. In these experiments the bacterial content of the skin grafts and their respective underlying tissues once again suggested an almost one-to-one relationship between the bacterial concentrations of the grafts and the underlying infected tissues when expressed in \( \log_{10} \) P. aeruginosa per milligram of (wet weight).

In similar experiments Hydron film or foam, nylon velour, and Epigard were applied to infected pannicular surfaces for 24 h. The control data (Fig. 4), again, represent the level of bacteria in the ungrafted, infected panniculus 48 h postcontamination. In these experiments two forms of Hydron used as graft materials gave similar results, and the bacterial counts in the panniculus muscles from these groups were combined to form one group with a \( \log_{10} \) of 4.2 which was significantly lower than the ungrafted controls (P < 0.001). There was, however, a significant difference in the bacterial contamination of the Hydron grafts themselves. The foam contained a \( \log_{10} \) bacterial count of 3.97, and the film was contaminated with a mean of 2.8. These means were significantly different at P < 0.025. Of particular interest were the mean values obtained for the bacterial concentration of the panniculus muscle covered with nylon velour grafts. The \( \log_{10} \) of 5.3 was similar to that obtained in the ungrafted controls, reflecting the inability of this graft material to modify bacterial flora of tissue with an established infection. This lack of effectiveness was also seen with the polyurethane foam grafts. The mean of 5.8 was not significantly different from the ungrafted controls. The bacterial content of both the nylon velour and the Epigard was considerably higher than the Hydron film (P < 0.001) with \( \log_{10} \) values of 5.2 and 6.0 P. aeruginosa per mg.
respectively. The variability in bacterial content of the nylon velour, as reflected in the large standard error, makes this material not significantly different from the Hydron foam. In addition, a comparison of the P. aeruginosa concentration of the panniculus under synthetics demonstrated that, under identical conditions, only the polyhydroxyethylmethacrylate dressings were effective in reducing the bacterial flora of the underlying tissue.

**Bacterial quantitation of the wound surface.** The surface-wash technique was carried out by using a modification (21) of Eade’s surface washing procedure (8) described above. This method was utilized to study the effects of graft materials on the bacteria in the infected wound. The technique provides an alternative to homogenization for sampling the bacteria present in the wound. Rat skin allografts, representing viable tissue, and Hydron film, as an example of a synthetic dressing, where chosen to evaluate their effects on established infections by using the surface-wash technique. The rat skin and Hydron were placed on pannicular surfaces 24 h postcontamination with P. aeruginosa and were removed for bacterial enumeration at 2, 4, and 24 h after application.

Figure 5 presents quantitative data on bacterial counts 2 h after graft application. Two groups of control data obtained 24 h after contamination are shown at the left. All of the data in the wash experiments are referred to a standard area of 3.9 cm². In nine rats the 3.9 cm² area of panniculus was excised for homogenization without a prior 5-min wash. The panniculus of an additional 25 rats were washed; then 3.9 cm² of the muscle was biopsied for homogenization and quantitation of residual bacterial content. These two control groups were utilized to insure that the total bacteria obtained by washing and homogenization of the panniculus was similar to that obtained on biopsy and homogenization of the unwashed muscle. A comparison of the unwashed control with the control wash fluid was sufficient to demonstrate that at 24 h postcontamination most of the infecting P. aeruginosa were extractable from the surface of the panniculus by washing. The unwashed muscle (3.9 cm²) contained 10⁷ P. aeruginosa. The control wash fluid in another group of rats contained a (log₁₀) of 9.22 organisms, and the small standard error of 0.0919 attested to the reproducibility of the wash procedure. The panniculus itself retained a log of 8.6 P. aeruginosa. Approximately 25% of the total detectable bacteria remained in the washed panniculus. For the wounds covered with allografts for 2 h the wash contained a mean of 8.41, significantly lower than the control wash fluid (P < 0.001). The panniculus contained a mean of 7.34, also significantly lower than the controls (P < 0.001). The wash fluid under Hydron grafts contained a mean of 8.56, and the panniculus contained a mean of 7.81, both values being significantly lower than the controls (P < 0.025 and P < 0.01, respectively). There was no significant difference in the (log₁₀) P. aeruginosa content of the wash fluids and panniculus muscles when the graft and synthetic were compared. The skin, however, contained significantly (P < 0.005) more P. aeruginosa, (log₁₀) of 7.8, than the Hydron film, (log₁₀) of 5.99.

In additional experiments the bacterial con-
tent of grafts, wash fluids, and panniculus muscles 4 h after graft application were compared with results obtained at 2 h for significant changes over the 2- to 4-h interval. The only significant alteration in bacterial numbers occurred as an increase in the bacterial contamination of the Hydron film from 2 to 4 h postgraft application (P < 0.05). All other compartments, graft, surface wash, and panniculus appeared to be stabilized during this interval.

Similar experiments were performed with rat skin allografts and Hydron film applied to established bacterial infections for 24 h. The results obtained were similar to those seen with allografts and Hydron at 2 and 4 h. The surface wash, however, at 24 h postgraft application contained significantly fewer P. aeruginosa than it did at 4 h (for skin, P < 0.001; for Hydron film, P < 0.006). Also, the Hydron was contaminated with fewer P. aeruginosa at 24 h than at 4 h (P < 0.01).

Having determined that a significant reduction in bacteria occurred within 2 h of either skin graft or Hydron film application to established wound infections, experiments were designed to determine the necessity of continuous graft coverage for more than the 2 h to maintain the reduced levels of bacteria over the 24-h period of observation. Rat skin allografts were applied to exposed, infected panniculus surfaces and left in place for 2 or 4 h. The grafts were then removed, and the rats were maintained, ungrafted, for the remainder of the 24-h interval. At that time the bacterial contents of the surface and muscle compartments were determined. Statistical comparisons were made with rats having allografts in place for 24 h and controls which remained ungrafted for 48 h (Fig. 6). There was no significant difference in the numbers of P. aeruginosa in the wash fluids or panniculus muscles when the allografts were in place for either 2 or 4 h. These groups were combined for presentation. The bar graphs demonstrate that after removal of the 2- or 4-h allografts, the numbers of P. aeruginosa on the wound surface increased over the remainder of the 24-h period to a (log10) of 9.65, a value not significantly different from the ungrafted controls and significantly higher than the (log10) of 7.29 found in those animals receiving allografts for the entire 24 h. The panniculus muscles which were grafted for only 2 or 4 h demonstrated an increase in P. aeruginosa to a mean of 8.71 at 48 h, which was not significantly different from the 48-h ungrafted controls. However, the presence of skin grafts for 24 h significantly lowered the bacterial content of the wound surface and panniculus.

The importance of host viability in the observed reduction in bacterial numbers was evaluated by killing rats and immediately applying skin allografts to the exposed panniculus surfaces containing established infections (Fig. 7). After application for 2, 4, and 24 h, the grafts were removed for quantitation of the wound surface and panniculus muscles. Control animals were killed but remained ungrafted for the duration of the test intervals. Statistical comparisons of the control data at 2, 4, and 24 h demonstrated no significant difference in the bacterial content of the surface wash or panniculus. These data were pooled to form an ungrafted control group of eight rats and are graphically presented at the left of Fig. 7. The wash fluid contained a (log10) of 9.44 P.
aeruginosa, and the panniculus contained a mean of 8.77. After allografts were removed at 2, 4, and 24 h the wash fluids contained means of 9.06, 8.88, and 8.97 P. aeruginosa, respectively. Similarly, the panniculus was found to contain means of 8.24, 8.06, and 8.39. Compared with the ungrafted controls, there was no significant difference in the quantity of bacteria eluted into the wash from the wound surface at any time interval. Within the panniculus, however, the P. aeruginosa content was significantly lower than the controls at 2 h ($P < 0.01$), 4 h ($P < 0.005$), and 24 h ($P < 0.05$). A comparison of Fig. 6 and 7 shows that grafts in the dead animal have essentially no effect in reducing surface bacteria, as compared to the substantial reduction seen in the live rat.

**Histology.** Representative photomicrographs of tissue sections from biopsies of the panniculus muscle illustrate cellular reactions seen in each treatment group. Figure 8a shows a section of panniculus carnosus which was not contaminated and remained ungrafted for 24 h. The inflammatory reaction is well demonstrated with numerous polymorphonuclear leukocytes restricted to the exposed muscle surface. The striated myofibrils are pictured in cross-section and are compact and normal in appearance. This histologic pattern remained stable through 48 h. Within 24 h of inoculation with approximately $10^3$ P. aeruginosa, the panniculus begins to show definite pathology (Fig. 8b). The degree of inflammatory cell reaction is increased, compared with the uninoculated controls, and these cells have begun to infiltrate below the muscle surface to approximately one-third of the distance to the base of the muscle mass. A granular exudate is present on the surface of the panniculus. The surface vasculature is engorged with degenerating red blood cells along with a few white cells. The individual myofibrils demonstrate pathology in the region of inflammatory cell infiltration. This is illustrated in the photomicrographs by the change in staining characteristics of the muscle tissue. In normal muscles the myofibrils are grey and segmented, the pathological tissue degenerates to form a uniformly stained mass with little identifiable internal structure.

The section of panniculus pictured in Fig. 9a is from a rat panniculus which remained ungrafted for 48 h after inoculation with bacteria. In this section the surface of the panniculus is relatively free of inflammatory cells and, similar to the previous section, these cells have migrated through the muscle. In this instance the inflammatory cells have become heavily concentrated throughout the subpannicular connective tissue. The panniculus occupies nearly all of the space in Fig. 9a from the top (upper left) to the heavy infiltrate (lower right).

Tissues with established infections, receiving rat skin allografts for 2 h, present white cells in the upper one-half to one-third of the muscle mass. In this respect within 2 h of graft applica-

![Fig. 8. (a) Cross-section of the exposed dorsal panniculus carnosus, 24 h after surgical removal of a 1-in. square (approximately 2.54 cm) full-thickness skin flap (hematoxylin and eosin, magnification = approximately ×200). (b) Cross-section of the exposed dorsal panniculus carnosus, 24 h after contamination with P. aeruginosa (hematoxylin and eosin, magnification = approximately ×100).](image-url)
tion, the histological findings are similar to the 24-h ungrafted control. With allografts in place for 4 h the histological picture is altered (Fig. 9b). The inflammatory leukocytes are now located primarily on the surface of the panniculus. From observation of the 24-h ungrafted control preparations, one might suggest that white cells had previously occupied the spaces between the irregularly shaped myofibrils. The amorphous condition of the muscle, two-thirds of the distance from the surface to the subpannicular connective tissue, is apparent. The basal one-third of the muscle tissue appears normal.

The sections of panniculus pictured in Fig. 10a and 10b were obtained from animals with established wound infections which received either a skin allograft or Hydron film graft for 24 h. To appreciate the effect of the wound coverage it must be recalled that in ungrafted controls, 48 h postcontamination with P. aeruginosa, leukocytic migration through the panniculus was almost complete and the muscle tissue was degenerating into a mass of unidentifiable tissue. The panniculus underlying both skin and Hydron film demonstrates a striking difference from these controls. In Fig. 10a the wound surface is covered with a granular exudate, and the relatively few inflammatory cells are located in the upper regions of the tissue. Individual myofibrils in this section appear abnormal, however; it is apparent that graft coverage has in some way averted complete degeneration of the muscle tissue which was observed in the ungrafted controls. In the section of panniculus grafted with Hydron film for 24 h (Fig. 10b) the inflammatory reaction is greater under the Hydron film than underneath the skin allograft. In this section the leukocytes are primarily on the surface of the panniculus. They can be seen to penetrate to the subpanniculus by infiltration of connective tissue. As under skin allografts, the myofibrils are irregularly shaped and, in comparison to the ungrafted controls, they do retain their individual unit structure.

The histological sections which have been presented suggest that graft coverage affects the distribution of inflammatory cells in infected tissue. The following experiment was designed to obtain verification of this observation. One rat with an established P. aeruginosa infection was treated with a rat skin allograft which covered only one-half of the exposed panniculus. Twenty-four hours later, biopsy specimens were obtained from both the grafted and ungrafted portions of the panniculus. A longitudinal section of the portion of the panniculus which remained ungrafted for the 48-h duration of the experiment (Fig. 11a) shows migration of the inflammatory cells deep within the muscle mass leaving the surface relatively free of these cells. The arrows point to myofibrils which have lost their normal staining characteristics. Between these myofibrils are intense accumulations of the leukocytes. The
surface is seen to be covered with a thick granular exudate, and the X indicates an engorged, occluded surface blood vessel.

The section presented in Fig. 11b is a longitudinal view of that portion of the wound receiving an allograft for 24 h. The spacing between myofibrils is normal, and the muscle tissue appears to stain normally. In contrast to the ungrafted portion of the same muscle which contains a diffuse distribution of white cells throughout the tissue, the polymorphonuclear leukocytes are heavily concentrated on the wound surface in the muscle segment covered with a graft.

**DISCUSSION**

Between 2 and 24 h after a standard contamination with *P. aeruginosa*, the bacterial concentration in the wound of the ungrafted animal increased tenfold when compared to the grafted control. Autografts of rat skin applied to freshly contaminated surface wounds for 1, 2, or 24 h kept the bacterial concentration of the exposed panniculus muscle constant during a 24-h period. The effect on surface contamination of split-thickness human and porcine skin xeno-grafts was similar to that observed with rat skin autografts 24 h after application to freshly contaminated surface wounds. Indeed, five
graft materials (rat, human, and porcine skin, Hydron film, and nylon velour) including the two synthetics mentioned were equally capable of stabilizing the bacterial concentration of this tissue when applied to the freshly contaminated wound surface for 24 h. This suggests that the presence of the grafts or dressings on the freshly contaminated wound stabilized the bacterial level, preventing the increase observed in the ungrafted controls during the 24-h test period.

The full-thickness rat skin autografts contained $10^4$ P. aeruginosa per mg whether they were grafted to the freshly contaminated surface for 1, 2, or 24 h. Both human and porcine split-thickness skin xenografts contained means of $10^4$ P. aeruginosa per mg at 24 h. By calculating the bacterial content as a concentration within these tissues it became evident that at 2 h after application, the concentration in the rat skin autografts and porcine skin xenografts was not significantly different from that of the underlying muscle tissue. This suggested the possible clinical use of such grafts for estimating the degree of bacterial contamination of infected surface wounds. Such a procedure gives an excellent estimate of the level of wound

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**Fig. 11.** (a) Longitudinal section of the ungrafted portion of the dorsal panniculus carnosus 48 h after contamination with P. aeruginosa. Symbols: arrows, myofibrils; X, engorged occluded blood vessel (hematoxylin and eosin, magnification = approximately x200). (b) Longitudinal section of allografted portion of infected dorsal panniculus carnosus 24 h after graft application (hematoxylin and eosin, magnification = approximately x200).
contamination and obviates the need for traumatic biopsies which are sometimes used to obtain such information.

Twenty-four hours after application of polyhydroxyethylmethacrylate to the contaminated wound, the synthetic contained only 600 bacteria per mg. The flat surface and hydrophobic nature of this synthetic probably kept it relatively free of bacteria compared to the nylon velour which contained $10^5$ bacteria per mg in a similar test. The low level of bacteria on the film appeared attached to the surface. The velour, on the other hand, provided spaces in its interstices for bacterial accumulation.

The experiments just discussed were repeated with graft materials applied to panniculus muscles contaminated with bacteria for 24 h before graft application. By our definition these were established infections. Twenty-four hours after grafting (48 h after infection), the mean concentration of bacteria in the panniculus had decreased significantly. The concept of reduction in bacterial numbers is emphasized because of the stabilization of bacterial concentration observed in the previous experiments with freshly contaminated wounds. Human and porcine skin xenografts and Hydron film were as effective as rat skin isografts in significantly reducing the concentration of bacteria within underlying tissues. However, nylon velour and polyurethane foam did not reduce the bacterial concentration in a panniculus with an established infection. In addition, the nylon velour and the polyurethane foam each contained at least $10^4$ P. aeruginosa per mg as compared to means of $10^4$ for the skin and Hydron film grafts.

On the basis of the experiments which have been discussed, the strength of the initial hypothesis that skin grafts play a direct and active role in the control of wound infection has been weakened. The use of synthetics as a control for viable skin grafts demonstrated that at least one skin substitute, polyhydroxyethylmethacrylate, appeared to approach the effectiveness of skin in modifying the bacterial flora of the infected surface wound.

Eade’s observation (8) of a reduction of viable bacteria in infected tissues under a graft were confirmed in our laboratory model with both skin and Hydron film grafts. After an initial significant reduction was observed at 2 h, the bacterial content of the wound surface and panniculus remained stable for an additional 2 h. Between 4 and 24 h, a second significant reduction occurred which was detectable by the wash procedure.

Experiments were designed to determine whether the bacterial reduction mechanism, once activated, could continue to maintain lowered numbers of organisms in the absence of graft coverage. The data from these experiments proved the need for continuous graft coverage over the 24-h period to lower the bacterial level in the contaminated wound.

The results obtained with grafts on dead rats suggest that the major effect in reduction of surface contamination produced by grafts requires a viable host. Although it was not investigated, it seems likely that the leukocyte migration observed in this study may not readily occur in the dead animal, thus eliminating any contribution this process might make toward control of surface contamination.

A histological examination of the panniculus in the model system was undertaken to obtain some understanding of possible mechanism responsible for the effectiveness of grafts in the control of surface contamination. In ungrafted, uninoculated control animals the surface of the exposed panniculus was found to contain a moderate accumulation of polymorphonuclear leukocytes. These cells, however, were restricted to the surface of the muscle for the 48-h observation period. The mass of striated muscle tissue remained compact, and there was no indication of the presence of edema fluid between individual myofibrils. Sections of these tissues demonstrated clefts within the myofibrils, characteristic of normal healthy muscle tissue (20).

Wound surface contamination with P. aeruginosa produced histological changes in the panniculus muscle. These tissues were examined 24, 28, 31, and 48 h postcontamination and showed progressive invasion or infiltration of inflammatory leukocytes into the striated muscle mass. At 48 h it appeared as though the white cells had migrated completely through the muscle tissue. The muscle fibers demonstrating some degree of pathology due to P. aeruginosa infection were seen to lose their stippled appearance (20) and no longer displayed the normal clefts within individual myofibrils. It appeared that the extent of pathology was correlated with the progression of leukocytes through the panniculus muscle. At 24 h postinoculation all fibers surrounded by leukocytes demonstrated the pathological changes. Below the plane of white cell migration, however, the individual fibers appeared normal.

The application of either skin allografts or Hydron film to these exposed muscle surfaces 24 h postcontamination was found to alter the progression of the inflammatory cells through the tissue. In sections obtained at 4 h after graft
application to established infections of muscle, the primary location of the white cells was on the panniculus surface. In these preparations wide spacing between myofibrils and the pathology of these fibers suggested that previous to graft application the leukocytes might have been located within the muscle mass. If this were true, the presence of the graft would have altered the direction of progression of these cells, perhaps attracting them to the muscle surface.

Within 2 h of application, both rat skin allografts and Hydron film were effective in reducing the total bacteria extractable from the wound surface and the residual bacteria remaining in the panniculus muscle. Bacterial analysis of the surface wash showed that early significant modifications in bacterial numbers could be detected under a graft. These data are in agreement with Eade’s observations (8) in burn patients of a reduction in bacteria extractable from burned surfaces within 2 h of skin graft application. In terms of percentage loss of bacteria from the wound after allograft application, the present data indicate that within 2 h, 70% of the bacteria present in the ungrafted control muscle disappears. Of this, only 4.0% can be accounted for by an uptake of viable bacteria into the skin graft. For Hydron film, the reduction in viable bacteria was not of the magnitude observed with skin. At 2 h after grafting, about 40% of the P. aeruginosa recoverable from the ungrafted controls was lost. No significant change in the bacterial content of either the surface or muscle compartments could be detected between 2 and 4 h of grafting. However, between 4 and 24 h, under both skin allografts and Hydron film, a second decrease in viable bacteria extractable from the muscle surface was observed.

The histological data suggest relationships between graft application and the distribution of inflammatory cells within the infected panniculus muscle. The white cell distribution in turn might be important in the reduction of surface contamination. Biopsy specimens of the ungrafted panniculus taken between 24 and 48 h postinoculation reveal a progressive migration of leukocytes through the mass of muscle tissue to the subpanniculus connective tissue. No reduction in viable bacteria accompanies this progression of inflammatory cells deep to the muscle surface. The point to be made is that the reduction in bacterial contamination and the white cell migration toward the muscle surface do not occur in the absence of wound coverage.

ACKNOWLEDGMENTS

The authors thank Norman Williams, John Williams, and Diane Lewis for technical help during the course of this work.

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