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Importance of Horizontal Plane Cell Mass Integrity in Wound Contraction.* (29630)

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Specialized cells in the deepest layer of the dermis at the edge of a full-thickness skin wound ("picture frame area") recently have been shown to perform an important role in the phenomenon of wound contraction. In their studies on the mechanism of wound contraction, Grillo, Gross, and Watts demonstrated that repeated separation of the "picture frame" area of dermis from underlying subdermal tissue interferes with the contracting process and causes an actively contracting wound to reopen to its original dimensions(1,2). Such data strongly suggest that wound contraction forces are developed in a vertical plane between the deep margin of dermis and the subdermal tissue, and that disruption of the dermis-subdermis interface prevents normal movement of the skin edges.

We have hypothesized that, even if contraction forces are oriented primarily in a vertical direction, horizontal plane changes within the "picture frame" area of dermis also must be occurring as the wound perimeter decreases. The possibility that a reduction in number and/or a more compact arrangement of cells in this area might be contributing actively instead of adjusting passively to the contracting process (horizontally oriented purse string effect) has been investigated by performing the following experiments:

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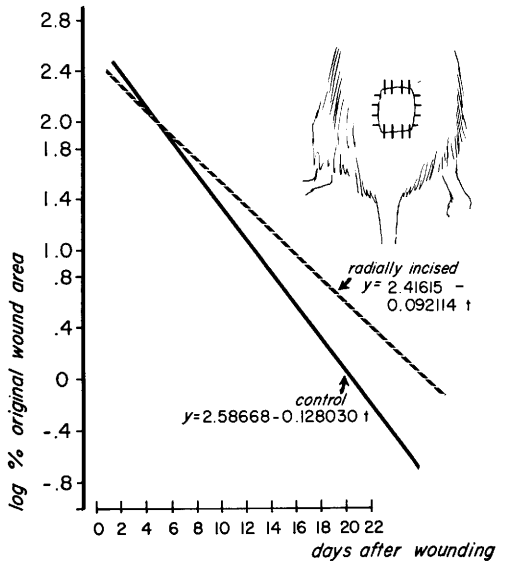
Materials and methods. Female, Sprague-Dawley rats weighing approximately 150 g each were used in all experiments. Full-thickness skin wounds were produced by excising a 2 × 2 cm area of skin from the mid-dorsal region. India ink marks were tattooed on the wound edges at 1 cm intervals around the circumference of the wound. As healing progressed, changes in wound area were measured by placing semi-transparent paper over the wound and outlining the area encompassed by the perimeter markers.

A group of 42 rats was subdivided into 4 groups and wounded as described above. Group I (15 rats) were allowed to heal without any other manipulation. Group II (15 rats) had 16, equally spaced, radially oriented, 5 mm, full thickness, skin incisions placed in the skin edge of the wounds every day after the fifth postoperative day (beginning of period of maximum rate of contraction). Group III (12 rats) were rewounded 7 days after initial skin excision (time of maximum rate of contraction) by excising a .5 × .5 cm area of skin from the cranial and caudal ends of both lateral wound edges. Removal of these portions of the wound created 2 centrally located opposing peninsulas of actively advancing wound margin, the leading edge of which contained an India ink mark. Rate of movement of these partially isolated wound edges was determined by measuring the distance between the tattoo

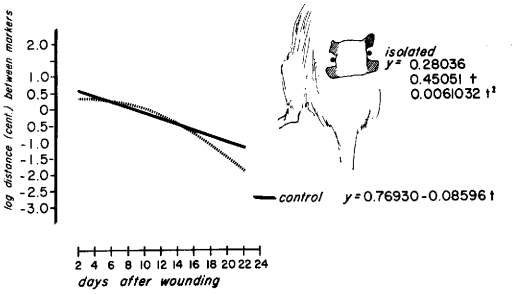


GRAPH I. Comparison of radially incised and normal wound contraction rates.

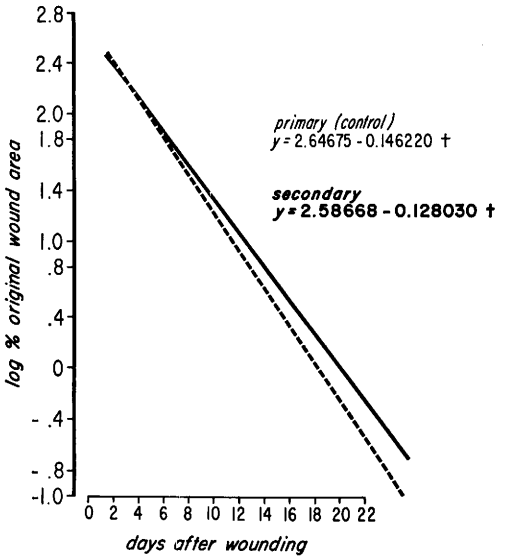
marks at regular intervals. The distance between coresponding marks in Group I rats was measured as a control for this experiment. Group IV (15 rats) were allowed to heal normally as the control group (Group I). Between the 20th and 24th day (or as soon as the wound was completely healed), scar tissue between the tattoo marks was cleanly excised and the wound edges allowed to retract to their original dimensions. The secondary wounds produced by this operation were allowed to heal without further manipulation, and rate of secondary contraction was compared with rate of primary contraction. In this experiment, each rat served as its own control.

Results. Comparison of the rate of wound contraction between Group II (radial incisions) and Group I (control group) is shown in Graph I. Analysis of the data reveals a significant reduction (P less than .15) in rate of wound contraction in rats which received multiple radial incisions as compared to rats with undisturbed wounds. Comparison of the rate of wound edge movement in partially isolated segments of wound margin (Group III) with non-isolated segments (Group I) revealed an even more significant (P less than .01) reduction in the rate of movement following interruption of the horizontally oriented peripheral cell mass (Graph II). Comparison of secondary wound contraction with primary wound contraction (Group IV) revealed no significant difference in the rate of the contraction process (P less than .10) (Graph III).

Discussion. Radially oriented incisions in the wound margins of Group II rats were envisioned as interrupting a possible sphincteric or purse string action of specialized cells in the "picture frame" area. Because cell regeneration and intercellular cementing is probably an extremely rapid phenomenon, the incisions were repeated at frequent intervals. The possibility that multiple radial incisions or isolation of sections of wound edge might interfere with blood supply at the



GRAPH II. Comparison of rate of movement of isolated and non-isolated segments of contracting wound margin.



GRAPH III. Comparison of rate of contraction of primary *vs* secondary wound.

dermis-subcutaneous tissue interface has to be considered. Decreased circulation does not seem likely, however, as the vessels are capillary in type, vertically oriented, and relatively profuse. No change in color or evidence of necrosis was observed following surgical manipulation. The possibility that repeated manipulation also might be interfering with the vertically oriented (dermal-subdermal) interface led to modification of the secondary wounding procedure in Group III rats. We reasoned that, by creating a single large secondary wound on either side of an advancing primary wound margin, the horizontally oriented cell mass would be disrupted more effectively and with less vertical damage than Group II wounds. More significant reduction in the rate of movement of such isolated segments strongly suggests that horizontal cell mass integrity has a measurable influence on the rate of movement of the wound edge.

The rate of secondary wound contraction (Group IV) was measured to document and confirm the impressions of Grillo that secondary manipulation of primary contracting wounds does not cause an acceleration of the contracting process similar to the accelerated rate of gain in tensile strength seen in incised and sutured secondary wounds(2,3).

Summary and conclusions. Repeated radial incisions which interrupt the horizontal integrity of the "picture frame" area of specialized cells in a full-thickness wound significantly decrease the rate of contraction of the wound edges. The rate of movement of a segment of advancing wound edge is retarded significantly by partially isolating such segments from the horizontally oriented picture frame cell mass. Rate of secondary wound contraction is not significantly different from rate of primary wound contraction. These data suggest that physical integrity of a horizontally oriented cell mass in the "picture frame" area of a contracting full-thickness skin wound exerts a measurable influence on rate of the contracting process.

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Culture Medium for Suitable Growth and Development of Embryonic Chick Femora *in vitro*.*† (29631)

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This laboratory has previously used a natural medium for cultivation of embryonic chick femora which consisted of rooster serum and embryo extract in a 3:1 ratio(1). It is clear that any attempt to define the hormonal and nutritional factors regulating growth in this organ culture has to begin with a definition of the medium. Biggers and his colleagues have taken a synthetic approach to the establishment of such a medium, adding compounds in a stepwise fashion (2,3,4). We have taken as our goal the identification of necessary growth factors in the natural medium.

Eagle and Peiz(5) have shown that in the presence of an adequate level of known water-soluble nutrients (minerals, amino acids, vitamins) only very small amounts of the protein synthesized by HeLa cell cultures were derived from protein in the medium. This suggested that we could replace the serum in the femur culture medium with one

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