Studies in Wound Healing:

II. The Role of Granulation Tissue in Contraction *

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MORPHOLOGIC STUDIES of contraction in healing skin wounds have led to the widely accepted hypothesis that the machinery for contraction lies in the granulation tissue. The component of this newly forming tissue implicated most frequently has been the collagen fiber. Recently, analytical studies of other elements of the granulation tissue have been made in an effort to isolate the responsible factors more definitively.

The suggestion that the process of contraction in healing skin wounds was due to shrinkage of the granulation tissue was first made by Carrel.[4, 5] This view was supported by Lindquist's experiments [9] where incision into the granulating wound appeared to release tension on the wound margins. Experiments by Abercrombie, Flint and James [1] where the granulations were excised yielded similar results.

The mechanism whereby the granulating area caused the wound margins to move remained unexplained. The view was widely accepted that the contraction resulted from shortening of collagenous fibers.[3, 10] The rise in collagen concentration reported by Abercrombie, Flint and James [1] and Dunphy and Udupa [7] might seem to support this theory. Abercrombie, Flint and James,[2] however, found that it was possible for contraction to occur even in scurvy where no collagen was formed and suggested that the force might be due to the connective tissue cells of the repaired tissue. On the other hand, Woessner [14] failed to observe contraction in wounds in scorbutic animals. Our studies of the chemical changes in contracting wounds, however, made us somewhat reluctant to accept the view that the granulation tissue filling the wound acted as the contracting unit.[8] No relationship to explain the process could be found between contraction and total tissue, water, collagen, hexosamine and tyrosine content and concentration. Our doubts were reinforced by observation of a "picture frame" of dense, white, connective tissue beneath the wound edge and strongly adherent to the base, which moved with the skin edge as contraction proceeded. This focussed our attention on marginal activity.

The experiments described here were designed to clarify the role of granulation

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tissue in contraction and to localize the site where the force for contraction is produced. The general procedure was to excise various parts of contracting skin wounds in guinea pigs and to measure the effect on the rate of contraction. As a result of these experiments, we have concluded that contraction is produced by activity in a narrow zone along the wound edge.

General Methods

White male guinea pigs weighing about 300 Gm. were used as described in the preceding report. The skin edges were marked as before with eight tattoo marks at the corners and midpoints of the sides of a wound 2.0 cm. square. Identical squares of skin down to the deep fascia (including panniculus carnosus) from each flank below the ribs were excised under ether anesthesia. The wounds were left exposed.

In every experiment one wound was used as a control. In addition, the baseline data for normal contraction of this type of wound was augmented by the results obtained from the unmanipulated wounds used in our previous chemical studies.[8]

Measurements were made by placing the animal in a standard position and tracing the wounds through translucent paper with a pen; the inner edges of the tattooed dots were used as markers of the margin of the normal skin. When the wounds were much contracted in the late stages, the inner borders of the marks were joined by straight lines, as the skin edge by then was indistinct. The area was measured with a planimeter; three readings were taken for each area and the average used in plotting results. In addition, the distance apart of the midpoints of the opposite sides was measured as an index of the distance the wound edge had advanced. For convenience, we have labelled the edges lying in the cephalocaudal axis of the animal "horizontal" and those at right angles "vertical." When incisions were to be made, the wound was

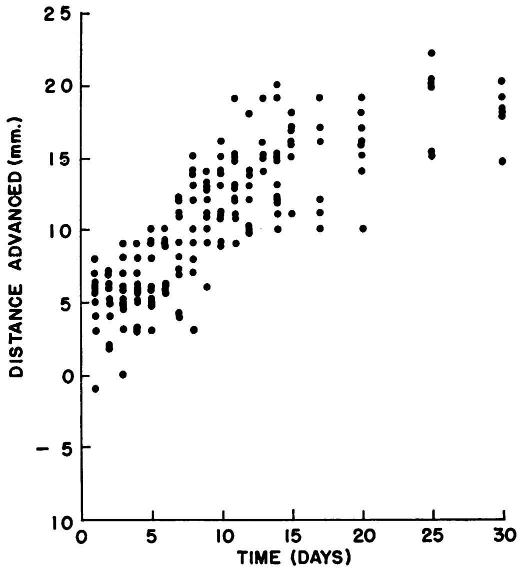

FIG. 2a. Linear movement of the cephalocaudal or "vertical" edges of the wound. These are the rapidly moving edges and result in the long vertical line of the final scar. Each point represents millimeters of defect across which the opposing edges have moved up to the day of measurment. Each point represents a separate wound.

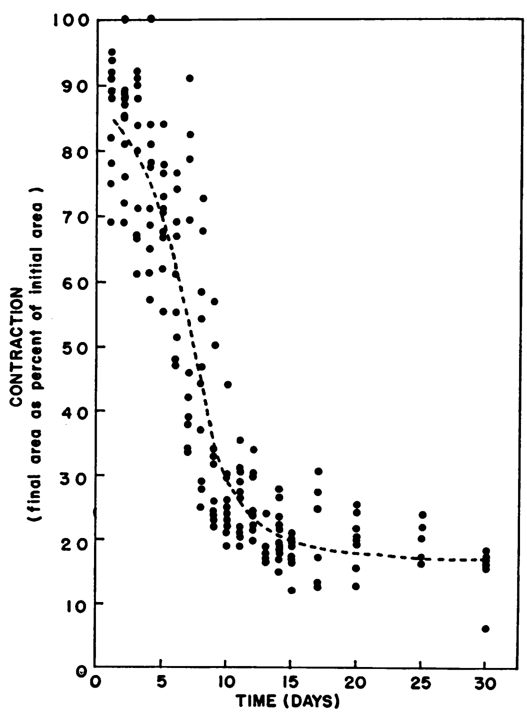

FIG. 1. Wound contraction with time. Contraction is calculated as per cent of initial wound area.

traced before and after making the incision, so that any change produced by the incision itself was recorded.

Results

Pattern of Healing in Non-manipulated Wound: Immediately after operation, the wound appeared as shown in Figure 2a in a preceding paper,[8] (page 147) but within a few hours the blood clot and exuded serum in the wound dried to form a thin scab. This steadily thickened and toughened during the next four days and eventually was cast off at about the sixth to the ninth day. It could, however, be easily separated from the third day onward without damaging anything except the epithelium. The latter showed signs of advance at once, but did not normally reach the base until about the third day. By this time, too, obvious granulations were present across the base of the wound.

The main process of uniform contraction was not established until after the third day. Prior to this, there was usually an initially rapid contraction in the first day in these undressed wounds followed by a lag. This was not clearly seen in the composite curve of contraction, but was evident in the individual plots. After the third day, contraction progressed rapidly until the twelfth to fifteenth day (Fig. 1). After this, the area reduced much more slowly. This contraction, however, did not occur in our wounds as a geometrically uniform decrease in area, as could be clearly demonstrated by measurement of the linear movements of the wound edges (Fig. 2). Before the fifth day, only the cephalo-caudal axis was reduced, the vertical sides moving inwards steadily, but the horizontal edges remaining stationary or actually moving outwards at the middle to form an irregular hexagon. The vertical sides advanced steadily until they met at about the twentieth day. The horizontal sides began to move inwards on about the fifth day and did so steadily until about the tenth, when they

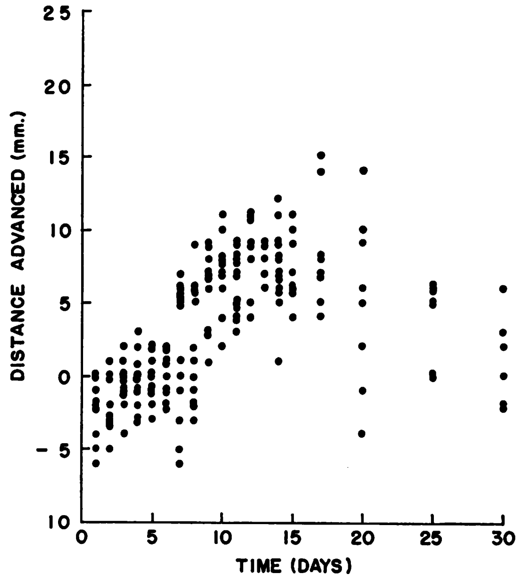

FIG. 2b. Linear movement of the dorso-ventral or "horizontal" edges. Initial increase in separation is commonly noted as evidenced by the many negative values in the first eight days. The process is actually reversed when the vertical edges come together.

stopped again. About the twentieth, they again retreated until they ended at approximately their original position. The wound by this time had assumed the characteristic stellate form described by others, although in our experiments we found a long vertical limb between the upper and lower parts.

During this process, a considerable amount of remodelling occurred in the wound edges as the changed length of the sides demanded. This was reflected in a changed shape assumed by the tattoo marks. The marks at the corners became elongated and pointed to the center of the wound; those in the middle of the fast moving vertical sides became elongated in the line of movement of the skin edge, whereas those on the alternate sides became compressed. A similar pattern was noted by Abercrombie, Flint and James.[1]

The effect of repeated scab removal was measured in two animals (Fig. 3) by removing the scab daily on one side of each animal and leaving the other side as a control. The epithelium was frequently dam-

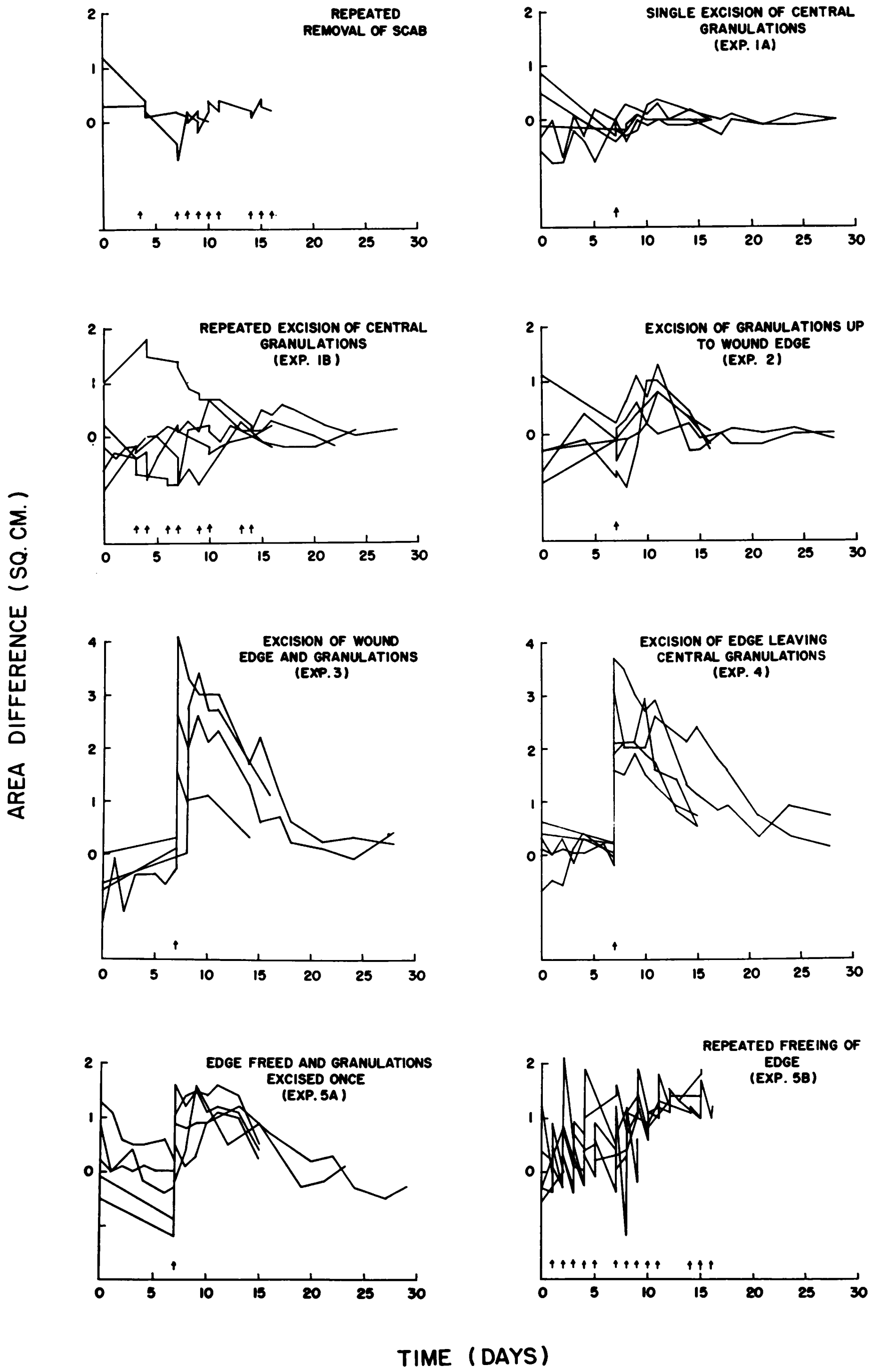

FIG. 3. Effect of manipulation on wound area and rates of contraction. Each of the eight graphs presents the results of a separate experiment involving manipulation of contracting wounds. Each line represents the area difference between the control and experimental wound in the same animal as a function of duration of wound. A starting value varying from zero merely indicates disparity in initial wound size. Arrows indicate times of manipulation of experimental wound. Maneuvers which affect contraction are manifested by increased positive values.

aged and epithelial closure was delayed markedly. This, however, had no effect on the contraction process. This was important as all the experiments described involved removal of the scab as a preliminary maneuver.

If the wound area was excised by blunt dissection of the outlying peripheral skin and panniculus carnosus from the deep fascia, there was no abnormal adherence to the deep fascia until the edge of the advancing skin was reached. This was found to be firmly bound down to the deeper structures. Once past this point, a further cleavage plane was found underneath the granulations which could be readily separated by blunt dissection. Gross examination of the base after removal of the skin and granulations showed a narrow (0.5 mm. wide) elevated ridge which lay along the line reached by the advancing wound margin.

Experimental Details: In general, the key experiments involved excision of different regions and amounts of the healing wounds. This was usually carried out on the seventh day of healing, a period when active contraction was still occurring. Area measurements were performed immediately and at daily intervals to chart the changes induced by the manipulations.

The results are illustrated in Figure 3 as the daily difference in area between the control and experimental wounds. Each curve represents a separate experiment in a single animal.

Experiment 1A (Five Animals)— Excision of Central Granulations: The excision of these wounds was carried out on the seventh day after wounding when the granulation tissue was well established. A narrow fringe of granulations (0.5 – 1.0 mm. wide) was left around the margin of the wound. The granulation tissue within this line was cleared completely down to the original base of the wound. There was no change in the rate or extent of contraction as compared with the control.

Experiment 1B (Five Animals)—Repeated Excision of Central Granulations: The excision here was the same as in 1A above, but was repeated whenever any granulations were visible; the average interval between excisions was three days.

It is clear that the rate of contraction was not influenced by these excisions (indicated by arrows on the graphs). Neither did the size of the wound change after the excision. (Unlike Lindquist,[9] we did not find that the central granulations contracted when they were incised.)

Experiment 2 (Five Animals)—Excision of Entire Granulating Area: Excision performed on the seventh day was carried up to the edge of the skin margin as indicated by the inner aspect of the tattoo marks and down to the original base. There was difficulty in excising the granulations without detaching the skin from the base outside the line of the incision. The results of this experiment were not as clear-cut as in the previous series. In some cases, contraction was delayed, but in others no change occurred and the wound continued to contract as though no intervention had taken place. It was not possible to decide whether the delay was caused by a detachment of the wound margin from the base, or whether the zone of activity actually extended in some cases into the outermost granulations so that it was damaged by the excision. Again, the wound did not enlarge at the time of excision of the granulations.

Experiment 3 (Four Animals)—Excision of Granulations and Skin Margin: Excision on day seven included 0.5 mm. of the original skin margin down to the deep fascia as well as all the granulations.

In sharp contrast to the results of experiments 1 and 2, excision which included only 0.5 mm. of the skin margin caused immediate distraction of the wound area to a size greater than that of the original wound (not the contracted wound) by about the area of additional skin removed (Fig. 3). The tissue encompassed by the incision did not get smaller in area either when in situ after the incision or after removal from the base. Following the incision, contraction began anew along a curve similar in slope to the original. It appeared that the skin away from the immediate wound edge acted to distract the wound; thus, it is unlikely that the elements causing contraction are acting outside the marginal skin.

Experiment 4 (Five Animals)—Excision of Wound Margin Only: The excision removed a narrow strip (1.0 mm. wide) of the wound margin, including skin edge and a very thin strip of the adjacent granulations down to the base, but left the central granulations still in situ. The results were, as one would expect, the same as in the previous experiment. The object, however, was to eliminate the possibility that the central granulation tissue might have some secondary role which was stopped by its excision.

Experiment 5A (Five Animals)—Elevation of Wound Edge and Removal of Granulations: On the seventh day after wounding, the granulations were removed down to the base as in experiment 2, but in addition, the margin was deliberately freed from the fascial base around the entire wound. There was immediate distraction of the wound and delay in subsequent contraction.

Experiment 5B (Five Animals)—Repeated Freeing of Edges: The edges of these wounds were freed repeatedly—usually daily, until the control wound had completed contraction. Granulations were not removed.

The effect of this procedure was to produce overall inhibition of contraction. It was found that at the end of fourteen days of repeated manipulation, the wound no longer contracted at the normal rate if left undisturbed. The final area was generally larger than the original excision. By this time there was a thick collagenous plaque in the granulating area over which the edge must advance. This suggested to us that the results obtained by some workers might have been considerably affected by using very large wounds which took many weeks to close. These wounds would also develop a similar sheet of collagen.

Discussion

These results show that the process of contraction in full thickness skin wounds is not caused by the granulation tissue filling the center of the wound. Neither are the edges pushed in by a process occurring in the peripheral tissue; in fact, there is a steady tension tending to distract the wound. Only manipulation of the wound margin interfered with contraction.

The machinery of contraction must, therefore, lie in a narrow zone underlying the advancing skin edge—the "picture frame." This thin strip of new formed connective tissue firmly binds the wound edge to the fascia of the wound base. Histologically, it appears to be a very cellular mass containing a little collagen but consisting mainly of fibroblasts. It appears that the inward moving "picture frame" pulls the skin along with it. One might speculate that the movement is produced by directional mass migration of connective tissue cells. Such migrations are well recognized in embryogenesis and have been described for epithelial cells in healing skin wounds in amphibia.[13] A rim of contracting connective tissue has been described before by Dann, Glucksmann and Tansley,[6] but a sphincter mechanism such as they proposed would not produce the stellate type of final scar, nor would separating the margin from the base interfere with contraction in the manner observed in this study. Abercrombie, Flint and James [2] suggested that the fibroblasts of the granulation tissue might be involved in pulling the edges together in a cellular contractile mechanism or by the rearrangement of cells. This was based on the observation that contraction could occur in scorbutic guinea pigs where collagen does not form. Woessner,[14] in contrast, found that the wound did not contract in guinea pigs suffering from scurvy. The point is obviously of importance. Using highly specialized preparations van den Brenk [11] found that the rates of wound contraction and granulation tissue formation were different and suggested that the processes were independent.

We did not confirm Lindquist's observation [9] or those of Abercrombie, Flint and James [1] that an incision at the wound margin gaped open and allowed granulations to contract. This, however, might be explained by the extreme narrowness of the "picture frame" zone and the smaller size of their wounds where the edge would be hard to detect. In addition, Lindquist's preparations were specialized by strapping of the edges and probably were disturbed when removing strapping.

The observed differences in rate of edge movements may result from any of several factors. We have shown, for example, in an unpublished study that tension can be a modifying factor. Weiss [12] has observed in tissue culture that orientation of the matrix can profoundly modify direction and rate of cell migration, a phenomenon which may apply here. Billingham and Russell [3] commented on the modifying effects of site of wounding on contraction.

It seems unlikely that only one process occurs in wound contraction. Probably in the later stages of cicatrization, a different mechanism is at work. The ultimate size and shape most likely depends upon scar tissue and its collagen component. It is probable, too, that in the very earliest phase, before new connective tissue appears, the contraction is of a different type, related to scab formation and drying.

Summary and Conclusions

1. Excision of central granulation tissue from a contracting open skin wound in normal guinea pigs did not interfere with the rate or extent of contraction.

2. Excision of the wound edge caused immediate distraction of the wound and delay in contraction.

3. Separation of the advancing skin margin from the wound base inhibited contraction.

4. It is concluded that the machinery for the major part of contraction of an open skin wound lies in the wound margin. The central granulation tissue is not required in the process of contraction nor is the peripheral skin actively involved.

5. It is suggested that contraction is not a single process. Late cicatrization is probably separate from the main phase of contraction which results in wound closure. It is also probable that other factors are at work in the earliest stages of contraction.

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