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Studies in Wound Healing:

I. Contraction and the Wound Contents *

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FULL THICKNESS skin defects in mammals heal in part by gradual approximation of the wound edges, a process observed as a reduction in wound area and described as contraction. The completeness of the process depends upon many factors such as location of the wound, size, the mobility of the skin and underlying tissues of the particular animal. Contraction has generally been attributed to changes in the granulation tissue of the wound bed. These have been described as tensile forces originating from collagen formation with subsequent fiber shortening, from diminution in wound contents, or from an as yet undefined mechanical pull of the granulating mass.[3, 5, 6, 8, 9, 12, 14, 15]

This study was undertaken to define more precisely the changes occurring in the composition and amount of the granulation tissue and to correlate this with the progress of the contraction process. In particular, we were interested in measuring water loss from the new forming tissue and changes in total wound content as possible contributors to the contractile mechanism, and in evaluating collagen production in relation to contraction.

These analytical studies did not identify changes in wound content and composition which could be correlated directly with the progress of contraction.

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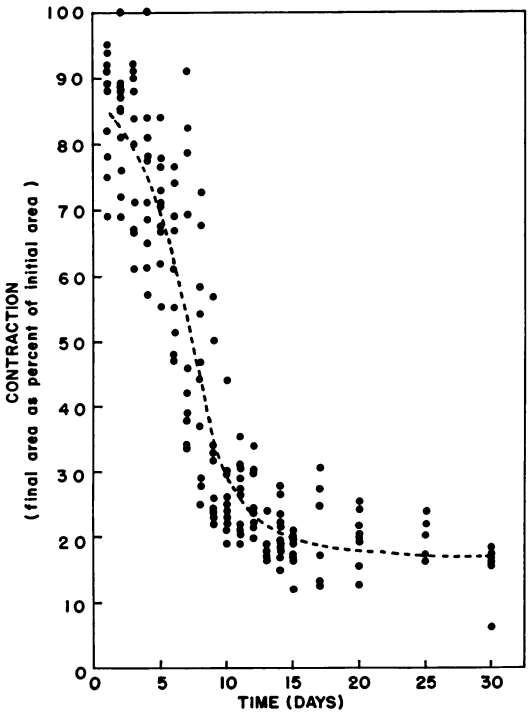

FIG. 1. The broken line is a mean curve of contraction. This fails to show the frequently seen distinct lag before the third to the fifth day which is evident in the individually plotted curves of contraction.

Methods

The studies were performed on white, male guinea pigs weighing about 300 Gm., receiving a standard laboratory diet of Purina rabbit pellets with lettuce and water supplement. The operative field was cleared with electric clippers and the remaining hair removed with a depilatory (Nair). Under ether anesthesia, using a modification of the tattooing technic of Abercrombie, Flint and James,[1] with the animal in a standard position, a square measuring 1.8 to 2.0 cm. on a side was demarcated on each flank. Four points were tattooed with India ink to mark the corners of the square and additional points were placed midway between each corner; the particles were deposited in the dermis. The wounds were made with sharp scissors by connecting the inner borders of the tattooed points, excising the full thickness of skin and panniculus carnosus, so that the deep fascia and its overlying loose connective tissue formed the base of the wounds. The tattoo points marked the advancing edge of the wound during contraction. No dressing was used. Scabs formed promptly and were left undisturbed. Sepsis was extremely rare; animals with infected wounds were discarded. The wounds were traced directly at the time of wounding and at intervals by marking the inner border of the tattoo points on translucent paper and interconnecting these points. Since the tattoo points move with the dermal edges of the original wound, a clear distinction can be made from the process of epithelialization which proceeds more rapidly. The midpoint tattoos are essential in order to obtain an accurate outline of the wound, because these move more rapidly than do the corners. The areas were measured with a planimeter, taking an average of three readings. Scabs were removed prior to final measurement.

The wound contents were excised totally along a line connecting the inner border of the tattooed points, so that the samples constituted as nearly as possible the new formed tissue within the wound edges. Analyses were done at three days, the earliest date at which wound contents could be dissected practicably and at four, five, six, seven, eight, 11, 14, 17, 20, 25 and 30 days. Three to eight wounds were analyzed for each time interval.

Analyses were done for total weight of tissue, water, hydroxyproline, hexosamine and tyrosine. Scabs were not included in the samples. Wet weight was determined immediately after excision and dry weight after dessication at 110° C. in vacuo in the Abderhalden drying chamber. The tissue was then ground cold in a Wiley mill and aliquots were again dried and analyzed.

Collagen was determined directly on a sample of ground tissue by hydroxyproline analysis * using the method of Neuman and

* Thus far collagen is the only known animal protein containing appreciable amounts of this amino acid. It has also been demonstrated in this

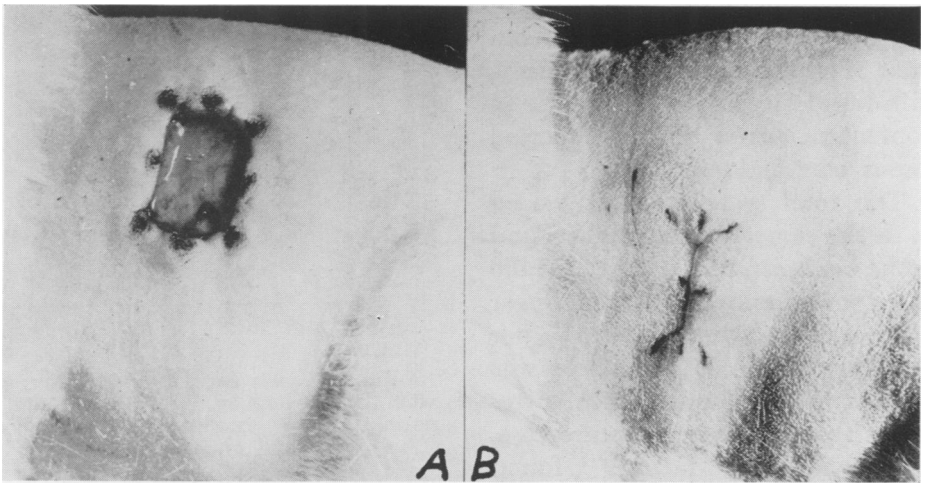

FIG. 2a. The fresh defect. The base is the deep fascia. The guinea pig's head is at the left. FIG. 2b. The fully contracted wound. The scar and tattoo points have assumed characteristic alignments.

Logan.[16] Hydrolysis was accomplished in 1 cc. of 6N HCl in a sealed tube in an oil bath at 138° for three hours. Hexosamine determinations were done by Boas' modification of the Elson and Morgan procedure [7, 11] as an indication of the content of hexosamine containing substances such as tissue and blood glycoproteins and mucopolysaccharides. Tyrosine determinations were done by the method of Bernhart [4] as an index of noncollagenous proteins, since purified collagen contains less than one per cent.[13] Determinations of these parameters were also made on normal skin, serum and on the exudate collected in Ivalon® sponges over the first 24 and 48 hours after wounding, before there is an easily dissectable layer of new formed tissue. The fresh wounds were covered with squares of polyvinyl sponge which were held in place by loosely placed sutures to the skin.

Direct comparison of total wound contents was made after correction for deviation of initial area from a standard wound size (four sq. cm.).

laboratory that analyses of the hydrolysates of whole skin give about five per cent more hydroxyproline than do analyses of gelatin obtained from alkali extracted autoclaved tissue. (Levene, C. I. and J. Gross: Evaluation of Preparatory Methods in the Analysis of Tissue Collagen. Laboratory Investigation, 7:258, 1958.)

Results

Contraction in Area. Contraction in wound area began in the first day, but did not become uniform until after the third to the fifth day. The wound was reduced by 30 per cent of its original area by the fifth day, by 55 per cent on the eighth day, was 70 per cent contracted on the tenth day and contraction was essentially complete by the fifteenth day (Fig. 1). Epithelialization was completed in most cases before full contraction but did not affect the continued approximation of the original wound edges. The wounds contracted in all cases in accord with a pattern, as pictured (Fig. 2a, b). The dynamic morphology of the wound is described and discussed in a subsequent paper.[18]

Total Tissue Content. When the total amount of wet tissue present within the confines of the wound edges defined by the tattoo points was measured, a rapid increase in total new formed tissue was noted from the third to the eighth day. The amount of wet tissue then declined sharply, the curve flattening out at three weeks

(Fig. 3). Comparison of the time curve of contraction with that of total wound content did not reveal a parallel relationship. In fact both rapid increase and decrease in wound content occurred during a period of continuous contraction.

Water. The total water content varied with time in the same way as total wound contents. *The concentration of water* in the whole tissue showed only a 4 per cent linear fall from 82 per cent at three days to 78 per cent at 30 days (Fig. 4). Thus, there was no dehydration of the wound which corresponds with the process of contraction. Paul, Paul, Taylor and Marsters [17] found essentially no loss of water in wound repair tissue in 12 days and a drop of only 8.2 per cent (from 82.9 per cent) in 18 days.

Collagen. Although the proportion of solids in the total wound tissue remained essentially constant, the distribution of components varied. No collagen was found in the first two days. A small but measurable amount of collagen was present in the wound on the third and fourth days.

The total amount of collagen began to rise sharply at five days to a maximum at

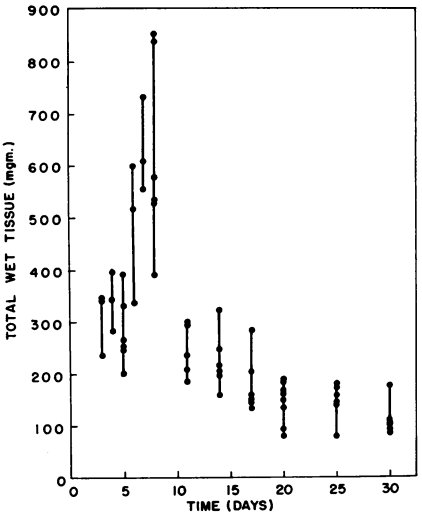

FIG. 3. Change in total wet tissue with time in days. Each point represents a wound. The difference between values for days 3, 4, 5 as compared with days 6, 7, 8 is clearly significant as judged by "t" test ($P < 0.005$).

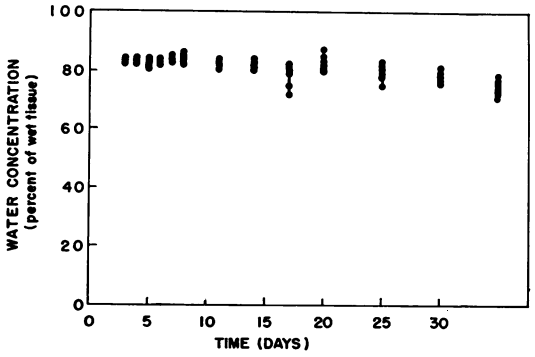

FIG. 4. Water concentration versus duration of wound. The data at the far right are values for normal guinea pig skin and subcutaneous tissue.

eight days; then the amount fell initially rapidly up to ten days, and then more slowly (Fig. 5). Collagen represented a smaller proportion of the total solids present when total wound content was maximum than it did later when total content fell. A lack of correlation between the contraction process and the total amount of collagen in the wound was evident.

The concentration of collagen (Fig. 6) rose rapidly from the fifth day to about the end of the second week after which it gradually approached the concentration found in normal skin. Since the water concentration of the wound was only very slightly and gradually reduced over the entire 30 day period, the contour of the curves was similar whether the collagen concentration was plotted in wet or dry tissue.

Hexosamine. Hexosamine concentration fell continuously in the first ten days from values in the range of those found in the serum in the first two days to levels found in normal skin and subcutaneous tissue (Fig. 7). The total hexosamine content of the wound increased just slightly prior to the peak of total tissue content.

Tyrosine. Tyrosine values fell in concentration from a level of 30 gamma per milligram of dry tissue on the third day toward levels found in normal skin and subcutaneous tissue (Fig. 8), probably reflecting a

fall in the total amount of noncollagenous proteins.

Discussion

The forces responsible for contraction of open wounds have generally been held to originate in the substance of the granulation tissue which forms in the defect.[6, 14] Our data, however, showed that contraction followed a course which was independent of the total amount of tissue filling the defect at various stages. While contraction proceeded continuously, the amount of tissue formed increased rapidly to a peak at eight days and then fell. This is of special interest since the hypothesis has been advanced that contraction is due to a reduction in the amount of granulation tissue in the wound.[1]

The relatively unchanged water concentration of the wound makes it apparent that dehydration is not the cause of the contraction process.

The early peak in total amount of collagen followed by a rapid decrease, lacking direct correlation with the curve of contraction, suggests that the two are unrelated. Although the total collagen in the wound followed the same pattern between the fifth and tenth day as did the total tissue, the concentration of collagen rose, indicating a loss of noncollagenous substances exclusive of water. The fall in concentration of tyrosine and hexosamine supports this contention.

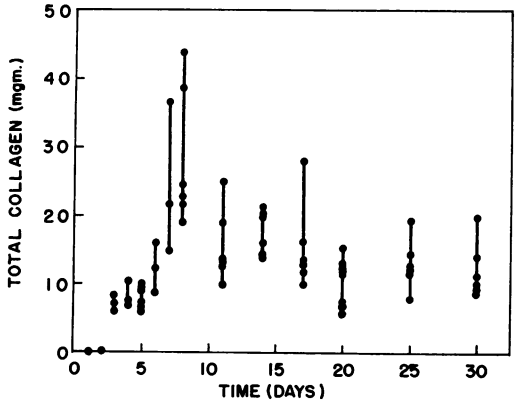

FIG. 5. Total collagen of wound as a function of duration. "t" test obtained for days 3, 4, 5 and 6, 7, 8 indicates significance of differences of values ($P < 0.005$). The differences for days 6, 7, 8 compared with 11, 14, 17 are also significant ($P < 0.01$).

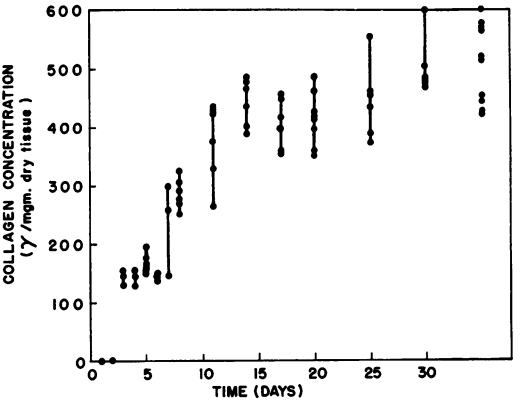

FIG. 6. Collagen concentration as a function of wound duration. The normal values for guinea pig skin are indicated at the right. The curve is entirely similar in contour if plotted for wet tissue.

The unexpectedly rapid decrease in total amount of wound contents after the eighth day may have been due either to resorption of new formed tissue or to its being covered by the advancing skin edges or a combination of both. Comparison of the rate at which total tissue diminished with the change in tissue weight per unit area suggests a combination of processes. In any event, the newly forming tissue within the advancing margins of the wound did not correlate directly either in composition or total amount with the rate of contraction. And it is this tissue within the wound edges which is under discussion when the role of the granulating mass in contraction is considered, not the regions of the wound bed already covered by the advancing skin.

Abercrombie, Flint and James [1] in their studies of contraction of open skin wounds in rats, observed a steadily increasing collagen concentration (on the basis of wet weight) at five, ten, 15 and 25 days. Our data generally agree with theirs in respect to concentration. However, in the later

stage determinations, their estimates of collagen concentration are high and show a continuing increase in total collagen content. This is probably due to inclusion of considerable amounts of normal skin because of the use of four corner tattoo points to mark the area excised for analysis. Normal rat skin contained almost twice as much collagen per unit as did their 15 day granulation tissue. The transient peak of production which we observed at eight days fell between two of their determinations. These authors concluded from the observed diminution in total wet weight and from their other data that active diminution of material in the wound area parallelled contraction and considered "that the content of the wounded area exerts a contractile force." However, they noted that collagen concentration continued to rise after contraction had ceased. Dunphy and Udupa [10] analyzed the collagen concentration of healing wounds up to 16 days on a dry weight basis and obtained similar results. They suggested that the apparent discrepancy between their data and that of Abercrombie, Flint and James was due to water absorption from the wound after the tenth day.

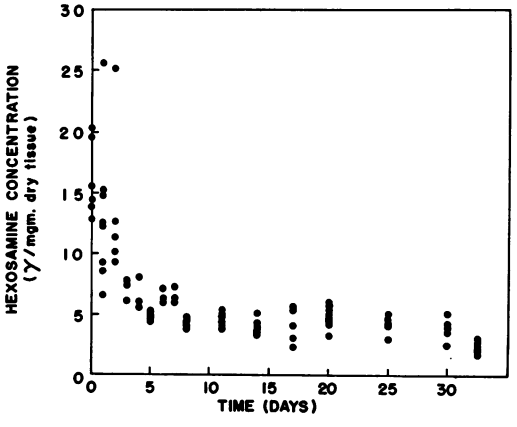

FIG. 7. Hexosamine concentration as a function of wound duration. The serum values are given on the time = 0 line, skin values at the extreme right. The 24 and 48 hour values are from exudate collected on sponges and represent lower than actual values.

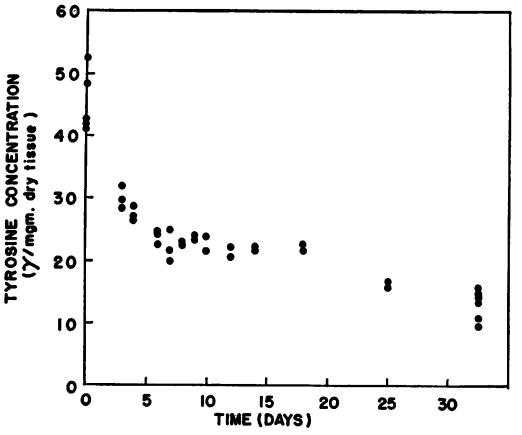

FIG. 8. Tyrosine concentration as a function of wound duration. Serum values are on the time = 0 line and normal skin values at the extreme right.

Numerous histochemical studies of healing wounds have indicated a temporal and geographical association between sugar containing high molecular weight compounds and connective tissue formation. The metachromatic reaction suggests that at least some of these substances are acidic mucopolysaccharides. Dunphy and Udupa [10] used hexosamine as a measure of tissue mucopolysaccharides and assumed that the hexosamine level rose rapidly from a baseline value of that in normal skin. However, hexosamine is a constituent of many substances and is present in relatively large amounts in serum as a component of glycoproteins. Thus aminosugar is a poor index of mucopolysaccharide in a complex tissue.* In our studies, the concentration of hexosamine fell continuously from 24 hour levels comparable with those of normal serum. This suggests that the hexosamine enters the wound primarily in the exudate and is in large part reduced as the exudate is removed. The analysis of exudate collected in

* In a recent publication Edwards, Pernokas and Dunphy recognized that hexosamine levels in whole tissue also measure glycoproteins. (Edwards, D. C., L. N. Pernokas and J. E. Dunphy: The Use of a Plastic Sponge to Sample Regenerating Tissue in Healing Wounds. Surg., Gyn. & Obst., 105:303, 1957.)

sponges from open wounds supplied only an approximation of the values of dry weight concentrations of wound constituents at any time and no information on total contents because of factors such as surface drying and cumulative deposition of materials whose composition may change with time. (In our hands, control analyses of Ivalon sponge plus added hexosamine and tyrosine have yielded values considerably less than the theoretical.) These data of course do not exclude mucopolysaccharides being involved in wound healing. More valid as an index of mucopolysaccharides would be determinations of nondialyzable uronic acid.

There is a progressive loss of tyrosine from the wound with time, closely following the curve of hexosamine. This suggests that the initially high concentration of noncollagenous protein comes from the blood as an exudate and is resorbed with time. Whether or not there is any noncollagenous protein synthesized by the fibroblasts to become an intrinsic part of the extracellular ground substance is not known.

Abercrombie, Flint and James [2] in later work found active contraction in the absence of collagen formation in wounds in scorbutic guinea pigs. This led them to propose that the connective tissue cells within the wound might be the causal agent of contraction. We do not propose that collagen formation is unrelated to the total healing of wounds and the development of tensile strength nor do we propose that the new forming connective tissue immediately beneath the wound margin is unrelated to contraction. But from the chemical data presented here, we believe that the mass of provisional tissue filling the open wound bed does not act as a unit to cause contraction. Chemical analyses have not revealed the mechanism of contraction. In our dissections we noted that the advancing skin edge was always firmly attached to a rim of newly formed tissue and that detachment of this rim resulted in edge retraction. This led us to suspect that the region of active contraction lies in this marginal area. Experiments designed to localize the site of the contraction mechanism are reported in a subsequent paper.[18]

Summary and Conclusions

1. Determinations of hydroxyproline (collagen), hexosamine (glycoproteins, mucopolysaccharides), tyrosine (noncollagenous proteins), water and total tissue weight are reported for the content of open healing skin wounds in guinea pigs and compared with the rate of wound contraction.

2. A rapid increase in total wound content occurred from the fifth to eighth day followed by an equally rapid fall which began to level off on the tenth day. Wound contraction progressed continuously during this period of rapid flux of wound content. These results are not consonant with an obvious causal relationship between total wound content and contraction.

3. The water content of the wound tissue fell very gradually over 30 days by only 4 per cent. Water resorption is therefore not a cause of wound contraction.

4. The total amount of collagen in the wound increased rapidly from the fifth day to a peak at eight days, followed by a rapid fall. This again is not reflected in the contraction process.

5. Hexosamine concentration decreased continuously from the level of normal serum to that of normal skin. This suggests that the wound hexosamine is mainly a measure of early exudate, and is not evidence for an early "productive phase" of wound healing. Tyrosine concentration diminished similarly.

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