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A METRIC STUDY OF SKIN HEALING

by
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Carrel in 1910 showed that the rate of wound repair was greater at the beginning of healing than at the end of it; also, the rate of repair depended not on the age of the wound but on its size, being directly proportional to it. This was confirmed by Spain and Loeb [1916] in the guinea pig.

To quantitate wound healing, Carrel and Hartmann [1916] developed a method of assessing wound healing by measuring tracings of wounds with a planimeter. Using this technique in animals, they found that contraction was a more important factor in the repair of a wound than epithelialization, which completed the work of contraction.

Using either direct measurements or the planimeter technique a number of studies of skin wound healing have been carried out in various species. In some of these studies the wounds were left open and scabs formed [Dann *et al.*, 1941; Abercrombie *et al.*, 1954; Grillo *et al.*, 1958; Watts *et al.*, 1958; Winter, 1964], while in others dressings were used and scabs presumably did not form [Billingham and Reynolds, 1952; Billingham and Medawar, 1956; Billingham and Russel, 1956; Sawhney and Monga, 1970].

The formation of a scab over a wound is a normal accompaniment of healing, its failure to form in a covered sterile wound is an artefact of surgical care [Billingham and Medawar, 1955]. As experimental studies could not be found in the literature comparing the healing pattern of wounds with and without scab formation, the present study was undertaken.

MATERIALS AND METHODS

Long-Evans rats weighing between 250 and 350 g were used in the study. They were anaesthetized with a droperidol/fentanyl mixture, Thalamonal (Janssen Pharmaceutics, Belgium—0.25 ml/100 g body wt by intramuscular injection), and approximately 25 cm^2 of the skin over the back of the animal was shaved.

Using aseptic techniques, a fold of skin was lifted up and clamped in a specially constructed perspex clamp which orientated a 7 mm diameter corneal trephine, set at a depth of 0.8 mm, at right angles to the skin (Fig. 1). The trephine was gently rotated between the fingertips and a circular wound made down to the level of the panniculus carnosus muscle but not through it; the vascular connective tissue just superficial to the muscle defined this depth (Fig. 2). In the same manner a total of 4 wounds was made on the back of each animal.

After many trial runs the dressings found most suitable in preventing scab formation were circles of sellotape (Sellotape and Adhesive Products, Johannesburg). Two layers of sellotape were placed so that their adhesive surfaces adhered to each other and then after being trimmed larger than the wounds, were placed one on each wound (Fig. 3). They were kept in place by strips of sellotape placed around the animal's body. This dressing, besides preventing scab formation, is transparent and enables one to inspect the wounds (Fig. 4). In a control series of animals the same operative procedure was used but no dressings were applied, the wounds being exposed to the air.

Two animals were killed with an overdose of barbiturate at intervals of 0.5, 1, 2, 4, 8 and 16 hours, daily up to 7 days and finally once the wounds had healed. All wounds were photographed under standard conditions immediately after being made and again at death. These photographs were enlarged to a standard magnification and the wound areas measured using a planimeter.

Statistical analysis was performed using the mean, standard deviation and Student's t test.

RESULTS

No animals died and no wounds became septic. In two animals from the experimental group scabs formed on three of the four wounds on each. These animals were replaced and no scabs formed.

a) *Healing of the wounds with scabs*

Immediately after being made the circular wounds had an area of 47.12 ± 6.1 mm^2 (mean $\pm$ standard deviation). There began now a slow ooze of blood into the wound until this was completely filled and the clot extended slightly above its edges. After approximately four hours there was an exudation of serum onto the clot surface, which soon dried. This together with the clot produced a firm scab.

As time progressed and the wound gradually reduced in area small flakes broke off from the first day. Between the fifth and seventh days large portions of the scab came off leaving only a small scab in the centre of the wound. The wound edge surrounding this had a smooth pale pink appearance which was felt to be due to epithelial migration. The scab finally disappeared on the twelfth day when epithelialization was completed.

The pattern of reduction in wound area is shown in Fig. 5. After an initial rapid decrease in area there was a slow steady reduction up to a total of 39.72 per cent in the first six days. The wound area then rapidly diminished over the next four days before slowing down again during the last two.

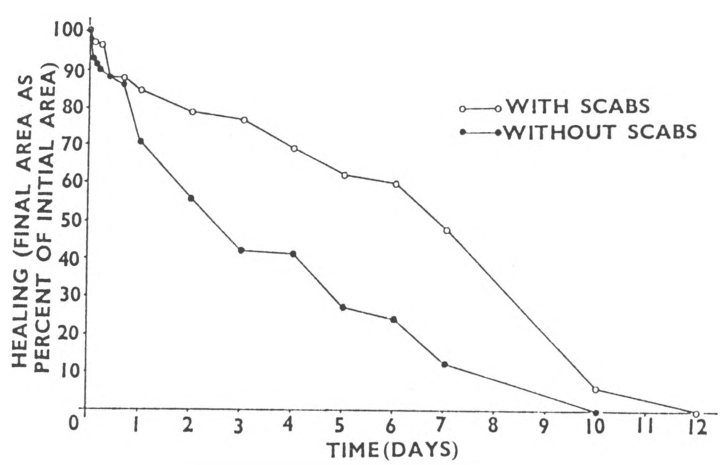

Fig. 5. Percentage healing related to time.

If the healing rate in mm²/hr is determined then two phases of healing can be seen (Table I). Beginning 30 minutes after operation and lasting eight hours, there is a rapid phase of healing having a mean value of 0.98 $\pm$ 0.39 mm²/hr. This is followed by a much slower phase which gradually reduces in rate and which has a mean value of 0.18 $\pm$ 0.08 mm²/hr. These two phases differ significantly from each other ($P<0.001$).

TABLE I

Phases of healing (mean $\pm$ SD mm²/hr)

	With scabs	Without scabs
Rapid (0.5-0.8hr) . . .	0.98 $\pm$ 0.39	1.62 $\pm$ 1.05
Slow (16hr-healing) . .	0.18 $\pm$ 0.08	0.32 $\pm$ 0.12

b) *Healing of wounds without scabs*

The mean area of the wounds immediately after operation in this group was 44.00 $\pm$ 2.2 mm². Contrary to the control group no scabs formed and within the first hour there was a fairly rapid reduction in wound area followed by a slight regression after eight hours (Fig. 5). From now until the third day there was a rapid reduction in area of 59.15 per cent. For the next day there was a lessening of the area reduction followed by an increase again until the wound was fully epithelialized by the tenth day. In this group epithelial migration was only seen one day prior to final closure.

As with the control group two phases were seen when the rate of healing in mm²/hr was calculated and which differed significantly from each other ($P<0.001$) (Table I). The rapid phase occurred earlier than in the control group and was of greater magnitude (Fig. 6). When the control and experimental groups were compared statistically there was no significant difference in the rapid phases but the rate of healing in the experimental group was significantly faster ($P<0.001$).

When the percentage reductions in area of the two series in Fig. 5 are compared there is an obviously faster reduction in the experimental group as compared with

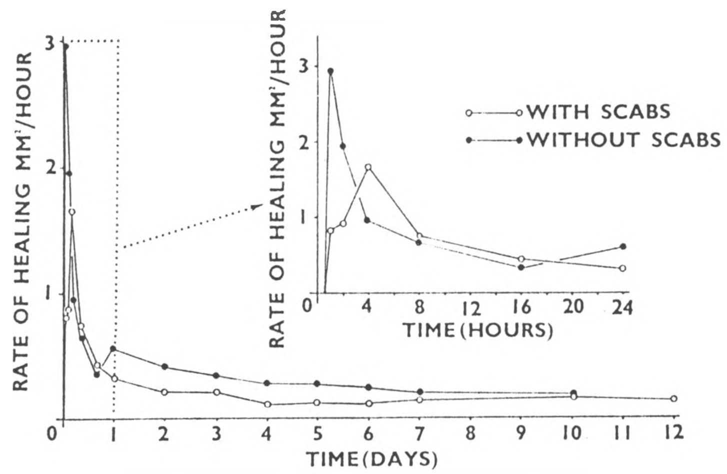

Fig. 6. Rate of healing in mm²/hr related to time.

the control group in the initial stages but these approach each other at 10 days. On the 10th day the control group did not differ significantly from the experimental group but did differ significantly from zero ($P < 0.05$).

Billingham and Russel [1956] suggested that for comparative studies the half-life, i.e. the time in which the wound area is reduced by 50 per cent, should be used. In this study the half life for the control series is 166 hours and that for the experimental group 41 hours which would suggest that the healing rate in the experimental group is four times faster than in the controls.

DISCUSSION

In this study healing was taken to mean complete epithelialization and not cessation of contraction. For this reason the india-ink marking techniques of Carrell and Hartmann [1916] and Abercrombie *et al.* [1954] which enable contraction to be measured were not used.

When cutting a circular wound with a corneal trephine of 7.00 mm diameter one would expect a wound area of 38.14 mm². In both the control and experimental series the wounds were larger. This is probably due to the natural elasticity of the skin retracting the wound edge. Any healing must first overcome this retraction and may possibly be a reason for the initially rapid rates of healing shown in Fig. 5.

The pattern of reduction in area in the control series was essentially similar to that described by Abercrombie *et al.* [1954] in rats and Grillo *et al.* [1958] in guinea pigs. The wounds without scabs however showed a much earlier reduction in area.

Although there was a significantly slower healing of the wounds with scabs over the first seven days, the area reduction approaches very closely that of the wounds without scabs on the 10th day. The fact that these reductions did not differ significantly although the wounds with scabs took a further two days to heal suggests that in a larger series both might be found to heal at the same time.

The rapid healing of the control wounds once the major part of the scab had fallen off suggests that in some way, possibly mechanical obstruction, wound healing is retarded by its presence. It might for example interfere with wound contraction although this appears to be balanced by an early epithelialization. Watts *et al.* [1958] examined the effect of repeated scab removals in skin wounds in two guinea pigs. They found that while epithelialization was delayed contraction was unaffected. We feel this was not supported in our study. The final slowing of healing between the 10th and 12th days could well be due to cessation of contraction followed by final epithelialization. This would agree with the findings of Abercrombie *et al.* [1961] and Carrel and Hartmann [1916].

When scabs are not formed there is a faster initial healing over the first two days followed by a slowing but with little epithelialization before the 9th day. This supports the possibility that the scab is a mechanical obstruction, interfering with wound contraction. One must also consider the role of the sellotape dressing itself. Winter [1964] covered superficial wounds with scabs in pigs with polythene film. He found that these wounds healed twice as rapidly as those exposed to air, something he felt due to the wound being kept moist. While moisture may have played a role, no effects of sellotape on tissues are documented.

Finally the half-life comparison time suggested by Billingham and Russel [1956] does not apply to the present study, as it suggests that wounds with scabs heal four times as slowly as those without which is not the true picture seen.

SUMMARY

The healing patterns of small, standardized, round, full thickness skin wounds in rats with and without scab formation, were compared. There was a significantly different pattern of healing between the two groups from one to seven days after operation. During this time the wounds not covered by scabs reduced more rapidly in area. The time of final healing differed between the two groups but this was not statistically significant. The different patterns of healing seen during the first seven days may be due to the scab acting as a mechanical obstruction thus interfering with wound contraction. The presence of moisture under the sellotape dressing used may play a role in accelerating the healing of the wounds without scabs. Any comparative studies performed during the first seven days should take these into account.

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Legends to Plate (overleaf)

PLATE 1

Fig. 1. Photograph of the orientating clamp holding the corneal trephine at right angles to a fold of skin.

Fig. 2. Freshly cut skin wound showing vascular connective tissue in the floor of the wound.

PLATE 2

Fig. 3. Circles of sellotape are placed on the individual wounds.

Fig. 4. The sellotape circles are held in place by sellotape strips right around the animals.