STUDY OF HEALING OF SKIN WOUNDS BY INDIA INK MARKING

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UDC 616.5-001.4-003.9-092.9-07 + 612.79 : 612.6.03

The possibility of using an India ink marking method to judge the state of wound healing was investigated in different animals. In adult rats displacement of ink marks applied around the wound edge reflects in its general features the course of wound contraction and the approximate position of newly formed tissues. In newborn rats and mice these processes cannot be judged from displacement of the marks because ink marks visible externally are located in the subcutaneous cellular tissue and the deep layers of the corium, and these layers of the skin are only slightly affected by wound contraction.

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Some investigators studying regeneration of the skin have used India ink marking techniques. From an analysis of the experimental data we have concluded that the behavior of ink marks in different animals shows certain special features, and failure to pay due regard to these may lead to serious mistakes when the results are analyzed.

In the present investigation the possibility of using ink marking to investigate the healing of skin wounds was studied in a series of mammals.

Ink was injected into the dermis of the animals either with two needles folded together or with a needle from a syringe. The marks were placed 1-2 mm away from the wound edge. Animals selected for the experiments included noninbred male albino rats weighing 120-150 g, newborn noninbred albino rats, noninbred male albino mice weighing 20-22 g, noninbred female albino mice weighing 18-20 g, and male CC57Br mice weighing 18-20 g. Altogether 216 adult rats, 435 mice, and 27 young rats were used in the experiments. Full-thickness skin grafts were taken from the mid-dorsal region of all the animals. The grafts were square or rectangular, with an area of 2.25-25 cm^2 in the adult rats, 1-6.4 cm^2 in the mice, and 1 cm^2 in the newborn rats.

The wound areas were measured at successive stages of the experiment. Pieces of tissue were taken for histological analysis from the region of the defect and adjacent areas of intact skin at definite stages of healing. The material was fixed in 12% formalin solution, passed through celloidin, and embedded in paraffin wax. The blocks containing pieces of skin embedded in paraffin wax were cut so that the section passed vertically through the ink mark. Histological sections 7-12 μ in thickness were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

The recent ink mark consisted of a sloping channel passing through all layers of the skin and reaching into the loose connective tissue of the subcutaneous fascia (Figs. 1A and 2A). This channel was completely filled with ink. It emerged on the skin surface where it formed a black dot (Fig. 3A).

In the early periods of regeneration (3rd-4th day) in the rats no ink was found in the surface epithelium. It lay as a heap of granules in the stratum pilare of the skin immediately beneath the epithelium. An accumulation of ink was also observed in the loose connective tissue of the subcutaneous fascia, where it was ingested by proliferating macrophages, gradually assuming the appearance of a black line directed toward the wound defect. In the other layers of the skin individual ink granules were observed, scattered at random.

Laboratory of Growth and Development, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR N. A. Kraevskii). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 2, pp. 123-125, February, 1968. Original article submitted December 23, 1966.

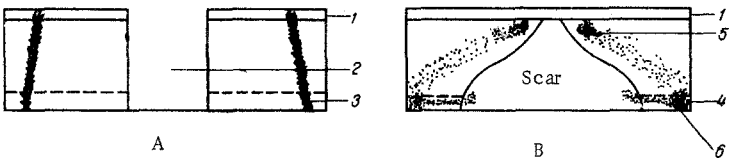

Fig. 1. Scheme showing displacement of ink granules in the corium in the neighborhood of a wound in rats. A, Initial position; B, at the moment of wound closure; 1) epithelium, 2) wound, 3) inner end of ink mark, 4) subcutaneous fascia, 5) collection of ink in upper layers of corium (outer end of ink mark); 6) collection of ink in subcutaneous fascia (inner end of ink mark).

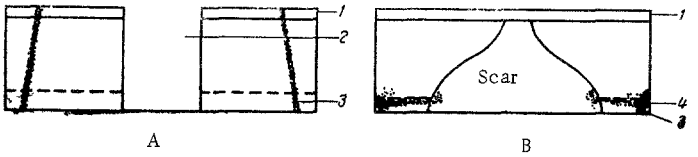

Fig. 2. Scheme of displacement of ink granules in corium in neighborhood of wound in mice and newborn rats. A, Initial position; B, at moment of wound closure; 1) epithelium, 2) wound, 3) collection of ink in subcutaneous fascia (inner end of ink mark), 4) subcutaneous fascia.

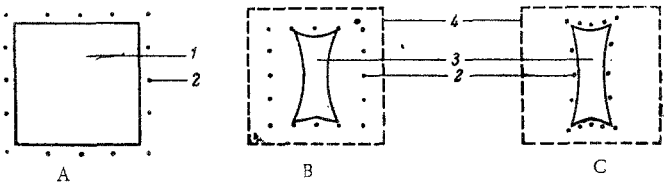

Fig. 3. Scheme of displacement of ink marks in rats and mice. A, initial position of ink marks in rats and mice; 1) wound, 2) mark, B, position of marks in mice and newborn rats near end of healing; 2) marks, 3) scar, 4) initial position of marks; C, position of marks in rats near end of healing.

On visual observation a collection of ink was seen (lying immediately beneath the epithelium), and throughout the subsequent periods of observation it resembled an indistinct dark spot. This spot gradually moved toward the wound, which diminished in size, indicating contraction of the wound.

The marks moved less in a cranio-caudal direction than transversely, indicating inequality of approximation of the wound edges in different directions.

In the case under examination, movement of the marks reflected accurately the course of the wound contraction, for in adult rats the marks remained for a long time in the upper layers of the corium next to the wound, and it was these layers which played an active part in the wound contraction process [1, 2, 7].

Near the end of healing, when a connective-tissue scar had formed, the marks came closer to the wound edge, lying 1-3 mm away from it; sometimes this distance was greater (Figs. 1B and 3C).

This arrangement of the marks in the later stages of regeneration may be explained as follows. Initially the marks were located in the skin immediately next to the wound. This area of skin was involved in the active repair process (intercalary growth). As a result of this process the area of skin between the marks and the defect increased in size and the marks moved away from the edge of the defect. In other

words, a small zone of "old" skin was always present between the scar and the marks, which were clearly visible on the skin surface, and its area varied considerably depending on variations in intensity of the intercalary growth [3-6].

Consequently, the area contained between the ink marks consisted not only of young tissues filling the defect, but also of an area of intact skin increasing in size because of intercalary growth.

If a full-thickness skin graft extending down to the cutaneous muscle was excised to include the wound defect and adjacent areas of skin, and it was examined on its inner aspect by transmitted light, a collection of ink could be seen in it in the subcutaneous fascia, from which radiated black lines becoming thinner as they came closer to the defect and became lost in the scar. The part of the mark (or collection of ink) lying on the inner surface of the skin has been described as the inner end of the ink mark. In rats this collection of ink cannot be seen with the naked eye, because of the comparatively thick skin of adult rats. In vertical sections passing through the ink lines radiating from these collections, a dense band of ink granules could be seen lying in the subcutaneous fascia, its end extending into the deep layers of the scar tissue, and in the earlier stages of regeneration into the deep layers of young connective tissue filling the defect. Some ink granules were found in the deep peripheral areas of young connective tissue.

Since the skin of mice and newborn rats is only between one-quarter and one-fifth as thick as that of adult rats, the defect caused by the needles used for injecting ink into these animals was much greater. For this reason a large quantity of ink spilled on to the skin surface with the blood and wound exudate soon after its injection into the dermis. In the early stages of regeneration (3rd-4th day) no large collections of ink could be seen in the stratum pilare of the skin, nor were they found in the surface epithelium. A large collection of ink was found only in the deep layers of connective tissue of the loose subcutaneous fascia.

In mice and newborn rats, because of their thinner skin, these collections of ink could be seen clearly with the naked eye over long periods of observation.

These visible collections of ink were essentially the inner ends of the original marks. In the course of healing of the skin, these marks became distorted, just as in the rats, into thin lines (bands) directed toward the wound defect.

All the original collections of ink in the subcutaneous fascia moved slightly during heating toward the wound for equal distances, but they remained a considerable distance away from its edges, and in some cases they remained practically in their original positions (Figs. 2B and 3B). This behavior of the marks was evidently due to the fact that wound contraction affects the subcutaneous fascia and deep layers of the corium only slightly, and displacement of the ink marks in this case cannot be used to judge the course of wound contraction, or the location of the newly formed tissues, for in such animals the area lying between the marks includes an extensive zone of intact corium.

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