A Histochemical Study of Skin Wounds

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The process of the healing of wounds has been investigated extensively (Blair, Slome and Walter, 1961). It is obvious that several changes take place during this process. The wound shrinks to smaller dimensions as healing advances, and biochemical substances increase or decrease at various stages of repair. The physical alterations or histological changes do not appear until several hours from the time of infliction of a wound. Investigations concerning the biochemical changes taking place in wounds have revealed no advance over the conventional histological methods in the detection of early reactions (Needham, 1952; Patterson, 1959). Nevertheless, it is logical to assume that some changes must be occurring in wounds from the time they are inflicted. A review by Raekallio (1961) of the physical, histological and biochemical methods showed that these methods can only detect vital changes in wounds more than eight hours after they are inflicted. Using histochemical methods in guinea pigs, Raekallio was able to detect enzyme changes as early as one hour after the infliction of a wound. In view of his striking findings, I decided to employ enzyme histochemical methods to study skin wounds in guinea pigs as well as in humans. Wounds inflicted after death were also examined for the presence of enzymatic reaction. Histologically, the skin of guinea pigs resembles that of humans, and the growth of hair in the two species is also similar. In guinea pig and in man, the growth cycle of each hair is independent of its neighbor, whereas in other animals, e.g., rat, mouse and rabbit, the hairs in one area are at the same phase of growth at any particular time (Rook, 1965).

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Materials and Methods

I used 50 healthy guinea pigs, both males and females, weighing 500–800 gm. The animals were anesthetized with ether, and the skin on the front and the back of the trunks and the limbs was shaved. Using sharp scissors, circular wounds about 1 cm in diameter were inflicted on the right half of the front and back of the trunk and on the right limbs, at 1, 5, and 10 minutes and ¼, ½, ¾, 1, 2, 3, 4, 8, 16, 32, and 64 hours before the animal was killed with ether. At identical periods after death, wounds were inflicted on the left half of the trunk and the left limb. Five sets of antemortem and postmortem wounds inflicted at these time periods were produced.

The human skin wounds were obtained from the autopsy room and the operating theater. In operations where removal of skin was a necessary procedure, a portion of skin was removed at 0, ¼, ½, ¾, 1, 2, and 3 hours after the infliction of the initial incision. These specimens were obtained from operative sites on the chest, abdomen and limbs of white male and female adults. One wound was inflicted on each person, and one set of skin samples was obtained from each wound, a total of five sets being obtained. Skin samples from wounds inflicted more than three hours before death were obtained at autopsy from white adults dying after a variety of surgical procedures. Five sets of postmortem wounds inflicted at corresponding intervals were obtained from the anterior, lateral and posterior aspects of amputated human legs.

One portion of each wound was frozen fresh with solid carbon dioxide immediately after removal. Sections were cut in a cryostat at −20° C and stained to demonstrate leucine aminopeptidase. Another portion was fixed in neutral-buffered 10% formalin at +4° C for 10-16 hours. Sections were cut in a cryostat at −20° C and stained to demonstrate non-specific esterase, acid phosphatase and alkaline phosphatase. Sections from a third portion were used to study histological changes and ribonucleic acid and desoxyribonucleic acid reactions. The details of the staining methods have been described by Pearse (1960).

Results

In the guinea pig skin wounds, the first unequivocal and constant histological change was an obvious leukocytic infiltration, in the form of a well-defined band, 100μ to 300μ thick, in the wound edge. This was seen from four hours onward. The nucleic acid reactions corresponded in time of appearance to the leukocytic infiltration seen in the histological preparations. Alkaline phosphatase and leucine aminopeptidase started to appear from

three hours, acid phosphatase from one hour, and the non-specific esterase from ten minutes after the infliction of a vital wound. The enzyme reaction, when present, was in the form of a gradually thickening band of staining, 100μ to 400μ wide, in the wound edge (Fig. 1).

In the human skin wounds, the first definite change in the histological preparations was the leukocytic infiltration in the eight-hour vital wounds, when the DNA and the RNA reactions also became obvious. Acid phosphatase started to appear after six hours, alkaline phosphatase and leucine and aminopeptidase after four hours, and the non-specific esterase after 30 minutes from the time of infliction of an antemortem wound (Fatteh, 1966a).

None of the postmortem wounds showed any histological or nucleic acid change or the enzyme reaction.

Conclusions

The experimental results in guinea pigs confirm Raekallio's findings. It is clear from the results that the enzyme reactions are the earliest detectable changes in the healing wounds. The non-specific esterase gives earlier staining reaction than any other enzyme studied so far. It is quite possible that a study of other enzymes may reveal even earlier detectable reactions.

The earliest appearance of the enzyme in the wound edge is probably due to the spillage of the intracellular enzyme following damage to the cells. A further noticeable increase of the enzyme may be accounted for by contribution from the blood stream with the increase of serum in the wound edges. It is also possible that local synthesis of the enzyme within the cells in response to trauma, and enzyme carried by the infiltrating leukocytes to the damaged area, are responsible for the increased reactions with the lapse of time.

It seems that the accumulation of the enzymes in the wounded zone is a defense mechanism. Enzymes appear in the wound edge earlier than the leukocytes. The infiltration by leukocytes in an area where the enzymes are already accumulating indicates that the enzymes may act as chemotactic substances and thus play a part in the healing process.

In both guinea pig and man, the wounds inflicted after death were completely devoid of any reactions. This fact is of medicolegal significance, as a positive enzyme reaction in a wound would help to label it as an antemortem wound (Fatteh, 1966b).

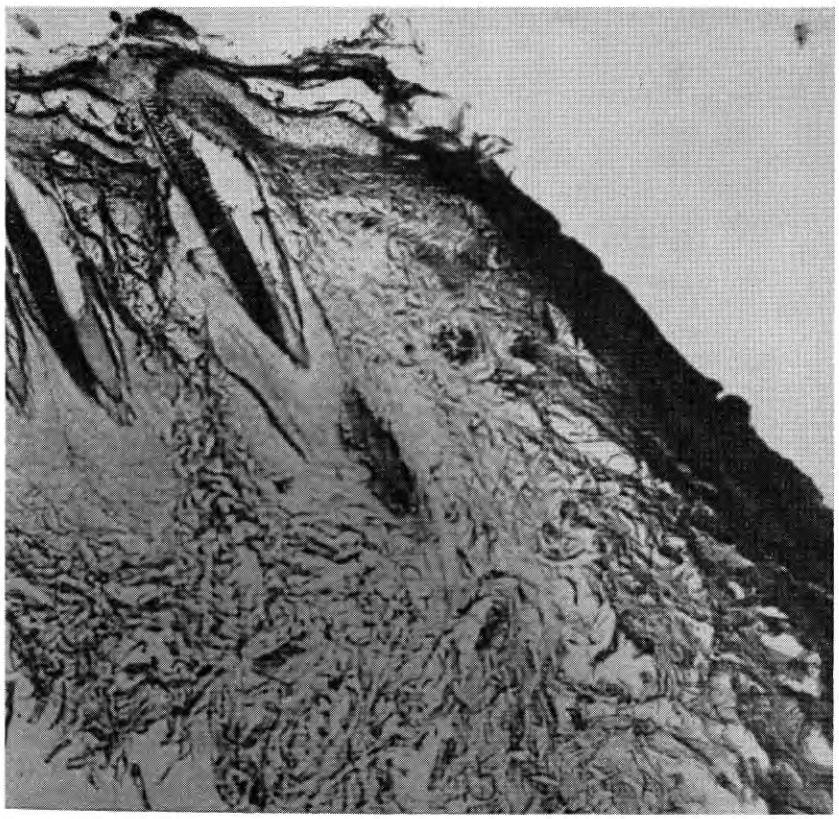

Fig. 1—Acid phosphatase staining of eight-hour antemortem wound of guinea pig skin.

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