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Cell Mitosis During Skin Wound Healing

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It has been well documented that an open (incised) skin wound through the epidermis and into the underlying dermis, results in a rapid, within 24 hours, and abnormal rise in the epidermal mitotic rate. The mitotic rate may be more than ten times the normal maximum, and is highest among those cells closest to the wound edge and lowest, near the normal rate, about 1 mm. from the wound edge. The decreasing mitotic rate within 1 mm. of the wound edge holds quite constant regardless of the size of the open wound. The theoretical explanation of this situation is presented by Bullough and Laurence (1 and 2) in the following three versions: 1. Following the theory that the epidermis is normally deprived of nutrients, it could be proposed that, since there is a marked hyperaemia around the edges of the wound, the increased mitotic activity may be due simply to a greatly increased supply of nutrients. 2. The high mitotic rate could theoretically be due to the stimulating influence of a "wound hormone" secreted from the wound itself. This has been the commonly accepted theory for nearly half of a century. However, the "wound hormone" theory was fully reviewed by Abercrombie (3), who concluded that in spite of many claims, no hormonal substance with mitogenic activity has been extracted from wound tissue, and the possibility must now be considered that no such substance exists. 3. The situation could be explained on the theory that the epidermis normally contains some substance which inhibits mitotic activity, and that the concentration of this substance is reduced in the immediate vicinity adjacent to the wound. This alternative is presently favored by most researchers in wound healing.

The work of this author gives evidence that the zone of the highest mitotic rate in wounds caused by dry ice burns is not immediately adjacent to the wound periphery, as is the acknowledged in the instance of open (incised) wounds.

Experimental methods: Dry ice burn wounds, of about 20 mm. in diameter, were made upon the shaved backs of 24 Sprague-Dawley rats which had reached the end of their growing period and were about 250-300 gm. in weight. In an attempt to use a quantitative approach to the study of mitosis in wound repair, tritiated thymidine (H^3T) was employed to indicate the areas of greatest mitotic activity. Cells that are replicating their chromosomal DNA during prophase of mitosis take-up the H^3T and become radioactive. Cells so labelled can be identified by autoradiographs of microscopic tissue sections.

The H^3T was prepared by New England Nuclear Corp., Boston, Mass., having a specific activity of 5000 millicuries per millimole, in an aqueous solution. Four millicuries per gram of body weight was injected into the rats via cardiac puncture. The individual rats were injected with H^3T 24 hours prior to being sacrificed. Three rats were sacrificed at each of the following post-burn intervals: 2, 4, 6, 8, 10, 12, 14 days, and autoradiographs were made of tissue sections from the edge of the wound area to a distance 8 mm. from the wound.

Results: Autoradiographs showed that no mitosis occurred immediately adjacent to the wound edge, but first took place at a distance about 1.0 mm. from the wound, this mitosis occurred within 24 hours of inflicting the burn wound. At a distance of 2mm. from the wound, the mitotic rate was very low for the first 2 days, reached a maximum on the fourth day, and then slowly reduced to a normal rate after 8 to 10 days. The migration of the dividing epidermal cells toward the center of the wound were blocked for 6 to 8 days by mechanical obstruction of the necrotic wound tissue. As a result the epithelial cells piled up from the normal five or six to ten or twelve layers. From the eighth to the twelfth day, at which time cell mitosis had diminished considerably, a rapid migration of cells of the epidermis takes place under the scab formation and closes the wound.

Conclusions: The above results sharply contrast with the mitotic activity of epidermis during the closing of an open wound. Whereas in the mitotic rate of an open wound the greatest activity is noted immediately adjacent to the wound, and decreases sharply within 1 mm. of the wound edge, the mitotic rate pattern of a dry ice burn wound is nearly opposite. In burn wounds there is little if any epidermis mitosis up to a distance of 1 mm. from the wound edge. The first active mitosis noted following the burn wound begins at a distance about 1 mm. from the wound edge and extends out 2 to 2.5 mm. In open wounds, regardless of wound size, no epidermal mitosis is recorded as far as 2 to 2.5 mm. from the wound edge.

Perhaps inhibitors produced from the necrotic tissue are responsible for the lack of epidermis mitosis immediately adjacent to the dry ice inflicted burn wound. Invesigation for the isolation of a possible mitotic inhibitor produced from necrotic burn wound tissue is presently underway by the author.

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ZOOLOGY

Nesting Habits of the Soft-Shelled Turtles (Trionyx Sp.)

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Introduction: Soft-shelled turtles (*Trionyx* Sp.) are common along the Mississippi River but, in spite of their abundance, the literature contains very few accounts of their life history, particularly with respect to nesting. The principal contributions concerning the ecology of *Trionyx* have been made by Breckenridge (1944); Cahn (1937); Carr (1952); Ditmars (1936); and Pope (1939 and 1955).

The present study was undertaken to learn more about the nesting habits of the *Trionyx* turtles in the area near Winona, Minnesota. Because of the great similarity between species of *Trionyx* turtles, and because close observation would have disturbed their normal nesting activities, no attempt was made in this study to identify the turtles to species.

Methodology: Field work was done during the entire month of June and the first half of July, 1962. The study area was a peninsula which extends downriver on the Minnesota side of U.S. Lock and Dam No. 5A on the Mississippi River (Figure 1). This sand beach provided steep and gradually sloping areas, and open to heavily brushed shorelines. Observations were made three times weekly on the average, and more frequently during the period of heavy nesting. Observations included all times of day from sunrise to sunset.

A boat was used to reach the study area and sometimes to observe from off-shore. A pair of 8 × 30 binoculars aided in observing the turtles as they were too wary to be observed at close range. Most observations were made from a camouflaged blind of mosquito netting. A compass and a tape measure were used to plot nest locations.

DISCUSSION:

Observation of the Beach. Pope (1955) has noted that a soft-shell preparing to nest first makes an observation of the beach from the water. In this study it was also apparent that the turtles surfaced to observe and then submerged several times before coming ashore.

Leaving the Water. The turtles seemed to prefer coming ashore where the beach was not heavily brushed, and yet not open for more than about 15 feet along the shore. Cahn (1937) saw a soft-shelled turtle leave the water and then return several times before nesting. This was not observed in this study, but since only six turtles were actually observed in the act of nesting, the habit of returning to the water several times may not be uncommon.

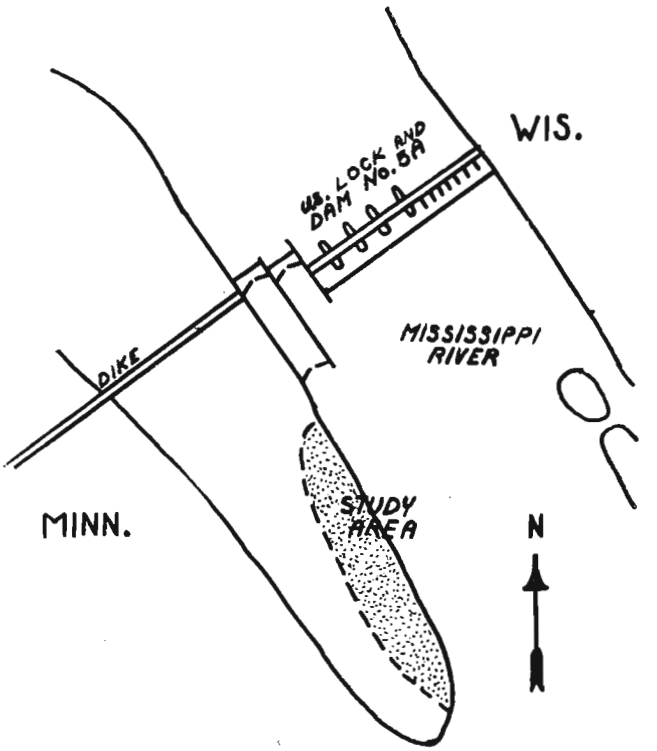

FIGURE 1. Mississippi River near Winona, Minnesota showing the location of the study area (shaded).

A factor which seems to play a part in the turtle's selection of the beach is the steepness of the beach. No definition of steepness will be used here as the study area only had steep and gradual beaches and none which were intermediate. Although other turtles were observed using the steep beaches, only gradually sloping beaches were used by the soft-shells.

Nesting activity was most intensive on warm, sunny days. All of the turtles which were seen nesting in this

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