

# THE MITOTIC RATE IN TADPOLE SKIN AFTER REPEATED INJURY<sup>1</sup>

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The epidermis of frog tadpoles usually shows a very low rate of mitotic division (Cameron, 1936a, 1936b). The present report is based on an attempt to set up a situation highly favorable to active mitosis. It was thought important to ascertain whether the consistently low rates obtained had been determined by specific inhibiting factors related to the age and development of the animal, the time of year, the existence of mitotic rhythms, or the manner of feeding and maintaining the tadpoles used in previous work.

## MATERIAL AND METHODS

Bullfrog tadpoles about six centimeters long were used. Care was taken to reject those showing signs of metamorphosis. Each was kept in a one-liter beaker half full of water, the beakers being surrounded by a water bath at 25° C. Eight 150-watt Mazda lamps, inside frosted, with reflectors, were grouped 18 inches above the water level in the beakers. Constant illumination was provided from 6:00 A.M. to 9:00 P.M. daily. Black curtains excluded stray light during the remaining hours. The bath and lights, with their electrical control system, were kindly supplied by Professor Albert Saeger.

After the tadpoles had been 24 hours in the bath, the posterior half-centimeter of the tail of each was cut off and fixed in Bouins fluid. Successive half-centimeter pieces were taken at 24-hour intervals. Each cut was approximately at right angles to the tail axis. Beginning with the second amputation each piece of tissue had for its anterior face a freshly cut surface and for its posterior face a surface which had been cut 24 hours earlier. The four lateral faces were covered with original epidermis which had contributed cells for the covering, by migration, of one or more "posterior face" surfaces.

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Each block of tissue was cut into vertical sagittal sections  $10\ \mu$  thick and stained with Mayer's hæmalum and orange "G." Thus each microscopic section of any piece, after the first or tail-tip piece, had along its posterior border a surface over which cells had migrated during the previous 24 hours, unless the section was a lateral surface section.

TABLE I

*Data from a tadpole in which each injury was covered by migration within twenty-four hours.*

Days	Previous injuries	Average cells per section	Sections counted	Mitoses counted	Mitoses per 10,000 cells
1	0	12,800	35	51	1.1
2	1	2,100	26	6	1.1
3	2	2,340	30	9	1.3
4	3	4,000	36	18	1.3
5	4	1,800	49	8	0.9
6	5	2,400	45	11	1.0
7	6	950	60	5	0.8
8	7	2,300	64	12	0.8

TABLE II

*Data from a tadpole in which all but the last two injuries were covered by migration within twenty-four hours.*

Days	Previous injuries	Average cells per section	Sections counted	Mitoses counted	Mitoses per 10,000 cells
1	0	10,800	14	56	3.7
2	1	10,040	11	35	3.2
3	2	4,800	12	18	3.1
4	3	2,150	13	5	1.8
5	4	3,800	21	15	1.9
6	5	5,100	22	22	2.0
7	6	3,800	31	151	13.2
8	7	3,500	42	732	50.0

The mitotic figures in all the epidermis of every fifth section of each piece were counted at a magnification of  $660\times$ . Mitotic figures were found in the original epidermis and very rarely in "new" epidermis formed by migration within 24 hours after injury. Rates per 10,000 cells have therefore been taken from the ratios of total mitoses to number of non-migrated epidermal cells. Average numbers of cells per section were obtained by counting selected sections from the region midway between the first lateral and the center sections of each piece of tissue.



*Findings*

Tables I and II are samples of the results obtained, one for each of the two classes into which the tadpoles studied can be logically separated.

## DISCUSSION

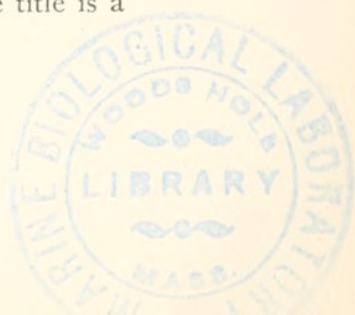
The experimental animals, living under conditions favoring a high rate of general metabolism, were subjected to successive injuries each requiring a greater number of cells to cover it on account of the increasing diameter of the tail cephalad from the tip. The epidermal cells anterior to any injury were subjected to some degree of stimulation to migrate over the cut surface and to divide, assuming that injury is the source of a stimulus to mitosis. The increase, if any, in relative frequency of mitosis in regions anterior to and near the successive wounds might be taken as an index of the degree to which mitosis supplied the new cells required.

In the first animals studied no increase in the mitotic rate was found. In cases where seven injuries were followed by seven complete coverages (see Table I), the rate remained around 1 in 10,000. Table I is a sample of counts made by K. O. Mills. The report (Cameron and Mills, 1936), made at the General Meeting of the Marine Biological Laboratory for 1936, was based on these data. The rate here is definitely lower than the rate found in adult frog skin adjacent to areas injured by X-rays (Cameron, 1936*b*, Table I<sup>3</sup>).

There were some individuals in which the latest and most extensive injuries were not completely covered by "new" epidermis after 24 hours. Counts of these specimens show that the mitotic rate rises sharply in the neighborhood of areas not covered in the usual manner. Table II is an example of records of this class. The injured areas of the second through the sixth pieces were covered, the seventh had an uncovered area about one-third the diameter of the notochord, and the eighth had an uncovered area about the diameter of the notochord.

The mitotic rate in the seventh is about five times the average rate of the previous six, and the rate of the eighth about twenty times the same average. The conditions associated with incomplete coverage within 24 hours may then be credited with a twenty-fold increase in the mitotic rate, and it is inferred that the low rates in the other cases indicate that cells were being supplied through migration. The same general picture is found in other specimens.

<sup>3</sup> This table is for 10,000 cells, not for 1,000. The figure 1,000 in the title is a misprint.



It is also possible that there is simply a cumulative or additive effect of all the injuries to a given tadpole which sets off a period of division as soon as the required threshold is attained. Certainly the conditions leading to failure of rapid coverage of the injured surface are closely related to those producing an increased mitotic rate. It also seems clear that even skin which has maintained a very low rate for a long period can be stimulated to active proliferation.

#### SUMMARY

Successive half-centimeter pieces were cut each day for eight days from the tails of bullfrog tadpoles. The epidermis maintained a very low rate of epidermal mitosis despite the great loss of cells by migration over the injured surfaces. Conspicuous exceptions with relatively high rates were found in cases where the epidermis failed to cover the seventh or eighth wound within twenty-four hours after injury. Here the mitotic rate reached a value twenty times the previous average rate.

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