THE EFFECTS OF X-RADIATION ON LIMB REGENERATION AND DEVELOPMENT IN AMBLYSTOMA[1]

W. O. PUCKETT

DEPARTMENT OF BIOLOGY, SOUTHWESTERN, MEMPHIS

It has recently been shown by several investigators, Curtis and Hickman (1926), Stone (1932, 1933), and others that regeneration processes in different invertebrate groups are inhibited by exposure to x-radiation. Butler (1933) found that limb regeneration in Amblystoma was also prevented by exposure to x-radiation and made a careful study of the histological changes associated with the non-regenerating tissues.

The present investigation was carried out to determine: (1) the effects of the radiation on limb regeneration of larvae of different ages, (2) the minimal dosage of x-radiation necessary to prevent limb regeneration, and (3) to secure additional information as to the histological changes associated with the failure of the limb to regenerate. As the investigation progressed other interesting results were obtained which will be outlined below.

Larvae of *Amblystoma punctatum* of different ages ranging from the early fore-limb bud stage to larvae with fully developed fore and hind limbs were used in this investigation. The limbs were amputated with iridectomy scissors with the larvae under chloretone anesthesia. The larvae were radiated "in toto" in small dishes of water. The source of radiation was a Coolidge medium-focus tube. The factors governing the radiations were as follows: 60 K.V., 6 ma., distance from the target to the larva, 25 cm. The rays were unfiltered. The output of the tube as determined by standard ionization chamber was 30 Roentgen units per minute. The only variable factor was the length of the exposure.

It was found that x-radiation prevented limb regeneration in all ages of larvae used. Regeneration did not cease immediately after exposure to x-radiation but a latent period of six days was found during which regeneration equaled that of control animals. At the end of this latent period regeneration processes ceased. Control larvae regenerated a complete limb in 25 to 30 days.

Histological studies revealed that in the case of young limb buds

---

[1]This investigation was carried out in the Biological Laboratories of Princeton University. The paper in full (50 pages and 35 figures) has been accepted for publication by the Journal of Morphology. The abstract as presented here was given as a paper before the Tennessee Academy of Science in session at Vanderbilt University, November 30, 1934.

before cartilage formation had set in, the failure to regenerate was due to a complete destruction of the undifferentiated mesenchyme cells making up the limb bud. This finding was in line with many other investigations which have shown the extreme sensitivity of undifferentiated cells to x-radiation. In older larvae, in which the amputations were made through the middle of the humerus, x-radiation completely suppressed the formation of the regeneration blastema. Later there followed a complete dedifferentiation of the remaining portion of the humerus and of all the other formed structures of the limb stump. This process of dedifferentiation spread into the cartilages of the shoulder girdle and the entire limb stump was left as a small sac filled with dedifferentiated mesenchyme cells. After 25 to 30 days these dedifferentiated cells regained their power of mitotic cell division but were never able to differentiate into the component structures of a new limb. The effect here seemed to be primarily one on cellular differentiation.

Data was also obtained as to the minimal dosage of x-radiation necessary to inhibit limb regeneration. It was found that a single exposure of 10 minutes (300 r) would effectively check regeneration processes. In most cases radiation was made immediately after the amputation of the limb. However, in other cases it was found that limbs radiated 30 days or more before the date of amputation lost their powers of regeneration if subsequently amputated. During the period after radiation they grew at a fairly normal rate, but appeared to be "castrated" against regeneration.

It was also found that limb development in young larvae could be controlled by properly governed radiations. The undifferentiated mesenchyme cells going into the formation of limb structures showed a marked sensitivity to x-radiation so that the differentiation of the limb could be arrested at any desired point by properly governed radiation. By radiating at the proper stages in development, larvae with permanent two, three, or four digit limbs could be produced. The effect here is primarily on the differentiation processes of the limb. Larvae in which digit formation has been suppressed grow at a fairly normal rate, but their limbs fail to regenerate if subsequently amputated.

The results as presented here do not appear to be peculiar to x-radiation. Investigations under way at present show that limb regeneration in Amblystoma is also checked by ultra-violet radiation. Treatment of the limb stump with ultra-violet radiation also leads to a complete dedifferentiation of the formed structures of the non-regenerating limb stump and gives a histological picture very similar to the non-regenerating limb stump of x-rayed limbs.

LITERATURE CITED

Curtis, W. C., and Jane Hickman. 1926. The effects of x-rays and radium on regeneration in Planarians. *Anat. Rec.*, 24:

Stone, R. G. 1932. The effects of x-radiation on regeneration in Tubifex tubifex. *J. Morph.*, 54:

Stone, R. G. 1933. The effects of x-radiation on anterior regeneration in Tubifex tubifex. *J. Morph.*, 54:

Butler, E. G. 1933. The effects of x-radiation on the regeneration of the fore-limb of Amblystoma. *J. Exp. Zool.*, 65:

ANNOUNCEMENT OF THE SPRING MEETING OF THE TENNESSEE ACADEMY OF SCIENCE

The Thirty sixth Meeting of the Tennessee Academy of Science will be held April 26-28, 1935, at Reelfoot Lake. *Headquarters* will be Walnut Log Lodge, the management of which has arranged a very reasonable special rate of $5.00 per person for the Meeting. This will cover room and all meals beginning with luncheon on Friday, April 26th (the first meeting day), and ending with breakfast on Sunday morning, April 28th. Members registered at the Lodge will not be charged extra for the *Academy Dinner.*

Members of the Academy who desire to present scientific papers should notify Doctor A. Richard Bliss, Jr., Chairman of the Committee on Arrangements, Crosstown Station, P. O. Box 6308, Memphis, Tennessee, at once. The Committee wishes to know the title of your paper, the length of time necessary for presentation, whether a lantern, moving picture machine, or other apparatus is desired, the place you prefer on the program, and, if possible, a brief abstract of your paper.

The sessions will be held in the Assembly Room of the Biological Station, which is equipped with 110-volt electric current, gas, running water, etc., for projection and demonstration purposes.

Hotel reservations should be sent to Mr. O. T. Wollaston, Walnut Log Lodge, Route 5, Hickman, Kentucky, as soon as possible.