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## ABSTRACT

Regeneration in the adult salamander, *Ambystoma annulatum*, parallels that of the adult newt (Iten & Bryant, 1973). However, a number of unique features become apparent upon examination of anomalies of adult regenerates. Two regenerates which displayed gross abnormalities revealed, upon histological examination, unique features which give insight into a possible pattern of digit formation in this species of adult salamander.

Normal regenerates show 4 or 5 digits radiating distal to the same respective number of bones (distal carpals) present in the distal row of wrist bones. The first anomaly showed only two large, fused distal carpals and two lateral digits. The second anomaly contained three bones in the distal row of wrist bones and three digits.

From the above observations, one might postulate that since the number of digits that will eventually occur corresponds to the number of wrist bones found in the distal row, then the presence of a proper number of wrist bones in the distal row is essential for normal digit development.

## INTRODUCTION

Regeneration, the process by which an organism can replace all or a portion of a lost appendage, initially involves the formation of a blastema which consists of mesenchyme-like cells dedifferentiated from tissues adjacent to the amputation site: bone, muscle, connective tissue, and dermis, but excluding the epidermal, nervous, and vascular tissues. After a growth phase during which an increase of blastema cells occurs, these mesenchyme-like cells redifferentiate into the missing tissues of the appendage.

Limb regeneration has been studied at the morphological level in aquatic urodeles: newt, both larval and adult, and the larval salamander. This has been reviewed by Iten and Bryant (1973). However, there has been no report of limb regeneration in any terrestrial urodele.

The purpose of this study was to determine if limb regeneration was possible in an adult terrestrial urodele and, if so, what period of time was required for this process. During the course of this investigation it was determined that regeneration does occur in these animals (Young and Bailey, 1978a). This paper concerns the distal carpal-digit interrelationships during regeneration in the adult *Ambystoma annulatum*.

## MATERIALS AND METHODS

Fifty adult *Ambystoma annulatum*, endemic to Northwest Arkansas, were collected during their September breeding migration. They were maintained in a terrarium, containing a mixture of moist peat moss and potting soil, at approximately 23° C, and fed bi-weekly with earthworms. After a six-month acclimation period, twenty-five salamanders of comparable size and weight, were selected as an experimental population. Two salamanders per month for the experimental twelve month period were subjected to the operation in which the right forearm was amputated through the wrist. The remaining salamander was designated as a control and underwent a sham operation. The sham operation consisted of a stroking motion across the right wrist rather than actual amputation.

At the termination of the experimental twelve month period, the regenerated right forelimb of all salamanders was reamputated 1-2 mm proximal to the original amputation site. The regenerated limbs were staged, examined at the gross morphological level, prepared histologically, and sectioned at ten microns. The longitudinal serial sections were examined to ascertain similarities and differences between newt regeneration (review, Iten and Bryant, 1973) and adult salamander regeneration (Young and Bailey, 1978a). The original twenty-four non-regenerated limbs and the control limb were either dissected or sectioned to ascertain a normal pattern of bone formation in the forelimb of the adult *Ambystoma annulatum* (Fig. 1).

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## RESULTS

The osteology of a non-regenerated forearm indicates two bones of equal length; a small diameter, laterally located ulna and a larger diameter, medially located radius. The bending seen between the forearm and the forehand occurs between the radius-ulna and the radialie-intermedium-ulnare. The first row of hand bones occurs distal to the radius, radius-ulna junction, and the ulna respectively. The second row of hand bones consists of the distal carpals. The third row of hand bones, specifically the proximal two-thirds of the metacarpals, comprises the region of the hand closest to the digits, while the distal one-third of the metacarpals and two rows of phalanges comprise the bones of the digits. Normal adult *Ambystoma annulatum* have forelimbs with either four or five digits, the number of distal carpals being equal to the number of digits present. Adult salamanders with forelimbs containing four digits show four distal carpals present, whereas a salamander with five digits shows five distal carpals. There is a greater occurrance of four-digit salamanders than of five-digit salamanders.

The normal bone pattern of the forelimb is specific in that for every digit there is a corresponding distal carpal and that the phalanges, metacarpals, and distal carpals are oriented along a specific distal-proximal axis. This pattern is apparent when one views the addition of the fifth digit (with corresponding structures) to the four digit pattern. But it is even more evident when one views anomalies to the normal digit pattern.

Anomaly A - Late Digit Stage (324-362 days post amputation), (Plate 1). The gross morphology shows only two lateral digits present. Histologically, the epidermis, dermis, muscle, and other assorted structures are the same as those seen during the Late Digit Stage of regeneration (Young and Bailey, 1978b). However, the osteology of this limb (Fig. 2) differs from that of the non-regenerated limb in the number and location of the distal carpals and in the number of digits present.

Anomaly A shows a small, laterally located ulna and a larger, medially located radius that make up the two bones of the forearm. The first row of hand bones consists of the ulnare, intermedium, and radialie and is located in the same position as that found in non-regenerated limbs. The second row of hand bones consists of two large fused distal carpals. These fused distal carpals are located in the same respective area as the lateral distal carpals from the non-regenerated forelimbs. Two lateral metacarpals and one row of phalanges comprise the two lateral digits.

Anomaly B - Middle Digit Stage (250-292 days post amputation), (Plate 2). The gross morphology shows only three digits present.

Histologically, the internal structures, other than the osteology, are the same as those seen during the Middle Digit Stage of regeneration (Young and Bailey, 1978b). However, the osteology of this limb (Fig. 3) differs from that of the non-regenerated limb in the number of digits present.

The radius and ulna comprise the bones of the forearm, while a large radialie, small intermedium, and large ulnare comprise the first row of hand bones and are located in relatively the same positions as those found in non-regenerated limbs. The second row of hand bones consists of three distal carpals with a corresponding number of metacarpals and two rows of phalanges to comprise the three digits.

## DISCUSSION

Significantly, in all limbs examined, non-regenerated, regenerated (Young and Bailey, 1978b), and anomalies, there is a direct one-to-one relationship of distal carpals to digits present.

In both the non-regenerated and complete regenerated hand, the number of digits always corresponds to the number of distal carpals, whether four or five. This relationship between the number of digits and the number of distal carpals is established as a direct correlation by the anomalies reported herein: Anomaly A with two digits and two distal carpals; and Anomaly B with three digits and three distal carpals. In regeneration of the adult salamander (Young and Bailey, 1978b) the bones of the distal carpals redifferentiate before the bones of the digits. The digit pattern is then, presumably, determined by the number of distal carpals which appear. Whether or not this relationship occurs in embryonic limb development remains to be determined.

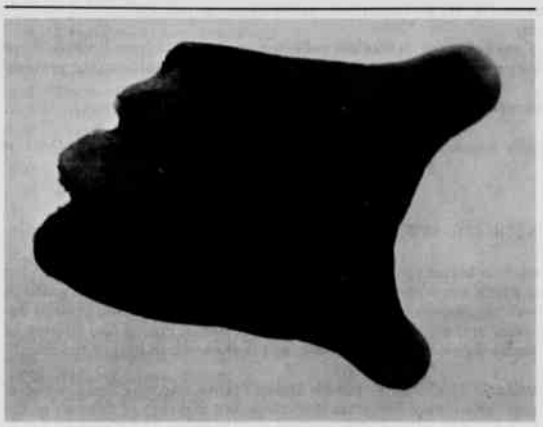

Plate 1. Anomaly A - Late Digit Stage, Ventral View.

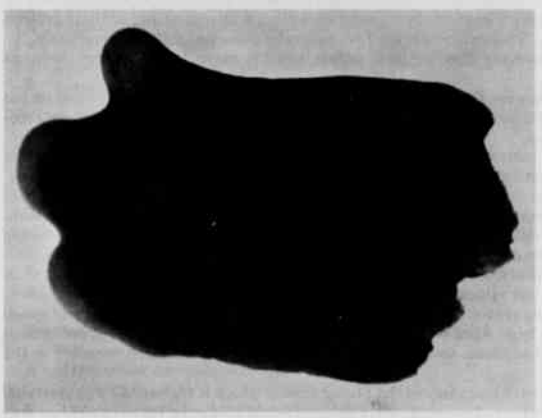

Plate 2. Anomaly B - Middle Digit Stage, Ventral View.

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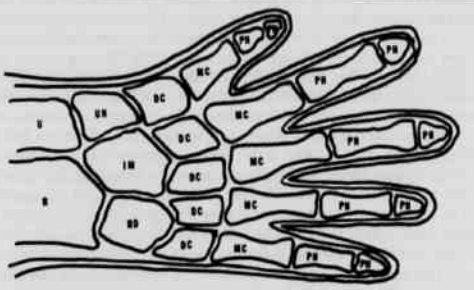


Figure 1. Normal Forelimb Osteology. U-Ulna; R-Radius; UN-Ulnare; IM-Intermedium; RD-Radialie; DC-Distal Carpal; MC-Metacarpal; PH-Phalange.

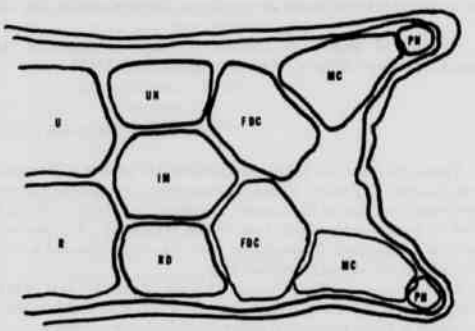


Figure 2. Anomaly A Osteology. U-Ulna; R-Radius; UN-Ulnare; IM-Intermedium; RD-Radialie; F-DC-Fused Distal Carpal; MC-Metacarpal; PH-Phalange.

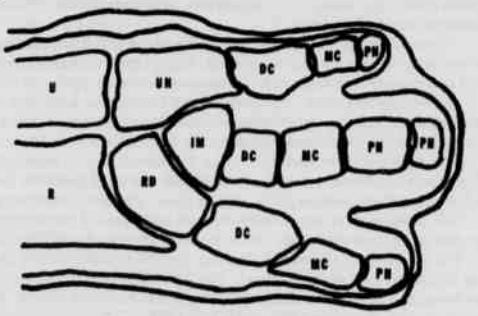


Figure 3. Anomaly B Osteology. U-Ulna; R-Radius; UN-Ulnare; IM-Intermedium; RD-Radialie; DC-Distal Carpal; MC-Metacarpal; PH-Phalange.