Induction of Regeneration of Body Parts in the Lizard, Anolis.*† (26549)

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The non-regenerating forelimb of the adult frog (Rana) can be caused to regrow after amputation if the fresh wound is repeatedly irritated by harmful agents(1,2). Regeneration can also be evoked if the number of nerve fibers at the wound surface is increased by deviating additional nerves from other regions into the forelimb(3); and, indeed, the method of traumatism does not call forth a growth if the stump is denervated(4). The theory behind my experiments emerged from previous studies on the important controlling influence of the nerve on regeneration of body parts in the newt and other lower vertebrates (5). The extremities of the newt regenerate after amputation provided the number of nerve fibers at the amputation surface satisfies certain threshold requirements; quantities of fibers below the threshold value are ineffective. The threshold studies raised the possibility that the loss of power to regenerate which the frog's limb suffers at metamorphosis may be due, among other causes, to quantitative inadequacy of the available nerve supply, a theory substantiated by the nerve augmentation experiments.

Vertebrates higher than the frog also do not regenerate their limbs. The present study reports experiments begun in 1957 to evoke regrowth in a representative of a higher vertebrate group, the reptile, *Anolis carolinensis*, by increasing the nerve supply at a wound surface. The lizard does not regrow§ limbs but does regenerate the tail provided the spinal cord is present at the amputation surface. The experimental design was based on the following questions: Do the limb nerves of the lizard also have the quality to support regeneration given appropriate circumstances, and, are the tissues of the limb stump able to respond to the nerve?

Materials and methods. To answer these questions 2 experiments were devised. In one case, hindlimb nerves were deviated into a surface wound area of the tail, namely, into an organ field capable of regrowth (Fig. 1); in the other, nerves of one hindlimb were deviated to the opposite amputated limb to increase the fiber quantity at the wound surface (Fig. 2). In each instance the sciatic nerve and its major branches were dissected to the upper thigh region and then deviated according to procedures adopted in my previous experiments with the frog(3).

Nerve deviation to the tail. In the case of deviations to the tail both sciatic nerves and their branches were dissected to the knee and then redirected under the skin to a dorsal region of the base of the tail from which approximately 5 mm^2 of skin was removed and the underlying tendons and muscles cut repeatedly to increase the severity of the injury (Fig. 1). In 18 animals, 8 showed growths beginning about 6 weeks after operation. The growths varied in shape and size; the largest was approximately 6.6 mm (Fig. 3) and the smallest about 1 mm. Initial histological preparations showed abundant growing nerve fibers, connective tissue and some regenerating muscle near the base of growth. In some of the negative cases a small pad of tissue developed under the regenerated skin. In 5 instances in which a wound was made but no nerves deviated ordinary wound healing ensued without any sign of enlargement.

Nerve deviation to the opposite limb. In the second experimental series, the left sciatic nerve and its branches to a level below the

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† The article of Simpson in this issue of the PROCEEDINGS, submitted for publication at the same time, reports induction of regeneration in a lizard by deviation of spinal cord to the hindlimb.

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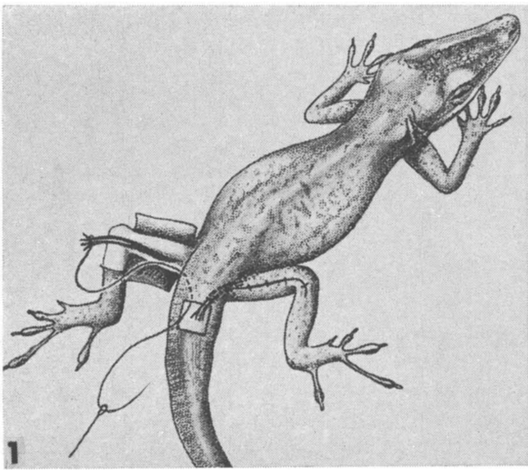
§ Kudokotsev(9) recently observed regenerative responses in the hindlimb of the desert skink which could be enhanced by enlarging the surface of amputation.



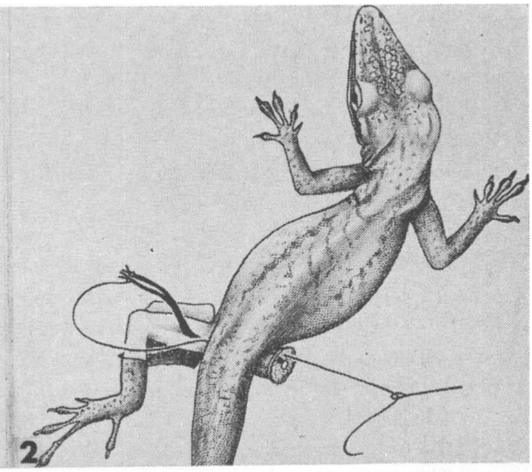


FIG. 1. Deviation of sciatic nerves to wound in tail. Right sciatic in place, left in process of deviation. × 0.8.
FIG. 2. Deviation of left sciatic nerve to right amputation stump. × 0.8.

knee were redirected under the spinal column and thence along the path of the opposite sciatic nerve to the freshly amputated stump (about 4 mm from base) of the right hindlimb (Fig. 2). In 9 of 21 cases, regeneration occurred from the amputation surface. The growth was initiated about 6 to 12 weeks after nerve deviation. The first signs of growth (Fig. 4) were swelling underneath a deeply pigmented wound epithelium and increased sensitivity of the wound skin to mechanical stimulation. Then growth was rapid for approximately 8 weeks. The largest growth achieved was approximately 4 mm and in this instance the distal end also became flattened suggesting a foot rudiment (Fig. 4). In general, the growths were smaller and less well-developed than those seen in the frog. In 9 controls involving either simple amputation or deviation of a resected piece of sciatic nerve there was no growth(6). The

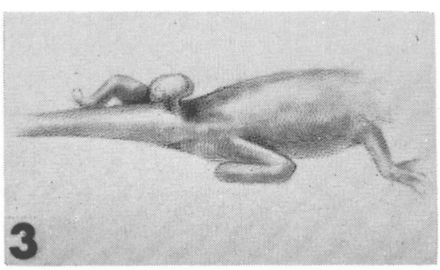


FIG. 3. Growth in tail evoked by sciatic nerve deviations (see Fig. 1). Anolis 16. × 1.

wound skin assumed a light grayish cast from the start and the end of the stump became atrophic.

Conclusions. The results show that the nerves of the lizard do have the necessary quality to evoke regeneration of a body part.

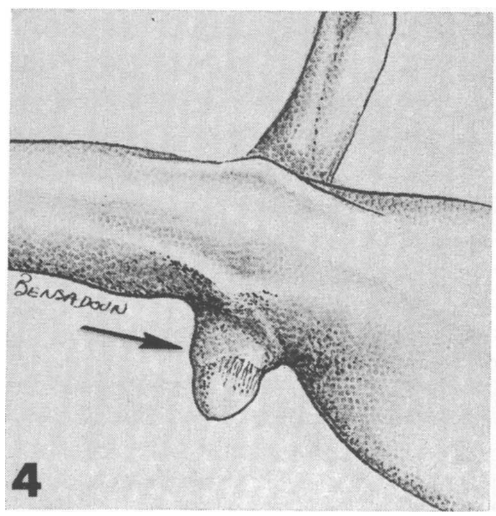


FIG. 4. Regeneration of right hindlimb after deviation of left sciatic to amputation surface (see Fig. 2). Arrow points to orignal amputation surface. Anolis 56. × 2.3.

Secondly, they show that the limb is capable of regeneration provided an adequate neural stimulus is available. The fact that fewer positive instances of limb regeneration were obtained than in the frog and that the growths were more limited and heteromorphic intimates that the lizard limb is more refractory to the nerve stimulus. The results bear out the expectation previously drawn(3,7,8) that adequate "dispositions" may be found to cause regeneration in forms higher than the frog and raise the hope that even the limb of mammals may some day be caused to regrow.

Summary. The amputated hindlimb of the lizard, *Anolis carolinensis*, incapable of regenerating, may be caused to grow by augmenting the nervous supply of the wound surface with additional nerves deviated from the opposite hindlimb. In addition, deviation of hindlimb nerves into the tail region also induced growth responses. The conclusion is drawn that the reptilian nerve does have the growth-evoking quality exhibited so well by nerves in lower forms and that reptilian body parts do have the power to regrow provided, among other conditions, that the nerve stimulus is quantitatively adequate.

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Induction of Limb Regeneration in the Lizard, *Lygosoma laterale*, by Augmentation of the Nerve Supply.*† (26550)

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Regeneration of appendages in urodele amphibians is dependent on presence of an adequate nerve supply(1). The same holds true for anuran larvae(2). Singer(3) showed that the influence of the nerves in Triturus limb regeneration was of a quantitative rather than qualitative nature. That is, the number of nerve fibers per unit area of tissue determines whether or not the limb will regenerate. He suggested that the absence of limb regeneration in the higher vertebrates might be due to lack of a sufficient nerve complement. To test this hypothesis, Singer(4,5) augmented the nerve supply of the normally non-regenerating forelimb of the postmetamorphic frog and obtained regeneration.

The adult lizard presents a unique case, in that the limbs do not regenerate, yet the tail regenerates freely. This apparent paradox can be most readily explained by the fact that the tail, which contains the spinal cord, obviously has a larger nerve supply than the limb. If this assumption is valid, one should be able to induce regeneration of the limb by augmenting its nerve supply.

The present study emphasizes the importance of nerves in lizard regeneration. The nerve supply of the rear limb of the lizard was augmented by deviating the spinal cord from the tail into the limb. A limited amount of true limb regeneration was obtained.

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† A more detailed account of the histological aspects of this study will be published later. The results were first presented as a progress report at Fourth Developmental Biology Conference, Alligator Point, Fla.