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.

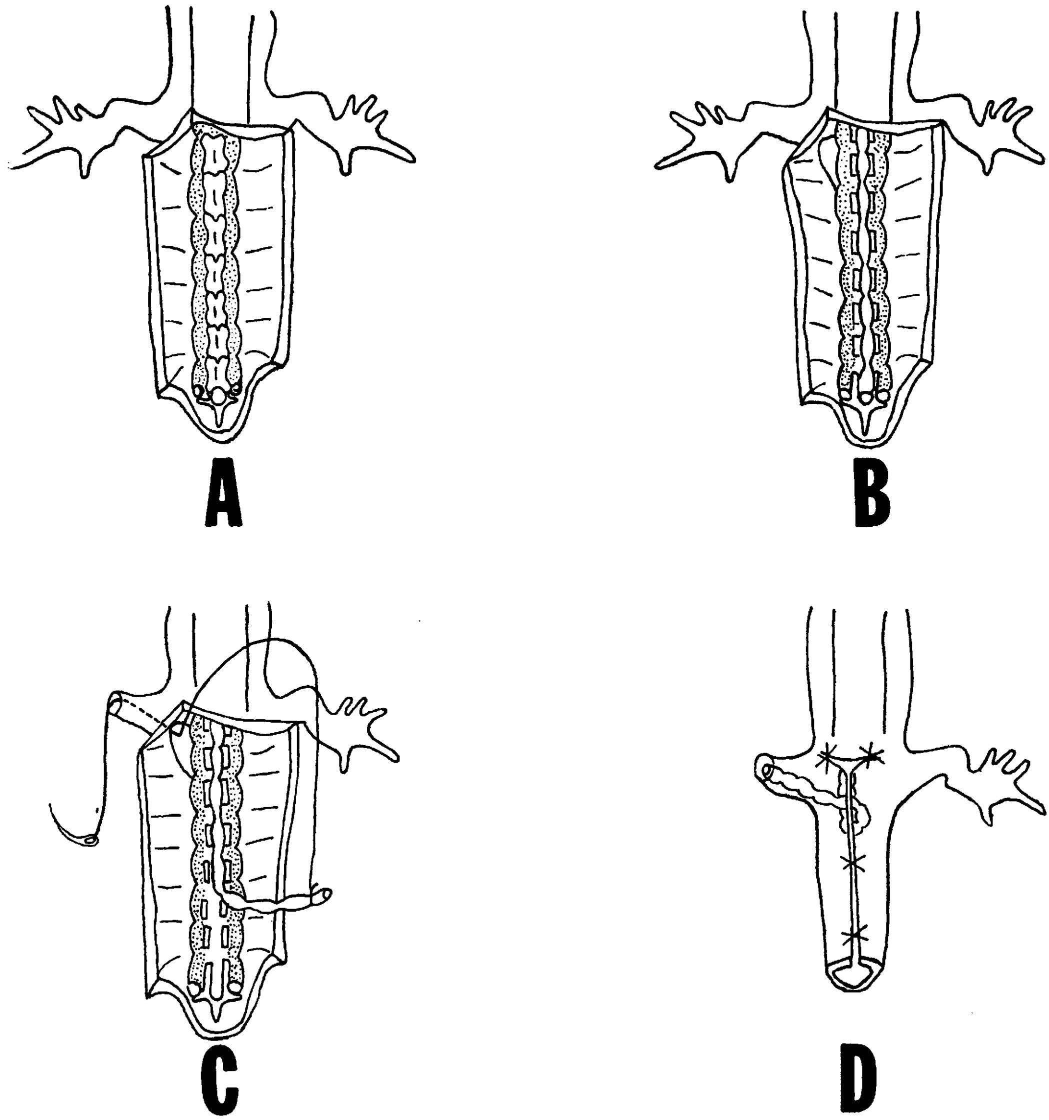

FIG. 1. Diagram of operation.

Materials and methods. In these experiments, adult brown skinks (*Lygosoma laterale*) were maintained in individual glass stacking dishes at room temperature (70-75°F) under controlled light conditions, and were fed a diet of meal worms, *Tenebrio* sp.

The experimental series consisted of 16 lizards in which the spinal cord was surgically deviated into the left rear limb. The major steps in the operation are diagrammed in Fig. 1. In each case, the lizard was first curarized with an intraperitoneal injection of tubocurarine chloride in saline. The tail was amputated approximately 2 inches from its base and a mid-dorsal incision was made in the integument. Working along this incision the vertebral column was exposed (Fig. 1 A). The roofs of the vertebrae were removed, exposing the spinal cord (Fig. 1 B). The limb was then amputated half-way between the body wall and the distal end of the femur. A suture was passed through a channel leading into the limb. The free end of the suture was tied to the posterior end of the spinal

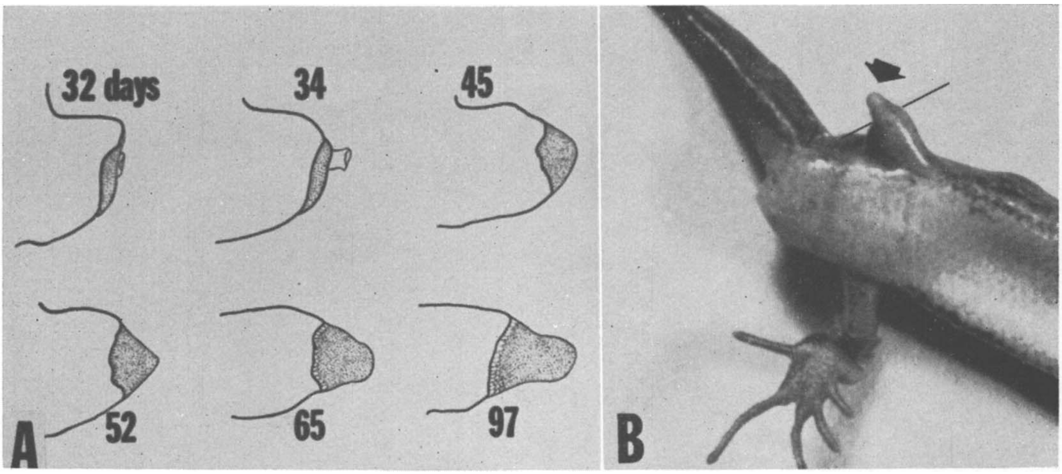

FIG. 2. Experimental lizard E-1. (A) Camera lucida drawings showing successive stages in development of the regenerate. × 7. (B) Photograph of regenerate (arrow) 106 days following operation. Broken line indicates original level of amputation. × 2.

cord (Fig. 1 C) and the cord pulled into the limb. The dorsal incision in the tail was then closed with sutures (Fig. 1 D).

Two control groups were set up. In both groups the control limbs were amputated at the same level as were the experimentals. In the first group (basic controls), simple amputation of the left rear limb was performed in 21 lizards. This series was established to stage the histology of healing in the normally non-regenerating limb. The second group (experimental controls) consisted of 17 lizards which underwent the same operation as did the experimentals, with the one exception that the spinal cord was not deviated into the limb. Tail muscle was transplanted to some of the limbs in this series. This was done to determine whether or not tail tissue, other than spinal cord, might possess inductive properties.

Results. None of the limbs in the 2 control series displayed a regenerative response. Histological examination of normal healing in the non-regenerating control limbs revealed considerable *de*differentiation of the limb tissues, with the exception of bone. However, these limbs were characterized by a premature *re*differentiation of the dedifferentiated cells into scar tissue. A cartilage callus also formed around the cut end of the unaltered bone.

Eight of the 16 limbs into which the spinal cord had been deviated, showed regenerative responses. Two of these limbs resulted in positive cases of limited limb regeneration. One of the regenerates is shown in Fig. 2. The first external indications of regeneration appeared 34 days following operation. The blastema was very soft and had assumed the shape of a small cone by 52 days. By 65 days, the blastema had elongated and was still soft and flexible. The regenerate had reached its maximum length (1.1 mm) by 97 days. At 106 days (Fig. 2 B) the animal was sacrificed and the regenerate removed for histological examination which revealed that bone and muscle had undergone extensive breakdown, and the regenerate fulfilled the requirements of a limb regenerate. Regenerated muscle fibers were present in the regenerate and were grouped in a manner normal for limb muscle. A regenerated cartilagenous model of the femur could be seen extending from the area of bone breakdown. The cartilage model had formed *de novo*, rather than by appositional layering from the periosteum. This was evidenced by the spatial arrangement of the chondrocytes and the presence of a well formed epiphyseal plate. The model was also undergoing periosteal ossification at its base.

The other 6 experimental limbs showed regenerative responses, but did not proceed as far as the one just described. Study of Bodian stained sections of the experimental series showed that the best regenerate was associated with a definite increase in nerve fiber content.

Discussion. Previous workers have succeeded in evoking growth responses in lizard limbs(6,7). In each case, however, the resulting growths proved to be tail-like in nature.

In the present study deviation of the spinal cord (a source of additional nerves) into the limb of *Lygosoma* induced a limited amount of true limb regeneration. Therefore, this study demonstrates that the limb tissues *per se* are still capable of responding to the inductive influences of nerves. It also emphasizes the validity of the neurological theory by extending it to the next higher vertebrate group, the Reptilia.

The results can be interpreted in at least two ways. 1) Possibly the nerves of the normal limb have lost their ability to induce regeneration, while the nerves of the spinal cord have retained this property. 2) Possibly the limb nerves have the ability to induce regeneration, but fibers are not present in sufficient numbers. Since a considerable amount of dedifferentiation occurred in the control limbs, it would seem that the limb nerves may still have the ability to take part in regeneration. If the latter is true, deviation of the spinal cord into the limb merely increased the nerve complement above the threshold that allowed regeneration to occur. Therefore, the results favor the second interpretation. Results of previous work on limb regeneration in Triturus(3) and induced limb regeneration in the postmetamorphic frogs(5) also favor this interpretation. This explanation is even more acceptable in light of recent findings of Singer(8).

Summary. The spinal cord was deviated from the tail into the rear limb of the lizard, *Lygosoma*. In those cases where the deviated cord retained its integrity and augmented the nerve complement of the limb, a limited regenerative response occurred. Therefore, the tissues of the limb are still capable of responding to the inductive influences of nerves. Histological examination of the regenerates revealed that they fulfilled the requirements of true limb regeneration. Experimental control limbs which underwent the same treatment with the exception that the cord was not deviated into them, failed to show a regenerative response. This study strongly suggests that lack of an adequate supply of nerve tissue imposes one, if not the major, restriction on limb regeneration in this lizard.

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