A COMPARATIVE STUDY OF THE HISTOLOGY AND GROSS MORPHOLOGY OF LIMB AND TAIL REGENERATES IN *LACERTA LAEVIS*

By

LAMIA T. HADDAD

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REGENERATION IN LIZARDS

L. Haddad

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ABSTRACT

This study is a test and comparison of the regenerative capacity of the tail and two levels of the limbs, forearm, upperarm, shank, and thigh in Lacerta laevis.

Simple amputations were performed on the left limbs, at the wrist, proximal to the elbow, at the ankle, and proximal to the knee, in addition to the mid-length of the tail. The experimental animals were kept in dampened containers at (25°-27° C) throughout the course of the experiment. At definite periods of healing and regeneration the amputated limbs and tails were cut off and fixed for histological study.

The regenerative capacity of the limb was found to be greatly reduced as compared to the tail. This regenerative capacity varied from specimen to specimen.

Gross morphological observation showed that regeneration in tails and limbs proceeded in the same manner to the stage of blastema formation. Limb regeneration did not proceed beyond the formation of a short conical outgrowth. Tail regeneration proceeded to the level of formation of a tail of normal appearance. Comparing the two levels of limbs (forearm, upperarm,

shank, and thigh), it was found that regenerative capacity is greatest in the forelimb, of the two segments of the forelimb, it is greatest in the upper-arm.

The initial histological changes in limbs and tails were similar. In both, limbs and tails, dedifferentiation resulted in the formation of mesenchymatous tissue. In limbs the mesechymal cells rapidly differentiated into dense, connective tissue (scar tissue) underlying the epidermis, and cartilage surrounding the end and distal surfaces of the bone. Only in the upperarm did muscle differentiate. In the tail the sequence of events was different. The mesenchymatous tissue became dense and compact and differentiated into cartilaginous tube surrounding the regenerated spinal cord, segmented muscle, dense connective tissue and other normal tail tissue.

Comparing the two levels of the limbs, histological study also revealed that the regenerative capacity is greatest and fastest in the forelimb, of the two segments of the forelimb the upperarm was better developed.

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INTRODUCTION

It is a common observation that, although some kinds of lizards readily regenerate their tails after a part of the original organ has been lost, the regenerative capacity of the limb is either entirely lacking or greatly reduced in the Lacertidae (Guyénot, 1928; Guyénot and Matthey, 1928; Marcucci, 1930). Marcucci found that limb regeneration in Lacerta muralis, L. viridis, Gongylus ocellatus, and Platydactylus mauritanicus after transverse amputation resulted at best in the growth of a short, conical stump. Histological examination of these regenerates showed that they consisted mostly of connective tissue, contained a few muscle fibers, and were supported by a cartilage rod attached to the bone of the original stump.

Most of the work done on regeneration in lizards has been aimed at determining the causes for failure of regeneration and methods for inducing it. The cause of failure of limb regeneration has been attributed to rapid closure of the wound surface by skin during healing (Barber, 1944) and to intrinsic factors in the local tissues coupled with lack of sufficient nerve supply (Rose, 1946). Attempts to induce regeneration in lizard limbs experimentally have, in general, supported these

ideas. Thus oblique amputation through the thigh, resulting in a large wound surface in Lacerta muralis will give a better regenerate than transverse section (Guyenot, 1928; Guyenot and Matthey, 1928; Marcucci, 1930), and transplantation of tail skin and spinal cord will induce regeneration in the non-regenerating forearm stump of Anolis carolinensis (Rose, 1951).

With the exception of Barber's work (1944) which was a comparative study of wound healing in the fore limb with regeneration in the tail in Anolis carolinensis, no other comparative studies of the regeneration process in lizards have appeared in the literature. Furthermore, none of the other reports has treated the subject in sufficient histological detail to warrant many conclusions concerning the role of histological factors in regeneration or its failure.

The present work entailed a comparative morphological and histological study of the tail and four limb segments (forearm, upper arm, shank, and thigh) in Lacerta laevis during wound healing and regeneration from 24 hours to 60 days post-amputation. In light of the data obtained, it has been possible to present a more complete histological picture of events during regeneration and the role of histological phenomena when regeneration fails.

MATERIALS AND METHODS

A preliminary study of limb regeneration in various lizards revealed that the kind best suited for the proposed investigation was a common brown species with blue spots along the sides of the abdomen. By comparing the coloration and scale pattern with descriptions given by Boulenger (1887) and Ditmars (1930), it was possible to identify these lizards as Lacerta laevis.

One hundred and fifty adult Lacerta laevis, abundant in the mountains of Lebanon, were used in the following experiments. The animals were collected as needed by the departmental collector. They varied in length from 4 to 8 cm. as measured from the tip of the snout to the anus.

All amputations were performed on the left limbs (forelimb and hindlimb) in addition to the tail, a single specimen usually undergoing simultaneous section of the tail and one or both of the limbs. Multiple amputations appeared to be no more harmful to the lizard than single ones, and possessed the decided advantages over single amputations in that regeneration rates between limbs and tails could be compared more significantly.

This would have been difficult if only single amputations had been performed because regeneration capacities in lizards vary slightly from one specimen to the next.

Simple amputations were performed at the mid-length of the tail, at the wrist, proximal to elbow, at the ankle, and proximal to the knee thus affording a test of the regenerative capacity of the tail and two levels of the limbs, viz. forearm, upper arm, shank, and thigh respectively.

The same operatory method was used throughout the course of the study. It consisted of the following:

Animals were etherized until gross movements of the body stopped, then chilled on ice. The operation was done under a dissecting microscope with the animal lying on its back on ice blocks. Instruments were sterilized with 70% alcohol. A ligature was placed around the proximal segment of the limb to prevent excessive bleeding during the operation. The desired segment of the limb was amputated at right angles to the bone. The end of the bone was cut a short distance proximally so as not to protrude from stump. The edge of the skin was trimmed back to leave a large open wound. After the ligature was untied sulfadiazine was applied to the wound surface to prevent infection. Recovery was very good in 95% of the cases.

Following the operations, the experimental animals were placed in individual moist chambers, made of fingerbowls lined with 3% agar, for the first five days. This technique had a number of advantages with respect to healing of the wound. In isolation the animals were found to be much less active than when kept in groups; and the moisture level, which could be controlled, was sufficient to prevent early rapid drying of the wound, but, at the same time, was not great enough to encourage skin infections to which these animals are prone. The fingerbowls were kept at room temperature (14 - 20°C) for the first 24 hours. A low temperature was found to facilitate recovery from the operation, and to reduce the animal's activity, thereby preventing possible injury of the stump during the initial phases of healing.

Beginning with the second day after the operation, the fingerbowls were placed at 25° - 27°C. This temperature was maintained throughout the course of the experiment. This approximated normal Spring and Fall temperatures in Lebanon, and accelerated healing and regeneration processes, thus ensuring the completion of the study within a reasonable length of time.

By the fifth day, healing had progressed sufficiently to allow the animals to be kept in groups in aquaria lined with dampened paper for the remaining period of observation.

Daily observation of the animals included morphological changes of the amputated limbs and tails and measurements of the regenerating parts.

Daily care of the animals consisted of changing the moist chambers, cleaning the aquaria, and force feeding the animals with ground meat.

The technique outlined above was found to be the best of several tried for the maintenance of the lizard in a healthy state. Inspite of this, the death rate of the experimental animals was comparatively high (approximately 30%) especially during the first post operative week. Some groups of lizards brought into the laboratory appeared to be hardier than others. While the high death rate was disappointing and necessitated the repetition of many experiments, it was not entirely unexpected, since many kinds of animals do not adjust well to laboratory conditions. This seems to be especially true when a major trauma, such as the loss of a limb, is suffered.

After definite periods of healing and regeneration, the limb and tail stumps of a number of experimental cases were amputated for histological study. An account of the total number of animal used in studying each phase of healing and regeneration is given in Table 1.

TABLE I: DISTRIBUTION OF EXPERIMENTAL CASES

Age of Organ	Number of Cases				
	Forearm	Upperarm	Shank	Thigh	Tail
24 hours	3	3	3	3	3
48 hours	3	3	3	3	3
72 hours	4	4	4	4	3
4 days	3	3	2	3	3
5 days	3	3	3	3	3
10 days	3	4	3	4	5
15 days	3	3	3	4	4
30 days	3	5	2	4	5
60 days	2	4	3	2	5
Total	27	32	26	30	34

During the initial months of experiments, tissues were fixed in Bouin's solution. Later, however, when the histological study was begun, difficulty was experiened in removing the picric acid from the sections, and AFA was used for all subsequent fixations with equally good results. The tissues were imbedded in paraffin, sectioned at 20 micra, and stained with Delafield's Hematoxylin.

RESULTS

Although studies were carried out on all limb segments and the tail, morphological and histological changes in these organs were so similar, that the main sequence of events can be presented by a detailed discussion of the forearm alone. Specific differences occuring at other limb levels and the tail will then be given.

During the first 24 hours after amputation of the hand, the forearm stump became edematous and swollen and the wound surface covered by a soft, white scab. Internally, mononuclear leucocytes were seen to have invaded the region. They were concentrated predominantly at the wound surface. Fluids had also accumulated near the region of amputation, thus causing the stump to swell and the tissues to be separated by fluid-filled spaces. Muscles, particularly those near the wound surface, were separated into discontinuous fibers, partly by the accumulation of fluid and partly by leucocytes which were either massed in the spaces between the fibers or were applied directly to the fiber surface. The discontinuity of the muscle was interpreted as representing an initial stage of tissue destruction as a result of injury (Fig. 1).

At the wound surface itself, a scab had formed as a low cap covering the end of the stump (seen as a thick, dark line over the wound surface in Fig. 1 and Fig. 3). The scab was composed of structureless material, presumably coagulated serum, in which many leucocytes were embedded.

The edges of the skin were widely separated by the swollen tissue. Epidermis had not yet begun to close the wound.

The epidermis began to migrate over the tissues beneath the scab on the second day, where it was seen in section as a layer 2 - 3 cells in thickness projecting from the cut edge of the skin toward the center of the wound (Fig. 2). By the end of the third day, the wound was completely closed by the wound epithelium which had slightly increased in thickness to 4-5 cells (Fig. 3).

Concomitantly, dedifferentiation of the muscle was observed, beginning with the distal fibers. The distal muscle in succeeding days presented a picture of increasing separation of the individual fibers by fluid and leucocytes, and disorganization of the muscle cells themselves. These areas were made conspicuous by the accumulation of cellular debris interspersed with small basophillic cells having large oval nuclei and scanty cytoplasm. In cases where the nerve, sciatic or brachial,

could be seen, dedifferentiation was especially marked in the region adjacent to its cut end.

The histological appearance of the limb had changed by the fourth day. Near the wound epithelium where the initial dedifferentiation products had accumulated, there were observed cells of a mesenchymal appearance, i.e. basophillic cells with prominent nuclei, little cytoplasm and cytoplasmic processes, forming a loosely organized tissue (Fig. 4). Concomitantly, dedifferentiation was proceeding proximal in the muscle, leaving behind basophillic cells and debris which formed a zone in transition with the mesenchymal cells. The bone remained intact throughout its entire length, and was not involved in the process of dedifferentiation. The distal end of the brachial nerve presented a frayed appearance, indicating some loss of organization of the fibers near the level of section.

By the end of the fifth day, it was noted that the area of the wound had slowly decreased. Histologically this was correlated with a slight closure of the intact skin over the dedifferentiating muscle, accompanied by a slight decrease in the area of the wound epithelium. In most cases, there was no further reduction of the wound area. The scab, meanwhile, had formed a hard cap over the end of the stump.

By the tenth day, the scab was shed, exposing a soft, light grey blastema protruding as a low mound approximately one millimeter beyond the end of the stump. The earliest of the blastemata appeared on the seventh day. In a few cases, the blastema was of a pink color, indicating that a high degree of vascularization had occurred. Initially, the pink blastemata were smaller than the others, but quickly increased in size and assumed a greyish cast. They were interpreted as cases in which the scab had been shed prematurely. No subsequent differences were found in the development of these as compared with blastemata of more normal appearance.

Histological examination revealed that dedifferentiation and the formation of disorganization products had not progressed much beyond the level seen in limbs fixed on the fifth day. However, the amount of mesenchymal cells showed a marked increase over the number observed previously. Moreover, these cells were beginning to condense as procartilage around the ends of the bones, and as a dense layer lying just beneath the wound epithelium. In the interim between the fifth and tenth days, the epidermis had continued to increase in thickness, so that it now formed a layer 7 -8 cells deep. Since mitosis were not observed in these cells, the increase in thickness was

presumably the result of continued migration from proximal areas of the skin.

Gross morphological and histological changes during the period between the fifteenth and sixtieth days were mainly quantitative.

During this period, the blastema increased in length and diameter very slowly, and acquired a conical shape by the end of fifteen days (Fig. 5). The increase in length of the blastema was approximately one-tenth of a millimeter per day. By the thirtieth day post-amputation, soft, weakly developed scales were beginning to appear at the proximal edge of the blastema. Upon the sixtieth day, when the experiments were terminated, the regenerate was a short, dark-colored, conical outgrowth approximately 2.2 millimeters in length, covered with immature scales (Fig.6).

Forearms fixed for histological study during this period revealed that dedifferentiation was proceeding at a greatly depressed rate at 30 days post-amputation and had almost stopped by 60 days. The amount of mesenchymal tissues decreased steadily during this period. Concomitantly, however, the differentiation and amount of regenerated tissue progressively increased. The procartilage noted previously around the ends of the radius and ulna was seen to constitute a larger mass and had started to differentiate as cartilage by the fifteenth

day. The layer subjacent to the wound epithelium had also increased in thickness, and now appeared as a zone of dense, fibrous scar tissue.

The trends in development already mentioned were continued in limbs 60 days post-amputation. By then, most of the mesenchymal tissue had disappeared, whereas, the amount of cartilage was slightly greater than before, as was the thick, collagenous scar tissue, which surrounded the cartilage and separated it from the epidermis. The epidermis was now 10-12 cells at its thickest part (Fig. 6).

A similar sequence of events occured in the upper arms, shanks, and thighs studied. Differences between these levels and the forearm involved mostly differences in the rates of growth as determined by daily measurements and rate of histological changes as determined by observation of slides.

In the upperarm, the extent and rate of dedifferentiation and differentiation was greater and faster than in the forearm. The maximum length attained by an upperarm regenerate was three millimeter. The main difference was that in addition to the formation of scar tissue and cartilage, muscle also differentiated in these limbs.

In the shank and thigh, a greater extent of wound

surface closure by adjacent skin than in the forearm was observed. In some cases, especially the thigh, complete closure of wound surface by adjacent skin was noted. The rate of dedifferentiation, differentiation and growth was slower in the shank and thigh than in the forearm. A blastema was observed to appear earlier in the thigh than in the forearm, upper-arm, and shank, but its rate of growth was the slowest as compared to the other three segments. The maximum length attained by shank and thigh outgrowth ranged from 1 - 1.5 mm.

The differences seen in the gross development of the tail stumps were striking in comparison with the limbs. Blastemata appeared within eight days after amputation in 18 cases out of 34. The blastemata were light grey or pinkish at first and then gradually darkened to a blackish color as pigment developed. The color change was initiated on the dorsal surface and gradually spread ventral, so that for a few days the blastemata were colored black dorsally and pink ventrally.

A blastema first appeared as a soft, low mound of tissue, which quickly lengthened into a blunt cone, and then into a gradually tapering outgrowth. The rate of increase in length was approximately 1 mm. per day, accompanied by a slight increase in thickness until the

normal diameter of the tail was reached. Scales started developing at the proximal edge of the blastema by the fifteenth day. As growth proceeded, scale development was gradually initiated at more distal levels, while the proximal scales continued their differentiation and finally assumed a more normal appearance. The maximum length attained by a regenerated tail was 4 cm. at 60 days.

The differences in the histological changes were equally notable between the forearm and tail. Within the first 24 hours, the epidermis had begun to migrate from the cut edges of the skin over the wound surface as a layer 2-3 cells deep. By the end of the third day, the epidermis completely covered the wound as a layer 6-7 cells deep (Fig. 8). In subsequent development, the epidermal layer became thicker than the corresponding wound epithelium in the limb. At 30 days, it had reached 14 cells at its thickest part, and at 60 days 13-16 cells in thickness (Fig. 9 and Fig. 10).

The tail stumps did not swell and become edematous as did the limb stumps. In most cases, no swelling occurred, while in the remaining ones, swelling was minimal. Histologically, the muscle masses were seen to remain in close proximity to each other. Leucocytes accumulated near the wound, but never to the extent observed in the limbs (Fig. 7).

Another difference between the behavior of limb and tail stumps was that in all of the limbs observed, the wound surface had been reduced to a greater or lesser extent within a few days by partial closure of the skin over it; whereas in the case of the tail stumps, the skin remained in its original position, and the wound surface was not reduced.

One of the major differences between regeneration in the tail as compared with the limb lay in the extent and rate of dedifferentiation. Histological comparison of a limb and tail at any time revealed that the zone of dedifferentiation extended farther proximal in the tail than it did in the limb.

The formation of blastema was initiated by the end of the first day after amputation. This resulted in the tail accumulating a far greater number of blastema cells than any of the limb segments had.

The second major difference between limb and tail regeneration was associated with the subsequent course of differentiation of the blastema cells. They became denser and more compact (Fig. 8) than in the limbs. Some of them collected around the regenerating spinal cord to differentiate into a cartilage tube containing the spinal cord (Fig. 9 and Fig. 10). The proximal end of the tube thus formed was continuous with the end of the vertebral column

in the stump. Segmental muscle also appeared as numerous isolated groups of cells differentiating within the mass of blastema cells (Fig. 9 and Fig. 10). In addition to these tissues, fibrous connective tissue, and capillaries also differentiated. By 60 days, differentiation of the blastema cells had not yet been completed. Much of the blastemal tissue, especially near the tip of the regenerate had not yet differentiated into definitive types of cells.

Differentiation followed a proximo-distal course; thus the most advanced stages of differentiation appeared at proximal levels, which were the oldest parts of the blastema. The same sequence occurred in the differentiation of the epidermis: proximal levels revealed various stages of scale development. Scales were progressively less well developed toward the tip of the regenerate. Dermis had not formed at any level by 60 days.

In a few cases limb regeneration did not proceed beyond wound healing. The muscle of the amputated surface shrunk, skin came in contact with the bone and closed the wound surface. No such cases were encountered in tails, though the rate of regeneration varied from tail to tail depending on the animal's regenerative capacity which varied from one specimen to specimen. In general an animal that proved to be a good tail regenerate, had better developed limbs outgrowths.

DISCUSSION AND CONCLUSIONS

The initial morphological and histological changes during wound healing and regeneration in the tail and limb of Lacerta laevis are similar. The immediate reaction to amputation is the accumulation of fluid and mononuclear leucocytes in the region of the injury. This causes an initial swelling of the stump. That swelling is much greater in the limb than in the tail may be attributed to the fact that the limb skin is much more flexible than tail skin, and is much more loosely attached by connective tissue to the underlying musculature than is tail skin. Thus the tail skin will not allow as great an expansion as will the limb skin.

The wound is first closed provisionally by a soft, white scab which gradually becomes hard and serves as a protective layer over the injured tissues. Epidermis soon migrates from the edges of the cut skin to effect a definitive closure of the wound. This migration begins earlier in the tail than in the limb and reaches a greater thickness. The role of the epidermis in regeneration has been a debated point. Recently the idea has been revived that epidermal cells may contribute directly to the formation of the regeneration blastema. Rose (1948) was able to correlate a sudden decrease in the number of epidermal

cells of the wound epithelium with a sudden increase in the number of blastema cells in Triturus viridescens. More recently, it has been shown that epidermis alone, without the direct participation of other tissues, may form a limb regenerate in Triturus (Rose, Quastler, and Rose, 1955 a, 1955b).

With respect to the lizard, it should be noted that the regenerating organ (tail) does accumulate a thicker epidermal cap than the poorly-regenerating organ (limb). Whether or not the epidermis contributes significant numbers of cells, if any, to the blastema has not been fully determined. Nevertheless, available evidence from the present study on this point indicates that it does not, because at all stages observed, the epidermal cap remained as a discrete layer which steadily increased in thickness concomitantly with the increase in numbers of blastema cells.

One possible hypothesis to explain the real difference in epidermal thickness at the two sites may be simply that it represents a case of recapitulation whereby an advanced organism repeats the morphological characteristic of a lower group, although the physiological function originally associated with the character is carried out by a different mechanism, namely dedifferentiation of the muscle.

A second hypothesis concerning the wound epithelium and its relation to regeneration involves the relationship between the wound epithelium, closure of the wound by the

skin, and dedifferentiation. It was usually the case that the limb skin would reduce the area of the wound surface somewhat by closing over the distal muscles. The degree of closure was quite variable from limb to limb, and in some cases closure was complete. In the latter event, simple healing resulted. The tail skin, however, never did close over the wound, for reasons already mentioned, and the surface area of the wound remained unreduced.

The extent of the wound epithelium could be correlated with the extent of dedifferentiation and the quality of regeneration. In limbs in which a great deal of wound closure had occurred by the limb skin, there was little dedifferentiation and poor regeneration; in limbs with less closure, more dedifferentiation occurred, and better regenerates ensued. In the tail, where no closure occurs, the tissues underwent extensive dedifferentiation, and regeneration was the best. These results are in essence with those recorded by Barber (1944), who compared forearm with tail regeneration in Anolis carolinensis, the American chameleon. In Anolis, closure of the wound surface is always complete in the limb, and few blastema cells are formed. These limbs do not regenerate, whereas Lacerta laevis limbs do to a certain extent. The causal relationship between the wound epithelium thus seems to be that the

dedifferentiation is dependent upon the presence of a sufficiently large wound epithelium, and regeneration is in turn dependent upon an adequate supply of blastema cells.

Such a relationship was suggested by the work of Gidge and Rose (1944) who studied the role of the epithelium in regeneration of adult Anuran limbs. Ordinarily, closure of the wound by skin occurs, and simple healing results. If, however, the skin is trimmed back from the amputation surface, a rather extensive wound epithelium is formed from epidermis migrating over the denuded muscle. In such cases, extensive dedifferentiation ensues, and partial regeneration results. A similar result was obtained when adult frog limb stumps were treated with hypertonic salt solution after amputation. The salt solution had the effect of preventing immediate closure of the wound. Partial regeneration also occurred in these limbs.

Guyénot and Matthey (1928) and Marcucci (1930) obtained similar results by increasing the size of the wound epithelium in four species of Lacerta. Ordinarily transverse section through the organ will result in the outgrowth of a small cone of tissue. Oblique section through the thigh, however, will produce a large wound. The regenerates in these cases were significantly larger than the previous ones, and took the form of tapering

projections, several millimeters in length.

It thus appears that dedifferentiation is dependent upon the formation of a sufficiently large wound epithelium. The size of the epithelium may be an important factor in determining the difference in regenerative ability of lizard tails as compared to the limbs. Therefore, although the epidermis appears not to contribute cells to the blastema in lizards, an epidermal layer of sufficient size is probably requisite for the necessary dedifferentiation which precedes regeneration.

The difference between regenerating and non-regenerating organs is not as clear cut as this, however. Other factors also play important roles in determining whether or not an organ will regenerate. One of these appears to be intrinsic factors in the tail and limb skin. Rose (1946) was able to suppress tail regeneration in lizards by transplanting limb skin to the tail stump, indicating an intrinsic difference between limb and tail skin in their abilities to take part in regeneration. This is independent of hormonal influence, since limbs and tails must receive the same hormones in the general circulation, unless one postulates that the response of the tail skin is different from that of the limb skin to circulating hormones.

A second factor involves the relationship of the nerve supply to regeneration. Rose (1951) showed that

regeneration could be induced in the forearm of Anolis carolinensis by the transplantation of tail skin plus spinal cord to the stump, neither tissue being effective in inducing regeneration when transplanted by itself. This work suggested although tail skin had the ability to take part in regeneration, the normal nerve supply in the lizard limb is insufficient to support regeneration, and it is necessary that the supply be augmented by spinal cord for the tail skin to be effective.

The dependency of regeneration on the innervation of the organ may be considered as established. Singer (1946 a, 1946b) and Singer and Egloff (1949) worked out the quantitative relationships between innervation and regeneration in Triturus viridescens. Rose (1948) showed that dedifferentiation was greatly reduced in denervated limb stumps of adult frogs. It has also been demonstrated that enervated lizard tails fail to regenerate (Kamrin, 1954).

The present experiments are in agreement with these findings. Dedifferentiation in the tail and limb is maximum in the area around the severed end of the nerve. In the limbs, where the main nerve trunk is ventral to the central axis, the initial center of dedifferentiation is also ventral in position. Moreover, the tail stump, possessing a relatively greater number of neurons in the spinal cord undergoes more extensive dedifferentiation than the limb with its smaller amount of innervation.

Therefore, it may be concluded that in addition to the effect of the more extensive wound epithelium on dedifferentiation, the tail stump is a better regenerator than the limb stump because of the effect of greater amounts of nerve on the dedifferentiation process.

In the tail and limbs, the initial stages of dedifferentiation are similar, and result in the formation of blastema cells that are mesenchymal in nature. The rate of dedifferentiation is faster in the tail, however, and results in the accumulation of a larger number of blastema cells than in the limb. In comparing fore- and hind limbs, the rate and extent of dedifferentiation was found to be faster in the forelimb; and of the two segments, faster in the upper arm. Barber (1944) in comparing forearm with tail regeneration in Anolis found that very few blastema cells were formed in the forearm. From her data it is clearly seen that the amount of blastema tissue forming in the forearm of Lacerta laevis is much greater than in Anolis carolinensis. This is undoubtedly the reason why Lacerta is a better regenerator than Anolis.

A major difference between the tail and limbs regeneration is the course of differentiation of the blastema cells.

In the limb the mesenchymal blastema cells are

rapidly converted into dense collagenous connective tissue which forms scar tissue underlying the epidermis and procartilage cells that surround the end and distal surfaces of the bone and later change to cartilage. Muscle differentiates only in the upperarm. Marcucci (1930) observed muscle differentiation in Lacerta limbs.

In the tail the sequence of events is different. The mesenchymal blastema cells become dense and compact and differentiate into a cartilaginous tube which surrounds the regenerated spinal cord replacing the vertebral column, dense connective tissue, segmental muscle between the dense connective tissue, capillaries and other tissues that normally take part in tail formation.

Regeneration in the tail and limbs results in morphologically different organs. In the limb the blastema results in a short conical outgrowth that does not exceed 2-3 mm. in length and fails to form a limb of normal appearance. Marcucci (1930) got the same results when he performed simple amputation on the limbs of four species of Lacerta. In the tail, the blastema changes gradually to form a regenerate tail of normal appearance, but imperfect internally.

In addition to the two mentioned explanations for the failure of limb regeneration in lizards, may now be added a third attempt.

The failure of a lizard's limb to regenerate itself may be attributed to the rapid conversion of the blastema mesenchymal cells into cartilage and collagenous connective tissue (scar tissue), at the expense of the formation of significant amounts of other limb tissues, without becoming dense and compact and then differentiating as they do in the tail.

It is concluded that the regenerative capacity of the two levels of the limbs as compared to the tail is greatly reduced in the lizard, Lacerta laevis. The regeneration rate varies from one specimen to the next.

The reduction of limb regenerative capacity varies from the formation of a short conical outgrowth 2-3 mm. in length to wound healing.

Tail regeneration resulted in a regenerate tail of normal appearance.

The forelimb has a greater regenerative capacity than the hindlimb, of the four segments of the limbs the upperarm shows the greatest regenerative capacity.

The initial gross morphological and histological changes in the tail and the two levels of the limbs are similar. Differences are in the time, rate and extent of these changes. The main differences are those concerned with the sequence of differentiation of the mesen-

chymal blastema cells.

The failure of a lizard's limb to regenerate itself may be due to the rapid conversion of blastema mesenchymal cells into collagenous connective tissue (scar tissue) and cartilage.

SUMMARY

Wound healing and regeneration at two levels in the left limbs (forearm, upper arm, shank, and thigh) of Lacerta laevis were studied and compared as to gross morphology and histology with regeneration in the tail.

Simple amputations were performed at the two limb levels and at the mid-length of the tail. The experimental animals were kept in dampened containers at 25° - 27° C. throughout the experiment. After definite periods of healing and regeneration, the organ stumps were re-amputated and fixed for histological study.

The results indicated the following:

1. Gross morphological changes during the first few days post-amputation at all limb levels were similar to changes in the tail. The only difference observed was the greater swelling of the limb stumps compared to the tail stumps.

2. Further morphological changes resulted in the development of short, conical limb blastemata which did not grow beyond a few millimeters in length. Tail blastemata, however, continued to grow until regenerate tails of normal appearance had formed.

3. The rate of morphological changes differed in the four limb segments, the fastest rate of change

occurring in the upperarm.

4. Initial histological changes were similar in the two levels of the limbs and the tail, although differences existed in the time at which the changes first appeared and in the rate of their subsequent development. The epidermis migrated over the wound surface within the first 24 hours post-amputation in the tail, while in the limb this migration was delayed, not occurring until the second day. The rate and extent of dedifferentiation was much greater and was accompanied by a larger number of leucocytes in the tail than in the limb.

5. Differentiation started earlier and proceeded further in the tail than in the limb. The initial stage of differentiation in both the two levels of the limbs and in the tail, was seen to be the formation of mesenchymatous tissue. In the limb, cells appeared to differentiate immediately into dense connective tissue (scar tissue) under the epidermis and into cartilage surrounding the end and distal surfaces of the bone. Only in the upperarm did muscle differentiate from the mesenchymatous tissue. In the tail, however, the sequence of events was different. There, the mesenchymatous tissue became dense and compact and differentiated into a cartilaginous tube surrounding the regenerated spinal cord,

segmental muscle, dense connective tissue, capillaries, and other normal tail tissue. The dermis had not yet differentiated by 60 days, when the experiments were terminated.

It was concluded that the regenerative capacity of the two levels of Lacerta laevis limbs is very low as compared to the tail. The ability of the limbs to regenerate varied from one individual to another, covering a spectrum of types from simple wound healing to the formation of a short, conical outgrowth. In the same specimen, however, the regenerative capacity of the limbs was the greatest in the upper arm.

Histological factors appear to play an important role in the reduced regenerative capacity of the limb. These factors seem to involve principally the rapid conversion of blastema cells into scar tissue and cartilage, at the expense of the formation of significant amounts of other limb tissues.

The role of other factors, such as reduction of the wound surface by closure of the adjacent skin resulting in a small wound epithelium, and lack of sufficient nerve supply is discussed.

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PLATE I

EXPLANATION OF FIGURES

Fig. 1: Thigh 24 hours after amputation. Frontal section. 45 X.
Note the scab over the wound surface, the separated muscle fibers and the leucocytes. Femur extends to scab.

Fig. 2: Forearm 48 hours after amputation. Frontal section. 45 X.
Note the epidermis migrating toward the center of the wound surface (dark, superficial tissue in the upper right of the photograph). Dense accumulation of leucocytes at the distal end of the stump.

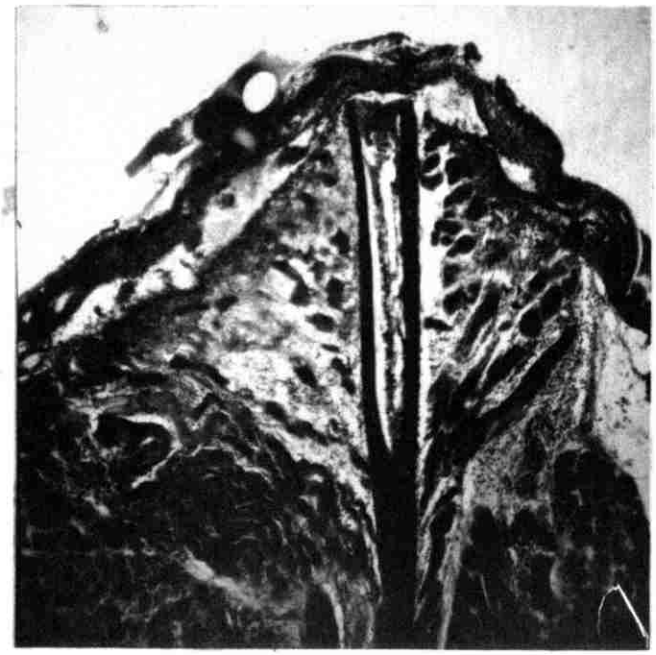


Figure 1.

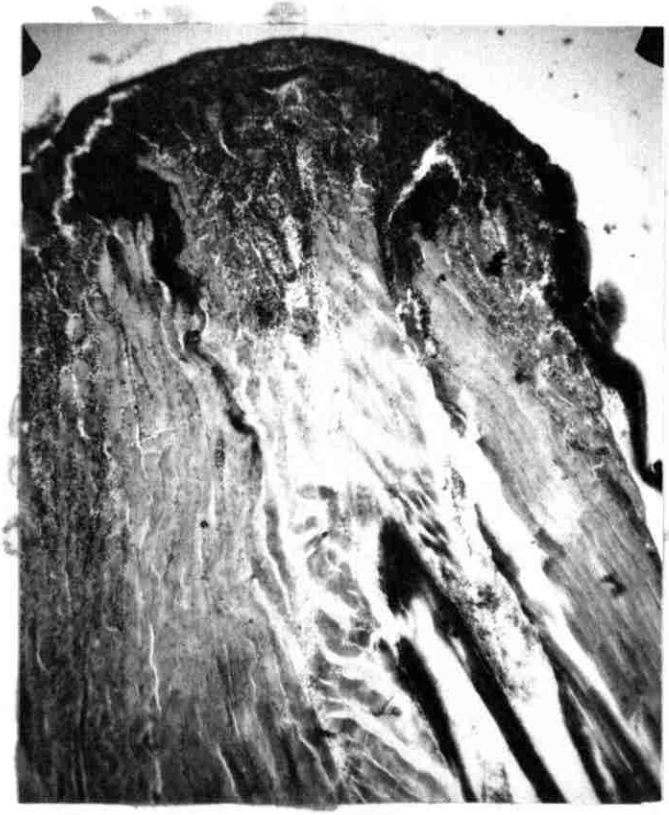


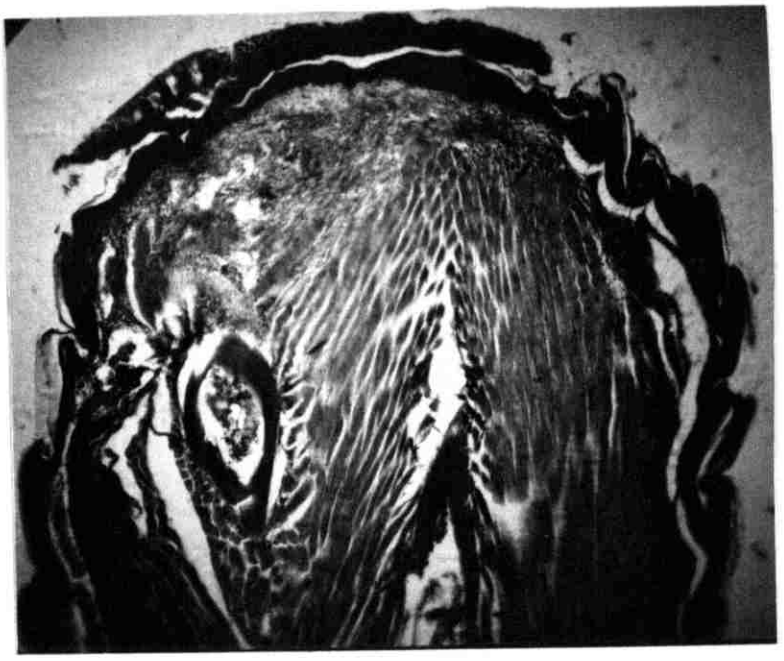
Figure 2.

PLATE II

EXPLANATION OF FIGURES

Fig. 3: Forearm 3 days after amputation. Frontal section. 45 X.
Note the epidermal layer over the wound surface, the scab above it and the dedifferentiated cells under the epidermis especially in the upper left of the section.

Fig. 4: Forearm 4 days after amputation. Frontal section. 45 X.
Mesenchymatous tissue under the epidermis. Distal tissue dedifferentiated.



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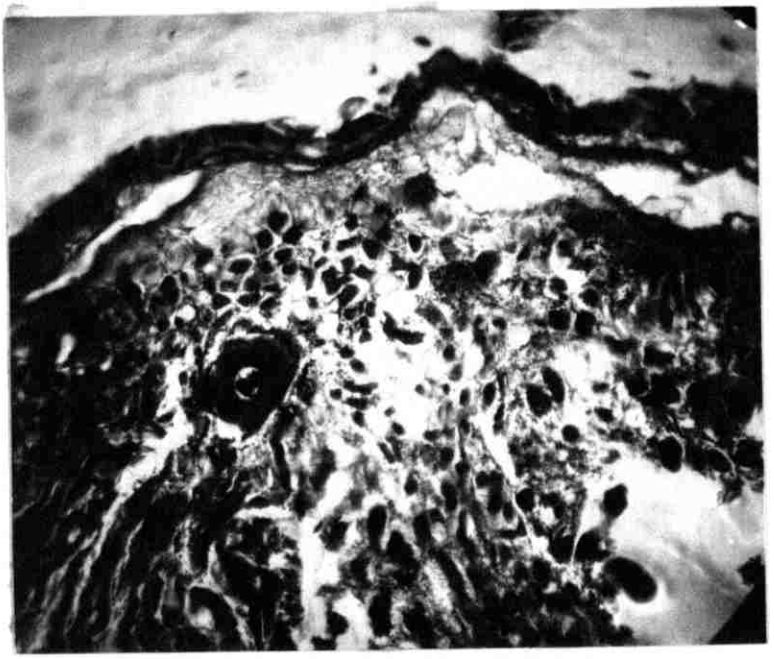

Figure 4.

PLATE III

EXPLANATION OF FIGURES

Fig. 5: Forearm 15 days after amputation. Frontal section. 45 X.
Epidermis 7-8 cells deep in the thickest part. Scar tissue forming underneath it. Procartilage cells differentiating around distal end of the bone.

Fig. 6: Upperarm 60 days after amputation. Frontal section. 45 X.
Cartilage covers end of humerus. Regenerated muscle can be seen in lower right of section. Nerve appears as dark tissue on left. Attenuated muscle distal to right. Dense scar tissue forming under epidermis.

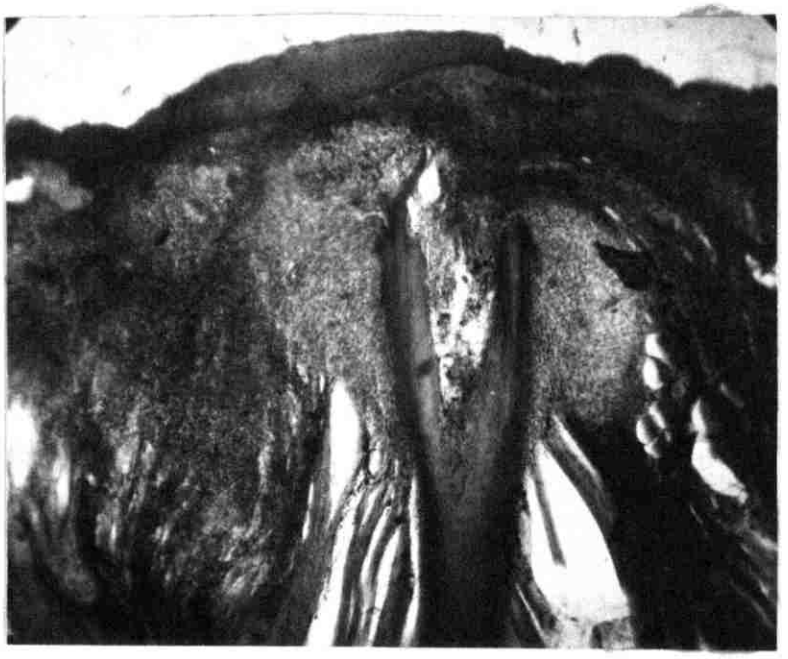

Figure 5.

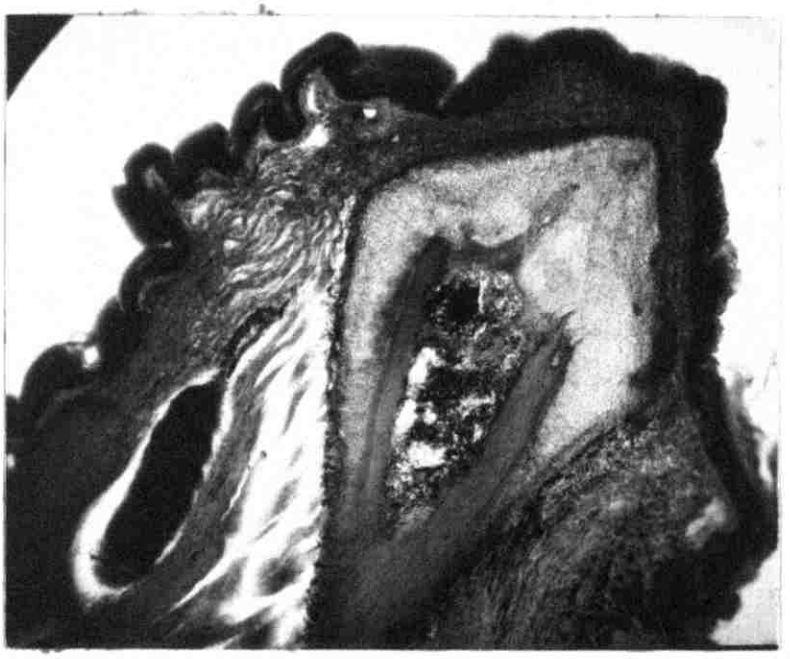

Figure 6.

PLATE IV

EXPLANATION OF FIGURES

Fig. 7: Tail 48 hours after amputation. Frontal section. 45 X.
Epidermis has closed the wound surface. Leucocytes have collected distally.

Fig. 8: Tail 15 days after amputation. Frontal section. 45 X.
Conical blastema consisting of thick epidermal and blastema cells lies distal to vertebra.

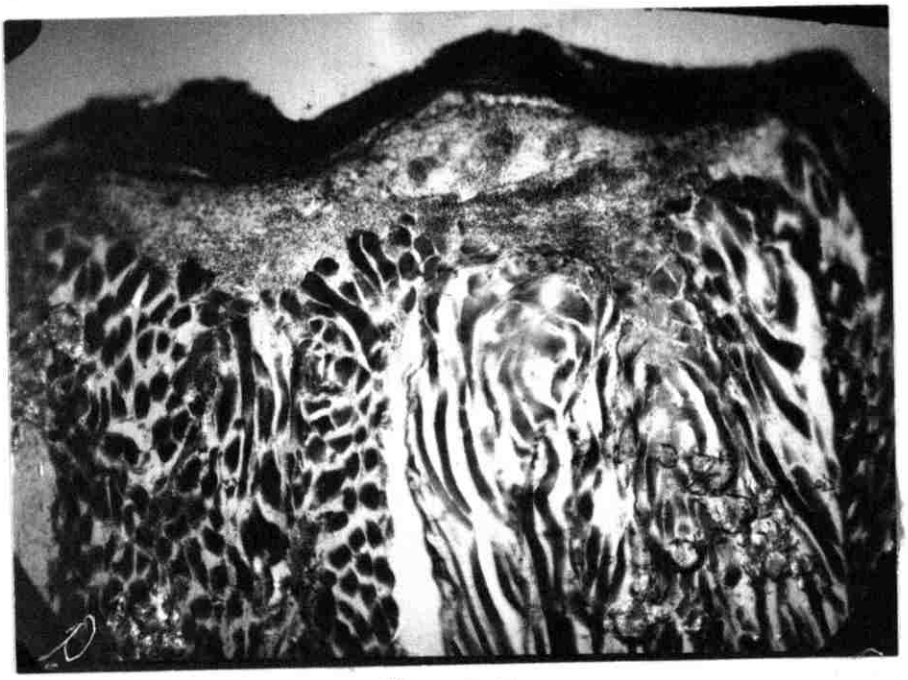

Figure 7.

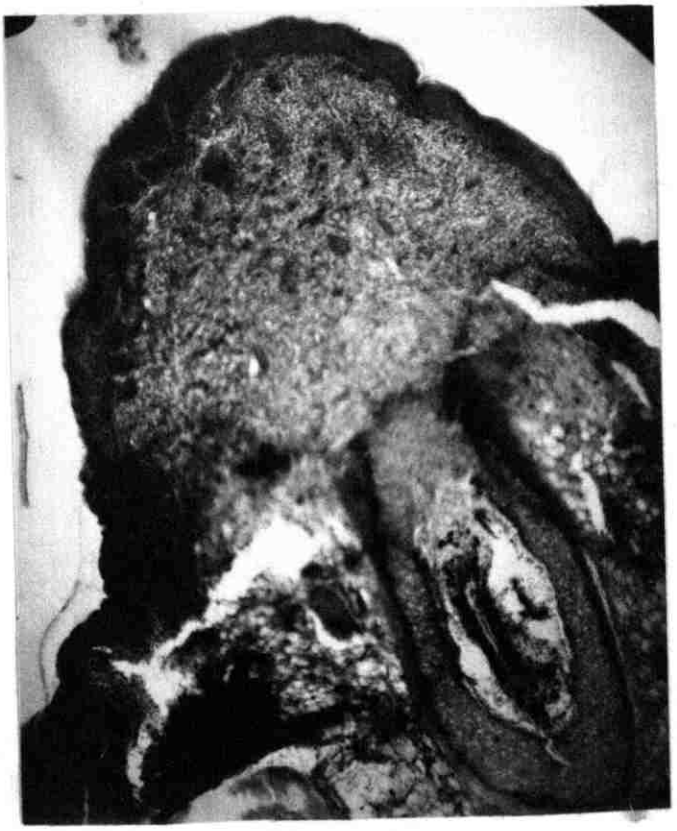

Figure 8.

PLATE V

EXPLANATION OF FIGURES

Fig. 9: Tail 30 days after amputation. Frontal section. 45 X.
Regenerating muscle can be seen as discontinuous masses of myoblasts. Section of cartilaginous tube appears at the extreme right of the photograph.

Fig.10: Tail 60 days after amputation. Frontal section. 45 X.
Note the cartilaginous tube, regnerated spinal cord, muscle, connective tissue and scales.

Figure 9.

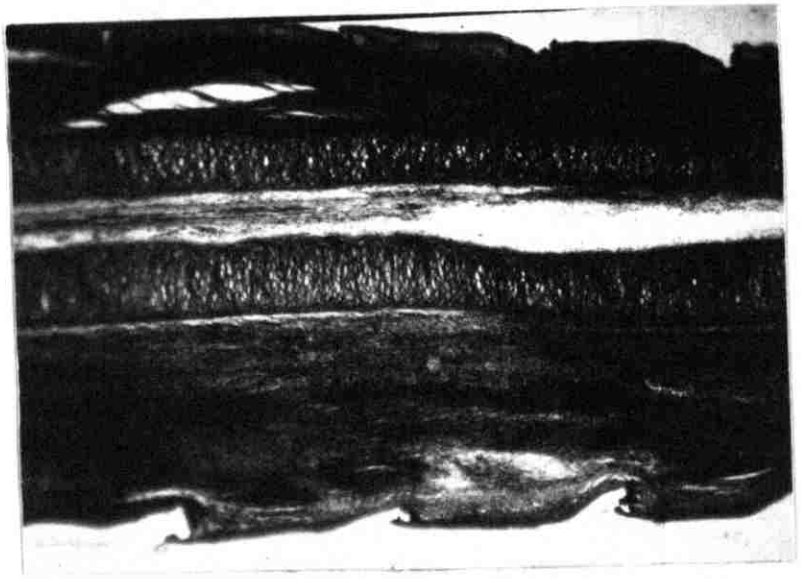

Figure 10.