A Thesis for Honors Presented to the
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Patrick R. Robins
Biology Major
Graduate: May 26, 1963

Approved by Dr. James J. Manion

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Regeneration of the Forelimb of the Salamander, Triturus viridescens, and the Adult Frog, Rana pipiens.

Introduction

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Introduction

Project yourself into the emergency room of a hospital in the not too distant future. A four year old girl is swiftly carried in after having her hand caught in a closing car door, and four of her fingers are missing. After a barrage of extensive and expedient treatment, the girl is soon released with her hand in a bandage. Following a period of a few short weeks, the bandages are removed to reveal the rudiments of newly growing fingers which in a short time will completely replace, in structure and function, her missing fingers.

Sound sensational? Today, yes, but tomorrow maybe not, due to the intensive investigations and research focusing around the regeneration of body parts. In fact, someday the above episode may be as common and successful as an ordinary cornea transplant of today.

Men of all ages have been intrigued by the processes and problems regarding regeneration, and have long speculated as to its' main mysteries of how and why. Actually, the most distinguishing characteristic of organic matter is regeneration. Belonging to the entire living kingdom, regeneration may be defined as the reproduction, or natural restoration, of parts of an organism lost through injury. In its' essense, regeneration is the displaying of universal properties of all organisms -- namely growth and differentiation.

We are all familiar with the degree or gradient of regenerative capacity of the animal kingdom, varying from ex-

treme regenerative capacity in lower animals to limited and slight regenerative capacity in higher animals. Animals low on the evolutionery scale are endowed with considerably greater powers to replace lost or injured parts. In some lowly forms, for example the hydra, the animal can be chopped into a number of pieces, and each piece will regrow the part it lacks. So ingrained are the powers of repair in some sponges that when one of these animals is broken up, by forcing it through a coarse cloth, its' fragments will reassociate into clumps which grow new individuals. A fish, or an amphibian, such as the salamander, can regenerate a tail and fins. A larval frog can regenerate limbs, but an adult frog cannot. Thus, we arrive at the line of demarkation of gross regenerative powers in the adult frog. How is it that lower vertebrates, such as the fish and salamander, are able to regrow a lost extremity, and higher animals, from the adult frog to man, cannot?

This is the purpose of my thesis -- namely, to present the problems of limb regeneration in higher animals, the various aspects and conditions relating to and influencing regeneration, and the possibility of its' eventual application to man.

Since the recent address of Dr. Charles W. Bodemer, University of Washington, renouned in this field of limb regeneration, I have become extremely interested in this phenomonon, and have carried out experiments of my own. The results I have

obtained will be interjected wherever suitable to the material.

We may ask ourselves, them, why a caudate amphibian can exhibit regeneration and an anuran cannot. Therefor, we will begin by explaining as much as possible just what happens in an amputated caudate limb during regeneration.

Regeneration in the Salamanders' Limb, Triturus Viridescens.

When a salamanders arm is amputated, the wound first heals: skin covers the stump and beneath the skin scar tissue forms.[1] Within ten days the scar tissue begins to disappear and the end of the stump enlargens. The tip of the swelling is really a bud, or blastema, containing many rapidly dividing cells which look like the undifferentiated cells of an embryonic limb. (Consult slide, 10 mm. pig) These cells spring from the old tissue at the wound surface, but their precise origin is not clearly known. According to Bodemer[2] "small nucleated masses of sarcoplasm detach from the free ends of the injured muscles; there is in addition, an apparent outwandering of connective tissue cells situated along the endomysial planes of the muscles. Histologically, their appearance suggests that these elements migrate toward the end of the nerve and may incorporate with the blastemal cells. Connective tissue cells

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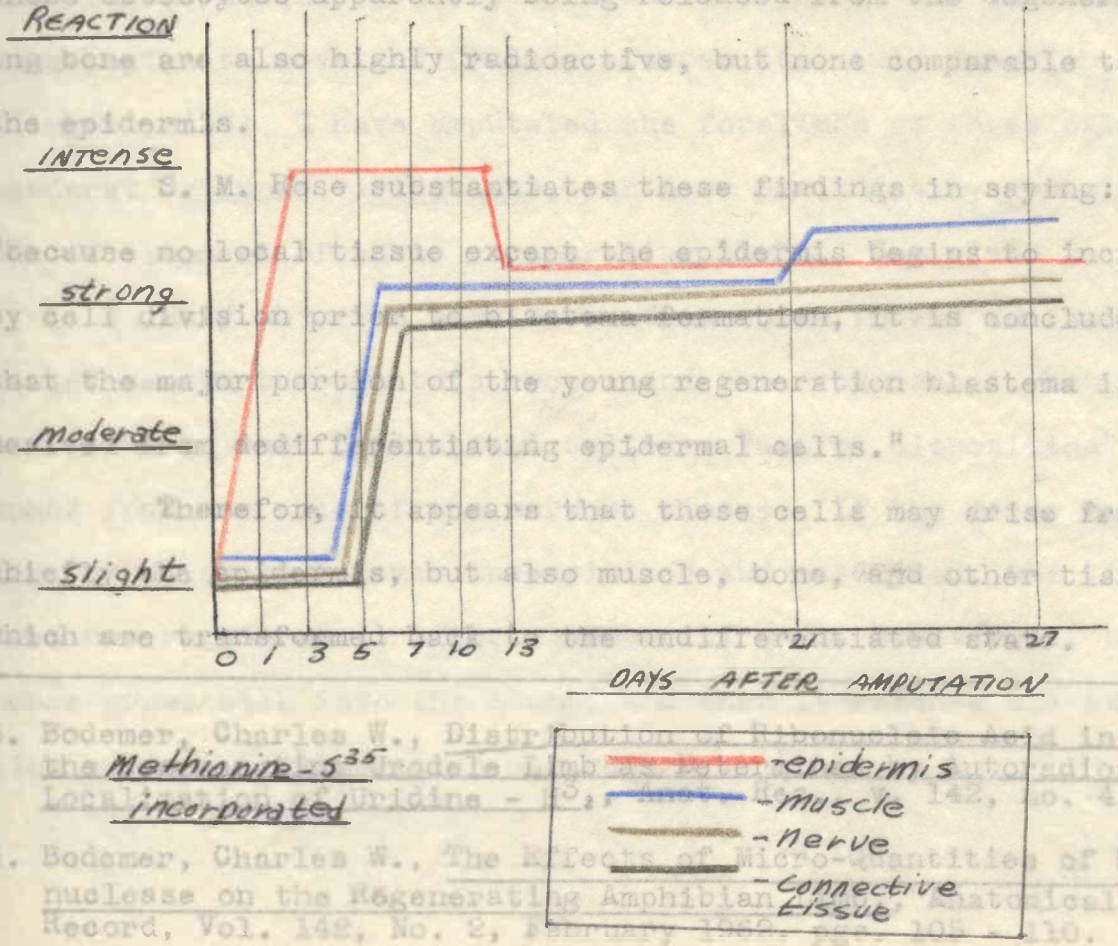
1. Singer, Marcus. The Regeneration of Body Parts, Scientific American, October, 1958, pgs. 79 - 88.
2. Bodemer, Charles W. and Newton B. Everett. Localization of Newly Synthesized Proteins in Regenerating Newt Limbs as Determined by Radioautographic Localization of Injected Methionine S-35. Developmental Biology, Volume 1, No. 3, Sept., 1959.

associated with the nerve sheaths comprise another possible source of blastemal cells." According to Thorten, "the mesenchymal components of the loose connective tissue and muscle sheaths have been averted to provide such cells."

Later on, however, Dr. Bodemer sought the use of radioactive isotopes to determine the extent of the origin of these cells. He employed the use of methionine - S^{35}, a labeled amino acid. He injected this amino acid into the stump area and later determined its' relative concentrations incorporated into proteins at various tissues. His results are as follows:

PLATE I



He also injected radioactive uridine, uridine - H^3, into the stump to show how it was distributed during the various regenerative phases.[3] As one would predict, its' distribution highly corrolated the above diagram, since RNA is vital in the synthesis of proteins from amino acids. If RNAase was injected into the limb, thus blocking the activity of RNA, regeneration would cease.[4]

Therefore, during the first few days after amputation, the epidermis only produces an intense reaction. Fragments of sarcoplasm detached from the damaged muscle produces a strong reaction, suggesting that they are not moribund fragments. Those osteocytes apparently being released from the degenerating bone are also highly radioactive, but none comparable to the epidermis.

S. M. Rose substantiates these findings in saying: "because no local tissue except the epidermis begins to increase by cell division prior to blastema formation, it is concluded that the major portion of the young regeneration blastema is derived from dedifferentiating epidermal cells."

Therefore, it appears that these cells may arise from chiefly the epidermis, but also muscle, bone, and other tissues, which are transformed back to the undifferentiated state.

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3. Bodemer, Charles W., Distribution of Ribonucleic Acid in the the Regenerating Urodele Limb as Determined by Autoradiographic Localization of Uridine - H^3, Anat. Rec., v. 142, no. 4, April.

4. Bodemer, Charles W., The Effects of Micro-Quantities of Ribonuclease on the Regenerating Amphibian Limb., Anatomical Record, Vol. 142, No. 2, February 1962. pgs. 105 - 110.

Whatever the origin of these cells, the process from this stage on resembles embryonic development. At first, the primitive cells multiply rapidly with a certain abandon resembling that of a cancerous growth; however, it grows slower and gradually takes the form of a functional organ. About a week after the start of regeneration, the bud already looks like the limb bud of an embryonic salamander. The bud elongates into a cone and by the third or fourth week the cone is transformed into the rudiments of an arm. During the next week or two, little nubs appear on the hand rudiment and grows into fingers. In the meantime, the embryo-like cells within change into specialized tissue-muscle, bone, tendon and so forth. The new limb soon begins to move and finally assumes its' full function. (Singer, 1958) I have amputated the forelimbs of three salamanders, Triturus viridescens, and have periodically photographed the development of their regenerating limbs. See plate 2.

Many theories have been advanced as to why the aalamander can go through this processand the frog cannot, but the best clue to what Spellanzani called a "useful disposition" comes from the role of the nervous tissue. If the nerve leading to the stump becomes insensitized and paralyzed, the limb does not regenerate. After a number of days, however, if the nerve grows back into the stump, and when it reaches the amputation surface, regeneration may resume.

The regeneration of the salaranders tail depends upon the nerve supply in the same way: the tail will not regrow after the spinal cord is destroyed. Therefor, it is a general rule that the regeneration of an external organ requires the presence of nerves at the would surface.

The Effect of Nervous Tissue Upon Regeneration

Just how the nerve assists regeneration is poorly understood, but most probably the nerve fibers give off substances which promote growth.(RNA ?) Also, the nerves effectiveness does not depend upon the kind of nerves present, but the number of nerve fibers is important. In the salamander, for instance, the nerves at the surface of an amputation in the upper arm usually contain about 2,500 fibers. If we destroy or reduce the number of fibers to a fourth or half, regeneration does not occur.[5] Therefor, the individual fiber itself is ineffective, but a number of nerve fibers -- the threshold number of fibers, act in concert to induce the new growth.

There is, then, a critical ratio of number of nerve fibers to the amount of stump tissue present in order to induce regeneration.

$$\frac{\text{Number of nerve fibers}}{\text{Mass of Tissue}} = \text{Threshold number, if regeneration proceeds.}$$

5. Singer and Egloff, The Nervous System and Regeneration of the forelimb of Adult Triturus VIII - The Effect of Limited Nerve Quantities on Regeneration., Journal of Experimental Zoology, v. 108, 1948. pgs. 295 - 315.

Anything below this ratio will not induce regeneration and adything above this number will induce regeneration. Therefor, the number of nerve fibers and amount of stump tissue play an extremely important role in regeneration.

Regeneration in Anuran Forelimb

With Augmented Nerve Supply

Now, supposing a nerve threshold also exists in the frog, is it possible that the animal loses its' ability to regenerate because its' normal nerve supply gradually loses its' effectiveness, or the threshold number is not attained? This would hold if in the course of the tadpoles development the tissues become less responsive to the growth factor of the nerve and require more nerve fibers than are normally available to provoke a response. It would also be true that if nerve fibers in the tadpoles limb do not increase in number in proportion to the volume of other tissues. As a result, the relative number of nerve fibers would gradually decrease and might fall below the minimum required for regeneration. Recent work by J. M. Van Stone as Princeton University indicates that the number of nerve fibers in the tadpole does indeed decline with respect to the growth of other tissues and parallels to the gradual loss of regenerative capacity.

It occurred to such people ~~such~~ as Singer, Rose and Bodemer, to determine what would happen in the frog if they in-

creased the number of nerve fibers available to the amputated limb. The frog is uniquely constructed for such experiments; the nerves of the hind limb can be freed and redirected to the forelimb to augment the nerve supply there.

After conversing with Dr. C. W. Bodemer, ~~here~~ at Carroll College this fall, I became extremely intrigued by this phenomenon and decided to perform experiments similar to his, namely, nerve augmentation in the anuran ~~and~~ forelimb.

In total, I have performed this operation on ten frogs, two of which developed to show encouraging and predicted results. Because of a fungus disease, "Red-leg" which spread rampantly through the frog population, the other eight frogs were lost before they could show desirable results.

Of the two frogs displaying favorable development of their forelimb, following amputation, I will present the record of one of these which has developed the farthest.

A small frog, (2 - 3 inches long), Rana pipiens, was used in my experiment. It was placed under anaesthesia using ether in an enclosed jar. It usually required approximately two to three minutes for the frog to become unconscious as was determined when it was not able to right itself when placed on its back.

After the frog was anaesthesized, it was placed on a paper wrapped board moistened with Ringers solution. Ample illumination was provided by two concentrated beam lamps.

Throughout the operation the frog was kept well moistened with Ringer solution, and an ether cup was always available in such cases when the frog began reviving.

To begin with, a small incision through the skin was made at the elbow area followed by a second incision at the knee area. Following this, a long glass needle was incerted in the incision at the elbow and gently maneuvered under the skin posteriorly to the frog until it emerged through the knee incision. Attached to the end of this needle was tied a red cotton thread, which will later be used to draw the dissected nerve back through the skin up into the arm. After this needle presented the thread at the knee, the thread was cut at its' end and the needle subsequently withdrawn.

Now, an incision was made around the entire thigh of the frog near the knee and the skin of the thigh was gently pulled up to reveal the fine muscles. The skin of the shank was cut down the side to the ankle and this skin was completely removed, thus, the skin of the entire leg of the frog is now removed or pulled back, revealing the muscles of the leg.

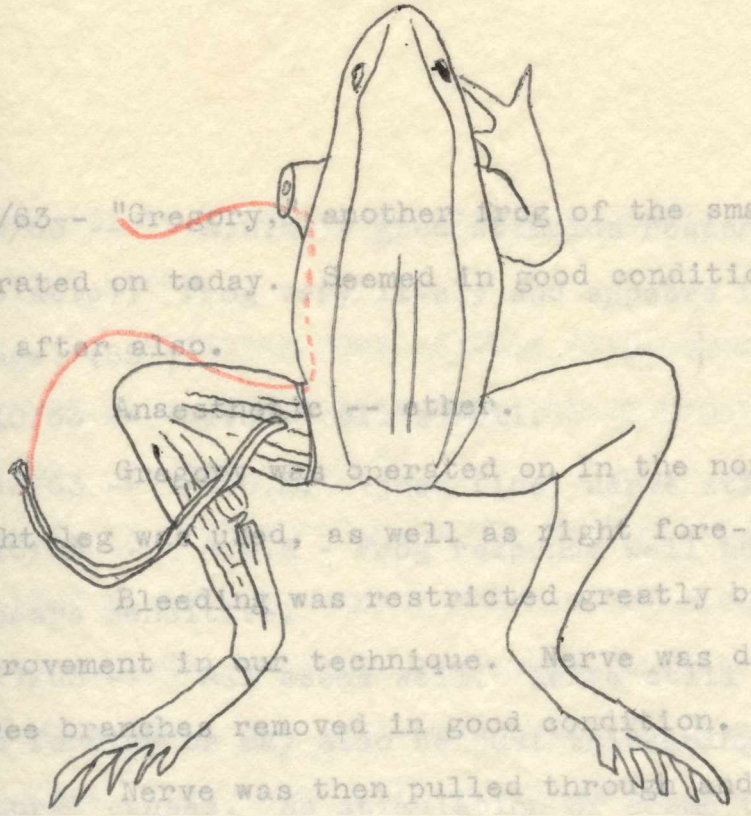
Next, the thigh muscles were separated dorsally to reveal the main branch of the sciatic nerve. Extreme caution was employed to prevent any undue bleeding, which could injure the nerve. If any blood vessels were broken, they were quickly clamped and tied off. After the two main branches of the sciatic nerve were freed from the surrounding tissues in the thigh area, the shank muscles were separated to reveal the four subsequent

branches of the two larger thigh branches. Because of the size of the frog and the tinyness of these four sciatic branches, there excision was extremely difficult. After these branches were freed, however, they were cut distally at the ankle with a glass slide. All branches were then brought together and the end of the thread was tied securely to their ends. Then, without tugging or pulling, the nerve was drawn up under the skin finally to appear from the incision earlier made in the arm.

The arm was now amputated, usually at the proximal portion of the elbow. The muscles of this stump were traumerized and the nerve implanted into these muscles which quickly regrow to surround the augmented nerve.

Because the leg is now paralyzed and useless, it is amputated immediately adjacent to the body and the skin pulled over the stump. After this skin was tied off, the operation was over, and the frog was allowed to recover. Usually the recovery period lasted from a half to two hours during which time the frog was under ~~the~~ close~~st of~~ observation and supervision. (See diagrams).

The following is the exact account I have recorded of one frog, "Gregory", from the time of operation to the time of his death, including photographs of his subsequent development:



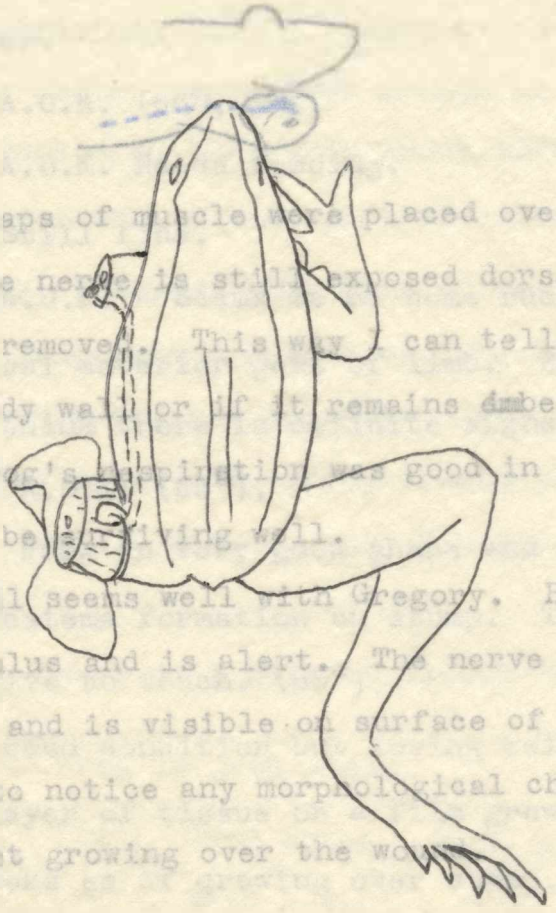
1/4/63-- "Gregory," another frog of the smaller batch, was operated on today. Seemed in good condition before operation-- and after also.

Anaesthetized -- ether.

Gregory was operated on in the normal procedure. Right leg was used, as well as right fore-limb.

Bleeding was restricted greatly by use of clamps-- an improvement in our technique. Nerve was dissected from leg and three branches removed in good condition.

Nerve was then pulled through and up to arm -- without stretching it, and placed in diagrammed position.



Flaps of muscle were placed over the nerve to anchor it; however, the nerve is still exposed dorsally and the skin covering it was removed. This way I can tell if the nerve slips back into the body wall or if it remains embedded in muscle.

Frog's respiration was good in post-op observation and appears to be living well.

1/6/63 --All seems well with Gregory. He responds readily to touch stimulus and is alert. The nerve is still imbedded in the muscle and is visible on surface of such muscle. Too early of course to notice any morphological change whatsoever...skin isn't as yet growing over the wo

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1/9/63 -- A.O.K. - good stimulus response when touched nerve (sciatic). Frog very lively and appears to be past critical stage. (65°)

1/10/63 -- 4:30 - all's well.

1/13/63 -- A.O.K. - just fine; nerve still there.

1/16/63 -- A.O.K - Frog responds well to stimuli and nerve appears sensitive.

1/17/63 -- All seems well. Nerve still in view. May be blastema forming or may also be just thickening of stump area due to natural causes. No stimulation of stump tried. (66°).

1/19/63 -- All is fine - nerve in view and skin not appearing to close over.

1/21/63 -- A.O.K. (66°).

1/22/63 -- A.O.K. Needs feeding.

1/24/63 -- Still fine.

1/25/63 -- A.O.K. - Seems to be some rudamentery blastema formation on dorsal anterior part of limb. Nerve still visible in muscle. I think there is definite signs now of blastema formation.

1/26/63 -- A.O.K. (65°).

1/29/63 -- Frog in very good shape and appears to have definite signs of blastema formation on stump. This blastema area is very sensitive to touch. (65°).

2/5/63 -- Good condition but losing weight apparently. There is a thin layer of tissue or a film growing over the stump area and skin looks as if growing over stump. The stump is very

sensitive to touch or pricks by a needly, especially in nerve augment area.

I removed some of this film covering stump but will observe it a couple more days before removing the rest.

2/5/63 -- A.O.K. (76°).

2/7/63 -- Conferred with Dr. Manion today about frog and we decided it best to remove layer of skin covering stump. This layer extended entirely over stump and it appeared that not only the epidermis, but also the dermis was beginning to cover stump. So, I put frog under mild ether anaesthesia and stripped off this outer film of skin. It bled a little bit, and the area does look completely like a blastema. It is ovally shaped and is orange in color (very light orange or even flesh colored).

I looked at this skin under 430x microscope but couldn't distinguish whether it was epidermis alone or with dermis. I am quite sure, however, that none of the blastenial cells' were disturbed. Temperature raised to 78°.

2/8/63 -- Good shape - blastema area quite sensitive to touch. It is a little dried and crusty around the surface of stump.

2/9/63 -- (75°). A.O.K., very lively. Skin not growing back over yet.

2/11/63 -- There is a film of tissue which has grown over the stump again. Frog in good shape.

2/12/63 -- (72°) Good shape. Film completely over stump again.

2/13/63 -- (75°). Skin covering stump once again removed. Area highly sensitive to touch, especially where nerve augment

is localized. Otherwise, frog in good condition and extremely lively.

2/14/63 -- A.O.K. with frog...is tiny blood spot on stump; good response to stimuli and frog very active.

2/16/63 -- All o.k. with frog - no skin growing over as yet.

2/18/63 -- (72°) - Gregory still doing fine. Has quick response to stimulus and quite active. No membrane or skin over stump, and stump itself is very sensitive to touch. It is sort of light in color, with a darker (blood, supposedly) mass in center.

2/19/63 -- A.O.K. - Thin layer of film over stump. (70°).

2/21/63 -- All fine. Nothing new (70°).

2/22/63 -- Today was a real fine day. I developed a new way of feeding the frogs and observed Gregory's stump and blastema formation under the dissecting microscope. Certainly, there is a well-defined blastemal formation, and attempts will be made to photograph it. Coming along very satisfactorily. (75°).

2/23-63 -- Again observed under dissecting microscope. Appears that dermal pigment cells are covering blastema. Will wait for two days before confirming it. Blastema has very good vascular supply. (72°). Frog very lively.

2/25/63 -- This morning, put frog under mild anaesthesia, (ether) and removed the skin covering blastema. This skin was widly invaded by dermal pigment cells, so thought it best to remove it as the large dermal element present.

After removing skin, I observed it under the microscope

and confirmed its' rich dermal layer present. Also, blastema was observed and seen to be dermal free, and very rich in vascular aspect -- tiny blood vessels enriching the blastema.

Frog still coming out of ether - respiration slow.(74°).

2/26/63 -- Frog very groggy and slow. Won't pull back leg when outstretched. Discoloring on ventral surface. Changed H-20. (76°).

2/27/63 -- Died. - Immediately immersed it in a fixitive solution not more than 2 to 4 hours after death. (3% formalin, 5% Glacial acetic acid, 20% Ethyl alcohol, 72% water). Frog was still in good condition. Took photomicrographs of stump area.

3/1/63 -- Photographs very good. Cut skin along body cavity and exposed nerve. It was still in tact and appeared as normal, functioning nerve. Still looked very lifelike and no signs of disintegration.

End of nerve in stump was not determined by microdissection, but it does grow into proximal end of stump and seems to enerate it. (Consult pictures, plate 3).

In other experiments where the frogs survived longer, they grew back new and functionally normal arms -- identical in outward appearance to the one they lost! (Singer, 1958).

These results support the view that the normal nerve supply in the frog is inadequate for regeneration. An increase in the number of fibers, however, is able to satisfy the threshold requirement.

The Effect of Traumerized Cells Upon Regeneration.

However, the augmented nerve supply is not alone adequate to cause regeneration in the frog. Recent work performed by C. W. Bodemer in 1961 has indicated that "not only a threshold number of nerves is sufficient, but also traumerized (broken) cells must be present in the regenerating area".

He performed the following experiment:[6]

He deviated the nerves of differing fiber content onto a traumerized area and noted of regenerate growth on each area. Those areas under the sub-minimal threshold number of nerves didn't grow. However, if he placed some other additional traumerized tissue onto this surface, the regenerate grew up to 3x as rapid as the normal threshold areas. If a nerve was deviated onto a surface of no traumerized cells, no regeneration occurred.

Bodemer: "It will be recalled that the implantation of fragments of newt liver or lung in the vicinity of the end of the deviated nerve increased the proportion of growth reactions from 15 - 47%. There thus appears to be a definite enhancement of nervous influence by the implanted tissue. It has been established that in their action, implants of various newt tissues are able to substitute for physical trauma, and it has been suggested that there is a mutual interdependence of the nervous system and tissue implants in the formation of

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6. Bodemer, C. W., The Importance of Quantity of Nerve Fibers Fibers in Development of Nerve Induced Supernumerary Limbs in Triturus and Enhancement of the Nervous Influence by Tissue

supernumeary limbs. Thus, a deviated nerve in the absence of trauma incapable of independently promoting limb formation, and tissue implants are equally incapable of inducing growth in the absence of a deviated nerve. Acting in concert, however, a deviated nerve and tissue implant often provoke a genuine growth reaction."

Physical trauma has long been considered as one of the primary stimuli to limb regeneration, yet little is known of its' specific action. This problem perplexed S. M. Rose, and he sought to induce regeneration by immercing the frog limb stump periodically into a strong NaCl solution. This was accompanied by no nerve deviation into the stump area, yet regeneration proceeded.[7] This may be because of one or two reasons: (1) The NaCl bath hindered closing of the wound, and caused unnaturally prolonged trauma. Also, in not allowing the wound to close, the dermis was not allowed to grow over. The NaCl baths kept the sensitivity of the nerve at a maximum, thus allowing the normal nerve supply to become adequate in initiating regeneration.

Most likely, it is a combination of the two -- prolonged and extensive trauma coupled with increased nerve sensitivity.

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7. Rose, S. M. The Effects of NaCl in Stimulating Regeneration of Limbs of Frogs. J. Morph., Vol. 77, 1945, pgs. 119 - 140.

Speculating on the results of these experiments, we may assume the release of chemical substances from cytolizing and perhaps normal implant cells which activiate the undamaged limb tissue to initiate the regenerative process. Indeed, the dedifferentiation in the undamaged muscles in the immediate vicinity of tissue implants suggests this represents the principle reaction of the traumerized cells.

Epidermal thickening is an additional response to tissue implants; in light of recent research upon the importance of an apical epidermal cap in normal limb regeneration, this, too, may represent an important corollary of the traumatic influence. Under certain circumstances, then, a submininal quantity of nerve fibers is capable of supporting regeneration. But just how trauma, or tissue implants, compensate for insufficient nervous tissue must at this time remain speculative. It may act as simply and mechanically as preventing dermal growth.

It is suggested, then, that cytolytic substances may emanate from the implanted tissues to encourage dedifferentiation in the muscles, enhance development of a thick wound epidermis, and provide, within a limited period of time, a favorable setting for the organization of a blastema by a deviated nerve.

Nervous Invasion of Epidermus During Regeneration

Still another clue is given to the riddle of limb regeneration. According to Singer, nerve fibers invade the epidermis of the regenerate of the blastema in numbers considerably greater than are observed in normal skin. These fibers appear within the epidermis a few days after amputation. The nerve fibers may enter the epidermis simply or in fascicles. Within the epidermis, their course appears to be entirely intercellular. The fibers wind among the cells, some reaching the outer cuticular layers, while others grow great distances tangentially. The fibers are naked, being unaccompanied by Schwann cells or a myelin sheath. Physiological observations show that the regenerate is much more sensitive than the normal limb, an observation commensurate with the above histological observations. The presence of fibers in the epidermis of the regenerate suggests a relation between nerve and epidermis important for the regenerative process.

The Effect of Dermal Growth During Development

It has also been demonstrated that for regeneration to appear and for formation of a blastema, the dermal layer cannot grow back under the epidermis. This is probably due to the fact that if the dermal layer does grow back, that its' fiberous nature will not allow for growth of nerve fibers into

the epidermal layer as mentioned above.

e.g. the epidermal layer is immediately subimposed by undifferentiated blastema cells. If the dermal layer separates the two, how are the epidermal cells supposed to dedifferentiate and drop down into blastema?

Therefor, the following factors seem to be detrimental in causing regeneration of a limb:

1. Threshold nerve supply.
2. Traumatic cells.
3. No dermal formation, at least in initial stages.
4. Nerve enervation of epidermal cap.

Composit Theory of Salamander Regeneration as Opposed to Frog Regeneration

SALAMANDER: Upon amputation, immediately a layer of epidermis covers over the wound and begins proliferating new cells at a tremendous rate, partially under the influence of traumatized cells and partially by nervous system. (Protein and RNA studies).

Below this epidermal cap are damaged cells of muscles, tendon, blood cells, etc., which have begun to differentiate themselves and also to induce normal tissues to also dedifferentiate, under the influence of the nervous system. The proliferating epidermal cells, coupled with the traumerized cells under the influence of the nervous system causes a rapid development of a group of unspecialized cells or a blastema.

This rapidly forming blastema may cause the dermal layer to be constricted so that it cannot grow back over the stump, and consequently a large number of fibers grow into the epidermis. Now, the action of the nervous system and threshold number is paramount, in that it by some means takes this unorganized blastema and guides or directs it into the formation of a structurally and functionally normal arm. Therefor, the role of the nervous system is two-fold:

1. Helping to initiate dedifferentiation.
2. Reorganizing dedifferentiated cells into distant tissues.

FROG: Again, the limb is amputated, and again a layer of epidermal cells grow over the wound. This layer begins rapid proliferation of unspecialized cells as in the salamander, and the traumerized cells also begin their function of self-dedifferentiation and that of surrounding tissues. However, the crux of the problem here lies within the nervous tissue. The quantity of nerve fibers present is below the threshold value and because of this dedifferentiation may be imcomplete and the dermal layer has a chance to grow back over the wound. Consequently, the rich supply of nerve fibers cannot penetrate the epidermal layer. Now, because the dermal layer has grown over the wound and because the nerve quantity is not sufficient to take and organize the unspecialized cells into new tissues, they merely form fibroblasts and lay down the dense fibers of scar tissue. Thus regeneration ceases.

These, then are the seemingly isolated clues of the intriguing and vital process of regeneration. It is a very complex phenomenon involving the basic fundamentals of life itself, namely wound healing, dedifferentiation, growth and differentiation. What, then, is their inter-relation and corrolation in the total regenerative process .... how do they act and work together to influence the molding of a new arm? What precisely is it about the nerve cell which causes the unspecialization and following reorganization of cells in forming new tissues? And how is it related to the traumerized cell? .... How can this process be applied to cancer?

These and many more questions are presently plaguing research workers the world over, and if their past findings are any indication of what they will produce in the future, we may be well certain that soon some answers will be turning up. The facts are pouring in, and their corrolation will be discovered.

And should the expectation of obtaining this advantage for ourselves be considered entirely fantastic? I'm certain that every organ has the power to regrow lying latent within it, needing only the "useful disposition" to bring it out.

Acknowledgment

I wish to express my sincere gratitude to Dr. Charles W. Bodemer, University of Washington, School of Medicine, for initiating my interest in this project and to Dr. James J. Manion for encouraging and assisting in the work of this thesis. Also, to Mr. Hilary Nickel for his assistance during the surgical operations and to Mr. Russell Munsell for his counseling in the photography of the specimens.

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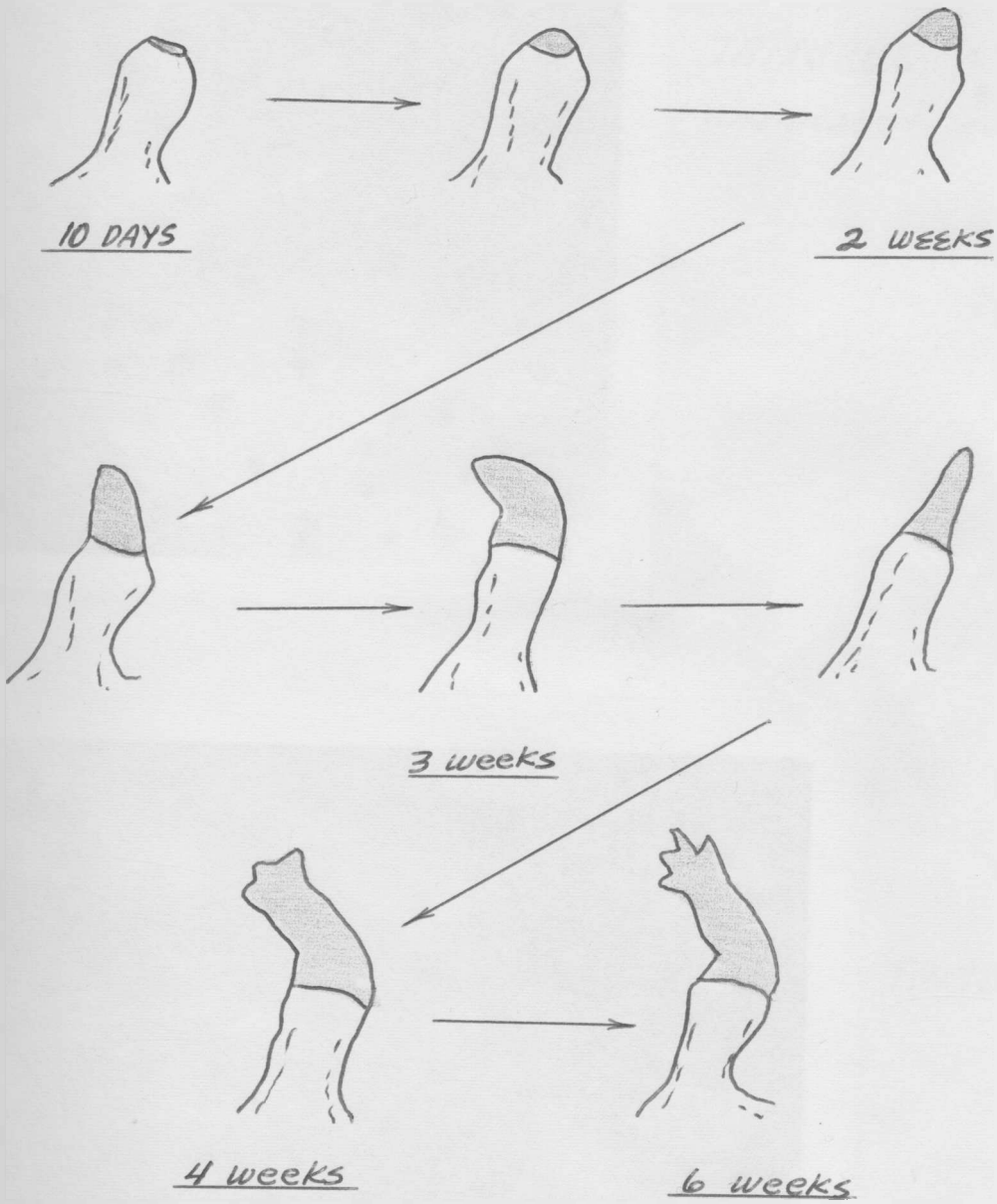
PLATE 2-A



from Singer,
Sci. Amer. 8/58

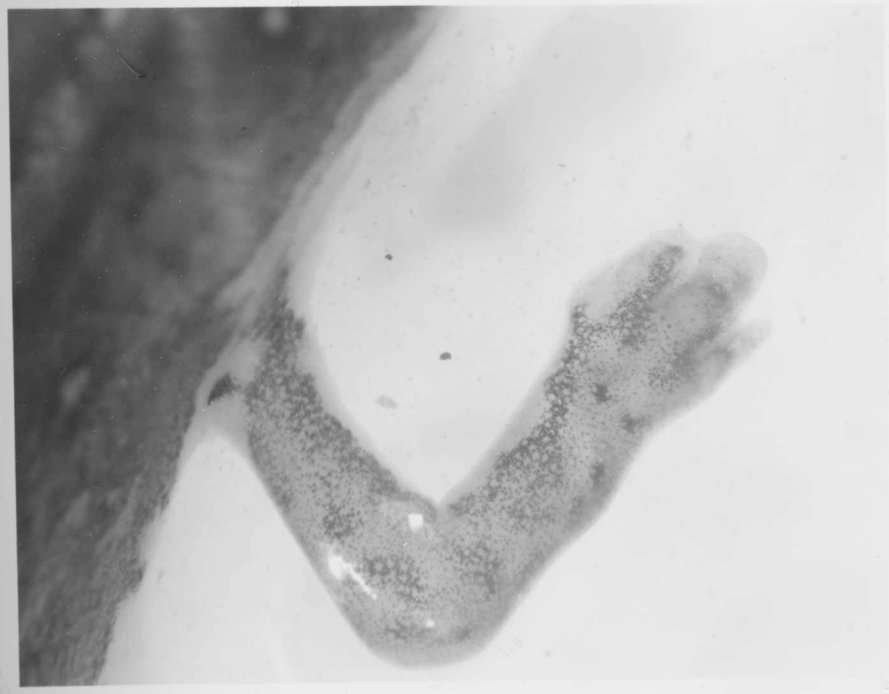
TRITURUS "A"
3-16-63
Amputated: 3-11-63

TRITURUS "A"
4-1-63

TRITURUS "A"
4-25-63

TRITURUS "A"
4-25-63

PLATE 2-B



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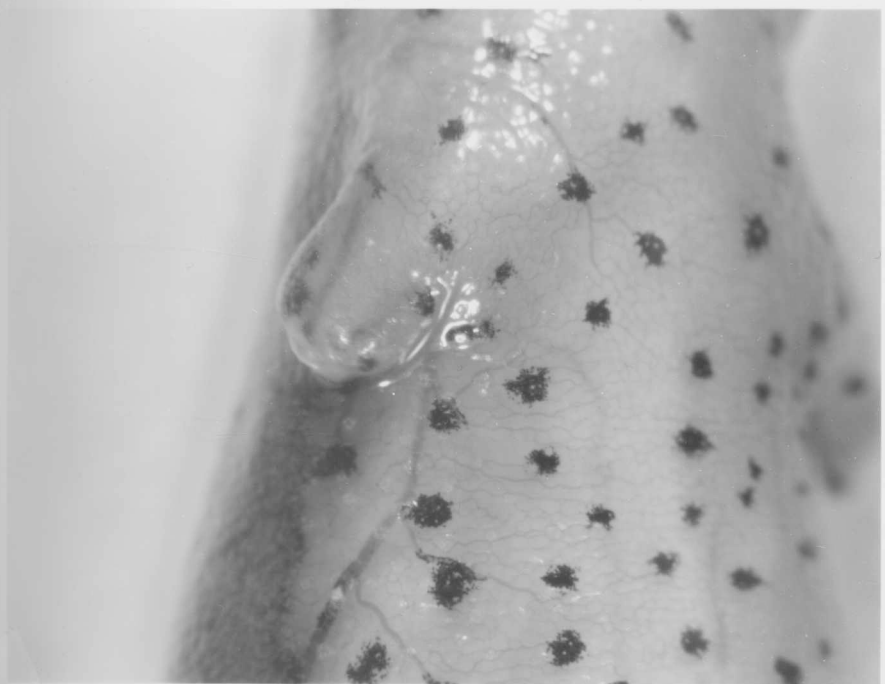
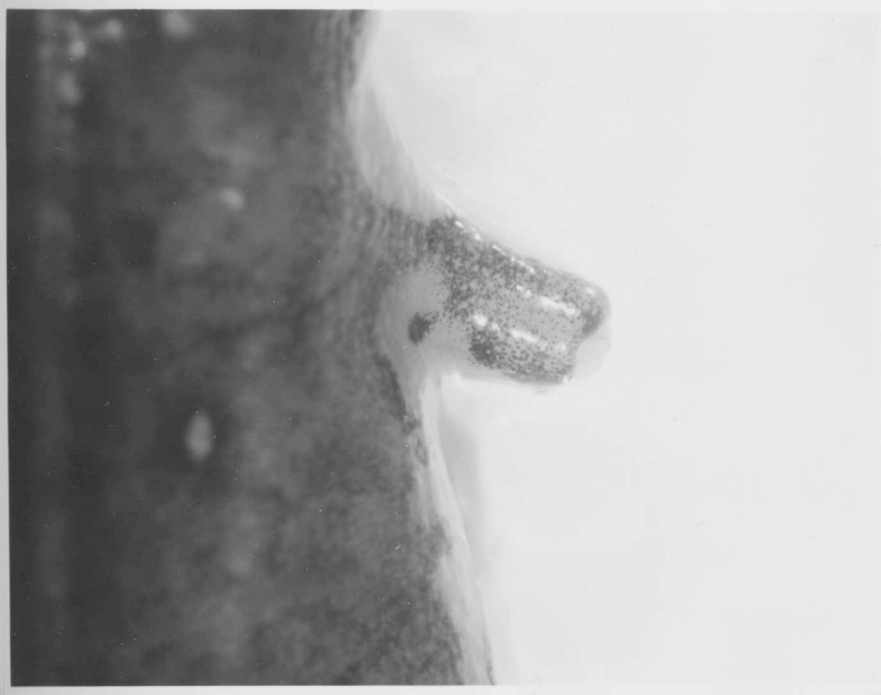
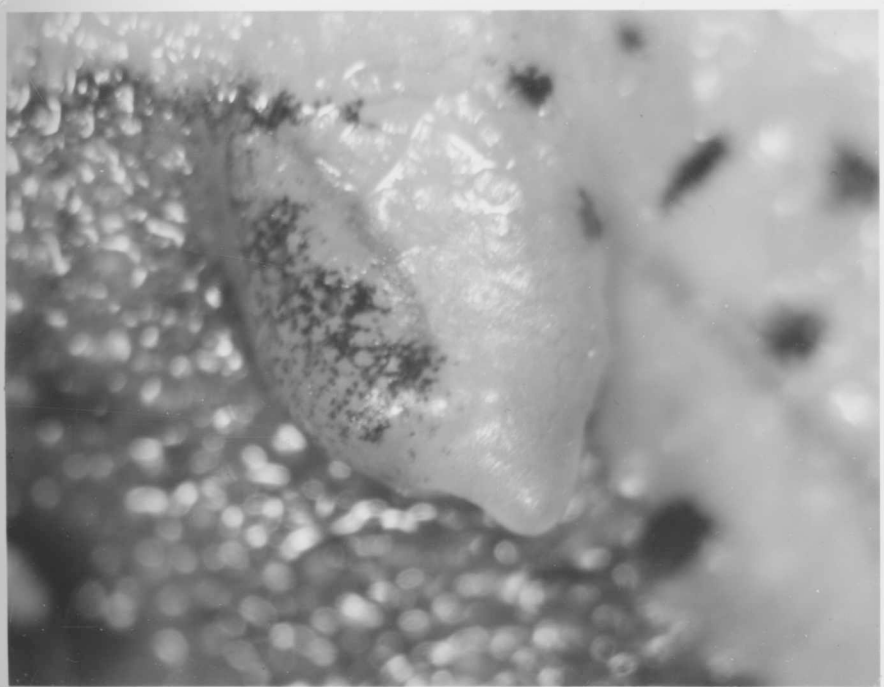
3-4-63



TRITURUS "B"
3-8-63

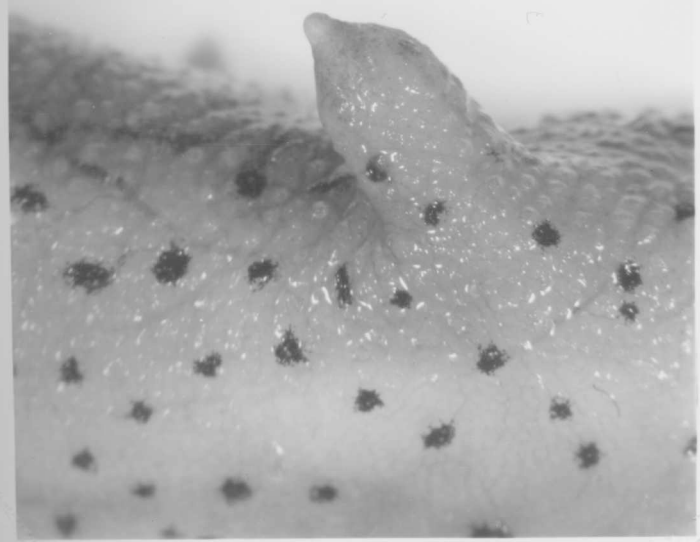


TRITURUS "B"
3-10-63



TRITURUS "B"
4-1-63

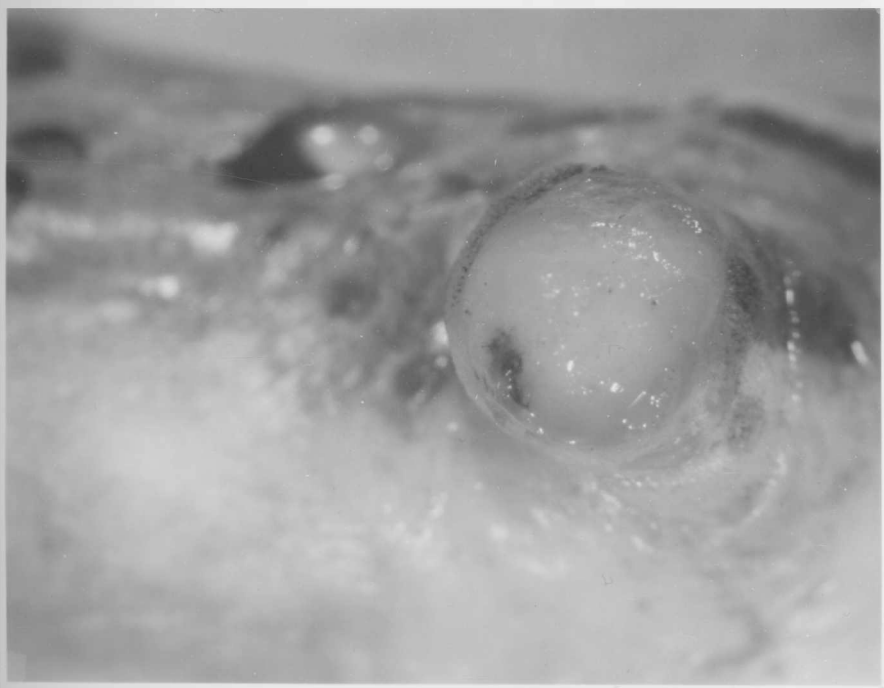
TRITURUS "B"
4-18-63
ENLARGED
VIEW



TRITURUS "B"
4-18-63

PLATE 3

FROG, "GREGORY", WITH NERVE AUGMENTATION, 54 DAYS AFTER AMPUTATION.



END VIEW OF ABOVE BLASTEMA FORMATION.

SIDE VIEW OF "GREGORY," SHOWING SCIATIC NERVE DIRECTED TO AMPUTATED FORE-LIMB.

EQUIPTMENT USED IN MICROPHOTOGRAPHY, INCLUDING BOX CAMERA, DISSECTING MICROSCOPE, STAGE LAMPS, AND EX-POSURE TIMER.