

Regeneration induced in the forelimbs by treatment with vitamin A in the froglets of *Rana breviceps*¹

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Summary. Young froglets of *Rana breviceps* do not possess the power to regenerate forelimbs after amputation through metacarpals. However, 5 min immersion of limb stumps in an oily solution of 150,000 IU vitamin A palmitate immediately after amputation, and once on each of the 3 subsequent days, induced definite though hypomorphic regeneration in 100% cases.

With few exceptions the anuran amphibians lose the power to regenerate limbs by the end of their larval life. However, several workers have succeeded in inducing at least some regeneration in the amputated limbs of adult frogs by various experimental procedures²⁻⁶. Polezhayev has suggested that one of the requirements for inducing regeneration in the otherwise non-regenerating limbs is intensification of dedifferentiation of cells of stump tissues⁴. Recent studies have shown that treatment with excess of vitamin A palmitate promotes early post-amputational regenerative changes in limb stumps of toad tadpoles by intensifying dedifferentiation resulting in the formation of good blastemas. While continued exposure to vitamin A excess inhibits post-blastemal developments⁷, good and even hyper-regeneration occurs if the vitamin treatment is discontinued after blastema is established. The present experiments were made to investigate whether vitamin A treatment could be a means to induce limb regeneration in young post-metamorphic frogs which otherwise do not possess this capacity.

Materials and methods. In young, 15-20-day-old froglets of *Rana breviceps* (Schneider), the left forelimb was amputated through the middle of metacarpal region. The vitamin preparation employed was Arovit (Roche, India) which is an oily solution of vitamin A palmitate. Administration of the vitamin by injection (75 IU and 300 IU per injection) or keeping the operated froglets partly immersed in 100 IU/ml solution of the vitamin resulted in heavy mortality within 2 days. However, the following technique proved successful: The operated limbs of experimental group of froglets were dipped in the oily solution containing 150,000 IU vitamin for 5 min immediately after amputation and then for the same duration once on each of the following 3 days with no further treatment on the remaining 12 days of the experiment. The froglets of both treated

and untreated (control) groups were maintained in aquaria resembling small ponds at room temperature (30-32 °C) with a light source to attract small insects to provide food at night.

Results. By the end of 15 days following amputation, forelimb stumps of all the 14 froglets of the untreated group had healed up indicating complete absence of regenerative power. 1 representative case of this group is shown in figure 1. There was, however, very definite though hypomorphic regeneration in all the 11 vitamin-treated forelimb stumps of the experimental group of froglets. The regenerates showed positive attempts at morphogenesis of distinct digit-like structures in all cases. In 4 cases there had developed 4 and in the remaining 7 cases 3 such digital structures per regenerate. 4 representative cases of this group are shown in figures 2-5.

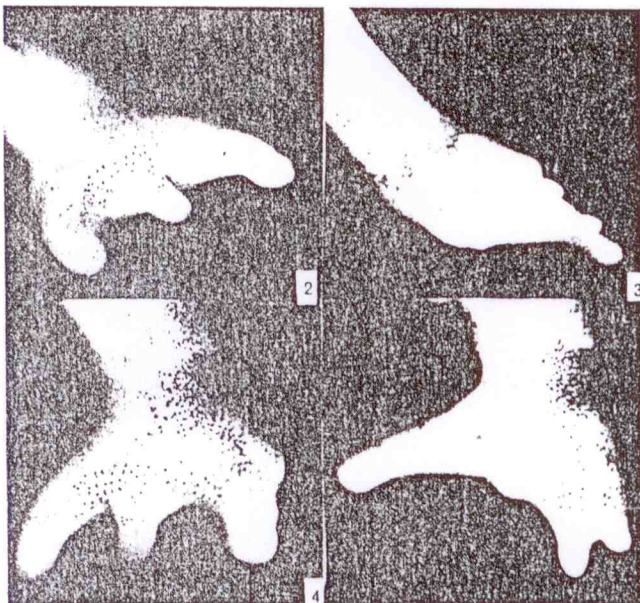
Discussion. The results provide very definite evidence that even very brief local-treatment of amputated forelimbs of



Fig. 1. A case of nonregenerating untreated stump of a forelimb amputated through metacarpal region in a froglet of *Rana breviceps*.

froglets with concentrated vitamin A palmitate for only a few days after limb transection is effective in inducing regeneration, although the regenerates obtained were all hypomorphic and sub-normal. To induce regeneration in adult frogs, Rose² had briefly immersed the limb stumps in saturated sodium chloride solution periodically; and Polezhayev³ had repeatedly traumatized the amputation surface with a needle to keep the wound epithelium of the limb stumps in a juvenile condition. Both these procedures prevented early differentiation of a fibrous dermal pad below the wound epidermis permitting prolonged interaction between the latter and the internal tissues of the stump and resulting in greater dedifferentiation and release of blastemal cells for regeneration. Similar changes in the pattern of wound healing were observed when regeneration was successfully induced in the limbs of adult frogs by implantation of additional adrenal glands in the lower jaw⁶.

It has been found that in tadpoles with amputated limbs vitamin A treatment maintains the epidermis in a larval condition, prevents dermal differentiation below the wound epithelium of the limb stumps and intensifies dedifferentiation as well as proliferation of blastemal cells derived therefrom^{7,8}. Vitamin A excess is known to enhance proteolytic activity in cartilage by inducing release of acid proteolases from chondroblasts probably due to its action on lysosome¹⁰. Increase in proteolysis is normally associated with the phase of dedifferentiation in regenerating limbs¹¹. Vitamin A is also said to promote cell proliferation by mitosis¹². Thus this vitamin promotes all those processes which, during the initial period following amputation, are conducive to regeneration. In fact, results of Niazi and Saxena⁹ indicate that this treatment results in the formation of blastema with greater than usual morphogenetic capacities equivalent to those of the original limb bud capable of forming a whole limb instead of only its distal and actually removed part. It may be inferred that vitamin A may have induced regeneration in the forelimbs of froglets due to its above-mentioned properties. It may have modified wound healing and caused sufficient dedifferentiation of the cells derived from stump tissues to enable them to reacquire morphogenetic potentialities to produce regenerates even though of sub-normal morphology.



Figures 2-5. 4 cases of hypomorphic regenerated hands with 3-4 digital structures from vitamin A treated stumps of forelimbs amputated through metacarpal region in froglets of *Rana breviceps*.

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