

**Spontaneous Regeneration of the Gastrocnemius Muscle of the Frog, *Rana tigrina***

The role of nerves in amphibian limb regeneration is well established<sup>1-4</sup>, but spontaneous regeneration of the muscle has been studied extensively only in Urodeles among the amphibians<sup>5-8</sup>. Muscle regeneration has been obtained in vertebrates, including the frog<sup>9</sup>, by mincing and implanting the ablated skeletal muscle where the nerve supply has remained intact<sup>9,10</sup>. The present work reports the spontaneous regeneration in the ablated gastrocnemius muscle of the frog *Rana tigrina*.

*Material and methods.* Adult frogs belonging to the species *Rana tigrina* were collected locally and maintained in the laboratory in aquaria, fed ad libitum on small frogs and insects. Frogs having a mouth to vent length of 8 cm were selected for the present study. In the first group of 6 anaesthetised animals, the gastrocnemius muscle of one of the hind limbs was excised for a length of 2 cm, with least injury to the nerves and vascular supply, leaving behind short stumps of the muscle at

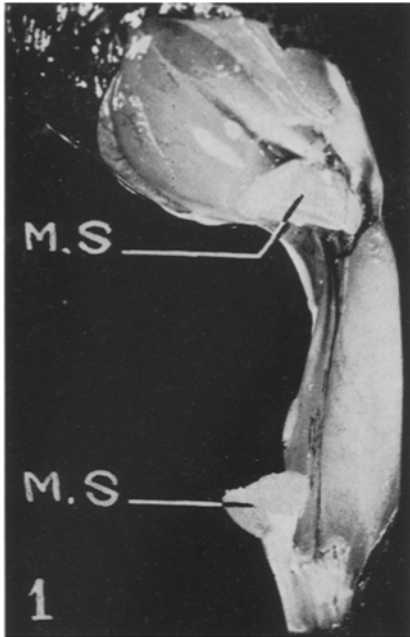


Fig. 1. The muscle stumps immediately after ablation M.S. = muscle stump.

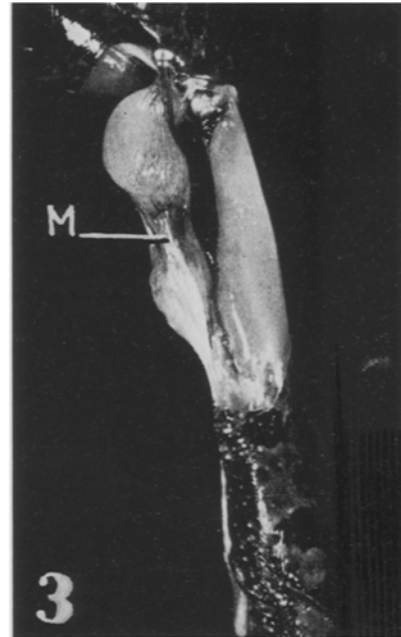


Fig. 3. Appearance of the muscle regenerate after 2 months of ablation.

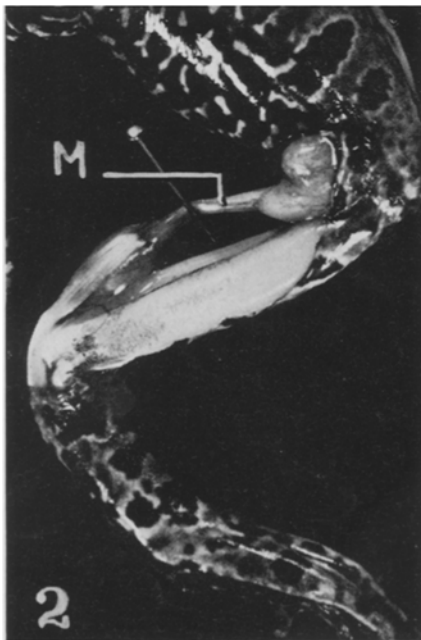


Fig. 2. Photograph of the regenerated muscle after 1 month duration.

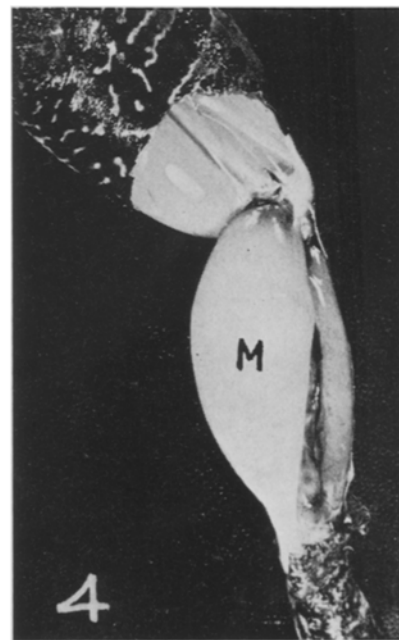


Fig. 4. Photograph of the fully regenerated gastrocnemius muscle after 7 months duration. M = Muscle regenerate.

origin and insertion (Figure 1). In another group of 6 frogs, a portion of iliac nerve from the middle of the femur to the posterior tibio-fibular branches was removed carefully without injuring the major vascular supply, followed by the removal of gastrocnemius muscle.

**Results and discussion.** Two animals from each group were sacrificed regularly 1, 2 and 7 months after the operation. In the group where the nerve supply was kept intact, 1 month after ablation the muscle regenerate was seen as a small band, 8 mm in diameter, extending between the origin and insertion, but connections were not established by most of the muscle bands (Figure 2). 2 months after the operation, the muscle regenerate appeared extending from the origin to the insertion. The regenerated muscle was about half of the original size, 13 mm in diameter, but lacked the normal shape (Figure 3). Regeneration was complete by 7 months after the removal. Here the muscle was almost identical to the control muscle of the opposite limb, except for its slightly reduced size (Figure 4).

Where the nerve has been removed before cutting the muscle, only a healing of the cut ends occurred and no trace of regeneration was evident, even after 7 months of ablation.

What has been recorded by other workers relates mainly to regeneration after mincing and implanting muscle fragments. The present study reveals for the first time the spontaneous regenerative ability of the gastrocnemius muscle of frog and also the significance of the nerve in muscle regeneration process<sup>11, 12</sup>.

**Zusammenfassung.** Die spontane Neubildung des Gastrocnemiusmuskels und die Rolle des Nerven in der Muskelneubildung des Frosches *Rana tigrina* sind untersucht worden.

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## The Cyclic AMP Response to Noradrenalin in Young Adult Rat Brain Following Post-Natal Injections of 6-Hydroxydopamine<sup>1</sup>

Catecholamines readily stimulate the formation of cyclic 3',5'-adenosine monophosphate (cyclic AMP) in incubated tissue slices of rat brain<sup>2-4</sup>. Moreover, an involvement of cyclic AMP in adrenergic transmission processes has been described<sup>5</sup>. Intraventricular injections of 6-hydroxydopamine (6-OHDA) which produce a selective destruction of catecholamine nerve endings in the rat brain<sup>6-11</sup>, subsequently lead to an enhanced stimulation of cyclic AMP by noradrenalin (NA) in vitro<sup>3, 4</sup>. Furthermore, during postnatal development of the brain prior to the appearance of monoamine containing nerve endings<sup>9, 12, 13</sup> the adenylyl cyclase receptor that is responsive to these neurohormones exists in a similar hyperactive condition<sup>14</sup>. Recently it was reported that injections of 6-OHDA into rodents at birth resulted in behavioral changes, weight loss and deficits in brain NA content when the animals reached adulthood<sup>9, 15-17</sup>. In the present study 6-OHDA was injected intracranially into rats at various times after birth in order to assess the effect of destruction of adrenergic neurons on the subsequent NA-induced stimulation of cyclic AMP in incubated tissue slices of cerebral cortex from young adult animals.

**Methods.** The experiments were carried out using Sprague Dawley-Holtzman rats. Littermates were injected intracranially with either a control solution (0.1% ascorbic acid) or 6-OHDA (250 µg in 0.1% ascorbate in 10 µl) at various times after birth and were maintained with their respective mothers. At 35 days postpartum the animals were sacrificed and the cerebral cortices removed, sliced and preincubated for 30 min in Krebs-Ringer bicarbonate buffer. The buffer was changed and after 15 min further incubation, NA (10<sup>-5</sup> M) was added.

6 min later the samples were homogenized and cyclic AMP was isolated and determined by methods described previously<sup>2-4</sup>. Cyclic AMP is expressed as picomoles per mg sample protein.

**Results.** The results of the present experiments are depicted in the Figure. In the presence of NA cyclic AMP levels were consistently elevated 2-3-fold in the

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