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Treatment of axolotls with retinoids for limb regeneration studies

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A great deal of research on the effects of retinoids on axolotl limb regeneration has been carried out because of the fascinating array of patterning anomalies induced by retinoids (Maden, 1982). Three basic methods of retinoid treatment have been employed to induce these pattern variations and duplications. These include immersion in retinol palmitate, implantation of retinoic acid (RA) in silastin blocks, and intraperitoneal injection of RA. The purpose of this report is to describe and evaluate these three treatment methods since the treatment method can influence the results obtained (Scadding and Maden, 1986a).

Immersion in retinol palmitate (RP) involves making a suspension of RP (Sigma R-3750) at a concentrations of up to 300 mg/L (= 75 IU/L) in the water in which the axolotl larvae are kept and then leaving them in this solution for 14 days following amputation. The RP solution is replaced every two or three days to offset the loss due to photodegradation. This treatment results in almost 100% of the treated animals showing either proximodistal duplications during limb regeneration or inhibition of regeneration (Scadding and Maden, 1986b; Johnson and Scadding, 1992).

Implantation of blocks of silastin (Dow Corning) containing RA was first employed by Maden *et al.* (1985). This procedure involves mixing a specified amount of RA with the silastin elastomer (up to 200 mg RA per ml silastin), adding a catalyst, allowing the silastin to set, and then cutting the resulting silicon material into suitably sized blocks (e.g. 0.3 mm cube contains 5.4 μg, sufficient to cause duplications in a regenerating limb). The block is then implanted into the amputated limb adjacent to the amputation surface with optimal effect when implanted on day 4 after amputation. The RA is slowly released over several days.

For injection treatment with retinoic acid, RA is dissolved in dimethyl sulfoxide at 50 or 100 mg/ml (Thoms and Stocum, 1984). This solution is then injected intraperitoneally into the axolotl larvae using a 26 gauge needle giving a single treatment of 50 to 100 μg per gram body weight on day 4 to 7 post amputation. A higher concentration is needed in smaller (2-3 g) axolotls than in larger axolotls (10-14 g) (Ludolph *et al.*, 1990).

Having used all three methods I can report that injection of RA gives the most consistent results. The response of amputated axolotl limbs to retinol palmitate immersion is more variable, probably due to variations in the rate of photodegradation and variability of uptake from one animal to the next. The advantage of local implantation of RA containing silastin blocks is that it allows much lower total dosages than other methods. However, due to difficulties with getting the RA uniformly mixed in the silastin, cutting blocks to a precisely uniform size, and placing them consistently in the limb, results are more variable than with injection treatment. Injection of RA offers the most consistent results with the least effort, since injection provides a precise dose which can be readily adjusted for animals of differing sizes.

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