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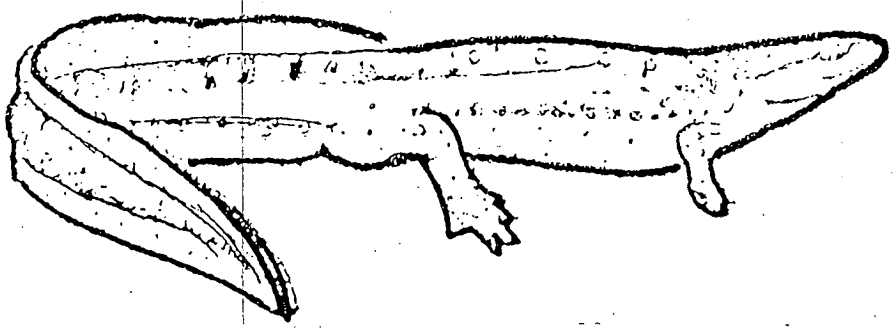

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CORRELATION OF THE PROXIMALIZING EFFECT OF RETINOID TREATMENT ON LIMB REGENERATION IN ANURAN TADPOLES WITH THE NORMAL CAPACITY TO REGENERATE - I.A. Niazi, Department of Zoology, University of Rajasthan, Jaipur 302004, India.

The unique effect of retinoids on limb regeneration in amphibians is by now well documented. Contrary to the general rule the blastema of retinoid treated anuran tadpoles and the urodele larvae and adults gives rise to the limb regenerate in which some or all of the stump structures are duplicated - a phenomenon described as P-D duplication or proximalization (1-7). In the urodeles this effect has been demonstrated after amputation through stylopodium, zeugopodium as well as autopodium of both fore- and hindlimbs in the larvae and also adults. In the anurans regenerative capacity gradually declines along the P-D axis of the growing hindlimb and finally disappears completely as the tadpole approaches metamorphosis. At our laboratory the effect of retinol palmitate treatment on hindlimb regeneration was investigated in Bufo melanostictus tadpoles of developmental stages when the ability to regenerate was high, when it was declining and finally when it had disappeared (8). Hindlimbs of the tadpoles of this toad at stages 30/31, 34, 36 and 38 according to Khan (9) were amputated through thigh, shank and ankle. The tadpoles were then immersed in 15 IU/ml aqueous suspension of retinol palmitate for 3 days and subsequently reared in ordinary well water for another 12 days at room temperature (30-32°C). The limb regenerates were examined after in toto staining with victoria blue or sectioned for microscopic study.

In the controls regenerates were of the usual type consisting of only the parts distal to amputation level. At stages 30/31 and 34 regeneration occurred in 100% cases but at stage 36 this percentage declined significantly at thigh, shank and ankle levels, in this order. In stage 38 tadpoles thigh and shank stumps simply healed up; in only a few cases (15%) small spikes regenerated at ankle level. Most regenerates in the youngest tadpoles were morphologically perfect but in older ones they were found to be increasingly hypomorphic.

In the treated groups 4 types of regenerates were produced in varying frequencies from the three levels of amputation in tadpoles of different stages : (a) Proximalized type possessing P-D duplications of stump structures; (b) usual control type; (c) small spikes containing some cartilage and (d) inhibited blastemas which did not redifferentiate. At stage 30/31 all good regenerates were proximalized type. This category included 90%, 95% and 55% cases of regenerates produced at thigh, shank and ankle levels, respectively. Remaining cases were those of inhibited blastemas. At stage 34 both proximalized and control types of regenerates developed. Good regenerates arising from thigh, shank and ankle levels, respectively, included 16%, 60% and 76% cases of proximalized type and 34%, 12% and 8% of control type. In the rest blastema was formed which did not differentiate. At stage 36 only control type regeneration occurred in 53% cases at thigh and in all 100% cases at shank and ankle levels. In the remaining 47% thigh level amputees only

blastemas were formed. In stage 38 tadpoles retinoid treatment increased spike regeneration to 50% cases at ankle level and induced such regeneration in 10% thigh and 20% shank level amputees also. In a number of cases of proximalized regeneration more than one (multiple) regenerates grew out of the same stump. Frequency of such multiple regeneration was greater in stage 34 than in stage 30/31 tadpoles and was the least at ankle level.

The following conclusions and suggestions emerge from this study:
(i) The ability of retinoids to cause P-D duplication is correlated with the presence of normal capacity to regenerate limbs. This is supported by the reported observations on Xenopus (10) and Rana breviceps (11). This may be the rule for all amphibians including the urodeles which possess this capacity throughout life.
(ii) Since retinoid treatment causes alterations in protein synthesis in the blastema (12) P-D duplication can be reasonably attributed ultimately to gene reprogramming in the blastema cells in which specific cytoplasmic retinoid binding protein(s) may be involved (13). Increased production of such protein(s) during blastema formation appears to be the normal event not caused by the retinoid (13); but perhaps the availability of enhanced quantity of a suitable retinoid together with the presence of sufficient amount of the appropriate binding protein in the dedifferentiating cells may affect gene transcription pattern in the blastema cells altering it to that of the original limb bud capable of forming a complete limb. Vitamin A is essential for proper morphogenesis and differentiation. It is known that levels of cytoplasmic binding proteins (CRABP, CRBP) have been found to be high in many tissues of foetal and neonatal stages of mammals but they drop in the adult stage (14). Investigations of the levels of CRABP and CRBP and vitamin A in the original limb buds and the limb regeneration blastemas of retinoid treated and untreated amphibians may be very useful. Such studies on the anuran tadpoles of various developmental stages may shed some light on the molecular basis of the decline and loss of the power to regenerate limbs in these animals.
(iii) The extent of P-D duplication is known to be related to dose and duration of retinoid treatment. This may be due to graded effects on gene activity related to dose and duration of exposure to the retinoid. Improvement in the morphological quality of the usual normal type regenerates of treated stages 34 and 36 tadpoles as compared to those of corresponding controls may be the expression of a lesser grade or a different type of effect of the retinoid on the genome in the blastema cells of older tadpoles in which the normal capacity to regenerate is on the decline.
(iv) Retinoids cause dissolution of cartilage matrix liberating cells that may remain healthy capable of redifferentiation on withdrawal of treatment. This may explain the regeneration of small spikes containing some cartilage in the most advanced tadpoles otherwise having no power to regenerate.
(v) Duplication in the A-P axis manifested in the production of multiple regenerates from the same stump may be due to mechanical causes emanating from increased destruction of tissues at the cut end by the toxic action of the retinoid, distortion in the formation of a smooth wound epithelium and consequent development of more than one apical cap on the amputation surface.

References : (1) Niazi, I.A. & Saxena, S. (1978) Folia Biologica (Krakow),26:3; (2) Jangir, O.P. & Niazi, I.A. (1978) Ind. J. Exp. Biol. 16:438; (3) Maden, M. (1982) Nature, 295:672; (4) Stocum, D.L. & Thoms, S.D. (1984) J. Exp. Zool., 232:207; (5) Lheureux, E. et al. (1986) J. Embryol. exp. Morph., 92:165; (6) Niazi, I.A. et al (1985) Roux' Arch. Dev. Biol., 194:355; (7) Koussoulakos, S. et al.(1986) Hell. Biochem. Biophs. Soc. Newsletter, 23:14; (8) Alam, S. & Niazi, I.A.(1988) - in press; (9) Khan, M.S.(1965) Biologia, 11:1; (10) Scadding,S.R. & Maden, M.(1986)J. Embryol. exp. Morph., 91:35; (11) Sharma, K.K. & Niazi, I.A. (1988) in "Monographs in Developmental Biology" (H.J. Anton ed.), Karger, Basel (in press); (12) Sharma, K.K. & Anton, H.J. (1985) In "Progress in Developmental Biology", Part A, p. 105, Alan R. Liss, New York; (13) Keeble, S. & Maden, M. (1986) Dev. Biol. 117:435; (14) Chytil, F. & Ong, D.E. (1984) In "The Retinoids" (M.B. Sporn, A.B. Roberts & D.S. Goodman eds), vol. 2, p. 89, Academic.