

Stage Dependent Effects of Retinoids on Limb Development
and Regeneration in Amphibians.

by I.A. Niazi

Department of Zoology, University of Rajasthan, Jaipur, India

Vitamin A and its derivatives collectively called the retinoids are involved in the differentiation of many cell types and tissues¹ and have a variety of effects on the developmental processes. They are known to produce severe teratogenic effects on the development of limbs in rodent embryos resulting in phocomelia or micromelia^{2,3}. Similar results have been obtained in the anuran tadpoles in our laboratory^{4,5}. On the contrary, in the regenerating limbs of amphibians the retinoids produce strange promotional effects so that more tissues and structures are formed than would have done so normally. This effect first discovered on the regenerating limbs of anuran tadpoles in our laboratory⁶ is manifested in the increase of the morphogenetic capacity of the regeneration blastema cells leading to serial duplication of some or all of the stump structures in the limb regenerate; often whole limb including the girdle is regenerated from the amputation surface irrespective of the level of amputation along the proximo-distal axis. In the anuran tadpoles frequently more than one limbs of this type are regenerated from the same amputation surface in response to treatment with retinol palmitate after amputation⁴⁻¹². This drug has also been found to improve the morphological quality of regenerates in anuran tadpoles and even restore to some extent the regenerative ability in the advanced prometamorphic tadpoles and postmetamorphic frogs¹³⁻¹⁶.

The teratogenic effects of the retinoids on limb development during ontogenesis are strictly related to and dependent on the developmental stage of the limb and the state of differentiation of its cells when the retinoid is administered. The cells are sensitive to the teratogenic action of vitamin A during a short period after which the retinoid has no effect on limb morphogenesis²⁻⁴. The teratogenic effect may be due to cytotoxicity but the questions still remain unanswered as to the exact nature of the state of differentiation of cells when they are sensitive or insensitive to the retinoids and the mechanism of action of these agents.

In the regenerating limbs also the kind of effect produced by the retinoids depends upon the stage of development of the blastema. In the anuran tadpoles (*Bufo* and *Rana* species) immersion of the tadpoles in 15 IU/ml retinol palmitate suspension during the phases of dedifferentiation and blastema formation results in the proximalisation of the developmental po-

tentialities of the blastema cells and regeneration of complete limbs contrary to the rule of the distal transformation of the blastema^{4,8,13}. If the tadpoles are given the retinoid after the blastema stage has reached regeneration is inhibited; the blastema does not redifferentiate or becomes necrotic^{4,17}. Detailed studies have been made on two urodele species (*Ambystoma mexicanum* and *Pleurodeles waltlii*) to investigate the morphogenetic effects of retinoic acid (RA) given at progressively advancing stages during the process of forelimb regeneration. In these experiments¹² RA (150 ug/g body weight) dissolved in DMSO (Dimethyl sulphoxide) was injected once intraperitoneally after amputation of the forelimbs through mid-zeugopodium at various stages of the regenerating limbs according to the staging system of Stocum¹⁸. The stages included dedifferentiation (DD), early bud (EB), mid-bud (MB), late bud (LB) and the beginning of redifferentiation (ER) of the blastema. It was observed that proximalisation of the blastema cells leading to serially duplicated stump structures differentiating in their regenerates occurred most readily and in good percentage of cases when RA was injected at DD, EB, and MB stages in this order. This effect was reduced or was absent when RA was administered at LB and ER stages; the regenerates showed progressively greater hypomorphism and malformation or there was no regeneration at all. Interestingly, while several of the regenerates of *P. waltlii*, in which RA was given at MB, MB⁺ and LB stages, contained some serially duplicated stump structures (partial or complete humerus and/or an extra radius-ulna) the distal parts (wrist, digits) were malformed or inhibited. The injection of RA at ER⁺ in *Pleurodeles* had no effect and the regenerates were normal as in controls. It seems that the retinoids cause some fundamental change in the blastema cells imparting to them greater morphogenetic potentialities than usual often equal to those of the original limb bud so that it can form the whole or nearly whole limb instead of only the distal, actually removed part. How is this change in the blastema cells brought about? Does it involve the reprogramming of the genome? Do the retinoids act on the cytoplasm, on the cell surface or on the extracellular matrix? Is this effect due to the influence on the cell cycle and mitotic activity? These questions are as yet unresolved. Whatever be the nature of the effect, it is clear that the retinoids produce it only if they are given when the stump tissues are liberating cells which are in the process of dedifferentiation and during the early phase of blastema cell accumulation. Beyond this stage the regeneration cells of the blastema are affected in the same manner as the cells of the developing limb in ontogenesis. The cases in which serially duplicated structures differentiated in the proximal part of the regenerate but the distal structures were malformed or inhibited indicate the gradual transition of regeneration cells from one kind of response to the retinoid to another kind in the course of development following amputational injury. It is obvious that the vitamin A derivatives influence the differentiating and dedifferentiating cells in radically opposite ways and the mechanism of their action may not be the same. The acquisition of greater morphogenetic potentialities of a specific nature by the blastema cells under the influence of retinoids cannot be due to cytotoxicity which could be considered to be a major factor in teratogenesis. The

two radically different types of responses can be elicited in the same individual. When the toad or frog tadpoles are treated with retinol palmitate for a short period after amputating the small hindlimb bud of one side and keeping that of the contralateral side intact, the amputated bud regenerates a good and complete limb but the intact bud of the other side does not develop further.

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