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BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON VITAMIN A INDUCED PROXIMALIZATION OF LIMB REGENERATION IN AXOLOTL.

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INTRODUCTION

In regenerating amphibian limbs only the structures normally distal to the level of amputation are regenerated. However vitamin A and its derivatives (retinoids) have been found to cause serial reduplications in the proximodistal axis in amphibian limb regeneration (Niazi and Saxena, 1978; Sharma 1982; Maden, 1982 ; Thoms and Stocum, 1984). We confirmed this dramatic effect further, and found that regenerating limbs of larval urodeles (Ambystoma mexicanum, Triturus alpestris, T. vulgaris, T. helveticus and Salamandra salamandra) and anuran tadpoles (Rana breviceps and R. temporaria) exposed to retinol palmitate consistently exhibited proximodistal duplication of limb segments after certain fundamental alterations in the growing blastema. This work briefly describes some biochemical and ultrastructural changes in the forearm blastema of axolotl growing under the influence of retinol palmitate.

EFFECT OF VITAMIN A

In 35 mm size axolotl 15 days treatment with 75 IU/ml vitamin A palmitate after forearm amputation resulted in proximalization of regeneration. Studies were made on DNA amount, cell cycle, proteins and ultrastructure of cells in the untreated and vitamin A treated blastemas on 10th or 15th day after amputation.

DNA Amount and Blastema Cell Cycle: Measurement of the DNA amount in nuclei (by Feulgen cytophotometry) show difference in the distribution of DNA classes in the mesenchyme of untreated and vitamin A treated blastemas (Fig.1). Vitamin A palmitate decreased 15 % mean DNA content and reduced the number of nuclei in the S phase from 37.4 to 17.7 % in the blastemal mesenchyme on 10th day. It seems as if some mesenchyme cells in the vitamin A treated blastema were arrested in the G1 phase being unable to enter into synthetic phase (S) of cell cycle.

Blastema Proteins: We found that vitamin A interferes during the early events of histodifferentiation. To study this effect of vitamin A on protein pattern, soluble proteins of blastema mesenchyme, epidermis and adjacent stump (without skin) tissues were separated on 15% SDS polyacrylamide gels on 15th day after amputation. The amount of proteins of 11.5, 16.5, 41.6 and 78 Kd molecular weight in blastema epidermis, mesenchyme and adjacent stump decreased after 15 days treatment. In the mesenchyme of treated blastema 12.4 and 13.9 Kd bands were faint and 12 and 38 Kd bands were absent (Fig.2). Similar effects of vitamin A palmitate on blastema proteins have been found in the larvae of Salamandra salamandra (Sharma and Anton, unpublished).

Ultrastructure of Blastema Cells: On 15th day, cells of untreated blastema represent prochondral condensation, the first visible sign of cartilage redifferentiation. These differentiating prochondral cells have oval and dense nuclei with very few nuclear pores in the nuclear envelope. In some cases the plasma membranes are closely aligned one against another, however, desmosomes or tight junctional structures have not been seen. The cytoplasm contains numerous mitochondria having dense matrix crowded with rough endoplasmic reticulum (rER). Free ribosomes are scattered in groups (polysomes) throughout the cytoplasm. The orientation of cisternae profile of rER is usually parallel to the nuclear envelope. Extracellular matrix constitutes condensed ground substance and occasionally oriented collagen fibrils (Fig.3). The cell organelles of vitamin A treated blastemas on 15th day differ in several respects from those of untreated blastemas. The nuclear membrane is porus and the cytoplasm contains numerous free ribosomes and comparatively less rER. Flattend Golgi vesicles which are a rather constant feature of differentiated cells