

## Effect of Dye-effluent on the Amino Acids of the Tadpoles of *Rana Hexadactyla*

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### Abstract

Metamorphosing tadpoles of *Rana hexadactyla* were exposed to sublethal concentration of dye effluent for different periods. The prometamorphic tadpoles registered elevation in the levels of FAA and depletion of BAA. But the tadpoles in metamorphic climax stage showed abnormal increase in BAA, while the post metamorphic individuals exhibited rise in BAA and fall in the FAA content.

**Keywords:** Dye-effluent; Metamorphosing; Essential Amino acid; Tadpoles.

## INTRODUCTION

The widespread use of chemicals in agriculture forestry, hydroponics and soil melioration to augment production has inevitably resulted in the pollution of water bodies (Metelev et al, 1983).<sup>6</sup> Moreover the industrial effluents are indiscriminately discharged into lentic and lotic systems.

Amphibians are the components of food webs in both aquatic and terrestrial communities (Porter and Mohanson 1976).<sup>7</sup> Metamorphosis of frog takes place in fresh water bodies which in Madurai city,

Tamil Nadu are fed up by the polluted Vaigai River. Exposure to polluted water results in accumulation by absorption through gills and skin in the case of amphibian tadpoles (Light, 1985)<sup>4</sup> thereby causing decline in frog and toad population across their range as suggested by Gibbs et al (1971).<sup>2</sup>

Regarding amphibians, studies on nitrogen metabolism in tadpoles during development alone were focused by Brown (1964)<sup>1</sup> and Weber (1967).<sup>8</sup> The effect of pollutant on amino acid metabolism in metamorphosing tadpole is totally neglected.

The present investigation is to study the impact of toxicity of sublethal concentration of dye effluent (0.009 ppm) on the levels of free amino acids (FAA) and bound amino acids (BAA) in metamorphosing tadpoles of *Rana hexadactyla*.

## MATERIALS AND METHODS

Tadpoles (10 days old) of premetamorphic stage were collected from Narayanapuram Tank, Madurai transported quickly to the laboratory and kept in cement water tanks filled with tap water

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and provided with aeration for 10 days prior to experimentation. During the period acclimatization these tadpoles were fed with fresh leaves of Hydrilla and Nelumbo. Subsequently the water was renewed and excreta as well as unfed were carefully removed daily.

### Dye effluent

Dye effluent was chosen for the present investigation. The concentrated dye effluent was obtained from a reputed dye factory situated by the side of River Vaigai running through Madurai City. The names of chemicals and dyes which are found in the dye effluent are specified.

By appropriate studies it was found that 0.009 ppm was the sublethal concentration at which no mortality occurred for 45 days

#### *Preparation of Experimental tissue*

Tadpoles of different stages after being harvested were killed and kept in oven maintained at 50°C + 2°C for 7 days. The dried materials were then thoroughly homogenized into powder and used later for amino acid analysis.

## **RESULTS AND DISCUSSION**

### Levels of FAA in healthy tadpole during development

In premetamorphic tadpole (40 days old) reared in untreated tap water at laboratory temperature for 20 days. The contents of FAA and BAA were found to be 16 mg and 13 mg respectively. In both pools all amino acids were found. In FAA pool,

arginine, lysine, glutamate and tyrosine were higher in concentration where as in BAA pool, arginine, histidine, glycine, hydroxyproline and proline were more. The total AA content was 29 mg (Fig. 1)

In premetamorphic tadpole (55 days old) of control group the content of FAA and BAA were found to be 13 mg and 14 mg respectively with an accumulation of 32 mg of total AA when compared to the level in premetamorphic tadpole, reduction in the concentration of FAA from 16 mg to 13 mg accumulation in the content of BAA from 13 mg to 19 mg ; depletion in the concentration of almost all amino acids except isoleucine, leucine, glycine and hydroxyproline in FAA pool and conversely higher concentration of almost all amino acids except histidine, glutamate, glycine and proline in BAA pool were observed in the healthy prometamorphic tadpoles.

According to Brown (1964)<sup>1</sup> new proteins are formed in the course of development and differentiation to yield specialized structural entities and special proteins including enzymes. Since the total AA level is enhanced from 29 mg in premetamorphic stage to 32 mg in premetamorphic stage and depletion in FAA level and elevation in BAA level than in prometamorphic stage it is assumed that protein synthesis seemed to be slowly promoted causing diversion of FAA to BAA.

In the normal tadpoles of metamorphic climax stage, the FAA and BAA and total AA contents were found to be 13 mg, 16 mg and 29 mg respectively. Eventhough the FAA level was retained the BAA level was slightly depressed from 19 mg to 16 mg when compared to previous stage. That depression was mainly due to the lower concentration of

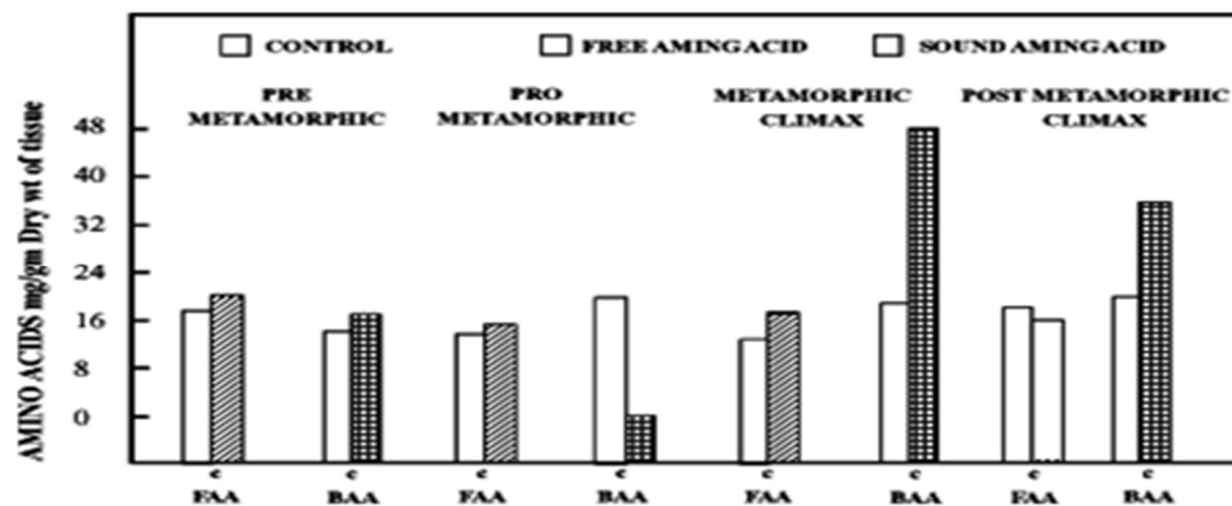


Fig. 1: Variations of the amino acid in healthy tadpoles during development

isoleucine, leucine, lysine, methionine, theonine, tryptophane, cysteine and tyrosine.

This is the period in which active regression and transformation are predominately taking places. Weber (1967)<sup>8</sup> suggested that protein is degraded during metamorphosis. The lower level of BAA without increase in the level of FAA is suggestive of active proteolysis as suggested in BAA pool it is assumed that these amino acids may be routed to glucogenic path way via pyrurate or to kreb's cycle via intermediaries of kreb's cycle to generate energy.

In the post metamorphic stage, the newly emerged frog had registered higher concentration of FAA (16 mg) and lower content of BAA (11 mg) with a total AA of 27 mg when compared to previous stage. The FAA level was slightly enhanced because of higher concentration of almost all AA except valine, proline and serine with a reduction in BAA level which was due to depression in the concentration of almost all AA except arginine, leucine. Methionine, tryptophane, cysteine, hydroxyproline and serine.

According to Brown (1964)<sup>1</sup> coupled with proteolysis, the amino acids of the newly created

pool may now be converted to postmetamorphic type of protein including new enzymes. Since BAA level is further depleted from 16 mg to 11 mg with a slight elevation in the level of FAA from 13 mg to 16 mg. It is suggested that protecolysis seemed to be continued even at this stage perhaps for the generation of amino acids which may be required for the synthesis of post metamorphic enzymes, as suggested by Brown (1964).<sup>1</sup>

**Effect of Dye effluent of metamorphosing tadpole**

When the premetamorphic tadpole (40 days old) was exposed to sublethal concentration of dye effluent for a period of 20 days. The toxic stress resulted in significant elevation in both FAA and BAA levels by 11% and 14% respectively (Fig. 2). Increase in the levels of FAA and BAA was effected by higher contents of EFAA (+13%) and NEFAA (+10%) in FAA pool and EBFAA (+16%) and NEBAA (+ 14%) in BAA pool. When compared to control the experimental tadpole had registred higher concentration of almost all amino acids in both pools except arginine, histidine, methionine, cysteine, glutamate and tyrosine in FAA pool and arginine, valine, glycine and proline in BAA pool.

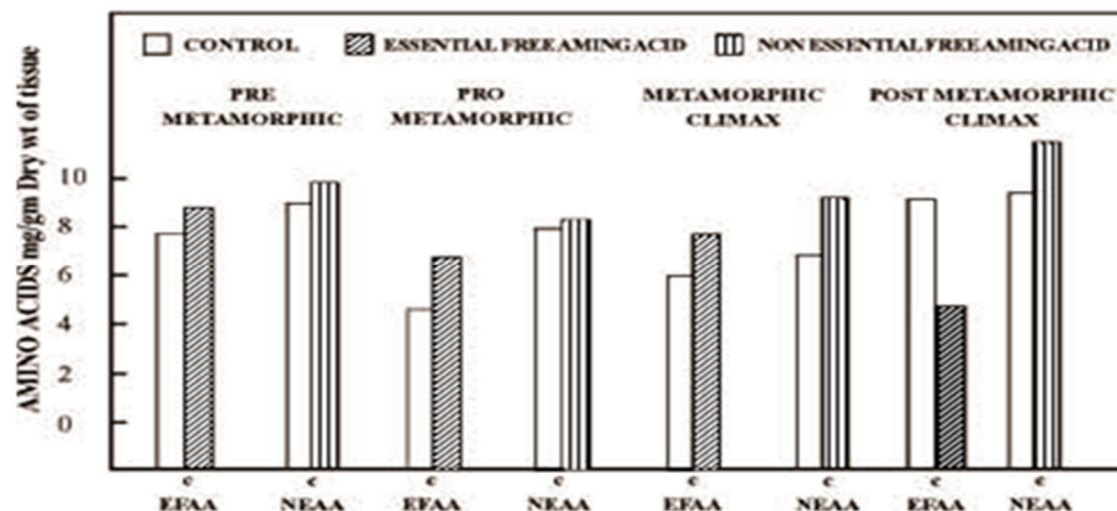


Fig. 2: Flucations of the amino acid in tadpoles exposed to sublethal concentration of the dye effluent

**CONCLUSION**

Subba Rao and Seyed Quadrin (1984)<sup>7</sup> observed that toxicity of toxicant can deplete the levels of protein and amino acids and enhance the activities of proteolytic enzymes. Similarly Madhusudhana Rao and Chari (1984)<sup>5</sup> reported that the toxicity of sevin

in Lamellidens causes decrease in total protein and FAA. Harris (1969)<sup>3</sup> also observed reduction in total FAA concentration in Isopoda due to exposure to 2% salinity. Exposure to pollutant elevates the basic metabolic rate to meet the energy requirements due to physiological stress, detoxification and tissue repair. The depression in the level of BAA due to toxicity is suggestive of activated proteolysis which can bring forth accumulation of FAA. Since this

degradation of BAA is not seemed to be inversely related to increase in FAA only it is assumed that most of the AA in the FAA pool may be diverted to metabolic pathways whether to generate non protein metabolites via pyruvate to Carbohydrate or Acetyl - COA to lipid or to generate energy via TCA cycle.

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