



## DEVELOPMENT AND DIFFERENTIATION OF KERATINIZED ORAL ARMATURE UNDER THE INFLUENCE OF VITAMIN A IN *Bufo melanostictus* STAGE 25 AND 30

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### Abstract

The effect of vitamin A on development and differentiation of oral armature with particular reference of *Bufo melanostictus* (schneider) tadpoles of stage 25 and 30 of this toad species. Stage 25 and 30 untreated group tadpole's shows well developed horny jaw and horny denticles. Treatment of Vitamin A to the tadpoles stage 25 and 30 cause's gradual degeneration in both horny teeth and jaws. Fifteen days continuous treatment causes complete degeneration of horny jaws and horny teeth. Tadpoles of discontinuous treated group shows reappearance of horny jaws and horny teeth and shows well recovery from the degenerating effect of vitamin A. It has been found to affect differentiation of oral armature in the toad tadpoles and the effect is more severe on younger tadpoles as compared to the older ones.

**Keywords:** Horny teeth, oral armature, vitamin A, denticles, Beak, Upper lip & lower lip Horny jaw.

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### Introduction

Vertebrates have skin derivatives such as epidermal derivatives, which are formed by the epidermis and comprises (1) Epidermal glands and (2) Hard horny structures including epidermal scales, sautés, beaks, horns claws, nails and hoofs, feathers and hair etc. and dermal derivatives which arise from dermis and comprised bony or dermal scales, plates or scutes, fin rays and antlers etc. All the hard horny structures together form the exoskeleton of an animal.

In amphibians, skin itself is relatively simple but its derivatives are numerous and complex. In Anuran amphibians generally mouth is surrounded by keratinized oral armature which helps in feeding at larval stages, the keratinized oral armatures are the epidermal derivatives. In Anuran larvae the mouth is provided with a keratinous beak supported by labial cartilages and various configurations of rows of keratinous denticles and labial papillae on the fleshy area of the mouth circumdistal to the beak. The larvae use their jaws and these keratinous structures to chop food into sizes that can pass through the small gaps of the mouth and to scrap or rasp food from surfaces. The mouth also acts as a value in a buccal-pump system in which water flows into the buccal cavity via the oral aperture. Simultaneously, food particles are trapped and gases exchanged from this flow of water.

In Anurans, the mouth usually is bordered laterally and ventrally by one or two rows of small papillae. The upper lip bears two or three rows of keratinized denticles, and there are three of four rows on the lower lip. The jaw bear keratinized beaks with fine serrations. The number of rows of horny denticles, found on the upper and lower lip has been used in

taxonomic identification of many anuran species (Dutta and Hejmadi, 1984; Duellman and Trueb, 1986)

In general, the horny beaks are visible for the first time when the external gills are well developed. The first traces of labial teeth appear at the stage when operculum is closed on one side. By the time the tadpoles reach metamorphic climax there is shedding of labial teeth. The distribution pattern of rows of horny teeth also helps in identification of Anurans in larval stages. In *Bufo melanostictus* position of mouth is sub terminal. The upper lip has got one incomplete rows of denticles and the lower lip has got three complete rows of denticles. Detailed structure and steps of differentiation of horny teeth in this road species is not yet known.

The epidermis of frog and toad tadpoles is generally mucold but in the mouth region it produces keratinized structures represented by rows of horny teeth of the labial fringe and black ridges over the larval jaws. The role of vitamin A in differentiation and maintenance of epithelia as a mucoid or keratinizing type is well known in mammals. To find whether vitamin A would affect the oral armature of Anurans at once or gradually and the effect is reversible or not. Sharma and Anton, (1984) carried out studies in the effect of vitamin A patmitate on the keratinized oral armature of young tadpoles of *Ranatemporaria*. Tadpoles of *Ranatemporaria* at stage XIXI (Taylor and Kollros, 1946) were reared in 30IU/ml solution of vitamin A palmitate for 1,2,3,4,5,6,7,8,9 or all 13 days.

In view of the above it is proposed to undertake studies on the effects of vitamin A on differentiation of skin of load tadpoles with the following objectives. Studies on the effect of vitamin A on development of epidermal derivatives such as keratinized oral armature on stage 25, 30

## General Material & Methods

The present studies were carried out on young and advance tadpoles of the common Indian toad, *Bufo melanostictus* Schneider (Bufonidae. Anura, Amphibia). This toad is found in abundance in and around Jaipur and Ajmer, It hibernates during winter and in other seasons it remains hidden during the day. From March onwards it comes out at dusk and can be collected easily during the nights. This toad species, like other many Anurans, breeds during monsoon. The spawning takes place shallow pool and ponds where the eggs are found in long double strings on the surface of water or entangled in between water plants. Generally these animals lay eggs in the early hours of the morning after a rain following a warm day. In laboratory conditions (29-32°C) hatching takes place in less than 24 hours after spawning and the larval period lasts for about four weeks from hatching to the end of metamorphosis.

The spawn collected from the field hatched in the laboratory aquaria. The tadpoles were maximally fed with semi-boiled spinach every day. The young tadpoles were distributed in several tanks and plastic troughs to avoid overcrowding. The water of aquaria and troughs was also changed every day to avoid pollution. The tadpoles grew well in such conditions and there was negligible mortality.

All experiments were carried out on young tadpoles of stages 25 and 30 (this toad species). The stagnation was done according to the normal table of development of *Bufo melanostictus* (Khan, 1965))

Following is the brief description of the various developmental stages of tadpoles used in the present study (After Khan, 1965).

### 1. Stage 25 (Spiracle stage or Hind limb bud stage)

At this stage of development the opercular folds are fused completely with the body wall on the right side, but on the left side the fusion is incomplete so that a spiracle is formed through which the gill filaments protrude out. The spiracle is situated on ventrolateral side of the body in the region where the first outer intestinal coil turns inwards to the mid ventral line. The preoral fleshy lip has an outer complete row of denticles and a second interrupted row. The post oral lip has got three complete rows of denticles. Mouth occupies subterminal position. The beak is well formed. Small hindlimb buds make their appearance at the junction of the belly and the tail on the dorsolateral side of the anal tube. The intestinal spiral is fully formed at this stage. Melanophores have developed in large numbers on the dorsal and lateral sides of the body.

### 2. Stage 30 (Length of Hindlimb 2½ times its breadth).

The general morphology of the body is well established at this stage. The hindlimb bud is 2½ times longer than it is broad. It is round near its base (stump) but its greater part is flattened from side to side (paddle). The free end is no longer round but somewhat narrow and conical and foreshadows the 4th toe. The constriction between paddle and stump marks the position of the ankle.

**The studies consisted of two main lines of research:**

1. Studies on differentiation of skin and keratinized oral armature of developing tadpoles under normal conditions and after treatment of tadpoles with vitamin A.
2. To study the effect of vitamin A on thyroid development during attention of skin differentiation.

### Experimental design

Tadpoles at each developmental stage were divided into three experimental groups:

- **Group A** Tadpoles of group A were reared in ordinary water throughout the period of experiment (control group).
- **Group B** Tadpoles of this experimental group were reared in vitamin A palmitate (1 IU/ml-sigma).
- **Group C** Tadpoles of group C were treated with vitamin A palmitate 1 IU/ml (sigma) for three days and then transferred to tap water for the remaining twelve days.

### Schedule of Fixation

Tadpoles of different experimental groups were fixed at 1 day, 2 day, 3 day, 4 day, 5 day, 6 day, and 15 day following treatment.

### Parameters of study

1. Temporal and spatial pattern of differentiation of skin and glands. Tadpoles fixed at different close intervals were sectioned serially and stained for visualization of various components of skin particularly the basement membrane and skin glands. The serial sections stained with modified Azan (Domagk, 1948) were also used for histo-chemical localization of mucin, collagen fibers etc.
2. Studies on keratinized oral armature under the influence of vitamin A, to find if vitamin A causes any degeneration of these structure and any possible recovery, once the treatment is withdrawn. morphological and histological observations were made on development of keratinized oral armature at selected stages of development.
3. Studies on development of thyroid glands of untreated and vitamin A treated tadpoles through serial sections to find the possible involvement of thyroid gland in differentiation of skin. For morphological studies, the tadpoles were examined under stereoscopic binocular microscope. They were sketched with the help of camera Lucida and representative cases were photographed.

For histological examination, the tadpoles were processed through the steps of dehydration and clearing and then embedded in paraffin wax.

The tadpole was sectioned transversely and serially at 6µ thickness and then stained with aniline blue and orange G according to the modified Azan staining technique (Domagk, 1948). The steps in sequence for this technique are given below:

- |                     |            |
|---------------------|------------|
| 1. Xylene           | 15 minutes |
| 2. Xylene           | 15 minutes |
| 3. Absolute Alcohol | 10 minutes |
| 4. 90% Alcohol      | 10 minutes |
| 5. 70% Alcohol      | 10 minutes |
| 6. 50% Alcohol      | 10 minutes |
| 7. Distilled water  | 10 minutes |
| 8. Nuclear fast red | 30 minutes |

9. Distilled water	Wash for 3-4 minutes
10. Phosphomolybdic Acid	1 minute
11. Distilled water	Wash for 2 minutes
12. Azan	5 minutes
13. Distilled water	Wash for ½ minutes
14. Differentiate in 90% alcohol	Few dips
15. Absolne alcohol	15 minutes
16. Xylene	15 minutes
17. Xylene	15 minutes
18. Mount in D.P.X using No.0 or No.1 cover glass	

Working solution of Nuclear fast red, Azan (Aniline blue, Orange G and Oxalic acid) and Phosphomolybdic acid are prepared as follows:

### Observation and Results

**Group A:** Untreated control; **Group B:** Treated continuously with vitamin A; **Group C:** Treated with vitamin A for three days and then transferred to water for the remaining twelve days of the experiment.

#### Stage 25

##### Untreated (control)

##### Group A

Morphological picture of tadpoles of stage 25 shows on complete outer row of horny denticles and a second interrupted (incomplete) row of denticles on the pre oral (upper lip) fleshy lip. The postoral lip (lower lip) has got three complete rows of horny denticles. The denticles are dark brown in colour. The dental formula of *Bufo melanostictus* is 1.1+1/3. These characteristics of horny denticles are similar to the description made by Khan (1965). The horny jaws represent as a straight line in the preoral region and V shape structure in the postoral region. The histological picture through the oral region shows pre and post oral cartilage showing horny jaws and upper and lower lip region having horny denticles.

The development of horny jaw has been shown in figure. The epidermis overlying the pre orbital cartilage showing gradual keratinization in the distal direction. Epidermal cells are transformed into keratinized type.

The structure of horny teeth is shown. Epidermal cells present in the upper and lower lip region at the prospective site of the teeth. Transforms into horny tooth. Every tooth is having a base, a neck and a broad flattened shaft. The margins of shaft are serrated.

##### Vitamin A Treatment (Continuous)

##### Group B

Treatment of vitamin A to the tadpoles of stage 25 causes gradual degeneration in both horny teeth and jaws. In the tadpoles treated with vitamin A for one day teeth are not as dense as are found in the untreated cases. Jaws are not affected as a result of one day treatment. Morphology of oral armature indicates beginning of disappearance of teeth from both the terminal ends of the rows. Three days of vitamin A treatment causes disappearance of teeth both in the upper and lower lips. Upper horny jaw although reduced but still shows its existence in the three day treated cases but lower horny jaw is lacking keratinization. Degeneration of teeth is continued both in the upper and lower lips. Histological organization of oral armature also supports these facts. Five days treatment of vitamin A causes almost complete

degeneration of interrupted 2<sup>nd</sup> row of horny teeth in the upper lip and inner most two rows of teeth in the lower lip. Both in the upper jaw and lower jaw keratinization has disappeared at the surface as a result of vitamin A treatment. Thus, cartilaginous core of jaw is present but its horny surface has degenerated. One such jaw without keratinization is shown in figure which cartilage supporting the jaw, epidermis is present but keratinization is absent in the epidermal cell present in the surface.

Fifteen days continuous treatment causes complete degeneration of horny jaws and rows of horny teeth.

##### Vitamin A Treatment (Discontinuous)

##### Group C

Those tadpoles which were transferred to water after three days of initial treatment shows gradual recovery related to reappearance of horny teeth and jaws. Those tadpoles which were reared in Vitamin A for three days and then reared in water for two days shows appearance of horny teeth in the upper lip region. In these cases horny jaws have not yet regenerated.

Three days rearing in water after initial three days of vitamin A treatment caused recovery in the horny denticles particularly in the upper lip region. Whereas the interrupted inner row of teeth has formed only on one side. Histological picture of the oral region shows horny denticles at the surface of lip epidermis.

Those tadpoles which were reared in water for twelve days after initial three days of vitamin A treatment shows reappearance of horny teeth and jaws. The respective epidermal cells of the lip region and jaw region begin keratinization. This is also evidenced by beginning of feeding by these tadpoles because as a result of disappearance of horny teeth and jaws where feeding on vegetation ceases.

#### Stage 30

##### Untreated (Control)

##### Group A

The structure of oral armature appears similar to the organization of stage 25 tadpoles. Horny teeth and jaws are well developed. Figure shows progressive development and differentiation of horny denticles from the epidermal cells of the concerning region.

The epidermal cells become elongated and their distal end shows extensive secretory activities. The denticles have a base, a neck and broad flattened shaft having serrations.

##### Vitamin A Treatment (Continuous)

##### Group B

One day treatment with 1 IU/ml vitamin A does not affect horny denticles and jaws. Treatment of tadpoles for three days causes degeneration of horny denticles as well as horny jaws. The degenerative effects of vitamin A on keratinized oral armature are more severe at stage 25 as compared to the stage 30.

Five days treatment of vitamin A causes almost complete disappearance of horny denticles in the lower lip region but these denticles are still present in the upper lip region.

Fifteen days treatment of vitamin A causes complete disappearance of horny jaws and teeth and tadpoles stop feeding due to these degenerative changes.

### Vitamin A Treatment (Discontinuous)

#### Group C

Those tadpoles which were transferred to water after initial three days of vitamin A treatment show scarce distribution of denticles both on the upper as well as on the lower lip. It is pertinent to mention here that even after withdrawal of tadpoles from vitamin A solution after initial three days of treatment effect of vitamin A continues as observed on 2<sup>nd</sup> days. The density of denticles and keratinization of jaws is poor in the tadpoles of group C on 5<sup>th</sup> days as compared to the tadpoles of group B on 3<sup>rd</sup> days. However, in those tadpoles which were reared in water for three days after three days of vitamin A treatment horny denticles starts their reappearance particularly in the upper lip region. In these tadpoles also regeneration of keratinization of jaws has not yet occurred. In the tadpoles of group C on 15<sup>th</sup> day there is good recovery from the effect of vitamin A on keratinized oral armature.

### Discussion

The epidermal derivatives present in and around the mouth are keratinized denticles and jaws. These structures represent adaptive features for aquatic mode of life and also demonstrate single cell transformation during morphogenesis. There are two rows of teeth present at the pre-oral fleshy lip and three rows are present on the post-oral fleshy lip. These rows of denticles also serve as a distinguishing feature for identification of Campbell (1931). Taylor (1942), Bragg and Bragg, (1959), Altig (1970), Dutta and Mohanty Hejmadi (1976), Agarwal and Niazi (1977), Altig and Pace (1974), and Lee (1976) have described the tooth structures of frogs from temperate climate. The epidermal cells present at the prospective sites of teeth, rows undergo differentiation and as a result of definite pattern of keratinization. The epidermal cells are transformed into a tooth structure having a base, neck and a serrated shaft. The horny jaws represent keratinization of epidermal surface of pre-orbital cartilage. There is gradual keratinization of epidermal cells from the base to the surface to form the horny jaws.

The role of vitamin A in differentiation and maintenance of epithelia as a mucoid or keratinizing type is well known in mammals (Reviews Fell and Rindini, 1965; Johnson and Scadding, 1991). Scadding (1989) found that immersion of Axolotls in vitamin A palmitate produced a number of changes in epidermis including the epidermal surface becoming irregular, mucoid and development of cilia in some of the cells. *Xenopus* tadpoles fed with vitamin A showed sloughing of epidermis. However, Koussoulakos *et al.* (1990) have found reversal of keratinization in the cells of outer layer of epidermis in the adult newts *Triurusalpestris* treated with vitamin A orally. Sharma and Anton, (1984) reported gradual degeneration of keratinized oral armature of tadpoles of *Rana temporaria* treated with vitamin A for varying periods. In this study 30 IU per ml dose of vitamin A (Roche) does not cause degenerating effect of the horny teeth during the first two days, and after 13 days treatment all horny teeth and most of the keratinized epidermal ridges over the larval jaws had disappeared in all treated tadpoles. Those

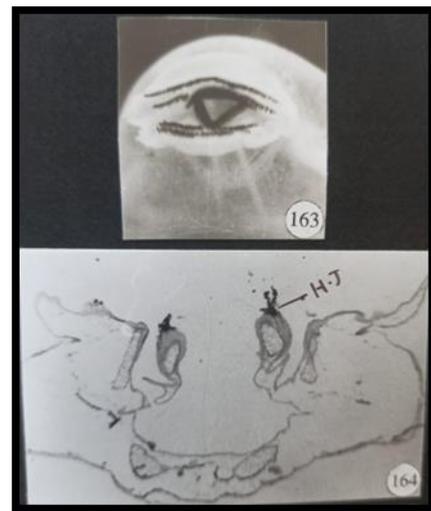
tadpoles which were transferred to water after eleven days showed beginning of recovery of keratinized oral armature from 26<sup>th</sup> day onward.

The present study confirms this and similar other observations on the effect of vitamin A on degeneration of keratinized epidermal derivatives and reversal of effects once treatment is withdrawn.

Another interesting feature observed in the present study on degeneration of epidermal derivatives of oral region was differential effect of vitamin A in degenerating teeth and jaws. Horny denticles and related epidermal cells are more sensitive to vitamin A than the epidermis covering the jaws. Further young tadpoles show more sensitivity to vitamin A than the older tadpoles. Stage dependent and tissue specific effects of vitamin A have been observed in other developing systems of Anurans also (Sharma, 1989).

### STAGE 25

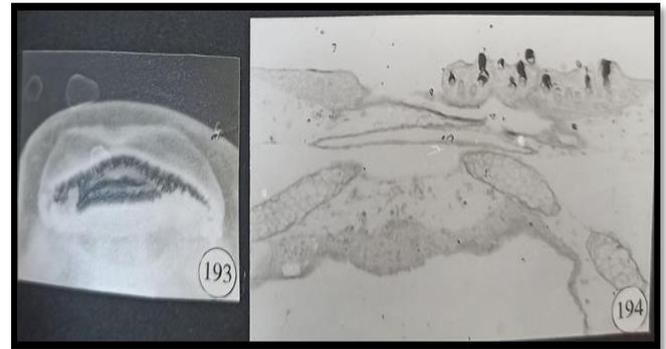
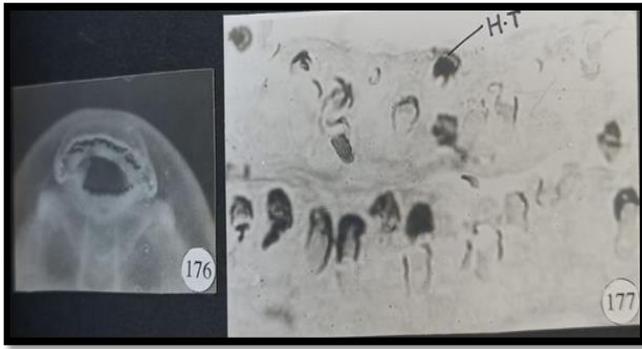
1. Morphology and histology of keratinized oral armature of untreated (Control) tadpoles of stage 25.



2. Morphology and histology of keratinized oral armature of Vitamin A treatment (Continuous) tadpoles of stage 25.

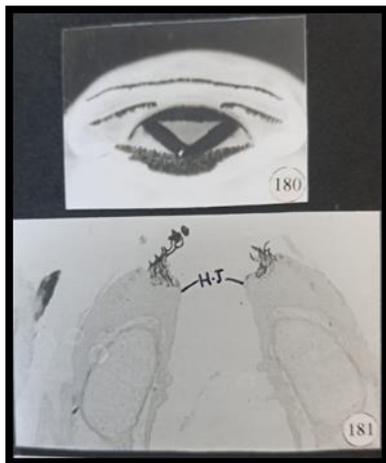


3. Morphology and histology of keratinized oral armature of Vitamin A treatment (Discontinuous) tadpoles of stage 25.

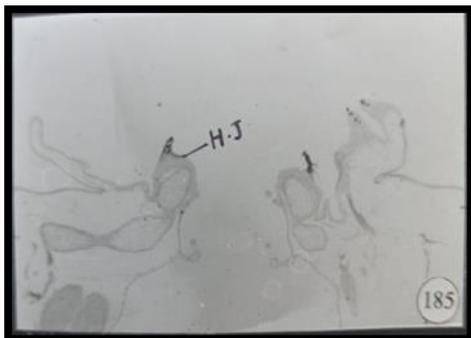


### STAGE 30

#### 1. Morphology and histology of keratinized oral armature of untreated (Control) tadpoles of stage 30.



#### 2. Morphology and histology of keratinized oral armature of Vitamin A treatment (Continuous) tadpoles of stage 30.



#### 3. Morphology and histology of keratinized oral armature of Vitamin A treatment (Discontinuous) tadpoles of stage 30.

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