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DEVELOPMENT AND DIFFERENTIATION OF KERATINIZED ORAL ARMATURE UNDER THE INFLUENCE OF VITAMIN A IN *Bufo melanostictus* STAGE 34 AND 36

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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 34 and 36 of this toad species. Vitamin A 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. In stage 34 and 36 untreated group shows well developed keratinized oral armature. Vitamin A treatment has been found to inhibit differentiation of oral armature and degeneration of horny denticles. Tadpoles of discontinuous treated group shows well recovery from the degenerating effect of vitamin A. Horny teeth and jaws are visible in mouth region of these tadpoles.
Keywords :- Horny teeth, oral armature, vitamin A, denticles, Horny jaw

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Introduction

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)

The labial teeth are some of the most distinguishing features for identification of amphibian larvae (Campbell, 1931; Taylor 1942; Wright and Wight, 1949; Stebbins, 1951 and 1954; Orton, 1952; Zweifel, 1955 and 1964; Bragg and Bragg, 1959; Starret 1960; Altig, 1970; Altig and pace, 1974; Lee, 1976; Dutta and Hejmadi, 1984; Duellman and trueb, 1986; and Agarwal and Niazi, 1977). These authors have reported on the teeth structure of the tadpoles of different species of frogs from temperate climate Orton (1953 and 1957) had proposed a classification of frog families based upon the keratinized mouth parts. A detailed report on the internal oral features of larvae from eight Anuran families of U.S.A. has been described by Wassersug, (1980), In a study on ontogeny he (Wassersug, 1976) has also described the internal oral features of Hylaregilla, Web and Korky, (1977) while studying the variation in the tadpoles of frogs of Rana tarahumarae group in Western Mexico have described the teeth row formula of that species and have given a formula for teeth structure.

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 34, 36.

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 stagination 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 34 (1st Toe Indentation stage)

On the ventral side of the paddle like food appears another indentation marking the position of the first toe, below the indentation for the second toe. The various digits (2-5) are better demarcated and separated from each other. The three parts of the leg, thigh or stylopodium, shank or zeugopodium and the foot of the autopodium are clearly demarcated. The area between the second and third toes has also been invaded by melanophores.

2. Stage 36 (Full Anal Tube Stage)

The anal tube has extended up to the ankle. The tail piece is fully developed. The palmar region of the foot is closely applied to the ventral fin on lateral sides of it. Eyes are almost black. The forelimb rudiments can be seen through the transparent body wall on the ventral side. Melanophores can be seen on the dorsal side of all the five toes and the anterior side of the limb. Melanophores can be seen aggregated in a circle round the outer border of the intestinal loop. Small groups of melanophores are present on the dorsal fin also.

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 1U/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 Azanstaining 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 than transferred to water for the remaining twelve days of the experiment.

Stage 34

Untreated (Control)

Group A

The tadpoles of stage 34 shows well developed keratinization oral armature. Mouth which is slightly protruding at stage 25 and 30 is now present in a triangular depression surrounded by lips. Horny teeth are present in two rows on the upper lip and in three rows on the lower lip, similar to the organization found in stage25 and 30 tadpoles. Structure of horny denticles and jaw has been shown in figure respectively.

Vitamin A Treatment (Continuous)

Group B

There is not much influence of one day treatment of vitamin A on horny teeth and jaws. Three days of vitamin A treatment causes degeneration of horny denticles both in upper lip and lower lip. The horny layer of jaws does not show signs of much degeneration in these tadpoles.

In the vitamin A treated tadpoles on 5^{th} days denticles are scanty both in the upper and lower lips and horny layer present on jaws has disappeared both on the upper beak as well as on the lower beak.

Continuous treatment of vitamin A to the tadpoles for fifteen days causes complete degeneration of horny denticles and jaws similar to the tadpoles of stage 25 and 30.

Vitamin A Treatment (Discontinuous)

Group C

Discontinuous treatment of vitamin A results in gradual regeneration of horny teeth and jaws. In these, tadpoles of group C two days rearing in water after initial three days of vitamin A treatment does not show much recovery rather there is further degeneration of horny of horny jaw due to persistent effect of vitamin A. There is good recovery in the horny jaws and teeth after three days of rearing of tadpoles in water after initial three days of vitamin A treatment, in these tadpoles horny denticles are present in good density both in the upper lip as well as in the lower lip region. Histological picture of mouth region shows again beginning of keratinization in the epidermal cells of the jaws.

Twelve days rearing of tadpoles in water after initial three days of vitamin A treatment causes well recovery from the degenerating effect of vitamin A in the tadpoles. Histological picture through the lip region shows reappearance of horny denticles in the lower lip region.

Stage 36

Untreated (Control)

Group A

The tadpoles of stage 36 show developmental changes towards metamorphosis. This phenomenon is also reflected in the mouth region as shown by natural degeneration of horny denticles. The depression of mouth along with over hanging fleshy lips indicates advanced features of metamorphic changes of the mouth region. The density of horny denticles has reduced both in the upper lip as well as in the lower lip region.

Vitamin A Treatment (Continuous)

Group B

Effect of 1 to 3 days treatment of vitamin A can be observed both on degeneration of horny denticles as well as keratinized jaw but five days treatment of vitamin A causes significant degeneration of horny denticles in the lip region. Horny jaws are still present in these tadpoles. This indicates differential effects of vitamin A on degeneration of horny denticles and horny jaws.

Continuous treatment of vitamin A for fifteen days causes degeneration of horny teeth and denticles in the preoral and suboral region of mouth as usual. Such type of effect of continuous treatment of vitamin A has been demonstrated in the tadpoles of earlier stage of development (stages 25,30and 34). The epidermis of jaw region stops keratinization and dermal denticles also have degenerated in these cases.

Vitamin A Treatment (Discontinues)

Group C

Similar to the observations of the effect of vitamin A on younger stage effect of vitamin A persist even after withdrawal from vitamin A solution and rearing in ordinary water for 2-3 days.

When compared with the tadpoles of three days continuous treatment the discontinuously treated group shows further degeneration of horny jaws and denticles. In the internal organization of these tadpoles horny denticles are present but keratinization of jaws has undergone degeneration.

Those tadpoles which were reared in water for twelve days after three days of vitamin A treatment show noticeable signs of recovery from the degenerating effects of vitamin A. Horny teeth and horny jaw are visible in the mouth region of these tadpoles.

Discussion

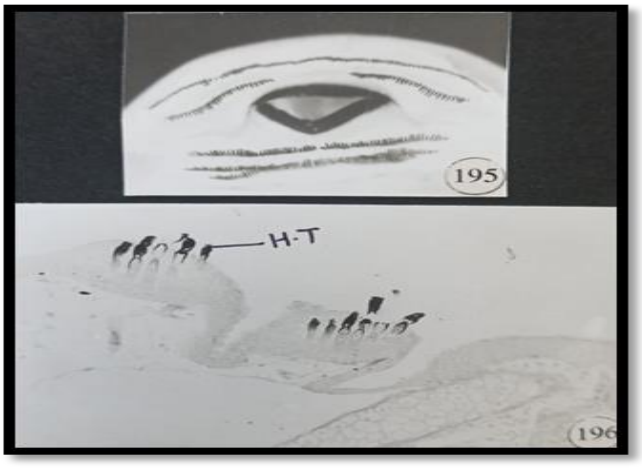
The epidermal derivatives present in an around the mouth are keratinized denticles and jaws. These structures represents adaptive features for aquatic mode of life and also demonstrates single cell transformation during morphogenesis. There are two rows of teeth present at the preoral fleshy lip and three rows are present on the postoral fleshy lip. These rows of denticles also serve as distinguishing feature for identification of camphell (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 tooth, rows undergo differentiation and as a result of definite pattern of keratinization. The epidermal cells is transformed into a tooth structure having a base, neck and a serrated shaft. The horny jaws represent keratinization of epidermal surface of proorbital cartilage. There is gradual keratinization of epidermal cells from the base to the surface to form the horny jaws.

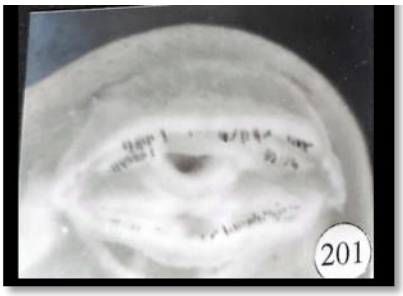
The role of vitamin A in differentiation and maintenance of epithelia as a mucoid or keratinizing type is well known in mammals (Reviews Fell And Rinldini, 1965; Johnson and Scadding, 1991) Scadding (1989) found that immersion of Axolotls in vitamin A palmitate produced a number of changes in epidermis 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 degenaraton 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 26th day onward. The present study confirms this and similar other observation 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).

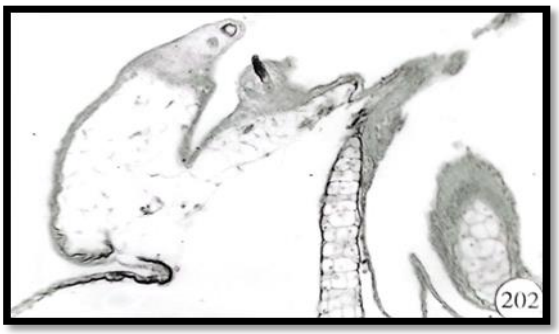
STAGE 34

1. Morphology and histology of keratinized oral armature of untreated (Control) tadpoles of stage 34.

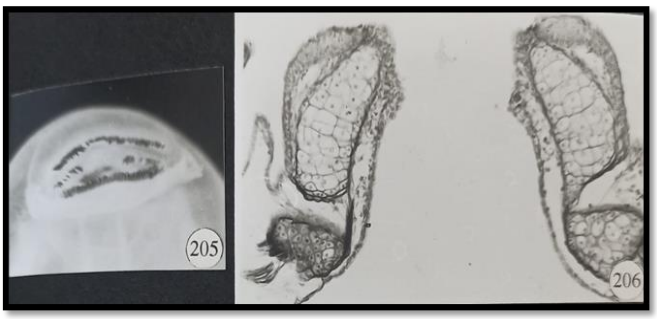


2. Morphology and histology of keratinized oral armature of Vitamin A treatment (Continuous) tadpoles of stage 34.



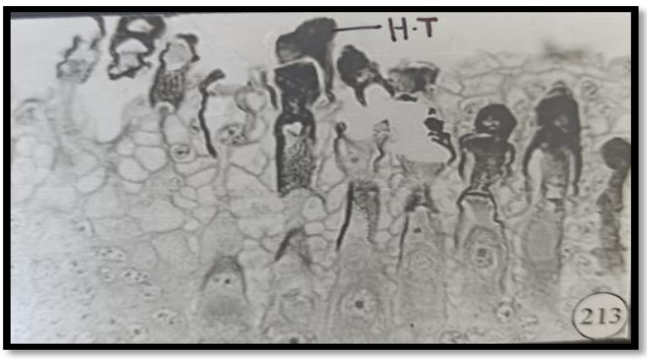


3. Morphology and histology of keratinized oral armature of Vitamin A treatment (Discontinuous) tadpoles of stage 34.

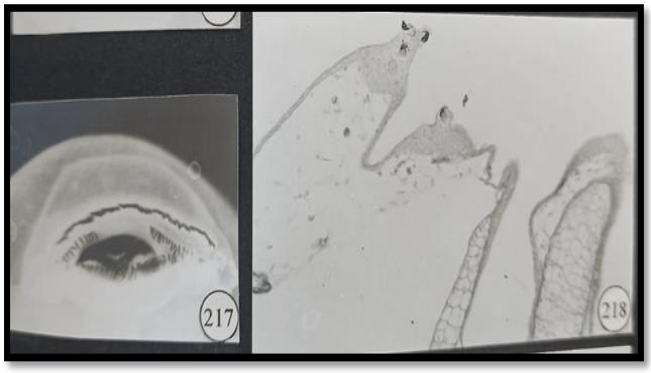


STAGE 36

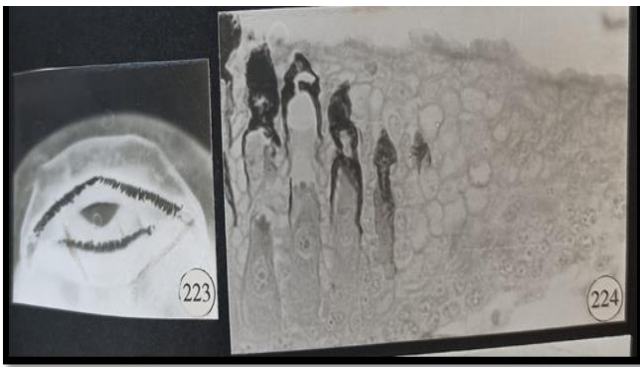
1. Morphology and histology of keratinized oral armature of untreated (Control) tadpoles of stage 36.



2. Morphology and histology of keratinized oral armature of Vitamin A treatment (Continuous) tadpoles of stage 36.



3. Morphology and histology of keratinized oral armature of Vitamin A treatment (Discontinuous) tadpoles of stage 36.



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