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EFFECT OF VITAMIN A ON SKIN AND METAMORPHOSIS OF BUFO MELANOSTICTUS STAGE 23

Kalpana Soni*

Department of Zoology, Govt. Raj Rishi (Autonomous) College, Alwar - 301001, Rajasthan

email: kalpanasoni2506@gmail.com

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Abstract

Vitamin A has been found to affect differentiation of skin in the toad tadpoles and the effect is more severe on younger tadpoles as compared to the older ones. The tadpoles of stage 23 when treated with vitamin A, distinct effect was swelling of tadpoles particularly in the ventral region. Integument became transparent and the cellular organization of integument has disrupted severely and cells appear to be swollen mucoid type. Such condition is known as mucous metaplasia. Vitamin A treatment causes mucous mataplasia both in the dorsal and ventral region in this stage (stage 23) of tadpoles of *Bufo melanostictus*. Vitamin A induced mucous metaplasia is greater on the ventral region as compared to the dorsal region of the body. Vitamin A treatment has been found to inhibit differentiation of skin glands and metamorphosiis in tadpoles of stage 23. The characteristic swelling as a result of vitamin A treatment is in not found in the tadpoles of older stages.

Keywords: Anuran, Dermis, Epidermis, Melanophores, Mucous metaplasia, Tadpoles.

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Introduction

Vitamin A and its derivatives (retinoids) are known to affect differentiation, morphogenesis and growth of vertebrates. Besides influencing morphogenesis in a wide variety of cells and tissues, retinoids have been observed to produce specific effect on cell differentiation particularly that of epithelial cells and appendages[1]. Vitamin A has been observed to cause complete suppression of epidermal keratinization and transformation of epidermal cell into ciliated and secretory type[2]. Similarly the transformation of avian foot scale into feather bearing scale by vitamin A[4]. It has also been found to affect metamorphosis of anuran tadpoles[5,6,7]. When frog and toad tadpoles were given exogenous administration of their rearing medium, besides, variety of teratological effects, their metamorphosis was delayed or inhibited[7]. The studies related to development of skin glands have been described with respect to particular region of skin. Treatment of tadpoles with exogenous thyroxine had revealed differences in the rate of development among the two types of glands i.e. mucous and granular glands[8,9]. The development of the skin glands had been found to be directly dependent upon thyroid in the tadpoles[8,13,14]. The sensitivity of the epidermis to thyroid appears very early but the response to the hormone manifested by development of these glands occurs much later and at different times in different regions of the body[8,11,13,15,16]. Verma[14] reported that the skin glands of *Rana pipiens* develop sequentially at different rates in different regions of the body. Very less is known how vitamin A influences differentiation of skin and its glands in amphibians. In view of the above the present study was carried out to study differentiation of skin and skin glands in different regions of the body of tadpoles of *Bufo melanostictus* (Schneider) at stages 23 of development.

Stage-23 Opercular folds stage

At this stage from the region of the head just in front of the first gill on either side arise two fold-like extensions of the body wall called opercular folds (OP.F.). They progressively elongate towards the posterior region covering the gills. The eye cup (E) is comletely circular, through still the Ventral portion of it is thin. The orbit and nasal openings (NA. OP) are clearly recognizable. The mouth is surrounded above and below by fleshy areas of lips which unite together to form the oral cup. The mouth is bounded pre-orally by a crescentic horny dark brown ridge. On the post-oral side also there is a horny ridge. Both these ridges form the beak (BE.). The margins of these ridges are not provided with the teeth-like projections as yet. Dental formula at this stage is 1/0. The pre-oral fleshy lip has one complete row of denticles and the post-oral lip has not developed denticles yet.

The cement gland (CE.GL.) has become greatly reduced and the remnants of its two bead-shaped moieties are losing their dark colour. Fourth gill bud appears just behind the third gill. Pronephric region which are quite prominent in previous stages has become indistinguishable here.

The demarcation between the head and belly has become further reduced than at stage 22. The region of head in front of gills shows considerable lateral expansion, so that the head appears to be dorso-ventrally compressed. The region of belly however is round ventrally. The head and belly have become almost transparent so that many internal

structures can be seen. Melanophores are present on the dorsal side of the head, belly and tail. The lateral line (LAT-Ll.) is clearly indicated along the lateral sides of the trunk and tail of tadpol.

Materials and Methods

The present study was under taken on young and advanced tadpoles of the common Indian toad, *Bufo melanostictus* (bufonidae, anura, amphibian). 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 and from March on wards it comes out at dusk and can be collected easily in the nights and like other many Anuran, it breeds during monsoon. The spawning takes place in shallow pools 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 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 contamination. The tadpoles grew well in such conditions and there was negligible mortality. All experiments were carried out on young tadpoles of stages 23 of this toad species. The stagination was done according to the normal table of development of *Bufo melanostictus*[17]. For observations living tadpoles were anaesthetized by immersing them in 1:4000 working solution of MS 222 (Ethyl-m-aminobenzoatemethanosulfonate Sandoz) within few minutes and they could remain under anaesthesia for about 30 minutes without any fatal results. On being transferred to ordinary water they revived in a few minutes.

Experimental design

Tadpoles at stage 23 were divided into two experimental groups: (1) Group A, tadpoles were reared in ordinary water throughout the period of experiment (control group), (2) Group B, tadploes were reared in vitamin A palmitate (1 1U/ml-sigma).

Chemicals used

(1) Nuclear fast red: 0.1 gm of nuclear fast red was dissolved it in 100 ml of 5% Alumimium sulphate in water. Heated and stirred the solution cautiously up to 2-3 hours and then cooled and filtered it. (2) Azan: 0.5 gms aniline blue, 2.0 gms Orange G and 2.0 gms Oxalic acid were dissolved in 100 ml distilled water, boiled for 10 minutes and allowed it to cool for some time and finally filtered it. (3) Phospomolybdic Acid: 1.0 gm phosphomolybdic acid was dissolved in 100 ml of distilled water. (4) Vitamin A palmitate, the preparation used was water dispersible powder of vitamin A palmitate, type VII (sigma). To study its effects, the tadpoles were immersed in aqueous suspensions of IU / ml of this vitamin for the required period of time according to the experimental design. One gram of this powder contains 250000 international units (IU/U.SP) of vitamin A palmitate. The aqueous suspensions containing 1 IU /ml of the vitamin A were prepared by dispersing 4 mg of the powder, respectively, in one liter of tap water. Normal development and differentiation of skin was observed in the tadpoles of *Bufo melanostictus* at stage 25, 30, 34, and 36 of development. Effect of vitamin A on skin gland differentiation was observed for these developmental stages. For vitamin A treatment tadpoles of different development stages were reared in 1 IU/ml solution of vitamin A palmitate (Sigma) for varying periods.

Schedule of Fixation

Tadpoles of stage 23 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

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 serical sections stained with modified azan[18] were also used for histo-chemical localization of muein, collagen and fibers. For morphological studies, the tadpoles were examined under steresoscopic 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 6u thickness and then stained with aniline blue and orange G according to the modified azan staining technique[18].

Results

Nornml Differentiation of Skin

Development and differentiation of skin was observed in the tadpole of Bufo melanostictus of stage 23 of development. Effect of vitamin A on skin gland differentiation was also observed on these developmental stages.

Stage 23

Untreated (control)

Group A

In the very young tadpoles of stage 23, differentiation of skin is not much advanced and is represented as a single layer of epidermis without skin glands throughout the body. In the tadpoles of stage 23 reared in water and observed during the subsequent three days skin is still represented by single layer of epidermis with a thin basement membrane but skin glands are absent. Melanophores are present in the skin of tadpoles of stage 23. These melanin containing chromatophores are present mainly in the dermis. Melanophores are generally absent on the ventral side.

Tadpoles of stage 23 reared in water have reached to stage 36 on 15th day. These tadpoles are having well differentiated skin with pigmentation. Skin is still made up of 1 to 2 cell layer thick epidermis on the dorsal side with occasional mucous glands.

Vitamin A treatment (Continuous

Group B

Those tadpoles of stage 23 treated with Vitamin A palmitate lor fifteen days demonstrated that Vitamin A treatment affects differentiation and development of skin of Bufo tadpoles. On 3rd day the distinct effect of Vitamin A observed was swelling of tadpoles particularly of the ventral region. In these, integument becomes transparent. The cellular organization of integument has disrupted severely and cells appear to be swollen and mucoid type. Such type of condition is known as mucous metaplasia. The skin of dorsal region does not show mucous metaplasia as compared to the

Ventral region. Subsequent treatment of vitami A causes disruption of skin. Most of the tadpoles of stage 23 treated with 1IU per ml dose of vitamin A die on 15 day post treatment and those who survived this treatment shows greatly retarted metamorphosis. Mucous gland and serous glands are generally absent in these treated tadpoles and melanophores becomes diffused.

Discussion

In the tadpoles of Bufo melanostictus both mucous and granular glands has been noticed in the present study. The skin glands observed in present study were similar to the general features of skin glands described by Dawson[19]. The granular glands of many amphibians particularly the toads protects them from being eaten up by many enemies because their secretion is repelling and irritating and hence are referred as poison glands. Abel and Match[20] described the poisonous secretion of some bufonids as Bufogin ($C_{18}H_{24}O_4$). In the present study serial cross sectional examination of skin in the dorsal, ventral and lateral regions in very young tadpoles of stage 23 has revealed certain interesting features regarding development and distribution pattern of skin glands. Although basic pattern of differentiation of skin is similar in this anuran species[21, 22] compared with other amphibians yet there are certain variations which are noticed only in this particular bufonid. Tadpoles of stage 23 showed one or two cell thick epidermis and very thin dermis. In these, glandular differentiation has been observed in the skin.

Verma[14] studied the skin of *Rana pipiens* around the jaw and posterior lip region and no sequential development was traced for the skin glands in other regions to traced priority of differentiation of mucous or serous glands. In the present study observation were made in *Bufo melanostictus* mainly confined to the skin of dorsal, ventral and lateral regions immediately posterior to the head region. In this bufo species during larval development the number of glands are more in the dorsal region as compared to the ventral and lateral regions. Among these two types of glands, mucous glands are more abundant in the dorsal skin then in the ventral and lateral skin. Similar observations were made in ranid amphibians. According to Le QuangTrong[23-25] the ventral skin of young tadpoles generally lacks serous glands. The distribution of glands in different amphibians may be related to differences in their habitat[25]. In *Bufo melanostictus* it has been observed that development of the two type of gland is independent without intermediate or transitional phase. During their development some glands contain both mucous and granular material at the same time.

The melanophores present in the dermis concentrated at regular intervals and gives black-brown colour to the dorsal skin. Dermal melanophores are generally absent in the ventral and lateral skin.

Retinoids (Vitamin A and derivates) have been observed to regulate vision, growth and differentiation, reproduction and healthy status of epithelium. It is well known that vitamin A is directly involved in the process of vision because it serves as important component of rhodopsin. There are reports that hypervitaminosis causes mucous metaplasia and other histological deviations on developing mammalian skin[28]. Vitamin A has been found to affect differentiation of skin and its glands and the effect is stage dependent. Tadpoles of stage 23 when treated with 1 IU/ml vitamin A continuously for fifteen days showed complete inhibition of differentiation of skin. Once the treatment is withdrawn cells come out of inhibitory influence of this drug. But when treatment is prolonged beyond three days the effects are irreversible. Similar effects of Vitamin A was also recorded in the tadpoles of stage 30 who demonstrated beginning of differentiation of skin glands on 6th day (equivalent to stage 34) after rearing in water. However, this experimental design does not answer the question whether effect of vitamin A is direct on epidermal and dermal differentiation or is mediated through systemic influences such as hormones

Vitamin A treatment causes mucous metaplasia of epidermis, decreases in number of pigments, swelling of abdomen due to accumulation of mucous and degeneration of horny teeth and jaw. Similar general effects of vitamin A have been reported by other investigators in different anuran species[6,7,30-32]. How vitamin A affects various components of skin of other anuran tadpoles at different developmental stages is not yet known. Studies concerning the effects of vitamin A during amphibian limb regeneration have been carried out in many amphibian species and it has been reported that prolonged exposure of blastema to vitamin A causes inhibition of regeneration[33-35]. Discontinued treatment results in whole limb regeneration[6,33,30,36-42]. The effect of vitamin A is stage dependent[36,43,44]. Vitamin A maintains healthy status of regenerating epithelium[45].

Legends of Figures

Fig. 1: General Morphology of stage 23 dorsal region.

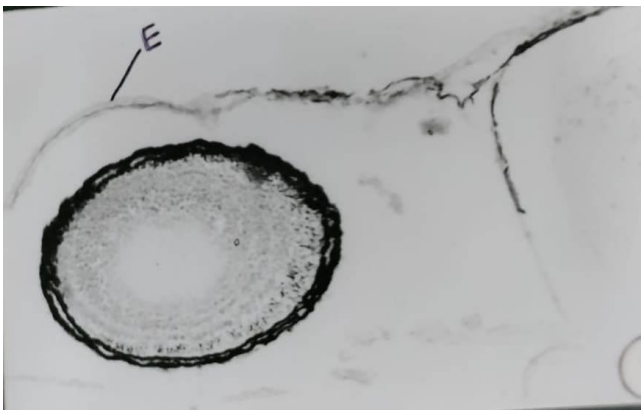

Fig. 2: Dorsal region of stage 23- skin represent as single layer of epidermis without skin glands.

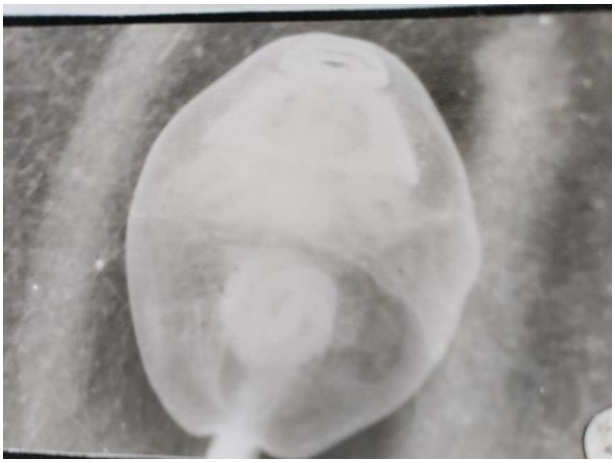

Fig. 3: 15 day ventral region-Swelling of the tadpole, skin becomes thin and transparent and show mucous metaplasia

Fig. 4: 15 day dorsal region- epidermal organization becomes wavy and irregular

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