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Ontogenic variation of ß-glucuronidase in the liver and intestine of *Labeo rohita*

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Abstract
β-Glucuronidase is a lysosomal enzyme, it is largely studied studied in mammalian species. In the present study the attempt was made to localize this enzyme in the liver and intestine during the development of teleost, *Labeo rohita*. For the demonstration of enzyme activity, Naphthol AS-BI-β-Glucuronide was used as a substrate along with diazotized pararosaniline. β-Glucuronidase shows variation in both the liver and intestine during the development of *L. rohita.* The enzyme activities in both the organs were increased gradually from 10 to 50 mm stage of development. In the sac-fry (5-7.5mm), the enzyme activity was confined in yolk sac and no enzyme activity was observed in the liver and intestine. It was concluded that the presence of β-glucuronidase in the yolk sac might be due to its role in the yolk metabolism. Gradual increase in enzyme activity from 10mm onwards in the intestine could be due to maturation of digestive tract and in the liver due to increase in the metabolic load with increase in growth and age.

Keywords: ß-Glucuronidase; intestine; liver; development; fish.

INTRODUCTION

Labeo rohita is one of the Indian major carp that occurs in both lentic and lotic ecosystem of India. It is an economically important, Fast growing fish that attends the sexual maturity at the age of two years.

Enzymes are the indicator of digestive physiology in fish species. The level of digestive enzyme activity in fish determines the capacity of digestion and absorption of nutrients, which influences the speed of growth and development in fish (Liu *et al,* 2010). In recent years, the research on digestive enzymes in fish with respect to its occurrence and change in its activity in the larvae and juvenile stages has focused the attention of researchers (Chen and Zhang, 2004).Studies on sea bream *Pagrosomus major* (Chen *et al,* 1998) and Amur sturgeon *Acipenseride schrencki* (Su and Zhao, 2005) found that types, time of occurrence and activity levels of digestive enzymes in larvae and juveniles differ significantly. Additionally, the occurrence of digestive organs in fish is not synchronous in early development and gradually improves during later development (Pan *et al.,* 2009). There are also differences and similarities in food composition for different fish species during individual development (Specker, 1988). Nutrient strategies and survival rates can be improved if the physiological mechanism of digestive enzymes is aquired during the fish larval stage.

β-Glucuronidase is One of the lysozymes. It's chemistry, function, cellular localization, synthesis and its relation to the physiological processes in general were studied for last many years. Fishman (1955) described β-glucuronidase as an enzyme with specificity for the β-glycoside linkage of variety of naturally occurring and synthetic glucuronide. Levvy and Chonchie (1966) later described β-glucuronidase as a group of specific enzymes which catalyses the hydrolysis of the biosynthetic β-D-glucupyronosiduronic acids (β-glucuronides) to yield their various aglycons and free glucuronic acid in the carbohydrate metabolism.

Advances in the biochemical assay and the histochemical techniques have helped in understanding the intracellular localization of β-glucuronidase and its physiological functions. This aspect has been excellently reviewed by De Duve (1959) and Fishman *et al.,* (1967). De Duve *et al.,* (1955) and Fishman *et al.,* (1967) added a new parameter to the cytological localization of β-glucuronidase. They demonstrated the dual localization of the enzyme in the lysosome and endoplasmic reticulum and further believed that this enzyme may serve as a structural protein of the biological membrane, particularly of endoplasmic reticulum. Paigen (1961) and Fishman *et al.,* (1967) confirmed histochemically that this enzyme has both lysosomal and nonlysosomal localizations in the cells. Though the enzyme is utmostly important important in cellular metabolism, no work has seen to carried out in the intestine and liver. Therefore, the present work was undertaken to study the role of β-glucuronidase in the intestine and liver during the development of Indian teleost, *Labeo rohita*.

MATERIALS AND METHODS

All the developmental stages of *Labeo rohita were collected* in monsoon season from Government of Maharashtra Fish Seed Center, Pench located 45Km from Nagpur. Live fish seeds were carried to the laboratory in oxygen filled polythin bags. Body length was measured prior to fixation in Bouin's fixative and ice-cold 10% Neutral buffer formalin (NBF) for 24 hrs. Sections for histology were cut at 8µm on Cambridge made rocking microtome. NBF fixed stages were washed with ice cold distilled water and then were cut 10µm thick sections on Leica cryostat at -20°C.

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11 mg Naphthol AS-BI-β-D-glucuronic acid (Sigma) was dissolved in 0.05 M Sodium carbonate and then diluted to 100 ml with 0.1 M acetate buffer (pH-4.5). This stock solution was preserved in the refrigerator at 4°C. It was then diluted with 0.1 M acetate buffer (pH-4.5) to get solution of variable molarities. It was observed that $1x10^{-4}$M substrate solution give the best result.

Immediately prior to incubation, 0.5 ml solution of sodium nitrate was added to 0.5ml acidic pararosaniline solution, mixed gently by inversion and was allowed to stand for 5 minutes. Later, 38 ml prewarmed deionized water, 5 ml acetate buffer (0.1M; pH-4.5) and 5 ml Naphthol AS-BI-β-D-glucuronic acid ($1x10^{-4}$M) were added to get the final incubation solution. The formation of precipitation was rejected.

Sections were rinsed with cold 0.01M phosphate buffer saline (PBS), pH-7.45. Later, sections were slightly allowed to dry at room temperature. The sections were incubated for 90 minutes at 37°C and during this process the light contact was avoided.The sections were rinsed for 2-3 minutes in double distilled water and then air dried for 15 minutes and later they were mounted with Glycerol jelly. Lastly the microphotographs were taken.

RESULTS

β-Glucuronidase activity during development of *Labeo rohita* was studied from 5mm stage upto 50mm stage after hatching (a.h.). It was observed that this enzyme exhibits tremendous variation from early to late stage of development after hatching.

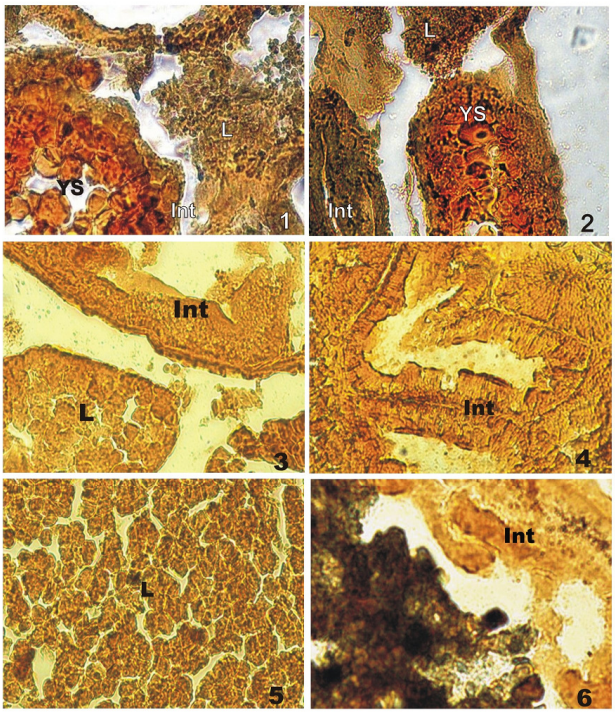

Fig.1. Sagittal section of 24hrs stage (a.h.) of *Labeo rohita* showing diffuse to granular staining in the yolk sac (YS) region and towards periphery X200.
Fig.2. Sagittal section of 72hrs stage after (a.h.) showing increased staining in yolk sac (YS), intestine (Int) and liver (L) is unstained X200.
Fig.3. Photomicrograph of 10 mm stage (a.h.) showing weak diffuse staining in the liver (L) and intestine (Int) X200.
Fig.4. Photomicrograph of 15 mm stage showing weak diffuse in the intestine (Int) X200.
Fig.5. Photomicrograph of 15mm stage showing weak diffuse staining in the liver (L) X200.
Fig.6. Photomicrograph of 20mm stage showing slightly increased diffuse staining in the intestine (Int) X200.

1. 5mm to 7.5 mm stages

5mm stage is a hatchling stage having yolk sac fully filled with yolk till 24hrs after hatching. 72hrs a.h. it attained the size of 7.5mm during which almost yolk gets absorb into the abdomen. Till 7.5mm stage, the intestine was in the form of straight tube. The β-glucuronidase activity was observed in the yolk sac (Fig.1 and 2) and not in the intestinal tube. However no reaction was also found in the liver till 7.5mm stage.

2. 10mm stage

At this stage yolk sac was completely absorbed. Intestine became elongated and was seen folded in the abdominal cavity. The β-glucuronidase activity was moderate both in the liver and intestine (Fig.3).

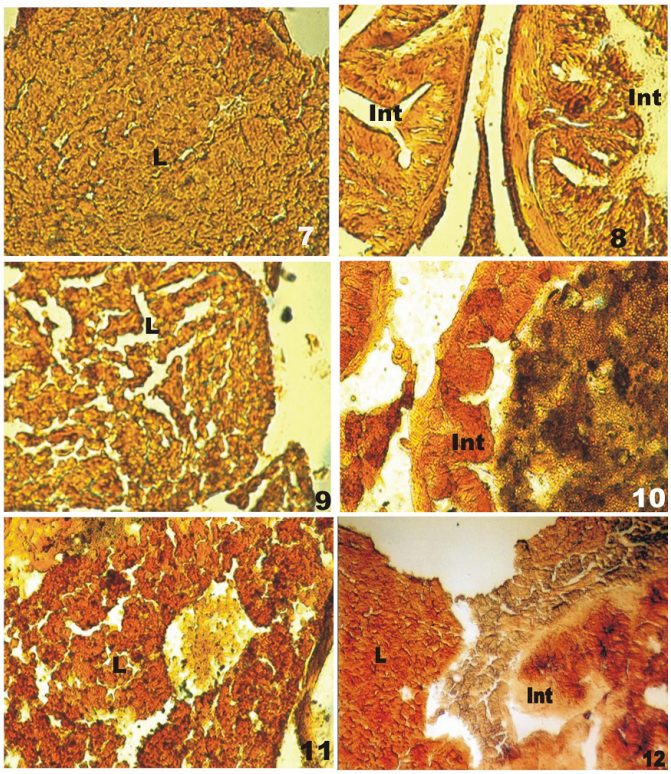

Fig.7. 20mm stage showing slightly increased staining in the liver (L) as compared to 15 mm stage X200.
Fig.8. 30 mm stage showing moderate increased staining both diffuse and granular in the intestine (Int) X200.
Fig.9. 30 mm stage showing moderate staining both diffuse and granular in the Liver (L) X200.
Fig.10. 40 mm stage showing increased intense staining in the intestine (Int) X200.
Fig.11. 40 mm stage showing increased intense staining in the liver (L) X200.
Fig.12. 50 mm stage showing highly intense staining in the liver (L) and intestine (Int) X 200.

3. 15mm stage

As compared to 10mm stage, ß-glucuronidase activity was slightly improved in the mucosa, sub-mucosa and in lamina propria of the intestine (Fig.4). Similarly the enzyme activity increased in the liver (Fig.5) as compared to previous stage.

4. 20mm stage

Staining for the enzyme intensified at this stage both in the intestine and the liver. Mucosal layer of the intestine became quite folded at this state, as compared to early one. The enzyme activity increased in the intestine, staining was seen in the mucosa, sub-mucosa and lamina propria (Fig.6). Compared to 15mm, in this stage the enzyme activity was noticed to enhance in the liver (Fig.7).

5. 30mm stage

In this stage, enzyme activity increased both in the intestine and the liver. The intestinal epithelium exhibited the diffuse reaction whereas the lamina propria showed granular reaction in the region of circular muscle bundle and connective tissues (Fig.8). In the liver, staining was observed both in diffuse and granular form (Fig.9)

6. 40mm and 50mm stage

Enzyme activity greatly increased at 40 and 50mm stage of development in both the intestine and the liver (Fig.10, 11 & 12). In these tissues, the staining was completely in diffused form rather than granular one.

DISCUSSION

Yolk is the main energy source for most of the fishes during endogenous feeding period which begins at fertilization and ends at the onset of exogenous feeding by hatched larvae (Kamler, 1992).

Upto 72 hrs after hatching, β-glucuronidase activity was observed in the yolk sac. By the end of 72 hrs, mouth opens and active feeding starts. Till 72 hrs after hatching negligible staining was observed in the intestine, but with the increasing stage of development, intensity of β-glucuronidase gradually increases from 8 mm to 50 mm stage. Cara *et al.,* (2003) reported the presence of acid and alkaline phosphatase activity at the moment of mouth opening during larval development of White Sea bream, *Diplodus sargus*.

Enzyme intensity suddenly intensified in the intestinal epithelium of *L. rohita* in 50 mm stage**.** The relationship between metabolic rate and temperature in early ontogenesis was studied in *Esox lucius* by Lindroth (1942, 1946) and in *Clupea harengus* by Holliday *et al.,* (1964). There are some recent reports about enzyme distribution and their localization in gastrointestinal tract during the larval period of fish such as sea bass; *Lates calcarifer* (Walford and Lam, 1993), *Theragra chalcograma* (Oozeki and Bailey, 1995), Winter Flounder (Baglole, *et al.,* 1988), *Solea senegalensis* (Ribeiro *et al*., 1999) and in Siberian Sturgeon (Gisbert *et al*., 1999). They all have reported a correlation between enzyme activities and maturation of digestive tract.

In general, liver is a center for metabolic activity in the body in all the vertebrate species. Liver upto 72hrs stage showed no β-glucuronidase activity. In 8 and 10 mm stage, the activity was weak, in later stages it was slightly improved (15 to 30 mm). However upto 40 and 50mm stage, the enzyme activity was greatly intensified.

It was concluded that the presence of β-glucuronidase in the yolk sac might be due to its role in the yolk metabolism. Gradual increase in enzyme activity from 10mm onwards in the intestine could be due to maturation of digestive tract and in the liver due to increase in the metabolic load with increase in growth and age.

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