

Histochemical localization of Acid phosphatase in the tissues of *Labeo rohita* (Hamilton)

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Abstract: Acid phosphatases (AcPs) are known to provide phosphate to tissues that have high energy requirements, especially during development, growth and maturation. AcPs enzyme shows variations in quantity in reproductive phases in gonads, kidney and liver. AcPs is highest in both the sexes in post-spawning phase and lowest in preparatory phase. In male, acid phosphatase activity is maximum in all three tissues i.e gonads, kidney and liver in post-spawning phase and lowest in the preparatory phase as in female. AcPs staining was either granular or in diffuse form in brown deposits. This study is aimed on the estimation of activity of acid phosphatase in kidney, liver and gonads of *Labeo rohita*.

Keywords: Acid phosphatase, *Labeo rohita*, Reproductive phases, Gonads

I. Introduction

Acid phosphatases which belong to hydrolases class catalyse the hydrolysis of various phosphomonesters in acidic medium (pH 5-6) to release an inorganic phosphate (Miteva *et al.*, 2010). These are present in animals (Sazmand *et al.*, 2011), plants (Tabaldi *et al.*, 2008) and lower organisms like protozoa (Amlabu *et al.*, 2009) and fungi (Leitao *et al.*, 2010). These enzymes are involved in many biological processes such as energy metabolism and signal transduction pathways (Shan, 2000). Acid phosphatases are frequently occurs in multiple forms (Fujimoto *et al.*, 1984) and can be differentiated according to structural, catalytic, tissue distribution and localization (Suter *et al.*, 2001). In vertebrates, three types of acid phosphatase have been described based on molecular weight and its localization within the cell organelle (Naz *et al.*, 2001). High molecular weight acid phosphatase (80-200 kDa) is localized in the lysosomes and low molecular weight enzyme (10-30 kDa) is present in cytosol fraction of the cell while intermediate molecular weight acid phosphatase (30-50 kDa) was found in the mitochondria of some mammalian tissues such as kidney (Naz *et al.*, 2006).

II. Material and Methods

The ovary, testis, liver and kidney of the adult were sectioned at 8-10µm thickness, and stained. For acid phosphatase activity tissues are fixed in cold buffered neutral formalin for 24-72 hrs. After fixation material transferred to 10% sucrose for 1hr, 20% sucrose for 1hr and 30% sucrose for overnight. The tissues were cut in transverse and sagittal section at 8-10µm on Leica cryocut. The activity of enzyme, acid phosphatase was demonstrated by Gomori (1952) method in the sections of the tissue fixed in cold buffered neutral formalin. 10-12µm thick sections were cut on a cryostat at -18° to -20° C. After washing with distilled water, the sections were incubated for 16-20hrs in incubation medium at 37° C. Sections were washed with distilled water and transferred to dilute yellow ammonium sulphide solution for 1 min, washed in distilled water and mounted in glycerine jelly. Brown deposit in sections confirms the activity of enzyme, acid phosphatase in the tissues (Pearse, 1968).

III. Result

A. Male

Acid phosphatase (AcPs) activity is observed as brown deposits in the tissues of *Labeo rohita*.

1. Kidney

The annual reproductive cycle, shows fluctuations in staining intensity of this enzyme in kidney. In resting phase, the glomeruli show moderate diffuse to granular staining. The brush border of intermediate tubules exhibit diffuse staining. The proximal tubules show diffuse moderate staining in the brush border and cytoplasm shows intense granular staining. However interstitial haemopoietic tissue (IHT) remains unstained (Fig. 1). During preparatory phase, granular deposition is noted in the proximal, intermediate and distal tubules. The brush borders of tubules show moderate diffuse staining. The glomeruli and interstitial haemopoietic tissue show granular reaction. The collecting tubules of kidney do not show any staining for AcPs (Fig. 2). In pre-spawning phase, granular to diffuse staining for AcPs is noticed in proximal, intermediate and distal tubules. The collecting tubules do not show any staining while granular staining is observed in interstitial haemopoietic

tissues. AcPs staining seems to increase in spawning phase (Fig. 3). It is however difficult to distinguish between granular and diffuse staining in the tubules. The granular staining could be seen in some tubules and also in the interstitial haemopoietic tissues (Fig. 4). Intense staining is observed in the kidney in the post-spawning phase of the reproductive cycle. Both granular as well as diffuse staining can be visualized in all the tubules of the kidney. Granular staining is noted in the brush border area as well as towards the periphery of proximal and distal tubules. The intermediate tubules show diffuse staining which is congregated at the centre while peripheral areas show dense granular staining. In the interstitial haemopoietic tissue more granular and less diffuse staining is noted (Fig. 5).

2. Liver

Diffuse and granular staining is noticed in the hepatocytes of liver. Blood vessel has diffuse staining at its inner side during resting phase (Fig. 6). Weak staining for AcPs is observed during preparatory phase (Fig. 7). In the pre-spawning phase, hepatic cells of liver exhibit intense granular staining (Fig. 8) while in spawning phase, liver shows moderate intensity for this enzyme, in less granular and more diffuse (Fig. 9). During post-spawning phase intense staining is noticed (Fig. 10).

3. Testes

AcPs activity could be detected at resting phase of the reproductive cycle in diffused as well as in granular forms. Wall and germinal epithelium of the testicular lobules show diffused AcPs activity while spermatogonia located closer to the wall of tubules show granular deposition in resting phase (Fig. 11). During preparatory phase, testicular lobules contain primary and secondary spermatogonia with few spermatocytes. Diffuse to granular staining of AcPs is observed in spermatogenic stages. Intense staining for AcPs is noted in preparatory phase in germinal epithelium of testicular lobules (Fig. 12). During pre-spawning phase, testicular lobules consist of primary and secondary spermatogonia, primary and secondary spermatocytes and spermatids. Both diffused to granular weak staining is noticed in spermatogenic stages. However, in the periphery, enzyme activity seems to be reduced compared to the preparatory phase (Fig. 13). In spawning phase, testes are full of spermatozoa where enzyme activity is very weak. As such no staining is noticed in the peripheral areas of the testicular lobules (Fig. 14). Histochemically, the intensity of staining for AcPs is found to increase in post-spawning phase than in the spawning phase. Boundary of testicular lobules, spermatogonia and spermatocytes show intense but diffuse and granular staining. Sperms show less granular deposition (Fig. 15).

B. Female

AcPs was demonstrated histochemically as brown precipitate in the tissues of Indian major carp *Labeo rohita*. According to the annual reproductive cycle of *Labeo rohita*, variations in staining intensity of AcPs enzyme are noticed.

1. Kidney

In resting phase, diffuse staining for AcPs is observed. The proximal tubules are moderately stained in brush border region. The glomeruli show moderate to intense staining for this enzyme. The interstitial haemopoietic tissues exhibit weak diffuse staining while the intermediate tubules are moderately stained for AcPs (Fig. 16). During preparatory phase granular to diffuse staining of this enzyme is observed. The brush border lining of proximal tubules show weak to moderate staining. The intermediate tubules exhibit weak staining while interstitial haemopoietic tissues show very weak staining (Fig. 17). During pre-spawning phase, proximal tubules show heavy deposition of this enzyme in brush border region in diffuse form. The intermediate tubules exhibit moderate to intense staining in diffused and granular form in peripheral and brush border region of the tubules. The interstitial haemopoietic tissues show granular to diffuse staining (Fig. 18).

Histochemically, enzyme activity is increased in spawning phase as compared to the pre-spawning phase. The proximal tubules are intensely stained for this enzyme in brush border region in diffuse form while granular deposition is noted in the cytoplasm. Intermediate tubules exhibit staining as noted for proximal tubules. The glomeruli and interstitial haemopoietic tissues show granular staining (Fig. 19). AcPs activity is found to increase in post-spawning phase than in the spawning phase. Staining is noted in the tubules in intense but diffuse form. Towards the periphery of tubules it is more or less granular. Some tubules exhibit only intense granular staining in the cytoplasm much towards the brush border area. Granular staining is prominently observed in the interstitial haemopoietic tissues (Fig. 20).

2. Liver

The brown deposition in the form of granules is located in the hepatic cells of liver. The intensity is moderate during resting phase (Fig. 21) which is reduced and weak in the preparatory phase (Fig. 22). In pre-spawning phase, enzyme staining for AcPs is slightly enhanced which is granular as well as in diffuse form in

the hepatic cells (Fig. 23). During spawning phase (Fig. 24) the staining becomes moderate but during post-spawning phase, the intense staining for this enzyme is observed in hepatocytes of liver (Fig. 25).

3. Ovary

Resting phase is dominated by both type-I and II immature oocytes. In both of them AcPs activity is observed. But the spaces adjacent to immature oocytes show reasonably moderate staining. Very few atretic follicles are observed during this phase which exhibit weak AcPs activity in diffuse form (Fig. 26). During preparatory phase, staining of this enzyme increases in immature and maturing (type-III) oocytes. In the immature oocytes, diffuse to granular staining is observed. Zona pellucida which becomes apparent in the maturing oocytes is intensely stained whereas the granular staining is noted in the cortical region just beneath the zona pellucida. Weak staining is noticed in the ooplasm (Fig. 27). In pre-spawning phase diffuse form of enzyme staining is observed in the peripheral wall of the maturing oocyte. Weak to moderate staining is noticed in the ooplasm (Fig. 28). In spawning phase, staining for this enzyme becomes more granular and less diffuse in theca layer, follicular epithelium and zona radiata of matured oocytes. Contrary to this, in central region of the oocytes more diffuse and less granular staining is observed during this phase (Fig. 29). In post-spawning phase, histochemically staining in the atretic follicles is more intense probably due to degeneration of atretic follicle by lysosomal activity. Staining is more pronounced towards the peripheral region of the atretic follicles which is intensely granular as well as diffuse. In the germinal epithelium, more AcPs staining in granular form rather than in diffuse form is observed (Fig. 30).

Photo Plate 1

Acid Phosphatase activity in the kidney of male *Labeo rohita*

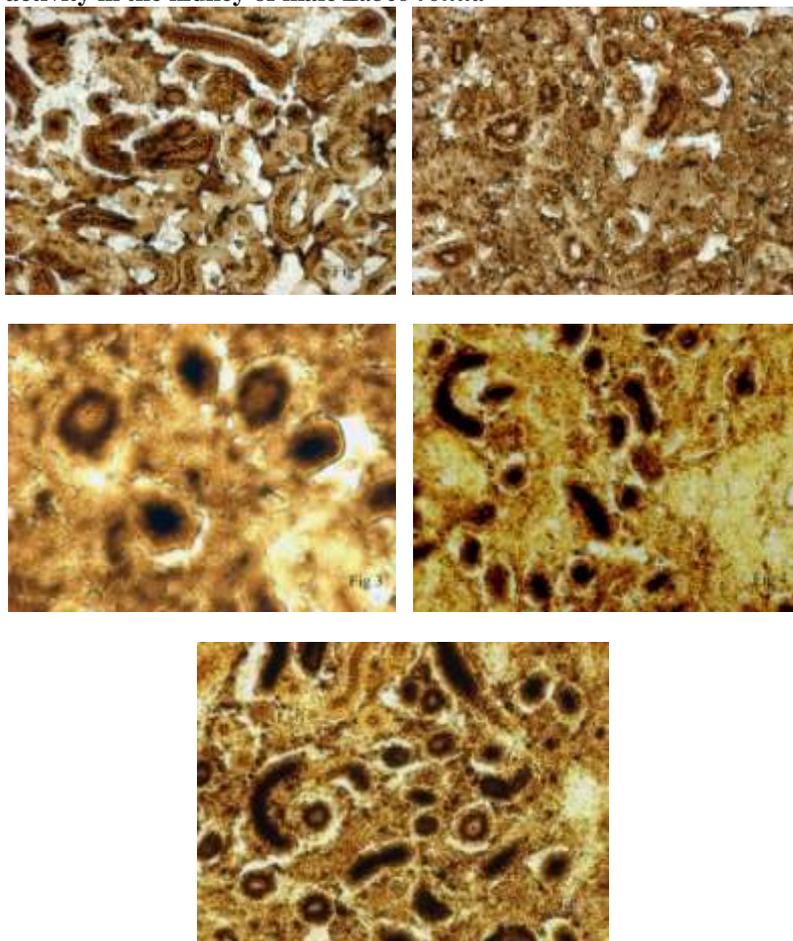


Fig.1. Weak to moderate and diffuse to granular activity of acid phosphatase in the glomeruli, proximal tubules and interstitial haemopoietic tissues (IHT) during resting phase of reproductive cycle X400.

Fig.2. Moderate activity of acid phosphatase in the kidney tubules during preparatory phase X400.

Fig.3. Granular to diffuse activity of acid phosphatase in the kidney tubules during prespawning phase X400.

Fig.4. Moderate to intense activity of acid phosphatase in the various tubules of kidney during spawning phase X400.

Fig.5. Intense activity of acid phosphatase in the tubules and IHT of kidney during postspawning phase X400.

Photo Plate 2

Acid Phosphatase activity in the liver of male *Labeo rohita*

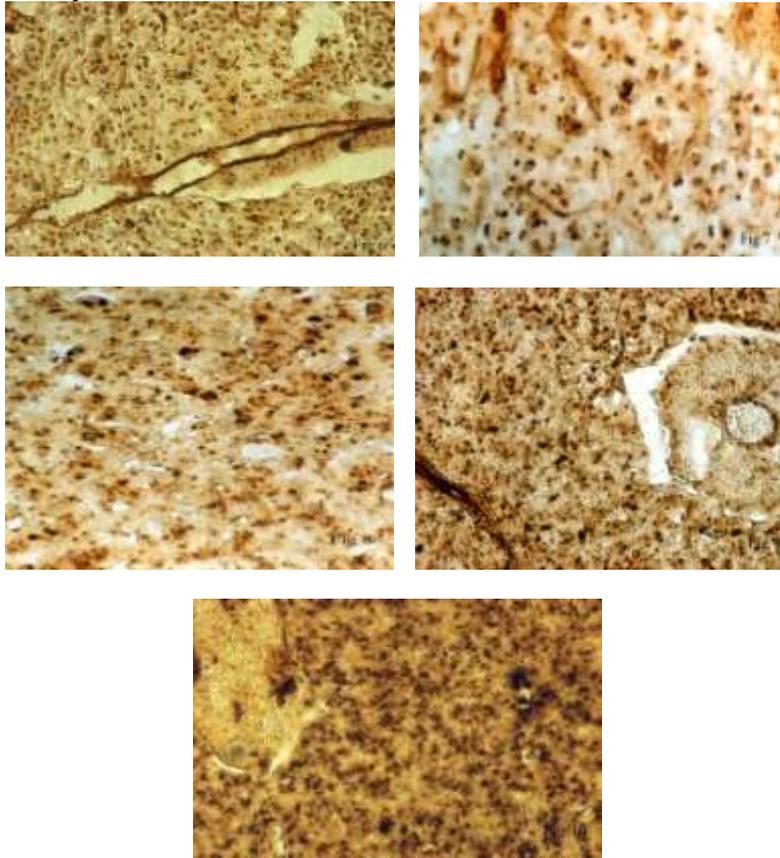


Fig.6. Diffuse to granular activity of acid phosphatase in the hepatocytes of liver during resting phase X400.

Fig.7. Weak activity of acid phosphatase in the liver during preparatory phase X400.

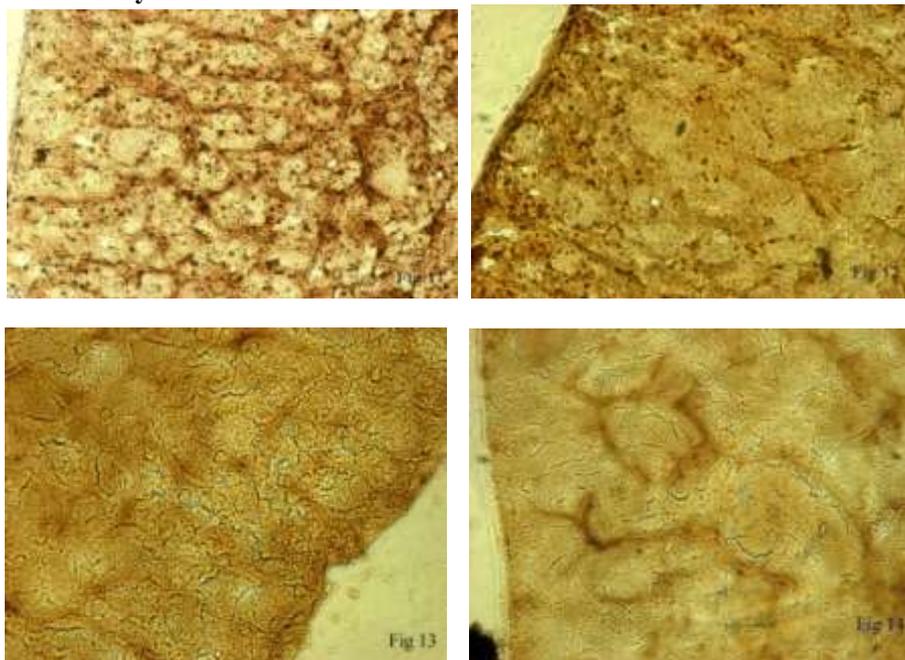
Fig.8. Weak to moderate activity of acid phosphatase in the liver during pre-spawning phase X400.

Fig.9. Moderate activities of acid phosphatase in the liver during spawning phase X400.

Fig.10. Intense activity of acid phosphatase in the liver during post-spawning phase X400.

Photo Plate 3

Acid Phosphatase activity in the testes of *Labeo rohita*



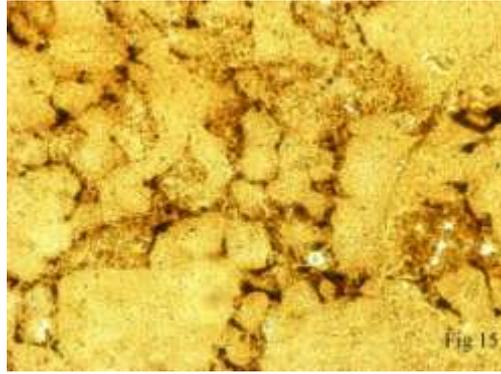


Fig.11. Wall and germinal epithelium of the testicular lobules showing diffuse acid phosphatase activity in resting phase X400.

Fig.12. Moderate diffuses to granular activity of acid phosphatase in spermatogenic stages during preparatory phase X400.

Fig.13. Diffused to granular activity of acid phosphatase in spermatogenic stages during pre-spawning phase X400.

Fig.14. Moderate to intense activity of acid phosphatase in the testis during spawning phase X400.

Fig.15. Intense activity of acid phosphatase in the testis during post-spawning phase X400.

Photo Plate 4

Acid Phosphatase activity in the kidney of female *Labeo rohita*

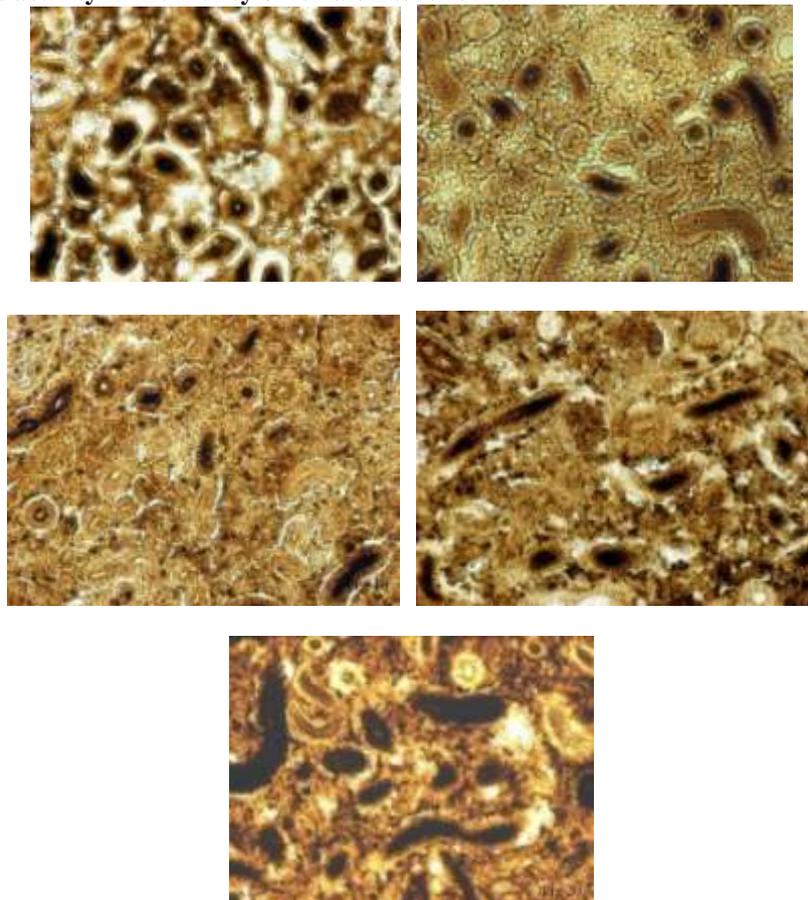


Fig.16. Moderate activities of acid phosphatase in the tubules of kidney during resting phase X400.

Fig.17. Weak granular to diffuse activity of acid phosphatase in the tubules and interstitial haemopoietic tissues (IHT) of kidney during preparatory phase X400.

Fig.18. Weak to moderate diffuse activity of acid phosphatase in the brush border region of tubules and IHT of kidney during pre-spawning phase X400.

Fig.19. Moderate to intense activity of acid phosphatase in the tubules of kidney during spawning phase X400.

Fig.20. Intense activity of acid phosphatase in the tubules of kidney during post-spawning phase X400.

Photo Plate 5

Acid Phosphatase activity in the liver of female *Labeo rohita*

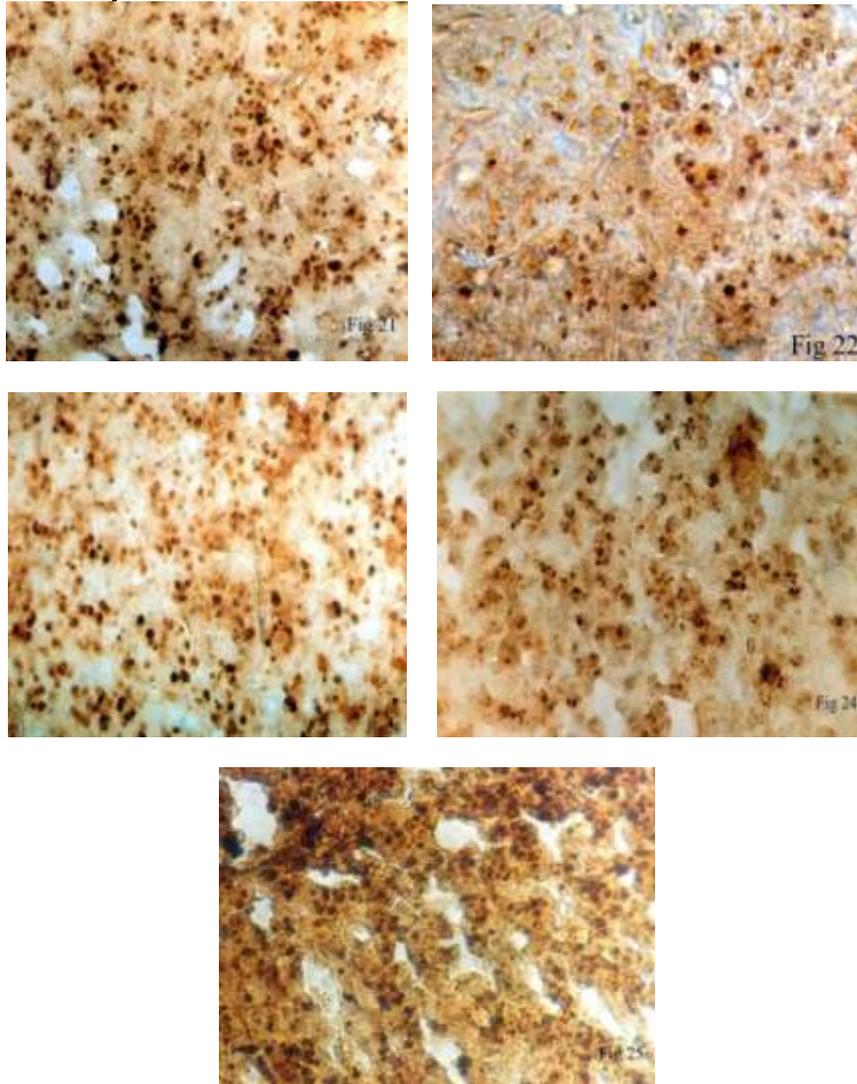


Fig. 21. Moderate activities of acid phosphatase in the hepatic cells of liver during resting phase X400.

Fig. 22. Weak activity of acid phosphatase in the liver during preparatory phase X400.

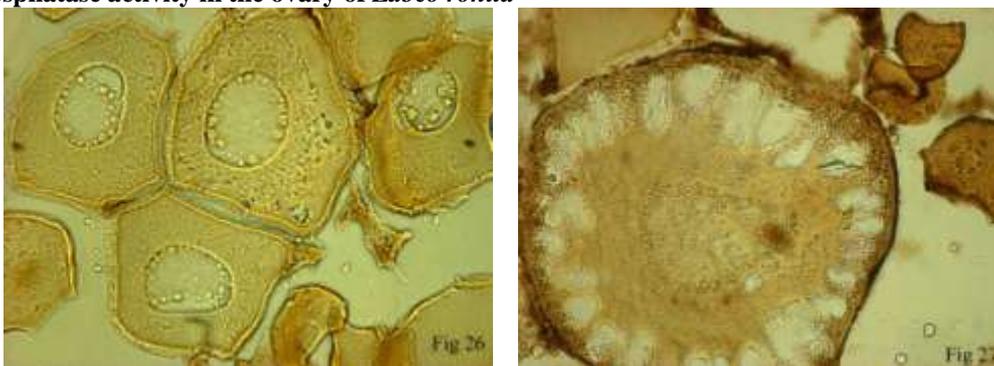
Fig. 23. Moderate activities of acid phosphatase in the hepatocytes of liver during pre-spawning phase X400.

Fig. 24. Moderate to intense activity of acid phosphatase in the hepatocytes of liver during spawning phase X400.

Fig. 25. Intense activity of acid phosphatase in the hepatocytes of liver during post-spawning phase X400.

Photo Plate 6

Acid Phosphatase activity in the ovary of *Labeo rohita*



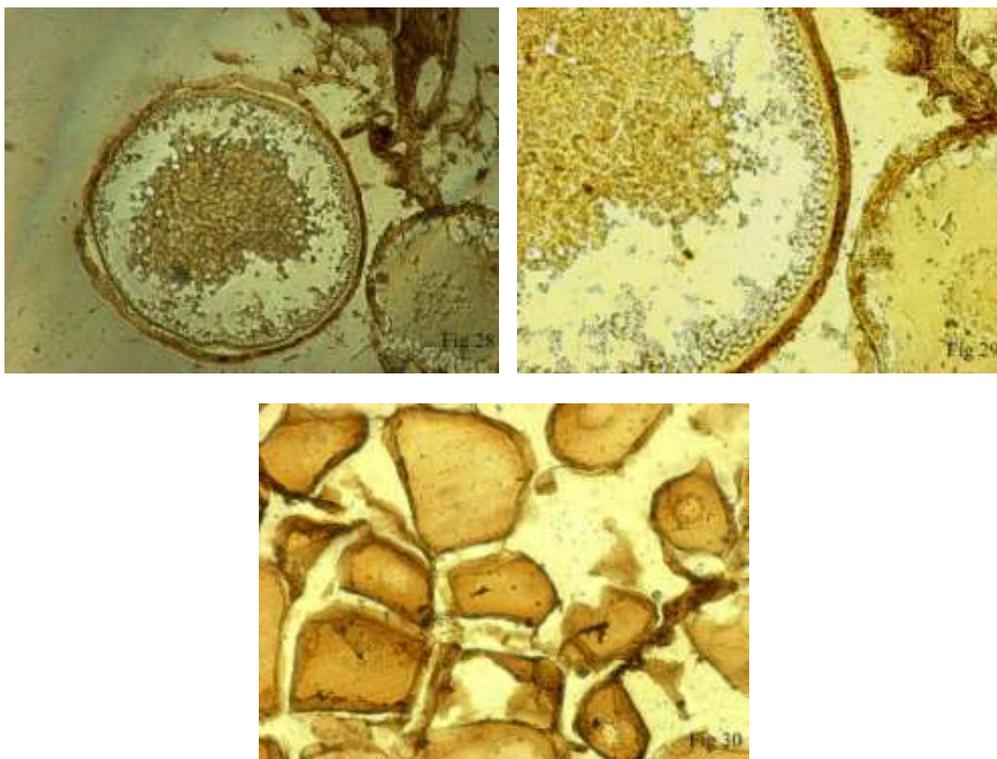


Fig. 26. Diffuse activity of acid phosphatase in the immature oocytes during resting phase X400.

Fig. 27. Peripheral wall of oocyte showing diffuse activity of acid phosphatase during preparatory phase X400.

Fig. 28. Diffuse activity of acid phosphatase in the maturing oocyte during pre-spawning phase X400.

Fig. 29. Moderate to intense activity of acid phosphatase in the wall of matured oocyte during spawning phase X400.

Fig. 30. Intense activity of acid phosphatase in the atretic follicle during post-spawning phase X400.

IV. Discussion

Studies on enzymes are important because a deficiency of or too low activity of enzymes and metabolites interrupt metabolic pathways and lead to the accumulation or loss of distinct substrates and metabolites (Lahnsteiner *et al.*, 1999). Enzymes such as β -D-glucuronidase, acid and alkaline phosphatase are implicated in lytic and degenerating processes especially in association with yolk protein degradation in Trout (Sire *et al.*, 1994). During resting phase, spermatogonia dominate in the spermatogenesis. In the wall of the lobules, granular to diffuse staining for the AcPs enzymes is observed. At these stages boundaries of the lobules lose their intensity for staining for this enzyme. In spawning phase when spermatozoa are most abundant, enzyme activity is less in spermatozoa. AcPs, a participant in cellular disintegration is a proven marker of lysosomal enzyme. Elevation in its activity is related to increase in lysosomal activity which occurs as a part of pre-necrotic changes, increased pinocytosis and due to enzyme induction which is associated with degenerative changes and cellular disorganization (De Duve, 1969). At the spawning time, wall of seminiferous lobules break down to release the milt and leftover spermatozoa are digested involving lysosomal acid phosphatase which accounts for the maximum activity during the spent phase. There is gradual rise for this enzyme from resting phase onwards in *Labeo rohita*. In *Schizothorax richardsonii* (Singh and Nauriyal, 1990), the activity was minimal during immature stage but increased again in spent phases. After spawning, testicular gland cells and ducts of testicular gland have a strong reaction for AcPs in *Salaria pavo* which is the histochemical proof for lysosomal processes (Lahnsteiner *et al.*, 1990). Such intense staining in testes of *Labeo rohita* is visible after spawning. As far as the ovaries are concerned in *Labeo rohita*, AcPs activities present contrasting results as seen in the testes. Acid phosphatase content is highest in the spent stages of the ovary. Histochemically also the staining intensity increases to a great extent in the pre-spawning phase. Zona pellucida is especially stained positively. Intense staining of this layer clearly indicates the role of this layer in the process of transport to differentiating oogonia. It is further substantiated by the fact that when the eggs are fully matured, intensity of zona radiata is reduced, the eggs are now about to be passed out of the body. Presence of acid phosphatase activity in the atretic follicles indicates that these follicles do not have an endocrine function in *Tilapia nilotica* (Yaron, 1971). Livni (1971) reported similar observation in the ovaries of *Cyprinus carpio*, *Mugil capito* and *Tilapia aurea*.

V. Conclusion

AcPs enzyme contents show variations in quantity in various reproductive phases in gonads, kidney and liver. For acid phosphatase activity, differential staining in various spermatogenic stages could be clearly identified. AcPs staining was either granular or in diffuse form. Increased AcPs activity in spent phase of testis may be associated with degenerative processes and cellular disintegration as boundary walls of seminiferous lobules are broken and spermatozoa are released out in the form of milt. For the digestion of leftover spermatozoa, lysosomal AcPs activity may be necessary which accounts for its maximum ($P < 0.001$) content during this phase. Acid phosphatase activity in kidney, unlike alkaline phosphatase is maximum ($P < 0.001$) in spent phases in both the sexes and minimum ($P < 0.001$) in preparatory phase. Proximal tubule is the primitive region of the nephron. It is reported to have well developed lysosomal system and high content of acid phosphatase which may be functioning in reabsorption of filtered proteins and other molecules. Acid phosphatase is highest in both the sexes in post-spawning phase and lowest in preparatory phase. This fluctuation is identical to that in the gonads. Histochemical localization supports these findings. Liver is known to be one of the richest sources of enzyme acid phosphatase. As maturation of gametes proceeds, this enzyme activity slowly picks up, maximum being in the post-spawning. When quantification of enzymes is compared in the three tissues in the males, acid phosphatase activity is maximum ($P < 0.001$) in all three tissues in post-spawning phase as in females. In kidney it is 38.0 ± 0.74 , in liver it is 24.3 ± 0.406 and in testes it is 26.3 ± 0.53 . In the females among kidney, liver and ovaries of *Labeo rohita*, enzyme acid phosphatase content is highest in all the tissues in post spawning phase and lowest in the preparatory phase. Highest content is in the kidney (36.8 ± 0.4) followed by liver (28.6 ± 0.62) and ovary (15.8 ± 0.4).

VI. References

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