CHAPTER 2

A HISTOCHEMICAL STUDY ON ISOENZYMES OF ALKALINE PHOSPHATASE IN THE INTESTINE OF MIGRATORY WAGTAIL, MOTACILLA ALBA

Presence of intestinal and liver type alkaline phosphatases in the intestine of rat was histochemically demonstrated by Rufo and Fishman (1972). Later Rufo et al. (1973) suggested a relationship between liver type alkaline phosphatase and fat absorption in the rat intestine. While studying activities of acid and alkaline phosphatases in the small intestine of two migratory birds viz., Wagtail and Rosy Pastor (Chapter 1) it was noticed that the alkaline phosphatase activity in the intestine of Wagtail, which feeds mainly on protein and lipid rich diet (insects) was relatively higher than that observed in the Rosy Pastor, which is known to feed mainly on carbohydrate rich diet (fruits and a few insects). The difference in the strength of alkaline phosphatase activity observed in the intestine of these two migratory birds appears to be due to difference in their diets. It is presumed that since more lipid is to be absorbed by the intestine of Wagtail, it may have liver type of alkaline phosphatase. To ascertain this contention, a

study on the histochemical localizations of the isoenzymes of the alkaline phosphatase in the intestine of Wagtail was undertaken.

MATERIALS AND METHODS

Wagtails were shot during their premigratory period (March) on the University Campus and were immediately brought to the laboratory. Intestine cleared of its contents and liver pieces were quickly removed and mounted on a chuck of a microtome mounted in cryostat chamber maintained at -20°C. 12 to 15 μ thick sections were cut and processed for histochemical study of the two isoenzymes; viz., intestinal and liver type of alkaline phosphatase. Histochemical method of Rufo and Fishman (1972) with slight modifications was adopted to demonstrate localization of the liver type of alkaline phosphatase reactivity. In the present study Zn^{++} salt ($ZnCl_2$) was used as an inhibitor of the liver type of alkaline phosphatase instead of L-homoarginine (as used by Rufo and Fishman, 1972). Intestinal type of alkaline phosphatase was studied using L-phenylalanine as an inhibitor. The incubation media were prepared as described by Burstone (1962), using Red Violet LB as diazonium salt and Naphthol AS-MX phosphate as the substrate (Sigma Chemical Co., U.S.A.).

Suitable control preparations were made to confirm the authenticity of the enzyme reactivity in the sample material.

OBSERVATIONS AND DISCUSSION

When the fresh frozen sections of the intestine were processed for histochemical localization of the reactivity of the alkaline phosphatase, it was observed that the enzyme reactivity was localized in the brush border and epithelial cells of villi, lamina propria and cells of intestinal glands (Fig. 1). On adding the inhibitor (L-phenylalanine) of the intestinal type of alkaline phosphatase to the incubation medium, the enzyme reactivity was partially inhibited in the brush border and epithelial cells of villi and the cells of intestinal glands (Fig. 2). However, in the lamina propria it was not inhibited (Fig. 2). In another set of experiments, where the inhibitor ($ZnCl_2$) of the liver type of alkaline phosphatase was added to the incubation medium the enzyme activity was inhibited only in the lamina propria but not in the other parts mentioned above (Fig. 3). Fishman et al. (1962), using biochemical method, reported that Zn^{++} inhibits the liver alkaline phosphatase activity. From these experiments, it became obvious

EXPLANATIONS FOR FIGURES

Figs. 1-3. Photomicrographs of sections of intestine of Wagtail showing alkaline phosphatase activity.

Fig. 1. Sections were incubated in medium without inhibitors. Note the enzyme activity in brush border, epithelial cells of the villi, lamina propria and intestinal glands. 125X.

Fig. 2. Sections incubated in medium containing L-phenylalanine. Note inhibition of enzyme activity in brush border, epithelial cells and intestinal glands. 125X.

Fig. 3. Sections incubated in medium containing $ZnCl_2$. Note inhibition of enzyme activity in the lamina propria. 125X.

Figs. 4-6. Photomicrographs of sections of liver of Wagtail showing alkaline phosphatase activity.

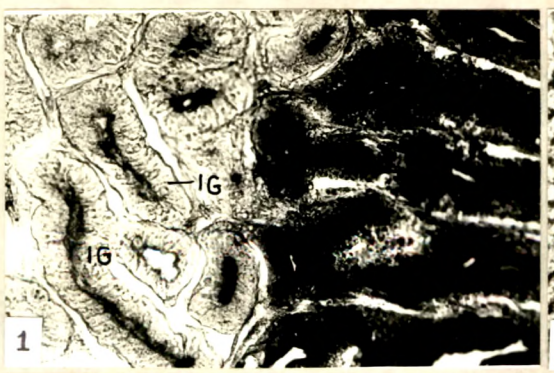
Fig. 4. Not treated with the inhibitors. 125X.

Fig. 5. Sections treated with L-phenylalanine. 125X.

Fig. 6. Sections treated with $ZnCl_2$. 125X.

ABBREVIATION

IG = Intestinal glands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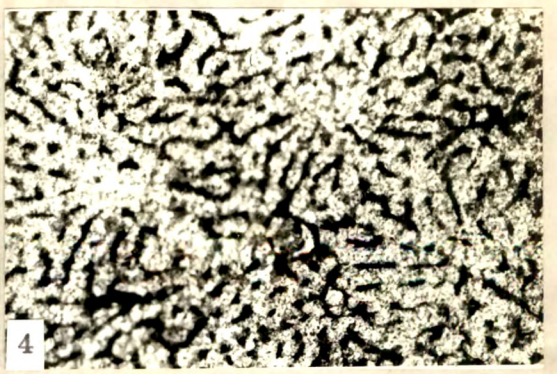
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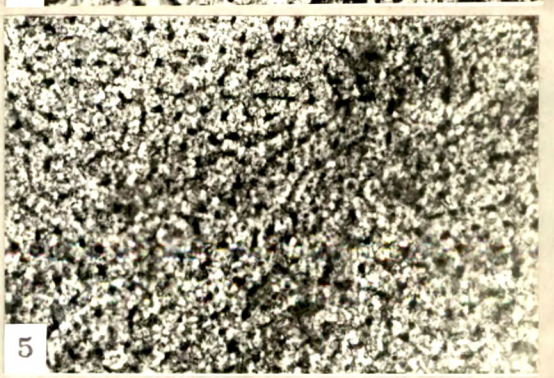
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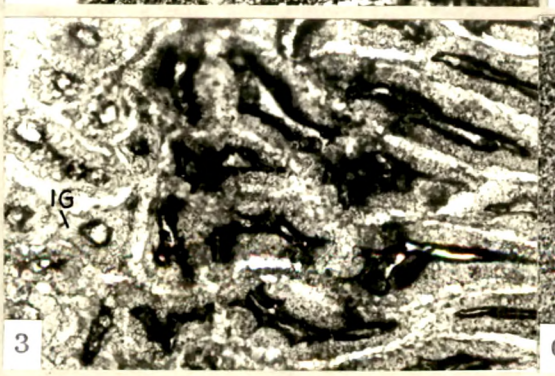
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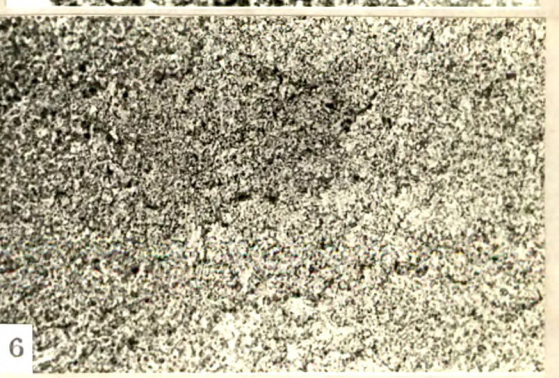
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that the intestinal type of alkaline phosphatase was present in the brush border and epithelial cells of villi and cells of intestinal glands, while the liver type of alkaline phosphatase was present in the lamina propria. The inhibitory action of $ZnCl_2$ on the liver type of alkaline phosphatase was confirmed by studying the histochemical localization of alkaline phosphatase activity in the fresh frozen sections of liver of Wagtail, incubated in the media with and without $ZnCl_2$. It was observed that in the sections incubated in medium without inhibitor ($ZnCl_2$) the enzyme reactivity was localized in the sinusoidal linings (Fig. 4) while it was found to be inhibited on addition of $ZnCl_2$ (Fig. 6). However, when the liver sections were processed in an incubation medium with L-phenylalanine (inhibitor of the intestinal type of alkaline phosphatase), the enzyme reactivity was not inhibited as much as was the case with $ZnCl_2$ treatment (Fig. 5). On the basis of the present study, it could be suggested that the liver type of alkaline phosphatase in the lamina propria could be well involved in the lipid absorption in Wagtail, as it has been reported by Rufo et al. (1973) in the rat intestine.

It is presumed that the liver type of alkaline phosphatase in the intestinal mucosa may be getting more

active during premigratory period when hyperphagia is induced in the bird. Since the thyroid has been known to be implicated in hyperphagia during the premigratory phase, one is tempted to assume that the thyroid hormone may be also influencing the activity of the liver type of alkaline phosphatase. It is possible that some other hormones may also be influencing the activities of these isoenzymes of the alkaline phosphatase. More work on these aspect is necessary and is being planned.