Histoenzymic distribution of phosphatases, dehydrogenases and esterases in ileal peyer’s patches of neonatal buffalo calves

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ABSTRACT

The histoenzymic study was conducted on ileum of neonatal buffalo calves (n=15) and their age was estimated by observing their temporary and permanent dentition. Histoenzymic distribution of different enzymes was studied on ileal peyer’s patches of neonatal buffalo calves. Strong activity of AKPase was observed at the dome and capsule of the peyer’s patch lymphoid follicles at this age. However, moderate Lactic dehydrogenase (LDH) and SDH activity was observed within the ileal lymphoid follicles. A strong activity of Glucose-6-phosphate dehydrogenase enzyme was observed in the follicles at their periphery and within center of few follicles. Also a strong positive activity of diaphorases was observed in peripheral region of the follicle as fine granular reaction. The activity of enzymes has been discussed with the physiological function of the tissue.

Key words: Buffalo, Enzyme histochemistry, Ileum, Neonatal, Peyer’s patch.

INTRODUCTION

To maintain homeostasis with gut microflora and protect body from infections invading via alimentary tube, a defence mechanism in gut is developed in the form of Gut associated lymphoid tissue (GALT). The lymphoid aggregates in ileum are known as Peyer’s patches. These develop as pear shaped lymphoid follicles in ileum at 231 days of fetal life in buffalo (Kapoor and Singh, 2015). There is specific distribution of different enzymes within the ileal lymphoid follicles as well as ileal epithelium according to their functional aspects. Alkaline phosphatase (AKPase) is associated with the ionic exchange across the membrane. It is found abundantly in endothelium of smaller blood vessels and also occurs in the cells specialized for endocytosis and pinocytosis. Glucose-6-Phosphatase (G-6-Pase) is related to carbohydrate metabolism. Succinic dehydrogenase (SDH) is found in all aerobic cells, and so is an essential part of Kreb’s cycle. Diaphorases activities are indicative of mitochondrial activity as well as cytoplasmic electron transport (Singh and Singh, 2014). Localisation of these enzymes in the tissues also serves as biochemical markers for tissue damage. The purpose to carry out this study was to observe the localization of these enzymes in the ileal peyer’s patches of neonatal buffalo calves. The literature regarding histoenzymic studies on the ileal peyer’s patches of neonatal buffalo calves is very scanty, so the present study was undertaken with to localize the distribution of various enzymes in the lymphoid follicles of ileal peyer’s patches in neonatal buffalo calves.

MATERIALS AND METHODS

The present study was conducted on ileum of neonatal buffalo calves (n=15). Their age was estimated by observing their temporary and permanent dentition. Cryostat sections of 10 µm of ileum were cut at -20ºC and incubated with different substrates to study distribution of different enzymes(Table 1) viz; Phosphatases: Alkaline phosphatase (AKPase) and Glucose-6-Phosphatase (G-6-Pase), Non-specific esterase (NSE) and Oxidoreductases: Monoamine oxidase (MAO), Succinic dehydrogenase (SDH), Lactic dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G-6-PD), Nicotinamide adenine dinucleotide diaphorase (NADH-D) and Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-D) (Chayen et al., 1969).

RESULTS AND DISCUSSION

Phosphatases:

i) Alkaline phosphatase: A moderate alkaline phosphatase activity was observed in villous epithelium of ileum just above the lymphoid follicles comprising the peyer’s patches (Figure A, Table 2), the same as observed by Nicander et al. (1991) in sheep and goat foetuses by 90 days of gestation and Raju et al. (2012) in lambs. Strong activity of AKPase was observed specifically at the dome of the peyer’s patch follicles (Figure B, Table 2). Landsverk (1981) reported that alkaline phosphatase activity over the luminal border of the entire follicle was more intense compared to adjacent villi in calves. The follicle-associated epithelium just over the dome of the follicle showed strong activity of alkaline phosphatase as observed earlier by Bjerknes and Cheng.

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In lymphoid follicles of ileum, moving towards its apical part (Figure C, Table 2). Poor activity of this enzyme was observed in the villous epithelium of areas of the lymphoid follicles at few places. Monoamine oxidase (MAO) enzyme was very weak. However, feeble enzyme activity was noticed at the marginal ileal peyer’s patches of neonatal buffalo calves, activity of MAO is an enzyme marker for outer membrane of lymphoid follicle (Figure B). Alkaline phosphatase activity was observed towards the periphery of lymphoid follicles, having moderate alkaline phosphatase activity. Halleraker (1981) in rat, Burns (1982) in domestic fowl and Owen and Bhalla (1983) in rat. Strong activity of this enzyme was observed towards the periphery of lymphoid follicles, having mature and developing B lymphocytes (Figs. A and B). This observation was in accordance with the findings on lymph node and spleen of buffalo by Singh et al. (2009). Intense activity of alkaline phosphatase was observed over the dome especially where lymphocytes move towards villi from the lymphoid follicle (Figure B). Alkaline phosphatase activity is correlated with ionic movements across the epithelium and cell differentiation. Correspondingly it was reported in rats and human fetuses, that the alkaline phosphatase activity increased as maturing epithelial cells migrated up to villi by Nordstrom et al. (1968) and Weiser (1973) respectively. The capsule of the lymphoid follicle was strongly positive for alkaline phosphatase activity. Halleraker et al. (1990) also observed alkaline phosphatase enzyme activity in the follicle capsule in ruminants. However, the center of lymphoid follicle had moderate alkaline phosphatase activity (Figure B) similar to that reported by Raju et al. (2012) in 6 month old sheep.

**Table 1: Histoenzymatic methods used on cryostat sections of ileum of neonatal buffalo calves**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Enzyme</th>
<th>Substrate</th>
<th>Method</th>
<th>Reference</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. Phosphatases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Alkaline phosphatase (AKPase)</td>
<td>Naphthol ASMX phosphate disodium salt in combination with Fast Blue RR</td>
<td>Coupling azo dye method</td>
<td>Chayen et al. (1969)</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td>B. Oxidoreductases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Lactic dehydrogenase (LDH)</td>
<td>Na-DL lactate</td>
<td>Nitro BT method</td>
<td>Chayen et al. (1969)</td>
<td>30 min</td>
</tr>
<tr>
<td>(ii)</td>
<td>Glucose-6-phosphate dehydrogenase (G-6-PD)</td>
<td>Glucose-6-phosphate</td>
<td>Nitro BT method</td>
<td>Chayen et al. (1969)</td>
<td>30 min</td>
</tr>
<tr>
<td>(iii)</td>
<td>Monoamine oxidase (MAO)</td>
<td>Tryptamine and adrenaline</td>
<td>Nitro BT method</td>
<td>Chayen et al. (1969)</td>
<td>60 min</td>
</tr>
<tr>
<td></td>
<td>C. Diaphorases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Nicotinamide adenine dinucleotide diaphorase (NADPH-diaphorase)</td>
<td>Co-enzyme NADPH</td>
<td>Nitro BT method</td>
<td>Chayen et al. (1969)</td>
<td>30 min</td>
</tr>
<tr>
<td>(ii)</td>
<td>Nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase)</td>
<td>Co-enzyme NADH</td>
<td>Nitro BT method</td>
<td>Chayen et al. (1969)</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td>C. Esterases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Non-specific esterase (NSE)</td>
<td>Naphthol acetate</td>
<td>Naphthol acetate method</td>
<td>Chayen et al. (1969)</td>
<td>15 min</td>
</tr>
</tbody>
</table>

Jervis (1963) observed only traces of this enzyme in jejunum of adult rats but in newborns slight enzyme activity in cells of all parts of intestinal epithelium was observed. On the other hand, moderate activity of this enzyme was evident in the crypt area of the villous epithelium in neonatal ileum. MAO is an enzyme marker for outer membrane of mitochondria.

**B) Dehydrogenases:**

i) **Succinate Dehydrogenase (SDH):** A strong activity was noticed in villous epithelium whereas moderate activity was present in cellular contents of lymphoid follicles, especially at their periphery (Figure D, Table 2) and weak to negligible in its center. The activity of SDH was also reduced moving towards the crypt cells and therefore mimicked the enzyme distribution of SDH in adults (Figure D). SDH is a mitochondrial enzyme associated with generation of energy by oxidation reduction reaction in cell. This enzyme might be associated with oxidation of fatty acids that led to formation of lipid pigment. Therefore the localization of enzyme product in the cells of intestinal villous epithelium and periphery of lymphoid follicles giving strong and moderate reactions respectively is consistent with mitochondrial localization of this enzyme. Similar observation was made by Walker and Seligman (1963) in rats.

ii) **Lactate Dehydrogenase (LDH):** A strong activity of this enzyme was observed in villous epithelium of ileum, but slightly more in supranuclear cytoplasm and lesser towards the basal portion of the villi. However, moderate Lactate Dehydrogenase (LDH) activity was observed within the ileal...
A. Photomicrograph of ileum of 1 month old buffalo calf showing weak to moderate AKPase activity in villous (V) epithelium and center of lymphoid follicle (LF) whereas strong activity (arrow) at the dome (D) of lymphoid follicle (LF). Azodye method X100.

B. Photomicrograph showing intense AKPase activity in the dome region (D) and capsule (Ca) and strong activity at periphery of lymphoid follicle (LF) in ileum of 1 month old buffalo calf. Azodye method X400.

C. Photomicrograph showing very weak activity (stars) of Monoamine oxidase (MAO) at the marginal areas of the lymphoid follicles (LFs) of ileal peyer’s patches, in the villous epithelium (V) and moderate activity in the crypt area (arrows) of ileum of neonatal buffalo calves. Nitro BT method X40.

D. Photomicrograph showing strong SDH activity in villous epithelium (V, arrow) and tunica muscularis (TM) and moderate activity in cellular contents of lymphoid follicle (LF) concentrated at the periphery and absent in its center. [Inset: showing lymphoid follicle (star) with granular SDH activity at its periphery]. Nitro BT method X40.

E. Photomicrograph of ileum of 1 month old buffalo calf showing uniformly strong LDH activity in villous epithelium (V) and its moderate activity on the periphery of lymphoid follicles (LFs). Nitro BT method X40.

F. Photomicrograph of ileum of 1 month old buffalo calf showing moderate G-6-PD activity in villous epithelium (V), lamina muscularis mucosae (arrow) and strong activity in lymphoid follicle (LF). Nitro BT method X40.

G. Photomicrograph showing intense activity of G-6-PD in myenteric plexuses (arrows), located in between the circular and longitudinal muscle layers of tunica muscularis (TM), just below the submucosal lymphoid follicles (stars) with moderate activity of this enzyme. Nitro BT method X40.

H. Photomicrograph of ileum of 1 month old buffalo calf showing moderate NADH-diaphorase activity in epithelium of villi (V) and strong activity in lymphoid follicles (LF) and tunica muscularis (TM). Nitro BT method X40.

I. Photomicrograph showing peripheral region of the lymphoid follicle with strong NADH- diaphorase activity as fine granular reaction around the lymphocytes that comprised the lymphoid follicles, Nitro BT method X400.

J. Photomicrograph showing moderate granular reaction of NSE in lymphoid follicles (LF) at their periphery in ileum of 1 month old buffalo calf. [Inset: fine granular reaction of NSE around a macrophage (yellow arrow) and lymphocytes at periphery]. Napthol acetate method X100.
lymphoid follicles comprising the peyer’s patches but was strong towards the periphery (Figure E, Table 2). LDH is a NAD dependent enzyme found in cells involved in glycolytic pathway. The presence of LDH activity suggests its role in differentiation of lymphocytes in foetal life as explained by Fennell and Pearse (1961) in chicken.

### iii) Glucose -6-phosphate dehydrogenase (G-6-PD):

Moderate activity of Glucose -6-phosphate dehydrogenase enzyme was observed in villous epithelium especially in its apical portion. However, strong activity of this enzyme in the form of granular reaction was observed in the follicles at their periphery (mostly) and within center of few follicles (Figure F, Table 2). Moderate activity was observed in lamina muscularis mucosae also. Moreover, an intense activity of this enzyme was observed in myenteric plexus (Figure G), located in between the circular and longitudinal muscle layers i.e., located just below the submucosal lymphoid follicles. Vollrath (1969) also observed a strong uniform reaction in ganglion cells of myenteric plexus of rats in their postnatal life.

### Diaphorases:

i) Nicotinamide Adenine Dinucleotide Diaphorase (NADH- D) and Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-D): A moderate activity of Nicotinamide Adenine Dinucleotide Diaphorase (NADH-D) and NADPH-D was observed in villous epithelium of ileum and strong activity in tunica muscularis (Figure H, Table 2). However, strong activity of NADH- D and NADPH-D was present in peripheral region of the follicle as fine granular reaction, specifically around the circumference of lymphocytes (Figure 1). NADH-D is a coenzyme dehydrogenase and acts in cell as a part of hydrogen transport chain. However, NADPH-D is associated with the metabolic activity of cell.

### Non Specific Esterases (NSE):

Activity of NSE was moderate granular reaction in lymphoid follicles. More intense and strong granular reaction was observed especially towards the periphery of the lymphoid follicles (Figure J, Table 2). NSE group of enzymes are associated with lipid metabolism and the presence of NSE activity in lymphocytes play a role in synthesis of lipids and phospholipids which were utilized for formation of cell membranes of lymphocytes. Fine granular activity at few places within the center and dome area of lymphoid follicle was observed that might be attributed to the presence and activity of macrophages as they react strongly for activity of Non Specific Esterase (Figure J). Similar activity of Non Specific Esterase (NSE) was observed by different researchers in thymic macrophages (Steer and Foot, 1987) as well as intestinal macrophages (Stadnyk et al., 1990) in rats.

### REFERENCES


### Table 2: Histoenzymic distribution in ileum of neonatal buffalo calves

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Villi</th>
<th>Lymphoid Follicle</th>
<th>Dome/Capsule</th>
<th>Crypt Epithelium</th>
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</thead>
<tbody>
<tr>
<td>AKPase</td>
<td>++</td>
<td>+++</td>
<td>++/++</td>
<td>0</td>
</tr>
<tr>
<td>SDH</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>LDH</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>G-6-P-D</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>MAO</td>
<td>0/+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>NADH</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>NADPH</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>NSE</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

0 Not observed; + Weak; ++ Moderate; +++ Strong


