Bulgarian Journal of Veterinary Medicine (2007), **10**, No 2, 83–93

HISTOCHEMICAL STUDY OF ALKALINE PHOSPHATASE ACTIVITY IN THE CHICKEN INTESTINE

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Summary

Sabatakou, O., E. Paraskevakou, S. Tseleni-Balafouta & E. Patsouris, 2007. Histochemical study of alkaline phosphatase activity in the chicken intestine. *Bulg. J. Vet. Med.*, **10**, No 2, 83–93.

The distribution of non-specific alkaline phosphatase (AP) in normal duodenum, rest small intestine and caecum of chicken has been studied by a catalytic histochemistry method. Embryos and chickens ranging between day 11 prior to and day 60 after hatching were used and the findings of AP reaction were correlated with the age. In the small intestine, during foetal life, the AP activity started to be weakly positive to the 11th day of incubation, becoming gradually stronger afterwards and after hatching. As far as in the caecum was concerned, a weak reaction was also observed during foetal life becoming stronger in its base (*Basis ceci*) after hatching. The body (*Corpus ceci*) and the tip (*Apex ceci*) of the caecum presented a slight or moderate reaction up the 21st day and a strong one from the 25th day onwards.

Key words: alkaline phosphatase, chicken, intestine

INTRODUCTION

Phosphatases are enzymes that hydrolyse the phosphate ester bond and are widely distributed in animal and plant tissues. Histochemical demonstration is limited to those phosphatases that liberate orthophosphate. Their enzymatic activity viz. their ability to liberate phosphate acid from certain compound substances is known since the beginning of last century (Burstone, 1962).

Historically, the phosphomonoesterase subgroups have been distinguished according to their pH optima. Out of them the phosphomonoesterases I subgroups or alkaline phosphatases, have a pH optimum activity between 9.0 and 9.6.

There appears to be no evidence in the literature about attempts of revealing histochemically the distribution of alkaline phosphatase (AP) in caecum of chicken before and after hatching.

The aim of this paper was to determine the distribution of AP activity in the developing intestine of the chicken before and after hatching. It deals with its location in small and large intestine, especially in the caecum.

MATERIALS AND METHODS

For this study 128 embryos and broiler chickens (Ross) ranging from 11-day old embryos to 60-day old chickens were used. They were obtained from an environmentally controlled commercial local farm. Samples were taken (a) from 51 embryos ranging in age from 11 to 21 days of incubation and (b) from 77 chicks and chickens ranging in age from day 4 to day 60 at intervals of 7 days. Details for the ages and the number of the samples for each age group are given in Table 1. The animals were sacrificed by decapitation under light anesthesia with ether and the abdominal cavity was immediately opened. The alimentary tract from the cardia of the stomach to the cloaca was removed. The intestine was moved away from the body, then unraveled and freed from mesentery. Segments of duodenum, rest small intestine and caecum (base, body and tip) were fixed in 10% neutral buffered formalin for at least 24 h and were constantly maintained at 4^0C or below. They were then rinsed in tap water, dehydrated with increasing concentrations of ethanol, cleared in xylene, impregnated and embedded in paraplast. The blocks were cut at a thickness of 4 μm (on a Reichert-Jung microtome-mod 1140/ Autocut).

Table 1. Age and number of sampled embryos/chickens

Embryos		Chicks and chickens	
Age (days)	Samples (n)	Age (days)	Samples (n)
11	20	4	1
14	1	7	10
16	10	11	1
18	10	14	10
21	10	21	10
		25	1
		29	10
		33	1
		35	10
		42	10
		46	1
		49	10
		53	1
		60	1

A modified coupling Azo dye method was used for revealing the sites of non-specific AP. This AP technique (Pearse, 1961) previously used on cold formalin, fresh frozen sections or freeze dried paraffin sections, was used on paraplast sections from the small and large intestine. The technique is based on the Azo dye method which was introduced by Menten *et al.* (1944). As a substrate in the present study, a simple organic phosphate e.g. sodium α-naphthyl phosphate was used (1-Naphthylphosphat Natriumsalz Monohydrat – $C_{1O}H_8NaO_4P$-H_2O, Merck 549VV276015). AP hydrolyses α-naphthyl-type substrate and the liberated α-naphthol reacts with the diazonium salt (Fast red TR salt C.I. 37085; GURR microscopy materials - BDH Limited Poole England) producing a brown insoluble azo dye at the site of enzyme activity. Also, some diffusion can be observed. The methodology was as follows: 10–20 mg sodium α-naphthyl phosphate were dissolved in 20 mL 0.1 M Tris buffer (stock solution), pH 10. To this, 20 mg of the stable diazotate of 5-chloro-o-toluidine (Fast Red) were added and stirred well. The resulting solution was filtered onto the slides. The well flooded sections were then incubated at room temperature for 1 h. Afterwards, they were rinsed in tap water for two min, stained by haematoxylin for five min and differentiated in acid-alcohol. Finally, after rinsing in dis-

tilled water and washed in running water for 30 min they were mounted in glycerine (coverslips). The sites of AP activity were stained brown.

RESULTS

Duodenum

At the 11th day of incubation, the AP reaction was either negative or weakly positive in small parts (discontinuous) along the outer edge of the brush border on the sides and towards the tips of the previllous ridges (Table 2). The reaction was stronger at the 16th day and even more at the 18th. At the 21st day of incubation however, a strong AP positive reaction was observed in the brush border of the tips and sides of the villous epithelial cells (Fig. 1). The bases of the villi and the crypts were free of reaction product. The reaction extended into the apical cytoplasm.

After hatching, at all ages, a strong AP reaction was observed along the brush border of the tips, sides and bases of the villous epithelial cells which extented into the apical cytoplasm (Fig. 2 and 3). AP reaction was also observed in the crypts while their deep cells were AP- negative.

Rest small intestine

At the 11th, 16th and 18th day of foetal life either a faint or a slight AP reaction was observed along the outer edge of the brush border in some parts (discontinuous) or it was AP negative (Table 3). At the 21st day, a strong AP reaction was observed along the brush border (tips, sides and bases) of the villous epithelial cells (Fig. 4). There was an extention of the reaction into the apical cytoplasm while the crypts were negative. After hatching (Fig. 5), at all ages the AP reaction was the same as in the duodenum. AP reaction was also observed in the crypts while their deep cells were negative.

Caecum

Base of caecum (Basis ceci): During foetal life there was either a weak AP reaction in some parts or there was no AP reaction in the rest part (Table 4).

After hatching at the 4th day and onwards, a strong AP-positive reaction was observed along the brush border of the epithelial cells of the tips and sides of the villi which was observed as well as at the bases of the villi extending also in the apical cytoplasm of the cells while the crypts were negative. A similar reaction was observed in the oldest animals examined (Fig. 6 and 7).

Body (Corpus ceci) and *tip of caecum (Apex ceci)*: During the 11th, 16th and 18th day of foetal life either a weakly positive or negative AP reaction was observed up to 21 days when a strong AP-positive reaction was displayed along the brush border of the tips and sides of the villi while the crypts were free of any reaction (Table 4).

After hatching, at the 4th day, a slight AP positive reaction was observed. The reaction became somehow stronger or moderate from the 25th day onwards (Fig. 8).

DISCUSSION

In the literature, there are data for AP distribution in the gastrointestinal tract of various animals – both foetal and adult.

After studying the gut of the adult rabbit, rat, pigeon and dog, Lafont & Moretti (1970) assumed that AP activity was not uniform throughout, but varied from animal to animal and that was independent of age and nutritional content.

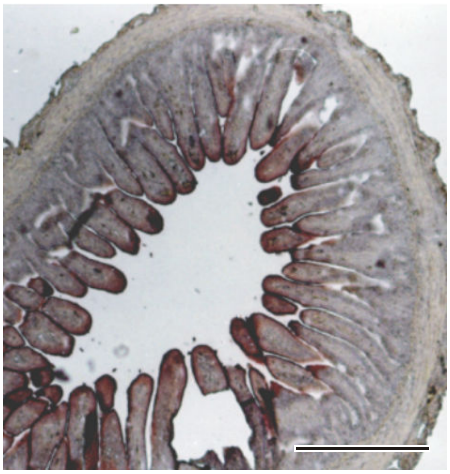

Fig. 1. Duodenum – 21-day-old foetus. Strong AP positive reaction along the brush border of the tips and 2/3 of the sides of the villi. Bases of villi and crypts are negative. Modified coupling Azo dye method for AP; bar = 0.5 mm.

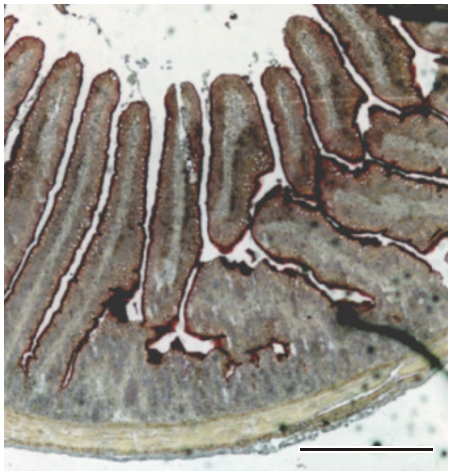

Fig. 2. Duodenum – 7-day-old chicken. Strong AP positive reaction along the brush border. Modified coupling Azo dye method for AP; bar = 1.0 mm.

Fig. 3. Duodenum – 14-day-old chicken. Strong AP positive reaction along the brush border. Cells lining the crypts are weakly positive. Modified coupling Azo dye method for AP; bar = 1.0 mm.

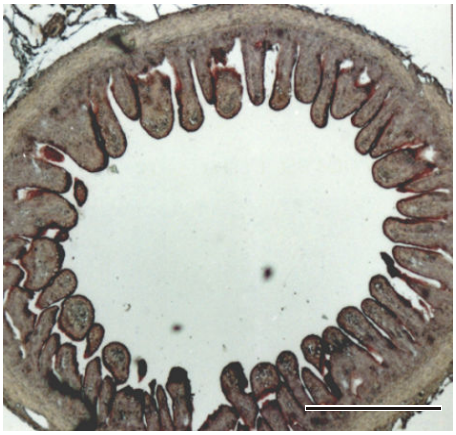

Fig. 4. Rest small intestine – 21-day-old foetus. Strong AP positive reaction along the brush border. Modified coupling Azo dye method for AP; bar = 0.5 mm.

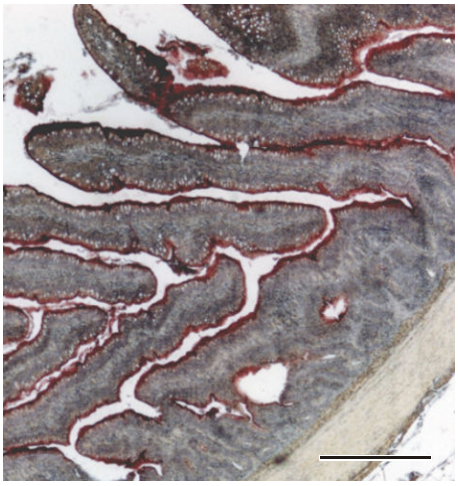

Fig. 5. Rest small intestine – 35-day-old chicken. Strong AP positive reaction along brush border. Modified coupling Azo dye method for AP; bar = 0.3 mm.

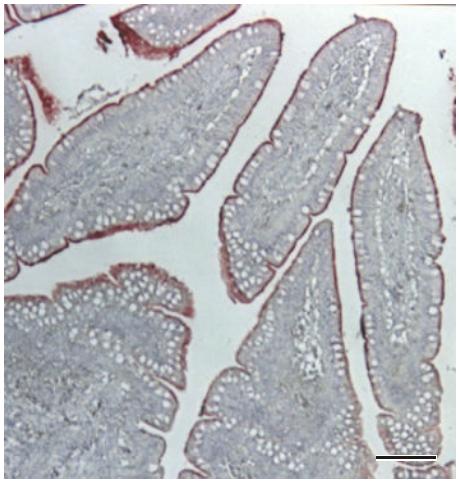

Fig. 6. Caecum (base) – 11-day-old chicken. Strong AP positive reaction product along the brush border of the cells of the villi; Modified coupling Azo dye method for AP bar = 0.3 mm.

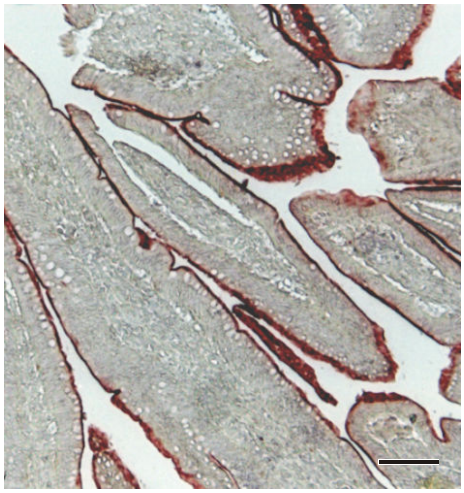

Fig. 7. Caecum (base) – 25-day-old chicken. Strong AP positive reaction product along the brush border of the cells of the villi. Modified coupling Azo dye method for AP; bar = 0.3 mm.

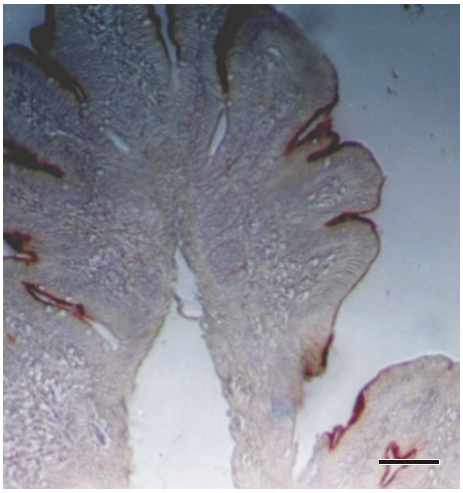

Fig. 8. Caecum (body) – 49-day-old chicken. Relatively strong AP reaction of variable thickness along the brush border of the epithelial cells. Modified coupling Azo dye method for AP; bar = 0.3 mm.

Table 2. Mean AP activity in chicken duodenum based on the intensity of the enzyme's reaction

	Embryos at 11–21 days of incubation					Chicks and chickens at 4–60 days after hatching									
Days of age	11	14	16	18	21	4	7	11	14	21	25	29	33	35	42,46,49,53,60
Villi															
Tip	±	±	+	++	+++	+++	+++ (+)	+++	+++ (+)	+++ (+)	+++	+++ (+)	+++	+++ (+)	+++ (+)
Sides	±	±	+	++	+++	+++	+++ (+)	+++	+++ (+)	+++ (+)	+++	+++ (+)	+++	+++ (+)	+++ (+)
Base	–	–	–	++	±	+++	+++ (+)	+++	+++ (+)	+++ (+)	+++	+++ (+)	+++	+++ (+)	+++ (+)
Crypts	–	–	–	–	–	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Base of crypts							-	-	-	–	–	–	–	–	-

Enzyme's reaction: ± negative or weakly positive, + mild, ++ positive, +++ strong, +++(+) very strong, – no reaction.

Table 3. Mean AP activity in chicken rest small intestine based on the intensity of the enzyme's reaction

	Embryos at 11–21 days of incubation					Chicks and chickens at 4–60 days after hatching									
Days of age	11	14	16	18	21	4	7	11	14	21	25	29	33	35	42,46,49,53,60
Villi															
Tip	±	±	±	± (++)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sides	–	–	–		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Base	–	–	–		++	+++	+++, ++	+++	+++	+++	+++	+++	+++	+++	+++
Crypts	–	–	–	–	–	+	+	+	+, ++	++	++	++	++	++	++
Base of crypts						–	–	–	–	–	–	–	–	–	–

Enzyme's reaction: ± negative or weakly positive, + mild, ++ positive, +++ strong, +++(+) very strong, – no reaction.

Table 4. Mean AP activity in chicken caecum based on the intensity of the enzyme's reaction

	Embryos at 11–21 days of incubation					Chicks and chickens at 4–60 days after hatching									
Days of age	11	14	16	18	21	4	7	11	14	21	25	29	33	35	42,46,49,53,60
Base *(Basis ceci)*															
Villi															
Tip	+	+	+	+	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Sides	–	+	+	–	–	++	+++	+++	+++	+++	+,++	+++	++	+++	+++
Base	–	–	–	–	–	–	+++	+++	+++	+++	++ (+)	+++	+	+++	+++
Crypts	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Body *(Corpus ceci)*															
Villi															
Tip	+	+	+	+	+++, +	++	++	+	+	+	++ (+)	+(+)	++ (+)	++	++
Sides	–	+	+	–	+++	–	++	–	-	+	++	+	++	++	++
Base	–	++ (+)	++ (+)	–	+++	-	++	–	-	+	++	+	++	++	++
Crypts	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tip *(Apex ceci)*															
Villi															
Tip	+	++ (+)	++ (+)	+	+++	+	+	+	+ (+)	+	++ (+)	++(+)	++ (+)	++	++
Sides	–	–	–	–	+++	–	+	–	+	+	+	+	+	++	++
Base	–	–	–	–	++ (+)	+	+	–	+	+	++	++	++	++	++
Crypts	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Enzyme's reaction: ± negative or weakly positive, + mild, ++ positive, +++ strong, +++(+) very strong, – no reaction.

Bourne (1943) found that in the lower part of the duodenal villi and crypts of the adult guinea pig the brush borders of the epithelial cells had a strongly positive bilaminar reaction fading in intensity and disappearing towards the tips of the villi. Also, the epithelial cells of the villi and crypts of the jejunum and rectum presented a positive reaction while the epithelium of Brunner's glands and colon were negative (Bourne, 1943; Deane & Dempsey, 1945).

Konopacka (1959) reported that in pig embryo, AP was highly active during the early stages of intestinal morphogenesis, then became inactive but recovered its activity just before the functional differentiation of intestinal epithelial cells.

The distribution of AP in normal duodenum, jejunum, ileum and large intestinal mucosa was studied in rabbits from the 26th day of foetal life to the 43th day of post natal life (Sabatakou *et al.*, 1999). At all ages studied a strong positive reaction was observed along the brush border of small intestine. In the caecum, during foetal life, no reaction was observed while after birth a discontinuous reaction along the brush border, weak from the 11th day and strong from the 27th day onwards progressed.

The mouse and the rat presented two saltations of phosphatase activity, one culminating at birth and the other 18 days later, when the young animal is ready to be weaned (Moog, 1962). The AP accumulation in the striated border happens during brief critical periods and in fact the development of the border as well as the increase in phosphatase activity are synchronous and apparently inseparable events. Although AP attains tremendously high levels of activity in the duodenum of the mature mouse or rat, the activity falls off sharply in more posterior patrs of the small intestine.

Singh (1975) referred to the histoenzymological demonstration of AP in the intestinal mucosa of three kinds of birds with diverse feeding habits and found that the activity tended to be greater in piscivorous and frugivorous and comparatively less in granivorous.

Moog (1944, 1950) reported that the intestinal epithelium in the chick embryo was devoid of AP activity as late as 8 days then AP accumulates slowly in the duodenum from 9 to 17 days of embryonic life. Moog & Richardson (1955) demonstrated a patchy phosphatase reaction on the surface of tips of villi in 17-days embryos using the Gomori technique. A biochemical-histochemical study revealed that as the chick prepares to be hatched, the duodenal epithelium passes through a critical period of no more than 60 hours in which AP activity rose to a peak above the adult level, becoming concentrated in the brush borders of cells that concomitantly acquired their characteristic columnar form (Moog, 1950). Also, Moog (1962) reported that the enzyme reaction reached its maximal level at 2½ days before hatching. Later, Moog & Glazier (1972) observed that AP was a perfect content of the continuous phase of the microvilli outer membrane. The timing of the critical periods of AP accumulation seemed to be controlled by the pituitary-adrenal axis but the thyroid hormone also played an essential role.

Hinni & Watterson (1963) also demonstrated histochemically AP activity in the embryos' duodenal striated border. The reaction of 18 and 19 day-old embryos was uniform and that of 20 and 21 day-old was very strong. Grey & LeCount (1970), studied the distribution of AP on the villi of the chick duodenum at the age

of 1–4 weeks and observed that AP activity was low or absent in the crypts and highest at the villi tips.

Schussler (1968) reported that intestinal AP from the adult chickens comprised at least four isoenzymes separable by chromatography.

Moreover, Hart & Betz (1972) communicated that generally, the changes of the duodenal AP activity in the chick embryo, were parallel to the morphogenetic pattern of it during the last 3–4 days of development (Moog, 1950; Moog & Richardson, 1955; Hinni & Watterson, 1963; Bellware & Betz, 1970). The final stages of differentiation were reported to depend on certain hormones (Moog & Richardson, 1955; Moog, 1961; Hinni & Watterson, 1963) while Bellware & Betz (1970) proved the role of pituitary gland in the completion of the duodenum development.

The present observations for the duodenum and the rest small intestine during foetal life from the 11th day of incubation revealed a weak discontinuous AP positive reaction in parts along the outer edge of the brush border of the tips and sides of the previllus ridges which was more intense at the 16th day, even more at the 18th day while at the 21st day a strong AP reaction along the brush border of villi and crypts was observed. The reaction was also present in the cytoplasm between the brush border and the nucleus. After hatching, the AP reaction along the brush border of villi and crypts was also strong. The above observations were in agreement with those described by Moog (1950;1962), Hancox & Hyslop (1953), Hinni & Watterson (1963), Hugon & Borgers (1969), Grey & LeCount (1970), Ono (1973), Michael & Hodges (1973), Uni *et al.* (1998) concerning the duodenum and the rest small intestine.

With regard to the caecum, during foetal life a weak AP reaction was observed along the outer edge of the brush border. After hatching, a strong AP reaction was observed in the base (*Basis ceci*) that lasted at all ages. The body (*Corpus ceci*) and tip (*Apex ceci)* of the caecum exhibited a slight or moderate reaction up to the 21st day and a moderate or strong reaction from the 25th day onwards. As far as we know there appears to be no evidence in the literature of attempts of revealing histochemically AP distribution in this part of the intestine.

It must be pointed out that in birds there is an abrupt change in the source of nutrients from the first day after hatch, when the yolk sac, an embryonic parental source of nutrients rich in lipids, is replaced by a carbohydrate-rich solid diet. The yolk sac is consumed until the 2nd week of age, when it is exhausted (Buddington & Diamond, 1989).

There are some suggestions about the physiological role of AP. It was thought earlier to be involved in sugar absorption and later was suggested to play a role in cell adhesion. However, Crane (1968) suggests the possibility of its being a digestive enzyme on the grounds that all the other enzymes found in substantial amounts in the brush border have a digestive function, that the intestinal AP catalyzes the hydrolysis of a wide variety of phosphorylated compounds which are ubiquitous and plentiful in nature and that the phosphate esters do not readily penetrate cell membrane, whereas nonmineral portion usually does and frequently by means of a specific transport process. On this basis it seems that the function of AP is that of a digestive enzyme cleaving a nonpenetrating molecule into transportable componets. Nevertheless, it is difficult to attribute a digestive role to the AP

in the foetus and the suggestion of Deren (1968) that it has a role in differentiation cannot be ignored. It is therefore possible for it to have a dual role viz. that of assisting differentiation on the one hand and participating in digestive activity on the other. In addition, Wieser (1973) and Traber *et al.* (1991) regard that AP expresses the maturity of the absorptive cell.

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Paper received 28.04.2006; accepted for publication 03.05.2007

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