

ISSN 1313-7050 (print) ISSN 1313-3551 (online)

**Original Contribution** 

# EXPRESSION OF TISSUE ALKALINE AND ACID PHOSPHATASES IN THE PARANAL SINUS (SINUS PARANALIS) OF THE DOG

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#### ABSTRACT

The localization of the alkaline and acid phosphatase expressions was studied in the paranal sinus of 14 non - thoroughbred dogs. The expression and determination of acid phosphatase were shown by the Gomori method which involved fixing and staining of the test materials from these animals. Tissue alkaline and acid phosphatase expressions were found in the stratified squamous cornified epithelium, in the apocrine and sebaceous glands, in the stroma of the perianal sinus and in the external anal sphincter of the dog. These results led us to suggest that these enzymes participate in the local homeostasis of the studied organ. Thus, the acid and alkaline phosphatase activities can be used as diagnostic markers in the monitoring of pathological lesions in this region.

Key words: alkaline and acid phosphatase activity, paranal sinus, dog.

## **INTRODUCTION**

The alkaline phosphatase (ALP) is a membrane - bound enzyme, which participates in membrane transport (1) and catalyses the hydrolysis of the organic and inorganic monophosphatic esters, whose activity occurs in alkaline medium, at pH range, 9,8 - 10,5 (2).

This enzyme has an important role apart from the active transport of substrates in the cell, (3).

A lot of authors (4) have studied the ALP participation in the regeneration of the cutaneous structures.

The isoenzymes of ALP are synthesized in the liver, bones, intestines, and placenta (2, 5, 6).

Some cells of the epidermis, called cells of Langerhans type, possess positive reaction for ALP activity; their dendrites penetrate between the cells of stratum spinosum (7). According to some other authors (8, 9), the ALP expression is connected with the sebaceous gland function and is an element for the diagnosis and monitoring of the apocrine gland tumours.

ALP participates in the transport of substrates through the capillaries and in the synthesis of the sebaceous secret (10).

Some other studies find that the increased expression is androgen – determined (11).

ALP localization is observed in human fibroblasts (12). The secretion and transport of calcium is ALP - dependent (13).

ALP participates probably in the pathogenesis of tumours, connected with calcium metabolism and described by in the dog paranal sinus (PS) (14).

Some authors have shown that ALP is associated with the sarcolemma and with the endothelial cells' plasmalemma of the surrounding capillaries (15).

The acid phosphatase (ACP) is a lysosomal marker It catalyses in acid medium at pH 4,5 (16, 17).

The enzyme is highly concentrated in the prostata, bones, blood cells, spleen and the cells of monocyte macrophagial system. This enzyme is localized subcellularly in the

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lysosomes and the cytoplasma. Some isoenzyme forms are known, the prostate one has the biggest importance in the laboratory diagnosis (3).

The localization of ALP and ACP expression is found in some organs of the animals and human (1, 17, 18).

This enzyme is observed not only in membrane - limited organelles; it is also diffusely spread in the cytoplasma (18).

ACP is presented in the intercellular space of stratum granulosum and stratum corneum. The authors suggest that it participates in degradation of the nucleotides and phospholipids (20).

According to some other authors (21) there is ALP and ACP expression in the anal, apocrine and sebaceous glands in the anal canal of the dog. The lipids in stratum corneum are supplied by exocytosis of the lamellar granules in the intercellular space. The lamellar granules contain ACP. The lipids and ACP participate in the function and desquamation of stratum corneum (19).

ACP is associated with the destruction and necrobiosis of the keratinocytes (8).

Increased ACP activity is observed in the changed smooth muscle cells with massive lipid deposition (atheromatosis aortae) of the rabbit. This is connected with the increased number and shape of the lysosomes (22).

In the human adrenal gland cortex there is ACP expression in the lysosomes and secretory vacuoles of the cells from the reticular zone, which is most active in the steroidogenesis (1).

The role of ACP has been shown in the catabolic processes around the glomerular basal membrane of the rats and in the glomerular ultrafiltration (23).

Other authors (24) have observed ACP and ALP expression in paranal sinus (PS) of the dog. The changes with the activity of the tissue acid phosphatase in the prostate gland are used like diagnostic and prognostic indicator for malignant prostate transformation (25, 26).

The decreased level of the tissue alkaline phosphatase is a positive biochemical indicator for disseminated prostate cancer (27).

We made this investigation with the intention to add the knowledge about the enzyme activity of ALP and ACP in PS, and to explain their functional importance in this organ.

## MATERIALS AND METHODS

Paranal sinus of 14 non - thoroughbred dogs (7 male and 7 female, aged 2 to 15 years) were used after the euthanasia of the animals with 500 mg Thiopental (Biochemie, Austria) IV.

Frozen slices with thickness 8  $\mu$ m were fixed in 10 % neutral formalin (for 24h, at 0 -4C°). The alkaline phosphatase activity was demonstrated by Gomori's method (28). Briefly, the slices were translocated in incubating medium for four hours at 37C°, edulcorated with water, treated with 2% cobaltous chloride for 3 - 5 min, edulcorated with distilled water and put in ammonium sulphide till they got a black colour. After that, they were edulcorated with distilled water, dehydrated, cleared and embedded in Entellan (Merck, Darmstadt, Germany).

The presence of the enzyme in the demonstrated was bv black tissues precipitations of cobaltous sulphide. The localization of the acid phosphatase activity was found by Gomori's method. The slices were translocated in incubating medium for four hours at 37C°, edulcorated with distilled water, treated with ammonium sulphide for one minute and embedded in glycerin gelatin. The black - brown sediment of plumbic nitrate was indicator for acid phosphatase activity.

## RESULTS

A high activity of alkaline phosphatase (ALP) was found in the basal (stratum basale) and middle layer (stratum spinosum and granulosum) of the stratified squamous epithelium of PS (Figure 1). In the stroma of the sinus was observed moderate enzyme expression in some cells. In the secretory cells of the apocrine glands was found a considerable alkaline phosphatase activity in the basal parts of the cells and near the basal membranes. The blood vessels of the microcirculatory region around the secretory tubules, demonstrated moderate and high expression of the investigated enzyme. Considerable activity was expressed in the peripheral cells (germinative cells) of the sebaceous glands and in the excretory ductus of PS, moderate and low enzyme activity was observed in the differentiated cells. High alkaline phosphatase expression was found in the endomysium and perimysium of the external anal sphincter.

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Figure 1. High expression of ALP in the basal and middle layer of the stratified squamous cornified epithelium (E), in the basal part of the cells of the apocrine glands (GA), in the blood vessels of the interstitium (arrowheads). Moderate ALP activity in some cells of the propria (PR) of PS (arrows). (Bar=50 $\mu$ m)

High and moderate activity of acid phosphatase (ACP) was expressed in all layers the stratified squamous cornified of epithelium (Figure 2), moderate and low expression was observed in some stromal cells of the sinus, in the cytoplasma of the apocrine gland cells, high expression - in the apical part of the secretory cells, in the lumen of the gland tubules and in the basal membranes (Figure 3). Low enzyme reaction was found in the differentiated cells of the mast glands, and in the peripheral ones (germinative cells) high activity. Intensive red - brown coloured granules were observed in the muscle cells, and diffuse brown coloration - in the sarcoplasma, and in the peri and endomysium.



Figute 2. High to moderate and activity in all layers of the stratified squamous cornified epithelium (arrowheads). Moderate to low expression in some cells of the propria (PR) of PS (arrow). (Bar= $50 \mu m$ )



**Figure 3.** Moderate to low ACD activity in the cytoplasma of the cells of the apocrine glands, High expression – in the apical parts of the secretory cells (arrowheads), in lumen of the glandular tubules and in the basal membranes (arrows). IC - interstitium. PR - propria. (Bar=50µm)

## DISCUSSION

We observed low expression of ALP in the apical parts of the cylindrical apocrine cells and high one in their basal parts, compared with ALP activity, observed by (18, 24) in the myoepithelial cells and the basal membranes in the secretory cells of PS of the dog. Sebaceous glands and their secretions demonstrated localization of ALP activity, compared with other investigations (24). The localization of ALP expression, observed by us in the stratified squamous epithelium in PS of the dog confirms other results about the enzyme expression in the epidermis (7, 9).

The localization of ALP activity, investigated by us in the PS apocrine glands of the dog, resemble the affirmations of (8, 9) about increased enzyme activity of ALP in the plasmalemma of the apocrine gland secretory cells and in the myoepithelial cells around them. The intensive ALP reactivity in the stroma of PS corresponds with the observed enzyme activity in the endothelial cells and the capillaries of the rats, in the cutaneous capillaries of primates (8, 9).

The cells of the sebaceous glands demonstrated positive reaction for ALP, as the cells in the cutaneous multiacinoses glands of the primates and in the sebaceous glands of the human (4, 9).

The low expression of ALP, observed by us in the endo - and perimysium of the external anal sphincter confirms the investigations of (15).

The localization of ACP activity, studied by us in PS of the dog, approaches the results of (19, 24) and, compared with them,

we described the ACP localization in the cells of the stratified squamous epithelium.

High ACP activity was found in the cytoplasma of the epidermal cells, in line with some earlier results (20).

The ACP localization in the stratified epithelium of PS makes us suggest its participation in the process of cornification, in line with some earlier result of another author (19).

Moderate and low enzyme expression was found in the stroma of the sinus. That could be connected with the observed localization of ACP activity in the fibroblasts of the human (12).

The apical expression of ACP in the cytoplasma of the cells in the apocrine glands and in the lumen of the glandular tubules could be associated with their secretion, which corresponds with the investigations of (5). The enzyme activity of ACP, observed by us in the basal membranes, was not confirmed by an earlier work (24). The ACP localization probably is connected with the transport of substrates through the basal membranes, which are necessary for the function of the glands, and that is confirmed by (1). In the same time we suggest that ACP in the apocrine glands of this organ is associated with the tumourigenesis, as its isoenzyme in the prostate gland (25). In the cytoplasma of the sebaceous gland cell in PS, we observed expression, which ACP fortifies the knowledge about the enzyme activity in the cutaneous sebaceous glands of the rat (17). The enzyme probably is responsible for the cholocrine secretion of these glands (10).

The results of (6) about the ALP and ACP expression in the skin motivate us to consider that the basic role of ALP in PS is connected with the transport of chemical substances, which are necessary for the normal function of different structures in this organ, while ACP participates in the destruction of phospholipids, in the necrobiosis of the keratinocytes, in the secretion of sebaceous and apocrine glands in PS. Probably these enzymes can be used as diagnostic and prognostic markers in case of pathologic processes in PS.

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