Haryana Vet. **50** (December, 2011), pp 5-8

Research Article

ULTRASTRUCTURAL LOCALIZATION OF PEROXIDASE, ALKALINE PHOSPHATASE AND ACID PHOSPHATASE IN THE BLOOD CELLS OF CAMEL AND BUFFALO

PARDEEP KUMAR YADAV and GURDIAL SINGH*
Department of Veterinary Anatomy, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar-125 004

ABSTRACT

The alkaline phosphatase activity was more in neutrophils of camel while it was more in eosinophils in buffalo. The acid phosphatase activity was confined to neutrophils in buffalo while in camel, the eosinophils also exhibited positive reactivity. The peroxidase activity was better seen in eosinophils as compared to neutrophils of both camel and buffalo.

Key words: TEM, peroxidase, alkaline phosphatase, acid phosphatase, leucocytes, buffalo, camel

The metabolic pathways are catalyzed and regulated by various enzymes within leucocytes. It has been shown that the metabolism of cells is altered due to disease, dietary manipulations and stressful situations. It is further believed that distribution of liposomal enzymes probably reflects the functional status of individual cells. Investigation of enzyme activity and cytochemistry helps not only in diagnosing and differentiating many diseases especially leukemia's but also aid in differentiating cell types of the blood.

MATERIALS AND METHODS

The present study was conducted on the leucocytes of three healthy buffalo calves and three camels. The blood from each animal was collected from the jugular vein in siliconized test tubes with ethylene diamine tetra acetate (EDTA) as an anticoagulant. From each animal, a total of 20ml blood was collected in equal amounts in two test tubes. Each test tube was centrifuged at 3000 rpm for 30 minutes. The buffy coat along with plasma was collected from each test tube and mixed. The test tube was again centrifuged at 3000 rpm for 30 minutes. The plasma was then expirated and subsequently drop by drop the fixative i.e. 1.5% glutaraldehyde in 0.1M sodium cacodylate - HCl buffer (pH 7.4) was added in test tube. The tube was allowed to stand for 30 minutes at 4^0C. After fixation, the buffy coat pallet was removed carefully and cut into pieces. These pieces were then processed and stained for localization of peroxdiase (Bentfeld *et al.,* 1976), alkaline phosphatase (Borgers *et al.*, 1978) and acid phosphatase (Bainton and Farquhar, 1968b).

After incubating, the pieces of pellets were fixed in 1% osmium tetraoxide for one hour at 4^0C, and dehydrated in acetone at 4^0C. After dehydration, the infiltration was carried out in one part absolute acetone + one part embedding medium for 2 hours, followed by embedding in embedding medium (Araldite Cy 212 and Epon 812) using beam capsule. The capsule was kept in an oven at 60^0C for 24 hours for polymerization and block making. Ultrathin sections were then cut and these were viewed as such or counterstained (if required) with uranyl acetate or lead citrate or both as per the procedure and then viewed under the transmission electron microscope (CM-10 Philips).

RESULTS AND DISCUSSION

Peroxidase Activity: The peroxidase activity was the best visualised within the eosinophils of camel. The activity was confined to electron dense centrum usually placed along longitudinal axis of the granule within the cell (Fig. 1). Rest of the granule was empty. However, the activity was quite reduced and less electron opaque around the electron dense centrum in a few granules (Fig. 1). Few granules exhibited a uniformly less dense

*Corresponding author: gurdivya@hotmail.com

activity throughout (Fig. 1), and such granules were few in number. The activity was less marked in the neutrophils as compared to eosinophils. Few granules exhibited positive reactivity which was confined towards the periphery of the granules and gave a dotted granular appearance.

In buffalo, the activity was more marked in the eosinophils. Few granules within the eosinophils were homogenously stained while majority of the granules were capped with electron dense area towards one of the end of the granule while rest of the granule was less electron dense. The reactivity was seen differently in different granules within the neutrophils (Fig. 2). Some of the granules were uniformly electron dense while a few exhibited less electron dense activity. Some granules although uniformly stained but had electron lucent periphery and some completely empty granules were also observed.

The reactivity of peroxidase was, however, much more in the eosinophil granules as compared to neutrophils. In camel eosinophils, the reactivity was more concentrated in the electron dense centrum as compared to whole of the granule. Dunn *et al.* (1968) in rabbit, Bujak and Root (1974) and Wever *et al.* (1980) in human being, Bentfeld *et al.* (1976) in rat, Singh *et al.* (1997b) in camel and Singh (2000) in buffalo also reported the presence of peroxidase in the eosinophils. However, Bujak and Root (1974) observed that the eosinophil peroxidase was genetically and biochemically distinct from neutrophil peroxidase, lactoperoxidase and intestinal peroxidase. Considering the higher number of eosinophils in camel (Singh *et al.,* 1997c), it appears that higher concentration of peroxidase in eosinophils reflects their role in bactericidal activity, in addition to their role in allergic conditions. Facklam and Kociba (1985) stated that peroxidase activity was more intense in granulocytes and further reported that peroxidase activity was negative in different type of leukemias in dog. Maxwell (1986) reported that eosinophils of Japanese quail were devoid of peroxidase activity.

The peroxidase activity seen in the platelets of camel and buffalo blood was in conformity with that of human platelets (Breton-Gorius and Josette-Guichard, 1972). Electron dense bodies which were evident in the buffalo platelets had also been reported by these authors. However, the reported presence of peroxidase in dense tubular system by these authors was not seen, probably due to the fact that such structures were rarely seen as reported by Singh *et al.* (1997a) in camel and Singh (2000) in buffalo.

In this study few granules of the neutrophils exhibited peroxidase activity. This finding is in agreement with those of Bainton and Farquhar (1968 a,b), Bainton *et al.* (1971), Bretz and Baggiolini (1973 and 1974), Bentfeld *et al.* (1976), Singh *et al.* (1997b) and Singh (2000). These authors also reported peroxidase activity in blood of different species. These granules being fewer in number can be classified as the azurophil (primary) granules as has been reported by the earlier authors.

Alkaline Phosphatase: The alkaline phosphatase activity within the neutrophils of camel was more prominent in circular as well as pleomorphic granules (Fig. 3). The round granules had more electron density as compared to elongated type of granules; however, few granules were devoid of any activity. The common type of eosinophils encountered did not exhibit any activity, however, within the other type of eosinophils, slight activity was observed

In buffalo, the eosinophils contained highly positive granules. Most of the granules had electron dense central portion surrounded by slightly less electron dense material and few were electron lucent. Within the neutrophils the alkaline phosphatase activity was either confined to the peripheral portion of the granules or these were homogenous in nature.

The alkaline phosphatase activity was more prominent in the neutrophils of camel and in the majority of the granules indicating thereby that it is localized in the secondary granules as has also been reported by Wetzel *et al.* (1967) in rabbit, Bainton *et al.* (1971) in human being and Jain (1986) in different animals species. The activity in the eosinophils was restricted to a type of eosinophils which usually are less in number as reported by Singh *et al.* (1997a) in camel.

Contrary to camel blood, the granules of the eosinophils were more reactive as compared to neutrophils in buffalo. Such variation in the localization

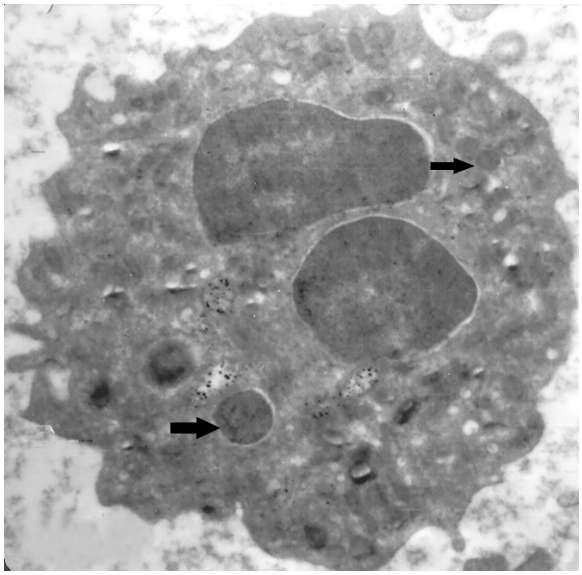

Fig 1. Electron photomicrograph of an eosinophil of camel showing granules with electron dense activity in the center of the granule along its long axis and rest of the granule is devoid of any reactivity (straight arrow), another granule showing electron dense activity surrounded by milder electron dense reactivity (curved arrow)) and a third type of granule having uniformly less electron dense activity (arrow head).
(Peroxidase x 3,050)

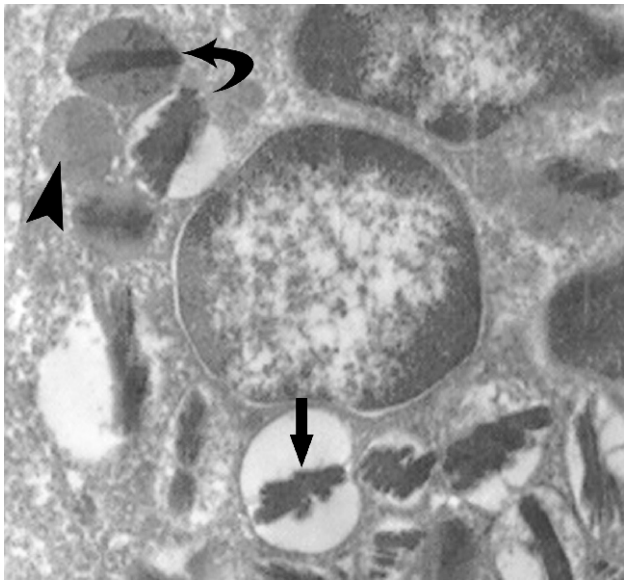

Fig 2. Electron photomicrograph of a neutrophil of buffalo showing few circular granules with electron dense reactivity (arrow head), some granules had mild reactivity (curved arrow), some were completely devoid of activity (double curved arrow) and few granules uniformly stained with electron lucent periphery (straight arrow).
(Peroxidase x 4,200)

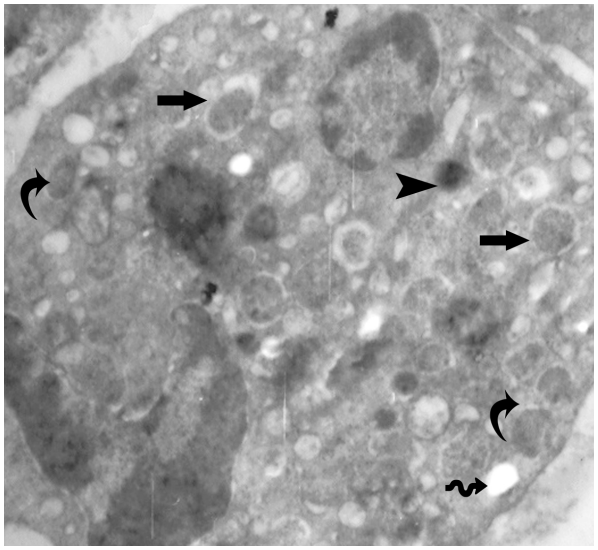

Fig 3. Electron photomicrograph of a neutrophil of camel showing circular granules with more electron density (curved arrow) as compared to pleomorphic granules (straight arrow) which showed mild activity.
(Alkaline phosphatase x 2,900)

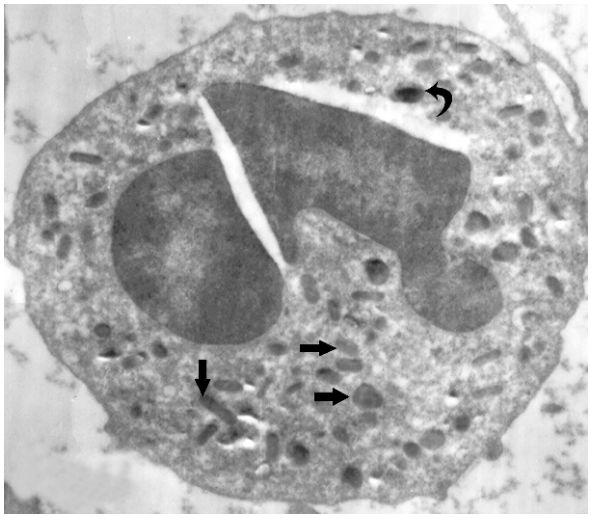

Fig 4. Electron photomicrograph of a neutrophil of camel showing few granules having mild and uniform activity (straight arrow). (Acid phosphatase x 4,200)

of alkaline phosphatase had also been reported by Jain (1986) in different domestic animals. As far as the platelets were concerned, camel platelets exhibited more reactivity as compared to the buffalo platelets and the electron dense bodies were, however, observed in both the animals.

Acid Phosphatase: The camel neutrophils showed acid phosphatase activity in some of the granules which had a uniform material within them (Fig. 4). Within the eosinophils, the central portion of the granules exhibited positive reactive material which was placed along the long axis while rest of the portion of granules did not show any reactivity. In buffalo, the eosinophils did not show any reactivity for acid phosphatase, while in the neutrophils, few granules circular in outline, exhibited positive reactivity. In some granules electron dense material was localized toward the periphery of the granules.

The acid phosphatase activity was observed in few granules of neutrophils of camel and buffalo indicating that this enzyme was present in the azurophilic granules as has also been reported by Wetzel *et al.* (1967) and Bainton and Farquhar (1968 a,b). Facklam and Kociba (1985) reported that total leukocytic acid phosphatase activity was markedly decreased in variety of hematologic diseases.

In consonance with the finding of Wetzel *et al.* (1967) in rabbit, platelets of buffalo and camel exhibited dot matrix like material within the granules and electron dense bodies with halo around them were only observed in buffalo platelets. It is felt that enzymic localization of various enzymes in the blood cells will help in precise cellular characterization, accurate identification of leukemic cells and clinical characterization of various leukemias.

REFERENCES

Bainton, D.F., Joan, Ullyot L. and Farquhar, M.G. (1971). The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. *J. Exp. Med.* **134**: 907-933.

Bainton, D.F. and Farquhar, M.G. (1968a). Differences in enzyme context of azurophil and specific granules of polymorphonuclear leukocytes. I. Histochemical staining of bone marrow smears. *J. Cell Biol.* **39**: 286-298.

Bainton, D.F. and Farquhar, M.G. (1968b). Differences in enzyme context of azurophil and specific granules of polymorphonuclear leukocytes. II. Cytochemistry and electron microscopy of bone marrow cells. *J. Cell Biol.* **39**: 299-317.

Bentfeld, M.E., Nichols, B.A. and Bainton, D.F. (1976). Ultrastructural localization of peroxidase in leukocytes of rat bone marrow and blood. *Anat. Rec.* **187**: 219-240.

Borgers, M., Thone, F., De Cree, J. and De Cock, W. (1978). Alkaline phosphatase activity in human polymorphonuclear leukocytes. *J. Histochem.* **10**: 31-43.

Breton-Gorius, J. and Josette Guichard, B.S. (1972). Ultrastructural localiization of peroxidase activity in human platelets and megakaryocytes. *Am. J. Pathol.* **66**: 277-286.

Bretz, U. and Baggiolini, M. (1973). Association of the alkaline phosphatase of rabbit polymorphonuclear leukocytes with the membrane of the specific granules. *J. Cell Biol.* **59**: 696-707.

Bretz, U. and Baggiolini, M. (1974). Biochemical and morphological characterization of azurophil and specific granules of human neutrophils polymophonuclear leucocytes. *J. Cell Biol.* **63**: 251-269.

Bujak, J.S. and Root, R.K. (1974). The role of peroxidase in the bactericidal activity of human blood eosinophils. *Blood* **43**: 727-736.

Dunn, W.B., Hardin, J.H. and Spicer, S.S. (1968). Ultrastructural localization of myeloperoxidase in human neutrophil and rabbit heterophil and eosinophil leucocytes. *Blood* **32**: 935-944.

Facklam, N.R. and Kociba, G.J. (1985). Cytochemical characterization of leukemic cells from 20 dogs. *Vet. Pathol.* **22**: 363-369.

Jain, N.C. (1986). Schlm's Veterinary Hematology. (4th edn.), Lee and Febiger, Philadelphia.

Maxwell, M.H. (1986). Ultrastructural and cytochemical studies in normal Japanese quail (*Coturnix coturnix japonica*) eosinophils and in those from birds with experimentally induced eosinophilia. *Res. Vet. Sci.* **41**: 149-161.

Singh, G., Singh, Y. and Nagpal, S.K. (1997a). Ultrastructure of formed elements of blood of camel (*Camelus dromedarius*) Part -I, II and III. *J. Camel Pract. Res.* **4**: 1-12.

Singh, G., Singh, Y. and Nagpal, S.K. (1997b). Cytoenzymic studies on the blood cells of camel (*Camelus dromedarius*). *J. Camel Pract. Res.* **4**: 15-17.

Singh, G., Singh, Y. and Nagpal, S.K. (1997c). Cytomophological studies on the blood cells of camel (*Camelus dromedarius*). *Indian J. Anim. Sci.* **67**: 499-500.

Singh, I. (2000). Light and ultrastructural studies on the blood cells of normal and dexamethasone treated buffalo calves. Ph.D. thesis, CCS HAU, Hisar, Haryana.

Wetzel, B.K., Spicer, S.S. and Hom, R.G. (1967). Fine structural localization of acid and alkaline phosphatase in cells of rabbit blood and bone marrow. *J. Histochem. Cytochem.* **15**: 311-334.

Wever, R., Hamers, M.N., Weening, R.S. and Roos, D. (1980). Characterization of the peroxidase in human eosinophils. *European J. Biochem.* **108**: 491-495.