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LOCALISATION OF ESTRASE IN THE BLOOD CELLS OF CAMEL AND BUFFALO AT ULTRASTRUCTURAL LEVEL – PART I

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ABSTRACT

The present study was conducted to localise ultrastructurally different enzymes in the leucocytes of buffalo and camel. The blood from each animal was collected from the jugular vein in siliconised test tubes with EDTA (Ethylene diamine tetra acetate) as an anticoagulant. The blood samples were then processed and stained for Non–Specific Esterase, Arylsulfatase C & Arylsulfatase B. The non-specific esterase activity using alpha-naphthyl acetate restricted mainly in the lymphocytes in the form of large globules in camel and buffalo blood while the activity with reference to alpha-naphthyl butyrate in addition to lymphocytes was also seen in few neutrophils and eosinophils of camel. Arylsulfatase B and Arylsulfatase C reactivity was more in eosinophils of camel as compared to neutrophils. Within the neutrophils the reactivity of arylsulfatase C was seen within the granules but for arylsulfatase B it was distributed within the cytoplasm and over the nuclear membrane. Few basophils of buffalo also exhibited positive reactivity for arylsulfatase B and arylsulfatase C.

Key words: Arylsulfatase B, Arylsulfatase C, buffalo and camel, leucocytes, non–specific esterase, transmission electron microscopy

Blood is a specialised, circulating, connective tissue, which plays a pivotal role in not only maintaining the homeostasis, but also in transporting oxygen and nutrients to cells and tissues, removal of metabolic waste products, maintenance of fluid volume, regulation of heat by variation of fluid volume and transportation of hormones for regulation of various functions. It thus helps in maintaining the constant environment for the optimum functioning of the cells and tissues.

Different enzymes, have diversified role like bactericidal or lytic in action and hence these blood cells tend to respond differently with different enzymes depending upon their status. It has also been seen that cytochemical staining for neutrophilic and monocytic markers provides helpful information to ascertain lines of cellular differentiation particularly in acute leukemia. Investigation of enzymes activity and cytochemistry therefore not only helps in diagnosing and differentiating many diseases, especially leukemia's but also aid in differentiating cell types of the blood.

Materials & Methods

The present study was conducted on the leucocytes and thrombocytes of three healthy buffalo calves and three camels. The blood from each animal was collected from the jugular vein in siliconised test tubes with EDTA (Ethylene diamine tetra acetate) 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 fixatives i.e. 2% para formaldehyde, 2.5% glutaraldehyde, 0.025% calcium chloride 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°C. After fixation the buffy coat pallet was removed carefully and cut into pieces. These pieces were then processed and stained for localisation of non-specific esterase using Alpha-naphthyl butyrate and Alpha-naphthyl acetate as a substrate (Monahan *et al,* 1981).

Non–Specific Esterase (Alpha-naphthyl butyrate)

1. For localisation of non-specific esterase with alpha-naphthyl butyrate as substrate the pieces of pallets

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were washed three times in phosphate buffer saline (pH 7.2).

2. The pieces were then incubated for 15 minutes at 20°C in the mixture composed of 10ml. (0.005 M final concentration) alpha-naphthyl butyrate (Sigma) dissolved in 0.5ml ethylene glycol monomethyl ether; 1ml 2% hexazotised pararosaniline, 3ml 0.5M phosphate buffer (pH 7.0) and a final solution adjusted to 10ml with distilled water for 5 minutes at 20°C. The pieces after incubation were washed three times in phosphate buffer saline (pH 7.2).
3. These were then put in a dilute Karnovsky's fixative for one hour at 20°C and then again washed in phosphate buffer saline (pH 7.2) and stored at 4°C.

Non–Specific Esterase (Alpha-naphthyl acetate)

1. For localisation of non-specific esterase with alpha-naphthyl acetate as substrate the pieces of pallets were washed three times in phosphate buffer saline (pH 7.2)
2. The pieces were then incubated for 15 minutes at 20°C in the mixture composed of 2.5mg (0.2M final concentration) alpha naphthyl acetate (Sigma) dissolved in 0.25ml acetone, 1 ml 2% hexazotised pararosaniline, 0.88ml 0.5M phosphate buffer and 10ml phosphate buffer saline (pH7.2). The pieces after incubation were washed three times in phosphate buffer saline (pH 7.2).
3. These were then put in a dilute Karnovsky's fixative for one hour at 20°C and again washed in phosphate buffer saline (pH 7.2) and stored at 4°C.

After incubating the pieces of pallets for the enzyme using both the substrates these were post fixed in 1% osmium tetraoxide for one hour at 4°C. This was followed by dehydration in acetone at 4°C. 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 Cy212 and Epon 812) using beam capsule and the capsule was kept in an oven at 60°C for 24 hours for polymerisation and block making. The semithin sections were prepared for the light microscopy and the area was marked. 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

Non-specific esterase: (Alpha-naphthyl butyrate as a substrate)

The alpha-naphthyl butyrate activity in the camel was seen to be localised in few lymphocytes. The reaction product was in the form of uniformly electron dense granules (Fig 1), which were placed around the nucleus at different places. However, close to the granules, intracytoplasmic activity was also seen (Fig 1). Although, the monocytes did not exhibit any globular reactivity yet their cytoplasm was more densly stained as compared to that of lymphocytes, and few granules with mild reactivity were also seen. Large number of small sized vesicles within the monocytes also exhibited reactivity on their membrane. The neutrophils also exhibited alpha-naphthyl butyrate activity within their granules (Fig 2) and very rarely an eosinophil with some activity was also seen (Fig 3). The few granules within the neutrophils, which were circular in outline, showed a very mild reactivity, whereas rest of the circular and pleomorphic granules exhibited intense electron dense reactivity within them. Within the eosinophils the electron dense material was seen in the centre of the granule (Fig 3).

In the buffalo calf the reactivity was seen only in lymphocytes (Fig 4). The membrane bound granules had a dot matrix like appearance. These granules were usually concentrated towards one pole of the cell.

Non-specific esterase (Alpha-naphthyl acetate as a substrate)

The alpha-naphthyl acetate esterase activity in camel blood was seen to be localised only in the lymphocytes. In few lymphocytes large number of granules were arranged along the periphery of the nucleus and were uniformly electron dense. In some of the lymphocytes few granules were seen in which the reaction product was uniformly and homogenously distributed with a peripheral area devoid of any reactivity (Fig 5). Such granules were usually seen towards one pole of the indented nucleus. Unlike, alpha naphthyl butyrate, intracytoplasmic and cell membrane reactivity was not seen.

The lymphocytes in the case of buffalo calf had positive granules which were homogenous in nature (Fig 6) and were concentrated at one pole of the nucleus. The number of granules were relatively less in buffalo calf as compared to camel for both the substrates used for localising non-specific esterase.

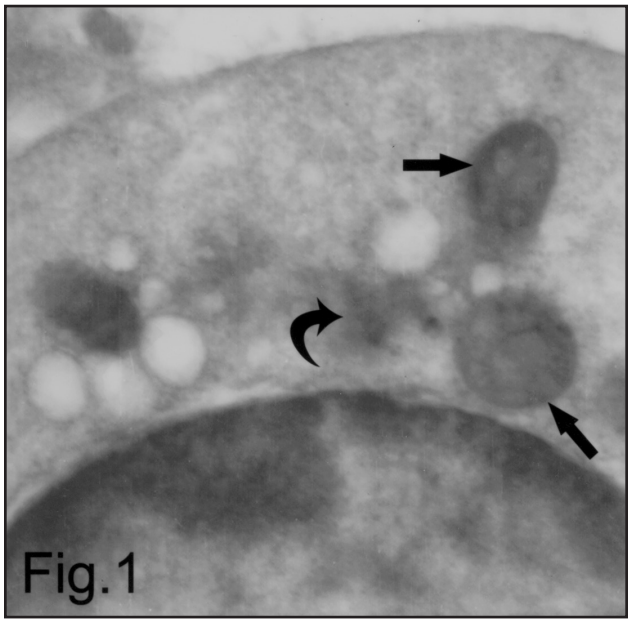

Fig 1. Electron photomicrograph of a lymphocyte of camel showing uniformly electron dense granules (straight arrow) around nucleus and intracytoplasmic activity (curved arrow). Alpha-naphthyl butyrate X 10,500.

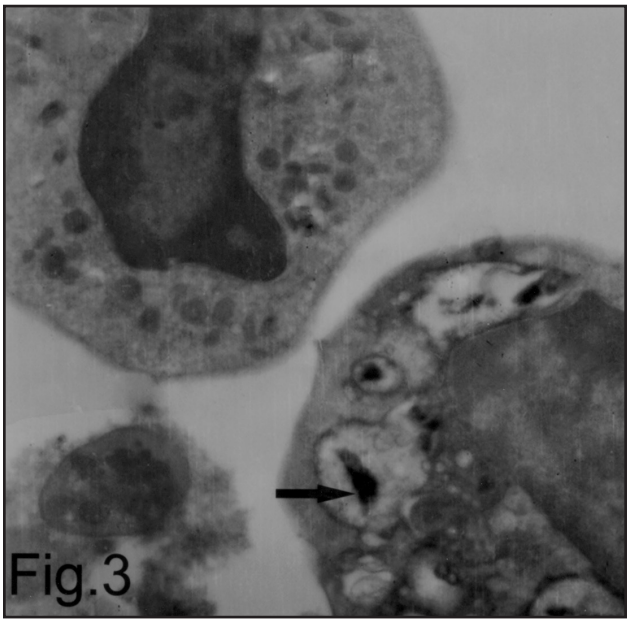

Fig 3. Electron photomicrograph of a eosinophil of camel showing electron dense material (arrow) within the granules. Alpha-naphthyl butyrate X 3,400.

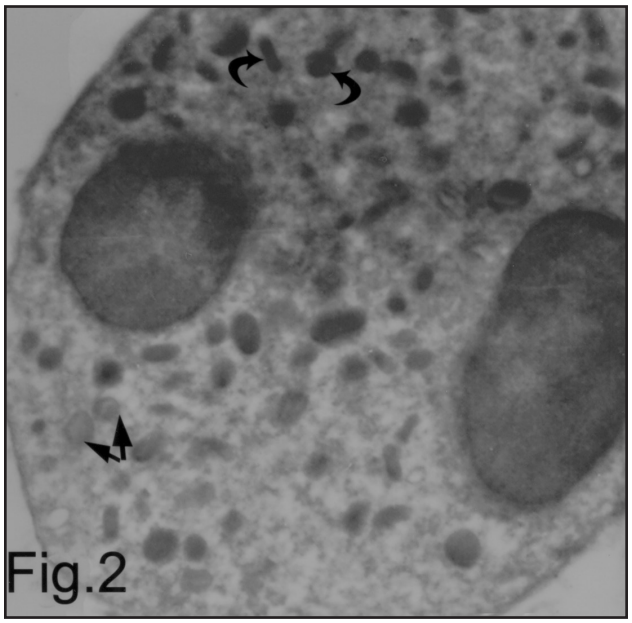

Fig 2. Electron photomicrograph of a neutrophil of camel showing few large circular granules (straight arrow) with mild activity and rest of the circular and pleomorphic granules (curved arrow) exhibited strong reactivity Alpha-naphthyl butyrate X 4,200.

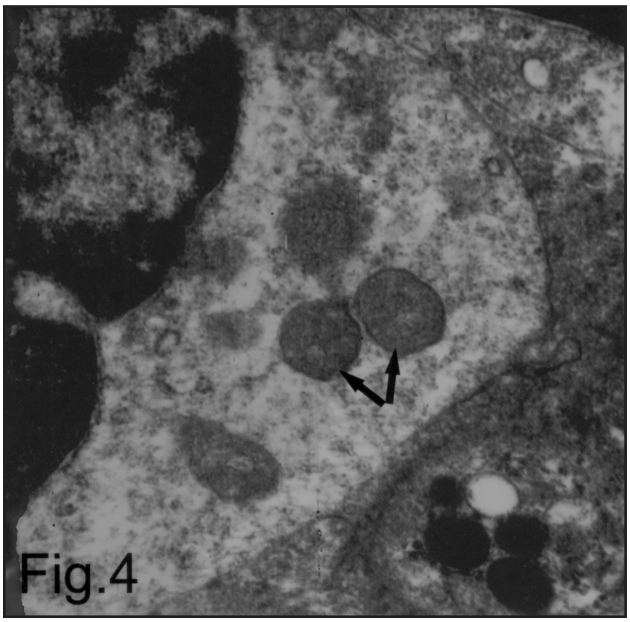

Fig 4. Electron photomicrograph of a lymphocyte of buffalo calf showing membrane bound granules (arrow) with dot matrix like appearance. Alpha-naphthyl butyrate X 8,200.

In the present study two substrates i.e. alpha-naphthyl butyrate and alpha-naphthyl acetate were used to study the localisation of non-specific esterase. Osbaldiston *et al* (1978) while using alpha-naphthyl acetate reported that the reaction product was seen in lymphocytes, neutrophils and eosinophils in the blood of human beings, rat, cat, dog, rabbit, guinea pig, hamster, sheep, goat and pig. However, Yoruk *et al* (1998) also, by using alpha-naphthyl acetate in van cats observed that 1-3 granules were present in lymphocytes while eosinophils and monocytes exhibited strong diffuse activity and the neutrophils

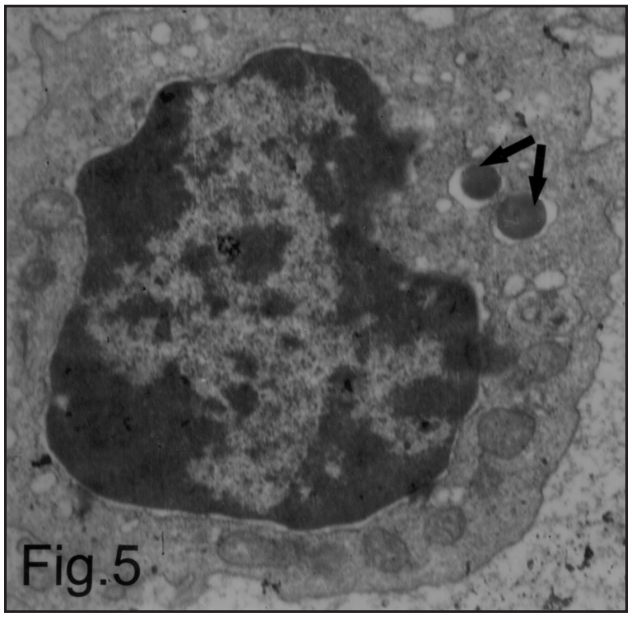

Fig 5. Electron photomicrograph of a lymphocyte of camel showing granules with homogenous type of electron dense material surrounded by rim of no activity (arrow). Alpha-naphthyl acetate X 4,600.

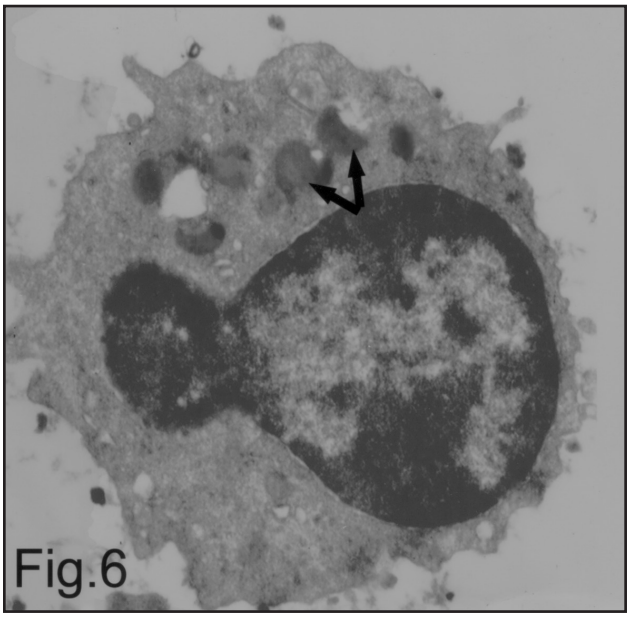

Fig 6. Electron photomicrograph of a lymphocyte of buffalo calf showing electron dense granules (arrow). Alpha-naphthyl acetate X 4,200.

were completely devoid of any activity. In the present study while using alpha-naphthyl acetate it was observed that the reaction was limited only within the lymphocytes whereas the reactivity for alpha-naphthyl butyrate was seen in lymphocytes as well as in neutrophils. However, very few granules in eosinophils of camel also exhibited reaction. Within the neutrophils the reaction product was seen more in the pleomorphic granules and to very less extent in some of the circular granules, which indicates that it is the secondary granules which exhibit positive reaction for alpha-naphthyl butyrate.

Contrary to Monahan *et al* (1981) and Zicca *et al* (1981) but in consonance with the finding of Osbaldiston *et al* (1978), Bozdech and Bainton (1981), Matutes *et al* (1983) and Yoruk *et al* (1998) the activity of the non-specific esterases with both the substrates i.e. alpha-naphthyl acetate and alpha-naphthyl butyrate, the reaction product was always seen within the granules and not in the cytoplasm or on the cytoplasmic surface of the vesicles. Even Monahan *et al* (1981) had pointed out that small lymphocytes generally had a single large paranuclear cytoplasmic vesicle cluster and the neutrophils displayed membrane bound granules. Bozdech and Bainton (1981) very clearly mentioned that the reaction product was always present within the membrane bound granules in lymphocytes while it was an ectoenzyme in monocytes. The cytoplasmic activity wherever observed could be because of diffusion of enzyme which originally might be present within granules as has been observed in few cells in the present study

Singh *et al* (1997) in camel and Singh (2000) at light microscopic level observed distinct granules in lymphocytes when stained for alpha-naphthyl acetate esterase and few monocytes gave a diffuse reactivity.

Matutes *et al* (1983) observed that the esterase activity was confined within the granules in T-prolymorphocytic leukemia and adult T-cell lymphoma leukemia while it was absent in patients with T-chronic lymphocytic leukemia and further suggested that in addition to these diseased conditions, differences could be found in normal T-lymphocyte subsets.

Facklam and Kociba (1985) also found that dogs with lymphocytic leukemia showed focal staining pattern while in granulocytic leukemia no reaction was seen and suggested that localisation of enzyme was a diagnostic aid in classification of leukemia in dog. This shows that there are different reaction patterns and probably firstly the reaction observed within the neutrophilic granules in the present study could be because of some isomer of this compound. Secondly as the presence is seen in secondary type of granules if there is production of immature cells, absence of localisation of this enzyme can be helpful in deducing the diseased condition. However, as reported earlier by Monahan *et al* (1981) and Zicca *et al* (1981) slight changes in processing also lead to

discrepancies. Li *et al* (1973) also commented that on the basis of gel-electrophorsis, it can be deduced that there are isoenzymes of non-specific esterases and these could be one of the causes for different staining behaviour with respect to alpha-naphthyl accetate and alpha-naphthyl butyrate.

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Dr. T.K. Gahlot attends the meet of International Association for Veterinary Editors at Estonia

International Association for Veterinary Editors (IAVE) invited Dr. T.K. Gahlot, Editor, Journal of Camel Practice and Research in its 9th annual meeting which held at University of Technology, Tallinn, Estonia from 7-8 June 2012. Dr.Gahlot presented a paper on "Genesis and Impact of Journal of Camel Practice and Research". Dr.Gahlot highlighted a big role of Camel Clinic located at College of Veterinary and Animal Science, Bikaner in developing the clinical aspect of camel diseases. Camel research was started in India since 1954 and the institutes undertaking camel research at Bikaner played a big role in nurturing the camel research. Journal of Camel Practice and Research was started in 1994 in order to provide a continuous source of camelid research. Publication of many subsequent books on camel physiology, nutrition, parasitology and anatomy were byproducts of the journal. Editor in Chiefs of many veterinary journals appreciated the efforts made for sustenance of JCPR.

Dr. Gahlot spoke on "Medicine and Surgery of Camelids" at LMU, Munich

Dr. T.K.Gahlot visited Ruminant Clinic of Ludwig Maximilians University, Munich (Germany) on 12th June 2012 and gave a lecture on Medicine and Surgery of Camelids to the students and faculty of this university. Dr.Gahlot showed them the over-view of biggest camel clinic of south Asia located at College of Veterinary and Animal Sciences, Bikaner and its far-reaching services across the desert state. He gave a powerpoint presentation about various clinical activities undertaken by the staff and students of RAJUVAS and camel diseases. He apprised them with the inventions in diagnosis and treatment of various camel diseases, particularly the surgical diseases out of which the treatment of mandibular fracture with interdental wiring was noteworthy. Dr. Gahlot presented them copies of books and journals on camels edited by him. Students of LMU were highly impressed by the ongoing clinical activities and showed interest in internship programme of CVAS, Bikaner to work in the multispecies clinic of this university.