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LOCALISATION OF ARYLSULFATASE C AND ARYLSULFATASE B IN THE BLOOD CELLS OF CAMEL AND BUFFALO AT ULTRASTRUCTURAL LEVEL - PART II

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The present study was conducted on the leucocytes of 3 healthy buffalo calves and 3 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.

A total of 20ml blood was collected in equal amounts in 2 test tubes from each animal. 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 fixative i.e. 1.5% glutaraldehyde in 0.1M sodium cacodylate - HCl buffer (pH 7.4) was added drop by drop 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 Arylsulfatase C (Kawano and Aikawa, 1987) and Arylsulfatase B (Bentfeld-Barker and Bainton, 1980).

Arylsulfatase C

1. The pieces of pallet before incubation were rinsed in 0.1M imidazole-HCl buffer (pH 7.5) containing 5% sucrose.
2. The fixed samples were dipped into media and incubated for 1 hour at 37°C. The total volume was 10ml of incubation solution which was made up of 1m Mus (4-methyl umbelliferyl sulfate), 1% Barium chloride and 0.1M Imidazole –HCl buffer pH 7.5 with 5% sucrose.
3. Incubated pieces were rinsed twice with cold 0.1M sodium cacodylate buffer (pH 7.2).

Arylsulfatase B

1. For localisation of arylsulfatase B, the pieces of pallet were washed with acetate-vernol buffer before incubation.
2. These pieces were then incubated at 30°C for 2 hours in the medium which consisted of 25mg para-nitrocatechol sulfate (Sigma) dissolved in 5ml acetate vernol buffer (pH 5.4), 0.16 ml 24% lead nitrate and the pH was adjusted to 5.5 with 0.1M HCl.
3. The pieces were washed by gentle centrifugation 4 or 5 times in acetate-vernol buffer (pH 5.4) with 7% sucrose.
4. Thereafter, these pieces were incubated in 2% ammonium sulfide in acetate vernol buffer (pH 5.4) for 10 minutes and again washed for 2 hours with at least 3 changes of pH 5.4 acetate-vernol buffer, followed by 3 changes of Michaeli's buffer (pH 7.4).

After incubating the pieces of pallets for the enzymes 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).

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Arylsulfatase C

The eosinophils of camel were distinctly positive for arylsulfatase C in which it was seen that within the granule along its long axis the reaction product was electron dense while all around, it was surrounded by homogeneously stained electron lucent area (Fig 1). The central electron dense area was not uniform and had granular appearance. Within the neutrophils (Fig 2), it was seen that only few granules exhibited a strong reactivity. In some the reaction product was limited to the peripheral portion of the few granules giving it slightly the appearance between crescentric and half moon. In rest of the large number of circular and pleomorphic granules, the staining was light and diffuse.

In case of buffalo calf within the neutrophils large number of arylsulfatase C positive granules were seen. Within these granules the peripheral portion was however devoid of any activity while the central large area was electron dense (Fig 3 and 4). The reaction product was homogeneously placed within these granules. However, few granules with very mild electron density were also encountered alongwith some granules having no activity. One of the cell probably a basophil had few granules placed close to the Golgi apparatus. Within this type of cell the central core of the granule was electron dense surrounded by comparatively electron lucent area which appeared to be homogeneous in nature (Fig 5). One or two granules which were completely electron dense were also observed but were placed away from the Golgi complex. The eosinophils also exhibited moderate activity within their granules.

Arylsulfatase B

The activity was more or less similar to that of arylsulfatase C, however, in the camel eosinophils the central elongated area of the granules exhibited more uniform and electron opaque activity while the rest of the granule was devoid of any activity (Fig 6). Rarely few granules having homogenous nature were also encountered. Within the neutrophils, the reactivity was confined to few granules (Fig 7) within which the activity was confined towards one part of the granule only. Some of the granules were however, homogenously stained.

In the buffalo calf the reaction product in the neutrophils was seen in the form of small dot like granules distributed all around the nuclear membrane and to some extent within the cytoplasm (Fig 8). The granules by and large did not exhibit any reactivity and appeared to be vacuolated. Similar to that described in arylsulfatase C the basophil like cells having reactivity for arylsulfatase B were also encountered. These cells also had very fine granular deposits over the nuclear membrane. However, within the cytoplasm very clear membrane bound granules were discernible (Fig 9). Few of these granules had electron lucent material while some of them had electron dense material. But the majority of granules had electron dense activity in the centre, which was either placed longitudinally or condensed circularly with electron lucent periphery.

Major mammalian arylsulfatases are classified into three types; arylsulfatase A, arylsulfatase B and arylsulfatase C. Arylsulfatase C (type I sulfatase) differs from arylsulfatase A and B (type II sulfatase) in microsomal distribution, alkaline pH optimum and sensitivity to certain inhibitors. As has been seen with various reports where arylsulfatase had been localised the substrate used was para-nitrocatechol which is meant for arylsulfatase B. In the present study the attempt was made to localise arylsulfatase B using para –nitrocatechol and arylsulfatase C using 4-methyl umbelliferyl sulfate which was used on the basis of histochemical studies by Kawano and Aikawa (1987).

In the present observations it was seen that the eosinophils of camel reacted positively with both i.e. arylsulfatase B and arylsulfatase C. The activity once again was seen to be more prominent in the electron dense centrum present on the longituidinal axis of the granule with respect to arylsulfatase B, whereas with regards to arylsulfatase C a mild electron lucency was also observed around the electron dense centrum indicative of some mild activity within the rest of granule in addition to electron dense centrum. Bainton and Farquhar (1970) in rabbit observed that the eosinophil reactivity for arylsulfatase was variable within the granules. No activity was observed in the eosinophils of buffalo calves in the present work.

Bainton and Farquhar (1968a and 1968b) in rabbit and human being reported that arylsulfatase activity was seen in the azurophilic granules. Similarly in the present study also, it was observed that in camel neutrophils the reaction product for both type of arylsulfatase was limited to only few granules indicating thereby that it is the azurophil (primary) granules which carry these enzymes.

However, contrary to camel neutrophils, within the neutrophils of buffalo calves, the reaction product was seen in large number of granules with respect to arylsulfatase C which indicates that the activity unlike in camel neutrophils, is present in secondary granules.

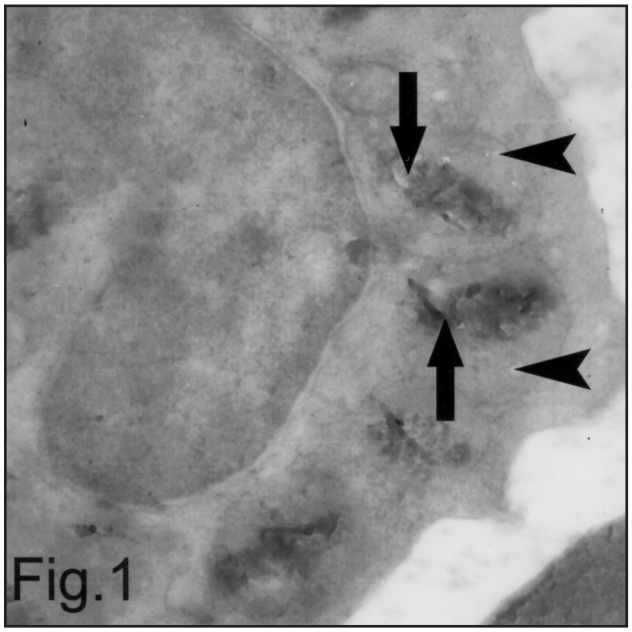

Fig 1. Electron photomicrgraph of eosinophil of camel showing membrane bound granules having electron dense centrum where the activity is not uniform (straight arrow) surrounded by electron lucent area around the electron dense centrum (arrow head). Aryl sulfatase C X 8,200

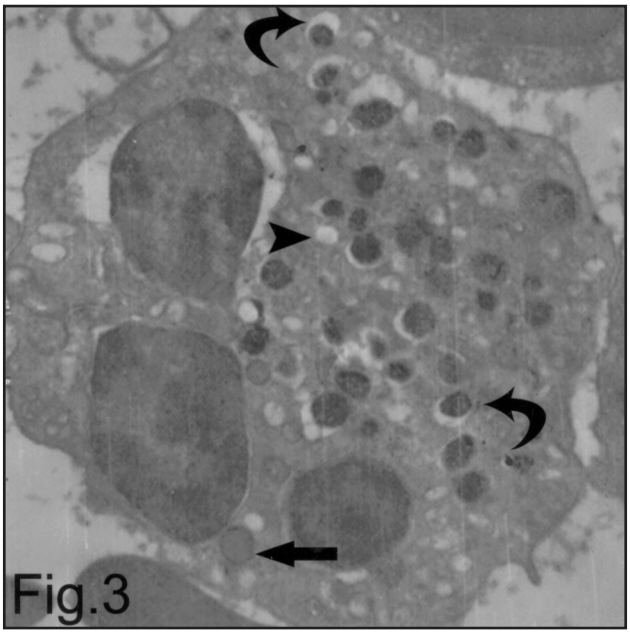

Fig 3. Electron photomicrograph of a neutrophil of buffalo calf showing electron dense granules with peripheral area devoid of reaction (curved arrow) and few granules having mild electron density (straight arrow) and some completely devoid of activity (arrow head). Arylsulfatase C X 2,900.

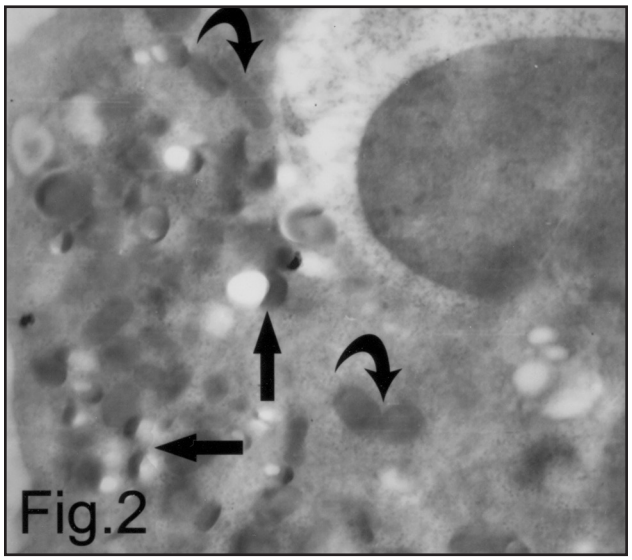

Fig 2. Electron photomicrograph of a neutrophil of camel showing uniformly but mildly stained circular and pleomorphic granules (curved arrows) and granules having dense reactivity towards periphery while rest of them is empty (straight arrows). Aryl sulfatase C X 8,200.

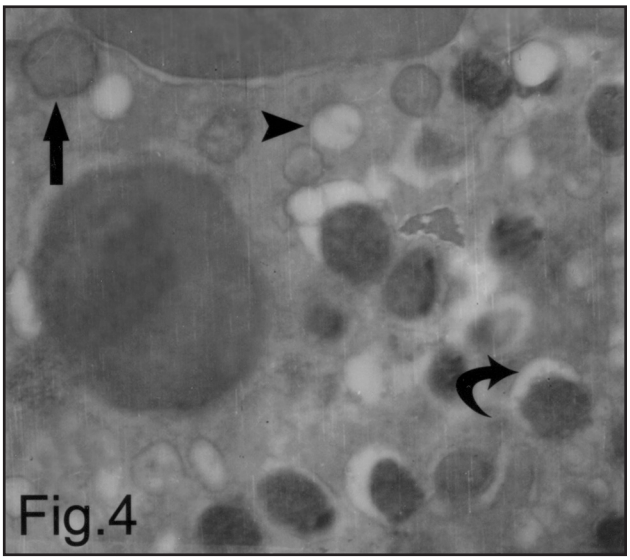

Fig 4. Electron photomicrograph of a neutrophil of buffalo calf showing electron dense granules with peripheral area devoid of reaction (curved arrow) and few granules having mild electron density (straight arrow) and some completely devoid of activity (arrow head). Arylsulfatase C X 10,500.

With respect to arylsulfatase B, the reaction product in the neutrophils of buffalo calves was seen in the form of small dots all around the nuclear membrane, cell granules and to some extent within the cytoplasm whereas the granules were empty and non-reactive. This could be the natural site for this enzyme or it could be due to the diffusion of enzyme from the granules during the processing.

In addition to neutrophils, the eosinophils exhibited moderate activity in buffalo calves. Another type of cell whose granules were bigger than those of neutrophils and eosinophils was also seen to

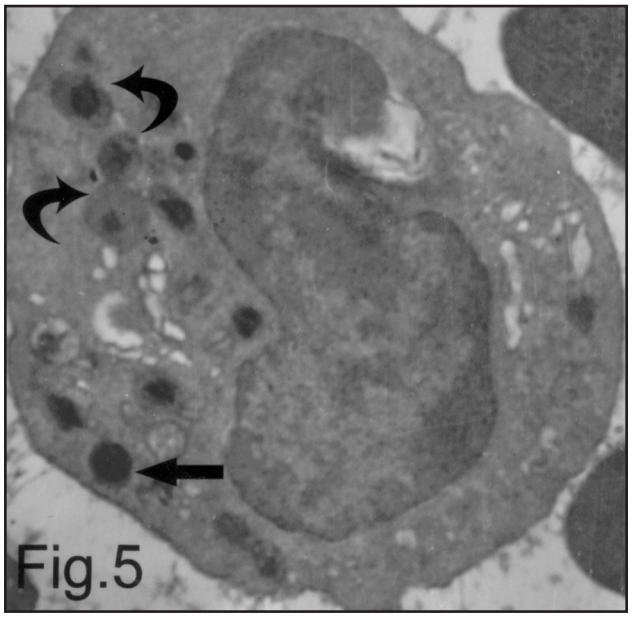

Fig 5. Electron photograph of a basophil of buffalo calf showing granules with electron dense core surrounded by electron lucent area (curved arrow) and few granules are completely electron dense (straight arrow). Aryl sulfatase C.X 4,200.

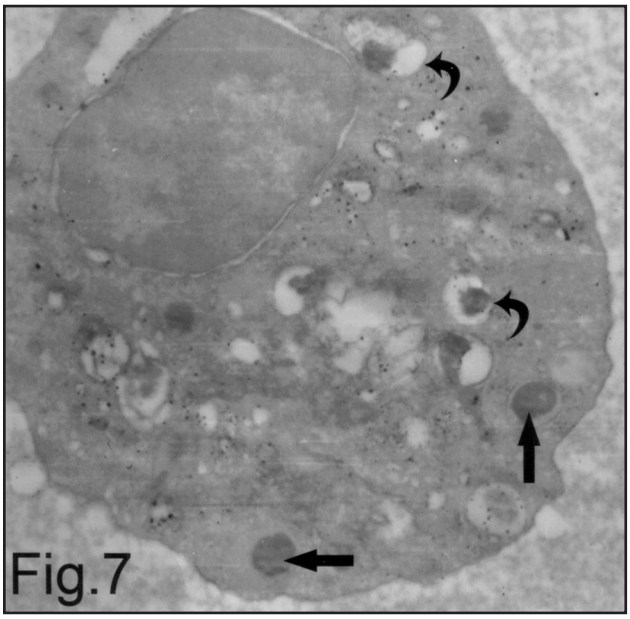

Fig 7. Electron photomicrograph of a neutrophil of camel showing uniformly stained granule (straight arrow) and a granule with electron dense activity confined towards one part (curved arrow). Aryl sulfatase B X 4,000.

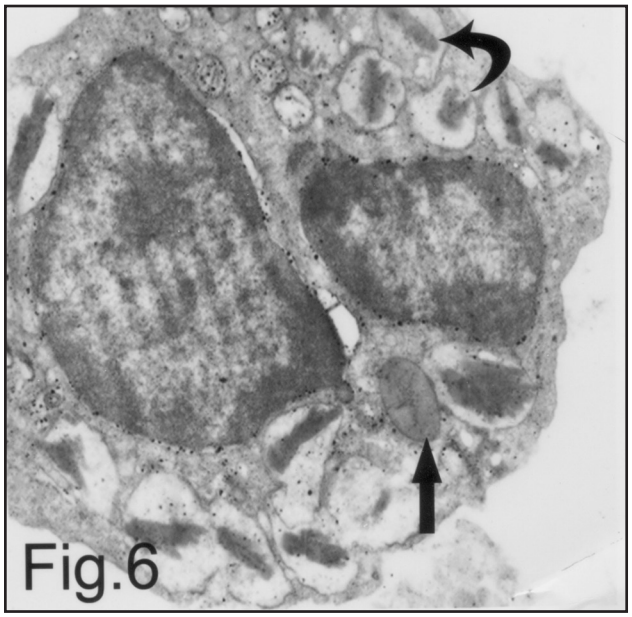

Fig 6. Electron photomicrograph of a eosinophil of camel showing granules exhibiting reaction in the central electron dense area while rest is devoid of any activity (curved arrow) and a granule showing homogenous reaction (straight arrow). Aryl sulfatase B X 4,200.

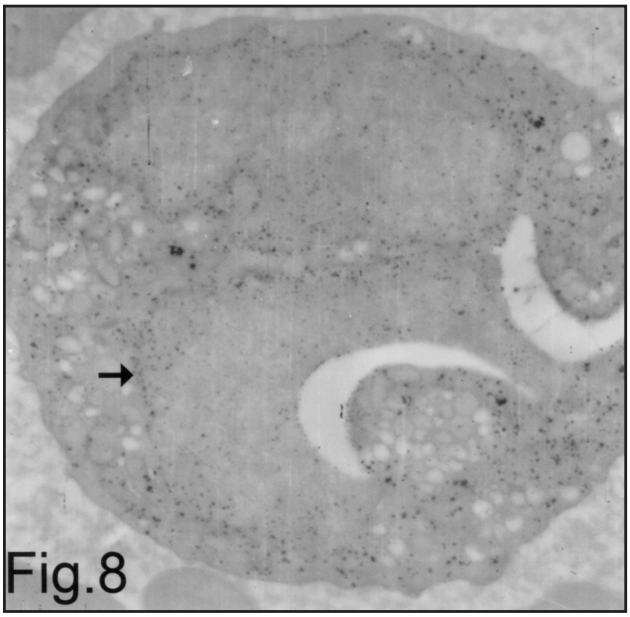

Fig 8. Electron photomicrograph of a neutrophil of buffalo calf showing dot like material over the nuclear membrane (straight arrow) and distributed within the cytoplasm. Aryl sulfatase B X 5,400.

be reactive for arylsulfatase B and arylsulfatase C, but the number of such cells was very less. The granules within these cells were circular in nature and had central core of electron dense material while the peripheral area was electron lucent. Like the neutrophils, in these cells also dot like reactive material was observed over the nuclear membrane when incubated for arylsulfatase C. These cells, on the basis of their number and size of granules can be the basophils. Bentfeld – Barker and Bainton (1980) also demonstrated the presence of arylsulfatase B reactive granules in rat basophils.

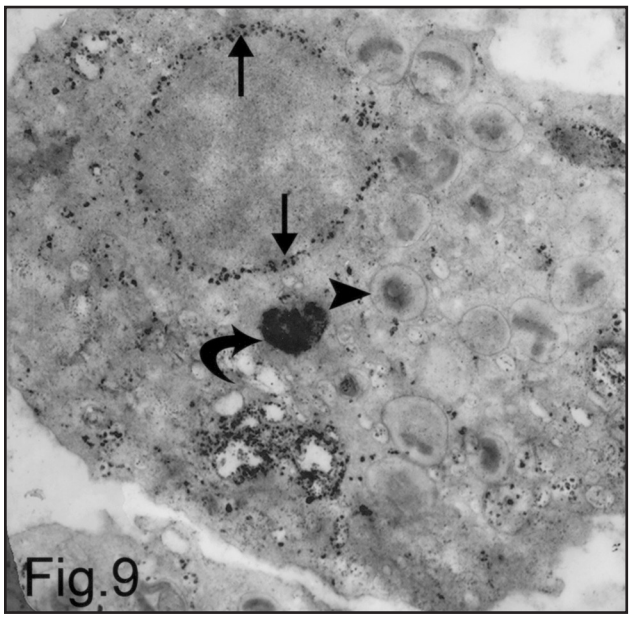

Fig 9. Electron photmicrograph of a basophil of buffalo calf showing dot like deposits over the nuclear membrane (straight arrow), granules having electron lucent periphery with central portion electron dense (arrow head) and uniformly electron dense granules (curved arrow) reactivity within them. Aryl sulfatase B X 5, 400.

Similar to the findings of Bentfeld and Bainton (1975) the platelets of camel and buffalo calves also exhibited positive reactivity for both the sulfatases i.e. B and C, however, dense bodies strongly reactive for arylsulfatase B and C were only discernible in buffalo calves.

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