

## CYTOCHEMICAL AND CYTOENZYMIC STUDIES ON BLOOD CELLS OF SHEEP

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### ABSTRACT

Cytochemical and cytoenzymic studies were undertaken on blood cells of ten normal healthy sheep. The eosinophils and neutrophils showed positive reaction for glycogen. The eosinophil granules stained black with Sudan Black B stain. The basophilic granules revealed positive reaction for mucopolysaccharides with toluidine blue stain. The lymphocytes showed intense reaction for acid phosphatase and the neutrophils and eosinophils showed moderately positive reaction for alkaline phosphatase. The eosinophils showed intense positive reaction for cytochrome oxidase while the neutrophils were weak positive. The nuclear DNA stained orthochromatically greenish yellow, whereas cytoplasmic RNA stained metachromatically red when stained with supravital stain of Wittekind. A few erythrocytes showed blue colored fine granules when stained with acid ferrocyanide stain.

**Key words:** Blood cells, cytochemical, cytoenzymic, sheep

Blood examination is an important weapon in the arena of disease diagnosis. Accurate identification of the cells and localization of various enzymes in the blood cells is of great importance in understanding body response to any kind of stress (Catovsky, 1975, 1980). Cytochemical studies can be used as a diagnostic aid in many hematological and nonhematologic disorders (Okun and Tanaka, 1978). The reports on cytochemical and cytoenzymic studies on blood cells of sheep are meager. Hence, the present work was undertaken to study cytochemical and cytoenzymic characters of blood cells of sheep.

### MATERIALS AND METHODS

Ten normal healthy sheep reared at the College of Veterinary Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar were selected to study the cytochemical and cytoenzymic characters of different blood cells. Blood was aseptically collected from jugular vein of sheep in a test tube containing EDTA as an anticoagulant. The blood films in duplicate were stained with toluidine blue stain for acid mucopolysaccharide, cytochrome oxidase, acid

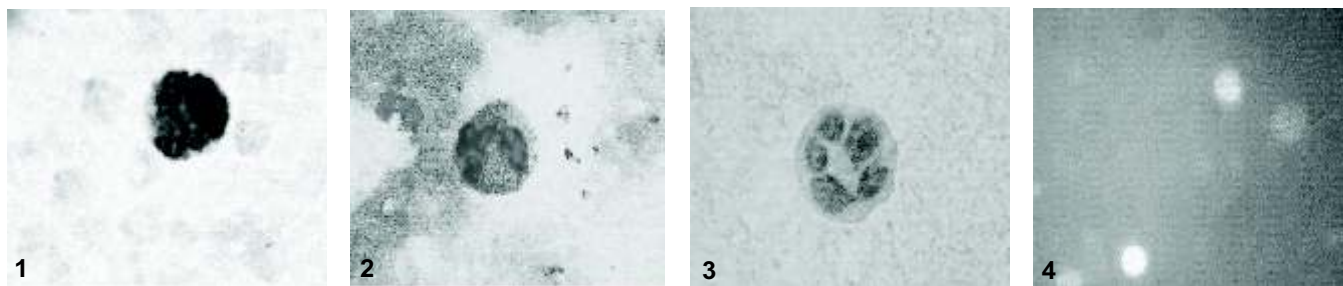
phosphatase and alkaline phosphatase (Bover, 1964). The slides were also stained with acid ferrocyanide for iron, Periodic acid Schiff's for glycogen and Sudan Black B stain for lipid (Jain, 1986). The stained blood smears were examined under oil immersion.

### RESULTS AND DISCUSSION

The basophilic granules showed positive reaction (blue coloured granules) with toluidine blue (Fig. 1). Singh *et al.* (1998), Menaka and Singh (2002) and Singh and Menaka (2004a) reported similar findings in camel, goat and horse, respectively. The eosinophil granules stained black with Sudan Black B, whereas neutrophil showed very weak to negative reaction for Sudan Black B. Similar findings have been reported by Singh and Menaka (2004b) in buffalo calves. In contrast, neutrophils and eosinophils were reported to be positive for Sudan Black-B in camel (Singh *et al.*, 1998) and goat (Menaka and Singh, 2002). Neutrophilic granules of horse also showed strong reaction with Sudan Black-B (Singh and Menaka, 2004a).

The eosinophilic (Fig. 2) and neutrophilic granules were PAS positive (pink colored granules). This finding is consistent with the observation of Singh

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Figs 1-4. 1. Photomicrograph of blood smear showing the basophil with metachromatically stained bluish purple colored granules. (Toluidine blue stain x 100). 2. Photomicrograph of blood smear showing strongly reactive eosinophil. (Periodic acid Schiff's stain x 100). 3. Photomicrograph of blood smear showing moderately positive neutrophil. (Alkaline phosphatase stain x 100). 4. Photomicrograph of blood smear showing greenish yellow nuclear DNA and red colored RNA. (Supravital stain of Wittekind x 100).

and Menaka (2004b) who also reported PAS positive granules in buffalo calves. Neutrophils of camel and horse have been reported to be strongly positive for PAS (Singh *et al.*, 1998; Singh and Menaka, 2004a). In another report in goat, cytoplasmic granules of neutrophils showed diffuse reaction with PAS stain (Menaka and Singh, 2002). The eosinophils showed intense positive reaction in the form of blue colored granules when stained for cytochrome oxidase, whereas the lymphocytes showed a negative reaction for cytochrome oxidase. More or less similar observations have been recorded earlier in buffalo (Singh, 2000).

The lymphocytes showed intense reaction in the form of brownish pink granules when stained for acid phosphatase, whereas granulocytes were negative for acid phosphatase. Earlier a positive activity of acid phosphatase in the lymphocytes of cow, horse, dog and sheep has been reported by Jain (1986). Facklam and Kociba (1985) reported neutrophils to be positive for acid phosphatase in dog. However, Singh *et al.* (1997) reported that the neutrophils of camel were devoid of acid phosphatase activity but the eosinophils were weakly positive and lymphocytes showed positive activity for acid phosphatase. The neutrophils and eosinophils were moderately positive for alkaline phosphatase (Fig. 3) as also reported in cow, horse and sheep (Jain, 1968) and in horses (Nigam and Singh, 1972). A few erythrocytes showed blue colored fine granules when stained with acid ferrocyanide. Similar observation has been reported earlier in buffalo calves (Singh, 2000).

The nuclear DNA stained orthochromatically greenish yellow, whereas cytoplasmic RNA stained metachromatically red when stained with supravital

stain of Wittekind and observed under fluorescent microscope (Fig. 4). The lymphocytic cytoplasm exhibited intense red fluorescence as compared to granulocytes. Singh *et al.* (1998) and Singh (2000) also reported intense red fluorescence in cytoplasm of lymphocytes in camel and buffalo calves, respectively.

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