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Transmission electron microscopic studies on agranulocytes and platelets of sheep

ADITYA KUMAR[1], ISHWAR SINGH[2] and MEENA MRIGESH[3]

Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145 India

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The lymphocytes and monocytes have key role in understanding the problems related to immunological status of the animals. Similarly platelets play a key role in understanding the clotting mechanism. The ultrastructural morphology of blood cells is documented in camel (Singh *et al*. 1997), buffalo calves (Singh 2000), goat (Menaka and Singh 2003) and guinea fowl (Gupta and Singh 2008) and macaque (Sakulwira *et al.* 2008). The reports on transmission electron microscopy on blood cells of sheep are meager. So the present study was undertaken to study the ultrastructural details of agranulocytes and platelets of sheep.

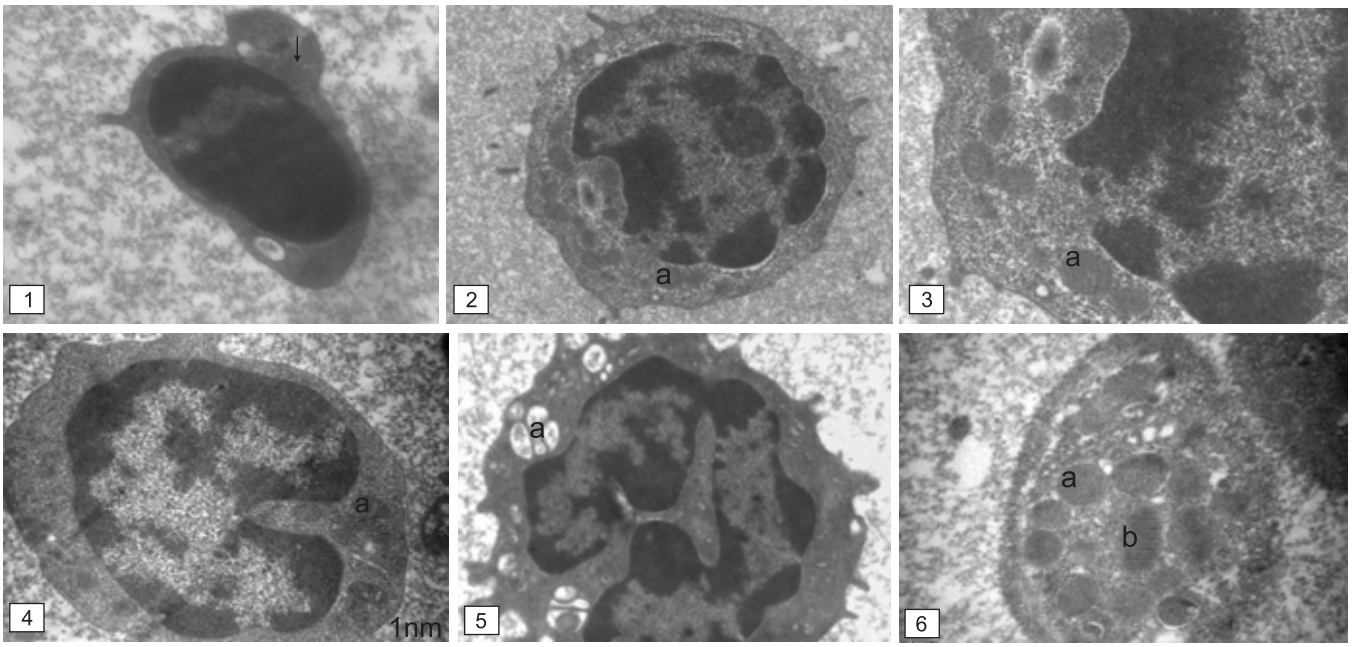
The study was conducted on 10 appearently healthy sheep maintained at livestock production and management farm of university. Blood (5 ml) was taken from the jugular vein of sheep in a sterilized and siliconized tube with EDTA as anticoagulant and centrifuged at 2500 rpm for 30 min. The excess of plasma was drained out leaving a small amount over the buffy coat. Then 2–3 ml of modified Karnovsky's fluid was poured along the sides of test tube drop by drop without disturbing the buffy coat for fixation and formation of buffy coat plug. The buffy coat plug along with a layer of red blood cells was taken out of the tube with the help of hooked needle and placed in petri-dish containing phosphate buffer. The plug was cut into thin slices of approximately 1 mm thickness. The samples were submitted in PBS at pH 7.4 for further processing for electron microscopy (David *et al*. 1973) at AIIMS, New Delhi.

The lymphocytes under TEM could be classified as small, medium and large, depending upon their size (Figs 1–4). The nucleus of small lymphocytes was occupying whole of the cell leaving a peripheral rim of cytoplasm. The cytoplasm was electron dense and had mitochondria with well developed cristae. The cytoplasm also had some vesicles enclosing lysed material. The medium sized lymphocytes were having more number of cytoplasmic processes. The cytoplasm was more in amount but comparatively less electron dense. The cytoplasm contained large number of mitochondria concentrated towards one area of the cell. The large size lymphocytes were mostly having indented nucleus. The cytoplasm was granular in appearance and having large number of well developed large sized mitochondria with clear septae. In the nucleus of both medium and large lymphocytes the electron-dense heterochromatin was peripheral while the electron lucent euchromatin occupied central position with patches of heterochromatin in it (Figs 2–4). Singh *et al.* (1997) found that the lymphocytes had very small dense granules spread all over the cytoplasm in camel. Large number of mitochondria were usually aggregated adjacent to nuclear indentation. Again, Singh (2000) reported that ultrastructurally the lymphocytes were almost circular in outline and had long cytoplasmic processes in buffalo. The cytoplasm was denser with large sized mitochondria and well developed cristae. The mitochondria were usually concentrated at one pole of the cells. Golgi-apparatus was better developed in large lymphocytes. Some of the large lymphocytes had membrane bound granules whose interior was homogeneously dense. In some of the lymphocytes, well developed cisterns of rough endoplasmic reticulum occupying the major area of the cytoplasm were also observed. Menaka and Singh (2003) reported that the goat lymphocytes were almost circular in outline with long cytoplasmic processes. The cytoplasm contained large sized mitochondria with well developed septae. The mitochondria were abundant in number.

The monocytes under TEM showed large number of various sized cytplasmic processes. The nucleus was varying in shape and generally deeply indented. The cytoplasm was having mitochondria, Golgi bodies and large number of pinocytic vesicles and vacuoles (Fig. 5). The heterochromatin was in the form of patches located peripherally while the euchromatin was located in the center of the nucleus (Fig. 5). Yamada (1970) reported horse-shoe shaped nucleus in sheep monocytes with large number of

Present address: [1]Ph.D. Student, College of Veterinary Sciences, CCSHAU, Hisar, Haryana, India.

[2]Professor and Head (singh_iswar@yahoo.co.in), [3]Assistant Professor, Department of Anatomy and Histology, College of Veterinary Sciences.



Figs 1–7. **1.** A transmission electron photomicrograph of small sized lymphocyte showing large nucleus occupying whole of the cell leaving a peripheral rim of cytoplasm and mitochondria (black arrow). Uranylacetate and lead citrate. × 7100. **2.** A transmission electron photomicrograph of medium sized lymphocyte showing rough endoplasamic reticulum distributed through out cytoplasm and mitochondria (a). Uranylacetate and lead citrate. ×3500. **3.** A transmission electron photomicrograph of medium sized lymphocyte showing mitochondria (a). Uranylacetate and lead citrate. ×7100. **4**. A transmission electron photomicrograph of large sized lymphocyte showing indented nucleus, granular cytoplasm and large number of well developed large sized mitochondria with clear septae (a).Uranylacetate and lead citrate.×3320c.**5.** A transmission electron photomicrograph of monocyte showing vesicles with septae dividing the interior into compartments (a). Uranylacetate and lead citrate. ×5000.**6.** A transmission electron photomicrograph of thrombocyte showing large, round and electron lucent granules (a) and elongated crystalloid granules with finger like impressions (b).Uranylacetate and lead citrate.×10800c. **7.** A transmission electron photomicrograph of thrombocyte showing large, round electron dense granule (a).Uranylacetate and lead citrate. ×13000.

mitochondria and endoplasmic reticulum in the cytoplasm. Singh *et al.* (1997) found that the camel agranulocytes had large number of mitochondria and vesicles. Some of these cells had electron lucent granules. Singh (2000) observed that the monocytes of buffalo calf had pleomorphic electron dense cytoplasmic granules of varying size. The granules were homogeneously dense. Mitochondria and golgi apparatus were well developed. Large numbers of pinocytic vesicles were observed towards periphery of the cell. Menaka and Singh (2003) reported that the goat monocyte appeared spherical in outline with slight distortions and had cytoplasmic processes of varying length and thickness. The cytoplasmic granules were pleomorphic and homogeneously electron dense. There were large number of mitochondria with varying shape and size surrounding the nucleus.

The platelets were anucleated, elongated, oval or circular in outline depending upon the plane of section. The granules showed great variation in their size and structure (Figs 6–7). One type of granules was electron dense in nature, while the second type of granules was electron-lucent. The third type of granules was crystalloid in appearance with finger like projections inside them (Fig. 6). The fourth type of granules was large in size and having centrally or eccentrically placed electron-dense material. Jain (1986) reported that circulating platelets of sheep in blood vessel were disc shaped and cytoplasm contained large, round alpha granules, smaller granules and occasional organelles. Also, Singh (2000) reported that in buffalo calves platelets appeared circular, oval or elongated depending upon the plane of section. The platelets could be clearly divided into 3 zones, i.e. peripheral, sol-gel and inner organelles zone. Different types of granules with different shapes, sizes and consistency were observed. In all type of granules the major part was made of homogeneously-stained-granular matrix, while part of these granules towards the membrane was made up of more electron dense material. Menaka and Singh (2002) reported that goat platelets

appeared as elongated, elliptical or oval in outline depending upon the plane of section. The platelet could be subdivided into 3 zones like peripheral, sol-gel and inner organelle zone. The organelle zone was having few organelle and different types of granules. First type of granules had electron dense margin. The second type was filled with granular material. The third type of granules were having an eccentrically placed highly electron dense material of variable size.

SUMMARY

Transmission electron microscopic (TEM) study was conducted on blood cells of ten normal healthy sheep (*Ovis aries*). The lymphocytes under TEM were almost round in outline with comparatively long cytoplasmic processes and could be classified as small, medium and large, depending upon their size. The monocytes under TEM showed large number of variable sized cytoplasmic processes characterized by large number of pinocytic vesicles and vacuoles having variable amount of lysed material. The platelets were elongated, oval or circular in outline depending upon the plane of section. The granules were generally round in shape with variations in size and interior detail.

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