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Transmission electron microscopic characterization of granulocytes of pig (*Sus scrofa*)*

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ABSTRACT

Transmission electron microscopic characterizations of granulocytes of pig were carried out on ten apparently healthy adult pigs. Ultrastructurally the neutrophils were roughly round in shape with number of small cytoplasmic processes. Cytoplasmic granules of the neutrophils varied greatly in shape, size and density. Populations of electron lucent granules were comparatively greater than electron dense. Eosinophils were round in outline and the cytoplasm had four types of cytoplasmic granules. The basophils were roughly spherical in outline with cytoplasmic granules. One type of granules was less electron dense and very small in size whereas second type of granules were highly electron dense and showed pleomorphism.

Key words: Basophils, Eosinophils, Neutrophils, Transmission electron microscopic study

The ultrastructural morphology of blood cells have been documented in canine (Sonoda and Kobayashi, 1970), camel (Singh *et al.* 1997), buffalo calves (Singh, 2000), goat (Menaka and Singh 2004), black bear (Salakij *et al.* 2005), fishing cat (Prihirunkit *et al.* 2007), guinea fowl (Gupta and Singh 2008) and macaque (Sakulwira *et al.* 2008). In spite of large quantum of literature available on domestic animals the reports on transmission electron microscopic studies on blood cells of pig are meager. Hence the present study was conducted to see its application in various fields of veterinary sciences.

MATERIALS AND METHODS

The study was conducted on 10 apparently healthy Large White York shire pig of 1–2 years age, maintained at livestock production and management farm of College of Veterinary Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar. Blood (10 ml) was taken from the ear vein in a sterilized and siliconized tube containing EDTA as anticoagulant and centrifuged at 3000 rpm for 30 minutes. The excess of plasma was drained off leaving a small amount over the buffy coat. Then 2–3 ml of modified Karnovsky's fluid was poured along with 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 or wire and placed in petri dish containing phosphate buffer. The plug was cut into thin and small slices of approximately 1 mm thickness. The samples were submitted in phosphate buffer at pH 7.2 to the electron microscopy facility at AIIMS, New Delhi for further processing and sectioning of the blood cells for the transmission electron microscopic studies. The samples were dehydrated in graded acetone solutions and embedded in beam capsule. Ultrathin sections of 60–80 nm thickness were cut and stained in alcoholic uranyl acetate and lead citrate. These sections were then placed on grids and viewed under transmission electron microscope (Morgagni 268) operated at 60–80 kv. (David *et al.*1973) at AIIMS.

RESULTS AND DISCUSSION

Neutrophils: The neutrophils were roughly rounded in shape with number of small cytoplasmic processes varying in size and shape. The nucleus was 2–5 lobed and surrounded by distinct nuclear membrane (Fig.1). At certain interval membranes had distinct nuclear pore. Beneath the nuclear membrane, there was a white line which was bounding the heterochromatin. The heterochromatin occupied major peripheral portion of the nuclear lobes where as the euchromatin was comparatively less contributed centrally

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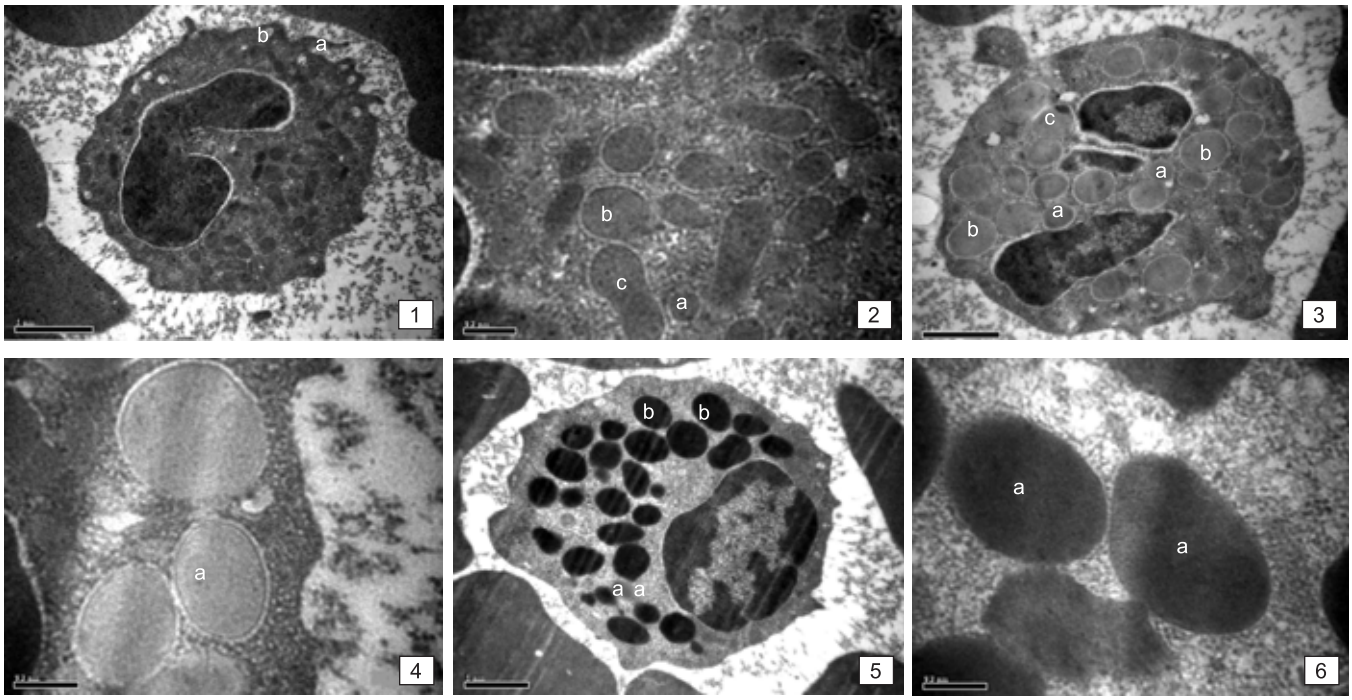
in the form of patches. Singh *et al.* (1997) observed that the camel neutrophils had 3-4 nuclear lobes with distinct outer densely packed chromatin underneath the nuclear membrane and central portion was occupied by the loosely arranged chromatin. Singh (2000) in buffalo calves and Menaka and Singh (2004) in goat observed that neutrophils were spherical in shape with 2 to 4 nuclear lobes, depending upon the plane of section. Prihirunkit *et al.* (2007) observed that neutrophils showed lobed nucleus in fishing cat.

Cytoplasm: The cytoplasm was laden with double membrane bound cytoplasmic granules. The granules were mostly rounded, oval and elongated in shape. Populations of electron lucent granules were comparatively greater than electron dense. The round shaped electron lucent granules were of two types, one types were smaller in size and second types were larger in size (Fig.2). The elongated shapes electron lucent granules were few in number and contributed in the middle area. The interior of all electron lucent granules was granular in appearance. The electron dense granules were few in number and mostly oval or elongated in shape. In addition to granules, the golgi apparatus and mitochondria were observed in the cytoplasm. The phagocytic vacuoles were found towards the periphery. The smooth and rough endoplasmic reticulums were occasionally seen. The free ribosomes were found scattered throughout the cytoplasm. The small vesicles of varying size were also observed. Sonoda and Kobayashi (1970) reported that the canine neutrophils had three types of granules. Jain (1993) stated that neutrophil contained at least two types of granules, azurophilic and specific granules. Salakij *et al.* (2005) stated that neutrophils of black bear had small specific granules and glycogen granule. Pothiwong *et al.* (2006) reported that the neutrophils of felis and panthera species showed highly condensed chromatin with relatively smaller granules, mitochondria, endoplasmic reticulum, ribosome and lysosome. Prihirunkit *et al.* (2007) observed that neutrophils of fishing cat. showed lobed nucleus with surface microvilli and numerous cytoplasmic granules. The vesicular bodies were found in cytoplasm of some neutrophils. Sakulwira *et al.* (2008) noticed that neutrophils of macaque contained numerous small rounded or oval specific granules and some mitochondria.

Eosinophils: Eosinophils appeared round in outline with thin and broad small cytoplasmic processes. The nucleus had two to three lobes with distinct nuclear membrane (Fig.3). The nuclear lobes were having less amount of



Figs 1–6. **1.** Transmission electron photomicrograph of neutrophil showing cytoplasmic processes (a) and vesicles with lysed material (b). Uranyl acetate and lead citrate. × 33000. **2.** Transmission electron photomicrograph of neutrophil showing small round electron lucent granules (a), large round electron lucent granules (b) and elongated electron lucent granules (c). Uranyl acetate and lead citrate. × 180000. **3.** Transmission electron photomicrograph of eosinophil showing small electron lucent granules (a), large electron lucent granules (b)and granules with single highly electron dense material placed eccentrically(c). Uranyl acetate and lead citrate. × 33200. **4.** Transmission electron photomicrograph of eosinophil showing granules with central electron lucent and a thin electron dense peripheral rim (a).Uranyl acetate and lead citrate. × 137000. **5.** Transmission electron photomicrograph of basophil showing electron lucent granules (a) and electron dense granules (b). Uranyl acetate and lead citrate. × 26500. **6.** Transmission electron photomicrograph of basophil showing large sized electron dense granules (a). Uranyl acetate and lead citrate. × 137000.

heterochromatin as compared to neutrophils. The heterochromatin was concentrated towards the periphery except nuclear pores. The loosely arranged euchromatin was located in the central area as compared to periphery of lobes (Fig.3). The nuclear pores were distinct. Singh *et al.* (1997) observed that the eosinophils in camel had the nucleus with densely packed chromatin at the periphery as compared to central part Singh (2000) reported that in the buffalo calves the eosinophils appeared roughly circular and had large number of small cytoplasmic processes with 2 to 3 lobed nucleus and distinct nuclear membrane.

The double membrane bound cytoplasmic granules were roughly rounded to oval in shape and distributed throughout the cytoplasm. One type of granules was smaller in size and electron lucent. The second types of granules were larger in size with homogenously electron lucent material inside (Figs 3, 4). The third type of granules were also larger in size with single highly electron dense material placed eccentrically. The fourth types of granules were smaller in size and oval in shape with central electron lucent and a thin electron dense peripheral rim. The other cytoplasmic organelles like mitohondria, rough endoplasmic reticulum, Golgi apparatus, pinocytic vesicles and vesicles enclosing lysed material were also seen occasionally. Menaka and Singh (2004) reported that cytoplasm of goat eosinophil contained mainly round oval and elongated membrane bound granules of different sizes. Salakij *et al*. (2005) noticed that eosinophils of black bear showed large pleomorphic granules, Eurell and Frappier (2006) reported that in eosinophil, Golgi complex elaborated many primary granules (azurophillic granules). The secondary granules (specific granules) of canine eosinophil vary from dense homogeneous granules surrounded by a narrow rim of lighter matrix to a clear vesicle with only a cap of dense material. Pothiwong *et al.* (2006) reported that specific granules of eosinophils of felis species were rod or oval shaped and contained a less electron dense matrix than that of the core and had an electron dense core crystalloid. Prihirunkit *et al.* (2007) stated that cytoplasm of eosinophils of fishing cat was primarily occupied with characteristic oval electron dense granules.

Basophils: The basophils were roughly spherical in outline with few small cytoplasmic processes. The nucleus was eccentrically placed and was having 1–2 lobes. The nuclear membrane and nuclear pores were distinct. The heterochromatin was distributed towards the periphery excepting at neuclear pore and loosely arranged euchromatin at the centre. The amount of euchromatin and heterochromatin were almost equal (Fig. 5). Singh *et al.* (1997) in camel observed that the nucleus had a densely packed chromatin material more at the periphery and occupied more space as compared to centrally placed loose chromatin. Singh (2000) reported that in the buffalo calves basophils were seen as circular shaped with few cytoplasmic processes. The nucleus was usually bilobed with condensed chromatin arranged at periphery and loosely arranged chromatin in the center. Salakij *et al*. (2005) stated that in black bear basophils showed lobed nuclei.

The population of the cytoplasmic granules were comparatively less when compared with neutrophils and eosinophils. One type of cytoplasmic granules was electron lucent, very small in size and few in number. The second type of granules which was highly electron dense, maximum in population and showed pleomorphism. Out of which few were very small in size and rounded in shape, few were oval or rounded in shape and few were medium sized round or oval in shape (Figs 5, 6). The interior of these granules were homogeneously highly electron dense. The other cell organelles observed were mitochondria, Golgi apparatus, vesicles and smooth as well as rough endoplasmic reticulum. Yamada and Sonoda (1970) reported three types of granules in sheep basophils. Singh (2000) reported that in the buffalo calves cytoplasm of basophils showed granules. Salakij *et al*. (2005) stated that in black bear basophils showed lobed nuclei, small heterogeneous electron dense granules with some mitochondria. Pothiwong *et al.* (2006) reported that specific granules in felis and panthera species were rounded. Gupta and Singh (2008) observed that in guinea fowl basophilic granules were round shaped and of same size. Sakulwira *et al*. (2008) noticed that macaque basophils revealed large rounded homogenous matrix and electron dense membrane bond specific granules.

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