



Original Research

Ultrastructural Morphology of Erythrocytes, Reticulocytes and Mononuclear Cells of Indigenous Fowl (*Gallus domesticus*)

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Abstract

The experiment was conducted to understand the ultrastructural morphology of the erythrocytes, reticulocytes and mononuclear cells (thrombocytes, lymphocytes and monocytes) of the Uttara fowl (local hill fowl of Uttarakhand). The study was conducted on 10 apparently healthy and adult fowl of either sex, reared at Instructional poultry farm, G.B.P.U.A&T, Pantnagar, Uttarakhand. The blood was collected from wing vein using E.D.T.A as anticoagulant. Transmission electron micrographs showed that fowl erythrocytes were elliptical in shape with oval nucleus and homogenous cytoplasm. The reticulocytes were nucleated and cytoplasm was homogenous with few polysomes and mitochondria. The thrombocytes were round with central nucleus and contained few granules in the cytoplasm. The small lymphocytes were rounded with few cytoplasmic processes. The medium lymphocytes were oval to round in appearance with prominent foot like cytoplasmic processes or pseudopodia. In the large lymphocytes ectoplasm appeared vacuolated due to presence of numerous small vacuoles. The monocytes were roughly round in shape with finger like cytoplasmic processes. The cell had bean shaped nucleus containing few patches of heterochromatin. The mitochondria, golgi apparatus, rough as well as smooth endoplasmic reticulum and large number of membrane bound empty vesicles of varying size were observed.

Key words: Erythrocytes, Mononuclear cells, Reticulocytes, Ultrastructure, Uttara Fowl

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Introduction

The Uttara fowl (local hill fowl) is said to be descended from the red jungle fowl. Its development took place through natural mating and selection. There is very little information available on characteristics of local hill fowl of Uttarakhand. However, existence of different strains of Uttara fowl has been reported (Pant *et al.*, 2007). Most of the hill fowls are unique in their adaptation to the agro-climatic conditions of



their habitat (Kaur *et al.*, 2010). Their germplasm is unexplored and possess various potential genes responsible for survival in rough conditions of Uttarakhand.

Blood cells can be categorized into erythrocytes, granulocytes (neutrophils, eosinophils and basophils), agranulocytes (monocytes and lymphocytes) and thrombocytes. The blood of birds have nucleated erythrocytes and nucleated thrombocytes. Birds also differ in coagulation, particularly in intrinsic system, because they appear to lack coagulation factor V, VII, X, XI and XII. Avian erythrocytes function similar to the mammalian erythrocytes with some notable biochemical differences (Jain, 1993). The 2, 3-bisphosphoglycerate is present only in chicken embryonal erythrocytes and disappears shortly after hatch (Bounous and Stedman, 2000). Avian thrombocytes are phagocytic in nature, participate in the hemostatic process and like mammalian platelets, tend to clump in blood film (Thrall *et al.*, 2004). Monocytes and lymphocytes are similar to mammalian cells. Monocytes have a crucial role in chronic inflammation. Monocytosis is usually a consequence of chronic disease such as granulomatus lesion, bacterial infection, parasitism, zinc deficiency etc. (Jain, 1993). Lymphocytes are responsible for the cell mediated immunity (T-lymphocytes) and humoral immunity (B-lymphocytes). The avian blood cell morphology differs significantly from the mammalian counterparts. There is some variation in blood cell morphology in different avian species. The ultrastructural picture of avian reticulocyte was not revealed. Keeping it in view the above facts, the present study was conducted to reveal ultrastructural morphology.

Materials and Methods

The experimental plan of the study was duly approved by the Animal Ethics committee G.B.P.U.A & T, Pantnagar, Uttarakhand, India. The study was conducted on ten adult apparently healthy Uttara fowl of either sex reared at instructional poultry farm, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar.

Sample Processing

For transmission electron microscopy (TEM) the blood samples were collected in 5ml syringe using EDTA as anticoagulant, transferred into siliconized centrifuge tubes and spun at 3000 r. p. m. for 45 minutes. The plasma was drained off and the buffy coat was fixed for 5 hours at 5°C in the standard fixative (5 % gluteraldehyde in 0.1M phosphate buffer, pH 7.4).The fixative was gently removed. Fragile buffy coat disc was carefully removed with the help of a sharp pointed scalpel. After removal, the disc was put in a petridish containing phosphate buffered saline (pH 7.4). The disc was cut into several blocks of about 1-2 mm size. The blocks were washed three times with 0.1M phosphate buffer (pH 7.4) for 15 minutes each. The blocks were again fixed at room temperature for one hour using 1% osmium tetra oxide. The blocks were dehydrated using graded alcohol. They were then placed into two changes of 100 % toluene for 5 minutes each, before being transferred to a mixture of equal parts of Araldite and toluene for overnight at room

temperature. Impregnation was carried out in the fresh changes of Araldite and continued for two days at room temperature. The blocks were finally embedded in another change of fresh Araldite and polymerized for 3 days at room temperature. The thin sections (60-70 nm) were cut on an ultramicrotome and placed on copper grids. The sections were stained in a saturated solution of Uranyl acetate in 50 % alcohol for 15 minutes, followed by lead citrate for 15 minutes and examined under TEM (JEOL JEM-1011) operated at 60-80 KV at TEM laboratory facility at GBPUA&T, Pantnagar.

For scanning electron microscopy (SEM) the samples of venous blood from the birds were collected from the wing vein in 5ml syringe using EDTA as anticoagulant. Few drops of blood were transferred into siliconized centrifuge tubes, equal amount of 5% gluteraldehyde was added and the blood was fixed for 1 hour. The mix was spun at 3000 r. p. m. for 30 minutes. The plasma was decanted and buffy coat was taken out in another tube. The blood cells were washed three times with 0.1M phosphate buffer solution (pH 7.4) by centrifuging @ 2000 rpm for 2 minutes. These samples of fixed cells were processed in SEM laboratory facility, G.B.P.U.A & T Pantnagar. There the cells were resuspended in distilled water and repeated washings were performed. The film of the blood cells was made on a clean circular glass cover slip. The blood film was coated with gold sputter coating using JFC-1600 Auto Fine Coater and observed under JEOL JSM-6610 LV scanning electron microscope.

Results and Discussion

The erythrocytes under scanning electron microscope were oval to elliptical in shape with rounded ends. The borders were generally smooth. The erythrocytes had raised central area which indicated the presence of nucleus (Fig. 1 and 2). The peri-nuclear area of the cell was darker as compared to the periphery of the erythrocyte. Kumar *et al.* (2012) reported elongated to round shaped erythrocytes in sheep. Yadav *et al.* (2013) reported elongated erythrocytes under scanning electron microscope in Kadaknath fowl. The thrombocytes under scanning electron microscope were roughly round to oval in shape showing numerous varying sized cytoplasmic processes projecting from their surface (Fig. 2 and 3). Narkkong *et al.* (2009) observed that thrombocytes of white-bellied sea eagle were oval to rod-shaped in appearance with a rough membrane. Yadav *et al.* (2013) reported round thrombocytes with cytoplasmic processes in Kadaknath fowl. Du Plessis and Wilpe (2009) observed that vulture thrombocytes were predominantly oval to elliptical with few pseudopodia. The surface of the thrombocyte was rough showing nodular appearance due to various small nodule like eminences (Fig. 2 and 3). Yadav *et al.* (2013) also reported irregular out growths on the surface of thrombocytes. The aggregated clumps of thrombocytes were also observed (Fig. 3). Mrigesh (2011) also reported aggregation of thrombocytes under scanning electron microscopy, in donkey blood. Scanning electron micrographs revealed four types of leucocytes. However, leucocytes having membrane ruffles are considered as agranulocytes. Burkhardt (1979) reported the cells, with ridge like

profiles having membrane ruffles, as polymorphonuclear leucocytes and cells, with more prominent membrane ruffles as monocytes in chicken. Some leucocytes were large in size and showed cauliflower like appearance. Their cell surface showed narrow ridge-like profiles and small ruffles and one category of leucocytes were also large with more prominent, membrane-like ruffles (Mohd *et al.*, 2016).



Fig. 1: Scanning electron photomicrograph of blood cells showing erythrocytes x 4,500

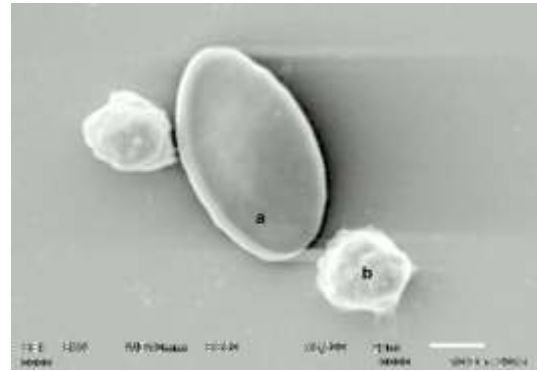


Fig. 2: Scanning electron photomicrograph of blood cells showing erythrocyte (a) and thrombocytes (b) x 6,800

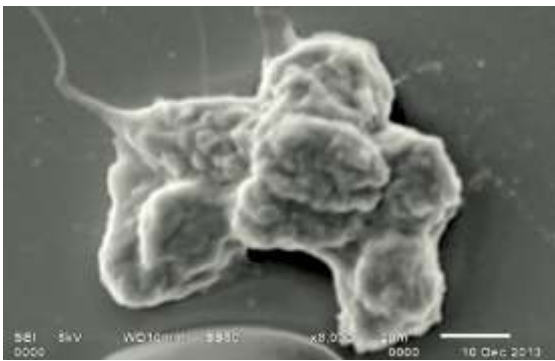


Fig. 3: Scanning electron photomicrograph of blood cells showing thrombocytes in clump x 8000

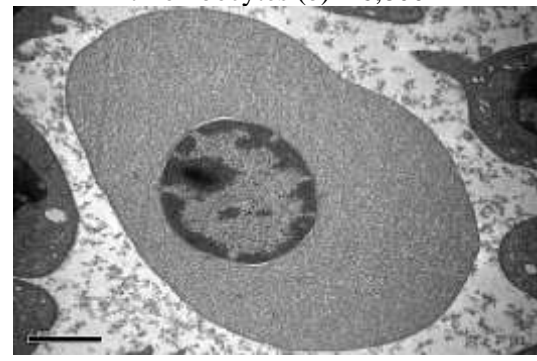


Fig. 4: Transmission electron photomicrograph of erythrocyte. Uranyl acetate and lead citrate x 26,500

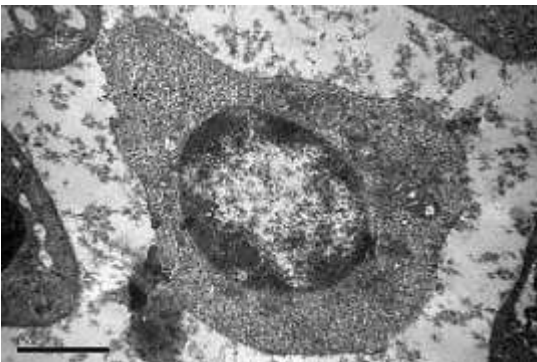


Fig. 5: Transmission electron photomicrograph showing reticulocyte with oval shaped mitochondria (a) and polysomes (b). Uranyl acetate and lead citrate x 33,200

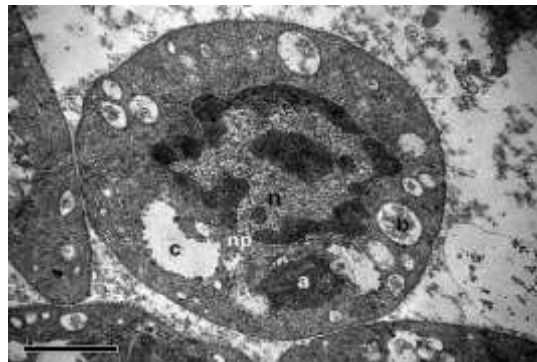


Fig. 6: Transmission electron photomicrograph of thrombocyte showing membrane bound electron dense body (a), vacuole enclosing lysed material (b), nucleus (n) with distinct nuclear pore (np) and granule (g). Uranyl acetate and lead citrate x 33,200

The TEM results showed that erythrocytes were elliptical or oval in shape as per plane of the section (Fig. 6). Elliptical shaped erythrocytes were also reported in Kadaknath fowl (Yadav *et al.*, 2013). The nucleus was oval or elliptical in shape. The perinuclear space was indistinct. The condensed chromatin was more concentrated peripherally and also distributed throughout the nucleoplasm in the form of patches (Fig. 4). Hodges (1977) in avian erythrocytes also reported oval shaped nucleus with masses of heavily condensed chromatin present both peripherally and throughout the nucleoplasm but according to that study there was well defined gap between nucleus and cytoplasm. Salakij *et al.* (2003) found that, painted stork's RBCs contained a nucleus, hemoglobin and some mitochondria. The cytoplasm was almost homogeneously stained (Fig. 4). No cell organelles were found in the cytoplasm of the erythrocytes. But Salakij *et al.* (2003) reported presence of hemoglobin and some mitochondria along with nucleus in painted storks. A few reticulocytes were seen under transmission electron microscope. The nucleus was large and oval in shape. The heterochromatin was arranged in the periphery of the nucleus in the form of patches and euchromatin was centrally placed. The cytoplasm was homogenous in appearance similar to the erythrocyte but a few polysomes and mitochondria were seen (Fig. 5). Koury *et al.* (2005) reported mice nascent reticulocyte with abundant ribosomes, intact mitochondria and vacuoles in an in vitro maturation study of nascent reticulocyte to erythrocyte. The thrombocytes were round in shape and contained more cytoplasm than small lymphocytes (Fig. 6). The nucleus of thrombocyte was centrally placed, dense and irregularly round to oval in shape. The heterochromatin was distributed more towards the periphery.

Maxwell (1974) also observed more cytoplasm than small lymphocytes in thrombocytes of duck, goose, pigeon, quail and turkey under electron microscope. Hodges (1977) observed irregularly round, oval or elongated thrombocytes with cytoplasmic processes or pseudopodia and round to oval shaped nucleus. Yadav *et al.* (2013) reported oval or elongated shape thrombocytes with round to oval shaped centrally or eccentrically nucleus having peripheral distribution of heterochromatin. Most of the cell organelles were arranged close to the nuclear membrane surrounded by an area of ectoplasm containing free ribosomes and a few glycogen granules. The mitochondria were small and oval in shape. Few granules were also observed. Hodges (1977) reported presence of mitochondria, Golgi complex, smooth endoplasmic reticulum and rough endoplasmic reticulum and many groups of microtubules in avian thrombocytes. Maxwell and Trezo (1970) reported presence of small round mitochondria, microtubules parallel to contours of the cell, very small fine granules close to nuclear membrane and a rare presence of Golgi apparatus in fowl thrombocytes. The cytoplasm had several vacuoles and lumen of the large vacuoles contained electron dense material inside it (Fig. 6). Presence of vacuoles was also reported in ostrich thrombocytes (Bonadiman *et al.*, 2009) and vulture thrombocytes (Du Plessis and Wilpe, 2009).

Transmission electron micrographs showed the lymphocytes were nucleated cells, roughly rounded in outline with cytoplasmic processes and pseudopodia. Depending upon their size they were classified as

small, medium and large lymphocytes. These findings were in corroboration with (Lucas and Jamroz, 1961) in chicken. Gupta and Singh (2008) observed only small and large lymphocytes in guinea fowl. The small lymphocytes were roughly round shaped with few cytoplasmic processes on the cell surface. The nucleus of the cell was placed at the centre of the cell occupying most of the cytoplasm. The cytoplasm surrounded the nucleus in the form of a rim. Few pinocytic vesicles and phagocytic vacuoles were also observed in the cytoplasm (Fig. 7). Gupta and Singh (2008) also reported that cytoplasm was surrounding the nucleus of small lymphocyte in the form of a rim. The nuclei of the small lymphocytes were generally oval in shape in the Uttara fowl. However, Maxwell (1974) reported that the nucleus showed considerable pleomorphism, being round, oval, irregular or indented in outline in domestic birds. The nucleus had distinct double layered nuclear membrane. The heterochromatin was distributed both at periphery and center of the nucleus (Fig. 1). Bonadiman *et al.* (2009) reported peripheral distribution of heterochromatin in ostrich lymphocyte. Maxwell (1974) reported nucleus with equal amount of heterochromatin and euchromatin, multivesicular bodies and some small clear vacuoles in small lymphocyte of duck, geese, turkey, pigeon, quail and guinea fowl.

The medium lymphocytes were oval to round in appearance with prominent foot like cytoplasmic processes or pseudopodia (Fig. 8). The nucleus was oval in shape occupying most of the cytoplasm. The heterochromatin was distributed both at periphery as well as at the centre of the nucleus in the form of patches. The distinct nuclear membrane was seen. The cytoplasm occupied the periphery of the cell in the form of ring. Few pinocytic vesicles were seen in the cytoplasm of the lymphocyte. Maxwell (1974) also observed round or oval shaped medium lymphocytes with small and thin pseudopodia and some lobopodia in six different species of domestic birds. He also reported high nucleus to cytoplasmic ratio and presence of pinocytic vesicles near the plasma membrane of medium lymphocytes. The large lymphocytes were roughly rounded in appearance and contained an oval nucleus. The nucleus occupied most of the cytoplasm and was placed at the centre of the cell. The heterochromatin was distributed both at the periphery as well as at the centre of the nucleus. The euchromatin region of the nucleus contained two distinct nucleoli, one small and other large sized (Fig. 9). The nuclear membrane was double layered with distinct nuclear pores. The endoplasmic reticulum was present near the peripheral region of the nucleus. The cytoplasm at the periphery appeared vacuolated in appearance due to presence of numerous small vacuoles. Gupta and Singh (2008) reported presence of narrow rim of heterochromatin beneath the nuclear membrane surrounding the euchromatin and no nucleoli were observed in large lymphocyte of guinea fowl. Salakij *et al.* (2015) observed that ultra-structurally, lymphocytes contained round nuclei with dense heterochromatin. A few lymphocytes (less than 5% WBC) contain azurophilic granules in Crested Serpent eagle and Shikra. However, no such azurophilic granules were found in lymphocytes of Uttara fowl.

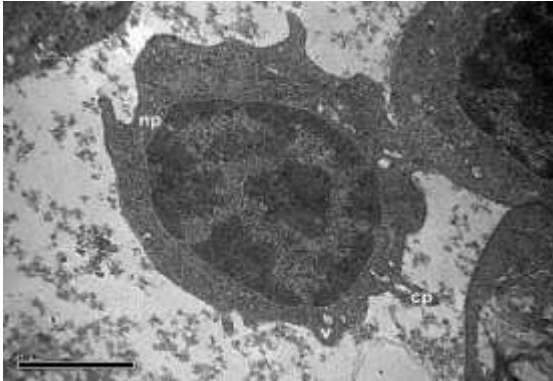


Fig. 7: Transmission electron photomicrograph of small lymphocyte showing nuclear pore (np), cytoplasmic process (cp) and pinocytotic vesicle (v). x 39,800

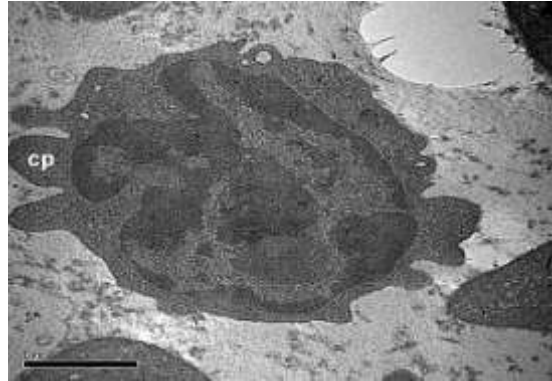


Fig. 8: Transmission electron photomicrograph of medium lymphocyte, showing cytoplasmic process (cp). Uranyl acetate and lead citrate x 39,800

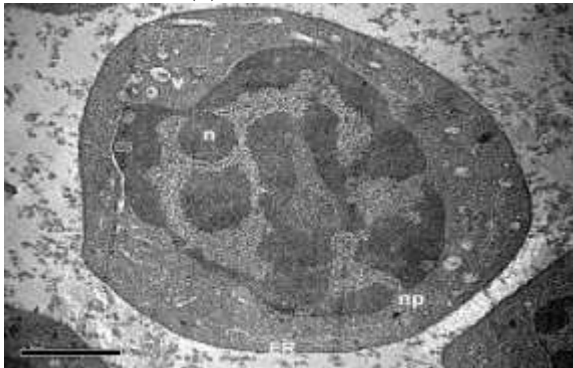


Fig. 9: Transmission electron photomicrograph of large lymphocyte showing nucleolus (n), nuclear pore (np), vesicle (v) and endoplasmic reticulum (ER). x 33,200

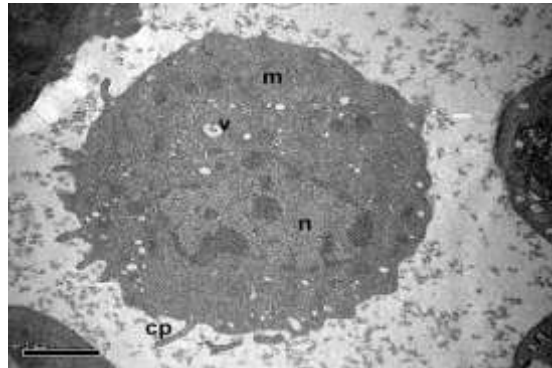


Fig. 10: Transmission electron photomicrograph of monocyte showing mitochondrion (m), nucleus (n), vesicle (v), cytoplasmic process (cp) and electron dense body (a). Uranyl acetate and lead citrate x 26,500

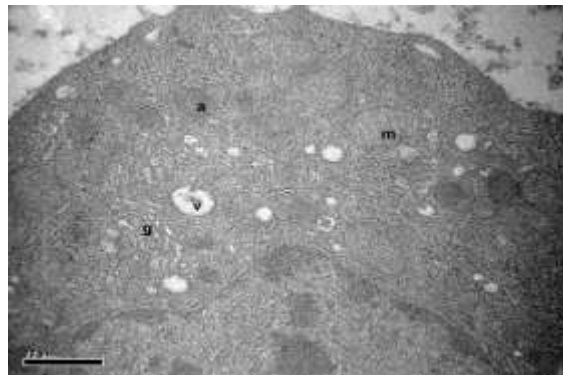


Fig. 11: Transmission electron photomicrograph of monocyte showing Golgi body (g), mitochondrion (m), vesicle with lysed material (v) and electron dense body (a). Uranyl acetate and lead citrate x 53,100

The monocytes were roughly round in shape with finger like cytoplasmic processes on cell surface. The cell had kidney or bean shaped nucleus containing few patches of heterochromatin (Fig. 10). The nuclear membrane was distinct double layered with nuclear pores. Gupta and Singh (2008) also reported kidney

shaped indented nucleus with well-defined nuclear membrane in guinea fowl. Suwannasaeng *et al.* (2014) reported that monocytes contained slightly indented unlobed nucleus, numerous ribosomes and many mitochondria in crested Goshawk. The mitochondria were oval in shape with well-defined cristae. The Golgi apparatus, rough as well as smooth endoplasmic reticulum and large number of membrane bound empty vesicles of varying size were observed (Fig. 11). The vesicles with variable amount of lysed material were found distributed throughout the cytoplasm. Bonadiman *et al.* (2009) reported that monocytes had a lower nuclear cytoplasm ratio when compared with lymphocytes in ostriches. Larger monocytes with higher vacuolar content and plasma membrane protrusions were also observed indicating blood monocyte heterogeneity. However, Gupta and Singh (2008) reported presence of only rough endoplasmic reticulum and mitochondria in the monocyte of guinea fowl. Few small electron dense bodies were also distributed in the cytoplasm. Similar observations were reported in the monocytes of different domestic fowls (Maxwell, 1974).

Conclusion

Scanning electron microscopic studies revealed four different types of leucocytes and thrombocytes presented nodular shaped surface eminences. Transmission electron microscopic studies indicated presence of reticulocytes with characteristic mitochondria and polysomes found within the cytoplasm. Lymphocytes of three types were observed *viz.* small, medium and large. Monocytes were highly vacuolated with well-developed cell organelles. Thrombocytes had an area of ectoplasm containing free ribosomes and a few glycogen granules.

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