Avian Erythrocytes and Agranulocytes - a Review

Ananya Bhattacherjee, PhD

Independent Researcher, Bhubaneswar, India; ananyabhattacherjee@gmail.com
https://orcid.org/0000-0002-3617-2125

https://doi.org/10.57098/SciRevs.Biology.2.2.2
Received June 19, 2023. Revised June 23, 2023. Published online July 04, 2023.

Abstract: This study reports on the morphology of red blood cells and agranulocytes in various types of birds, such as rheas, emus, ostriches, chickens, and geese. Though all of them have the typical avian similarity in blood cells, yet there are some differences. Along with normal and mature erythrocytes, lymphocytes, and monocytes, the present study also focuses on rubricytes, reticulocytes, poikilocytes, polychromatophilic erythrocytes, reactive lymphocytes, pleomorphic monocyte nuclei, etc.

Keywords: Erythrocytes, lymphocytes, monocytes, blood cells, hematology, birds, Aves, morphology

Introduction

Birds inhabit a vast area from sea to mountains and deserts to rainforests. Their physiology differs based on habit, habitat, age, sex, feed, and breeding season. Blood cell morphology plays an important role in diagnosing physiological and pathological aspects of birds' health. The study of the blood of birds revealed various types of cells just like other vertebrates. These cells are red blood cells or erythrocytes, white blood cells or leukocytes, and thrombocytes which are equivalent to mammalian platelets. There are two types of leukocytes, viz., agranulocytes and granulocytes. Agranulocytes are of two types, namely, lymphocytes and monocytes. Granulocytes are of three types, heterophils same as neutrophils in mammals, eosinophils, and basophils. In the following paragraphs, facts about avian erythrocytes and agranulocytes are discussed.

Erythrocytes

An erythrocyte carries oxygen and carbon dioxide and the exchange of gases depends on the cell's surface area to size ratio. Avian erythrocytes are large, ovoid, or elliptical with homogenous eosinophilic cytoplasm and a similarly shaped nucleus at the center containing condensed chromatin (Narkkong et al., 2011 and Lazăr et al., 2012). Such red blood cells (RBCs) are more efficient than spherical ones of the same volume. Aves are the most active vertebrates and have a high metabolic rate. Due to this, the demand for gaseous exchange is higher than other animals. Among Aves, aquatic birds have a higher metabolic rate than terrestrial birds. As a result, their erythrocytes are larger so that sufficient gaseous exchange can take place as reported by Hartman and Lessler, 1963. Nucleated RBCs of birds maintain better homeostasis thus helping in better utilization of water. Due to this reason, aquatic birds' erythrocytes bear larger nuclei compared to others which help them to survive during osmotic stress.

A comparative study on erythrocytes of Vanaraja chicken (terrestrial bird) and White Embden goose (aquatic bird) performed by Tripathy and Acharya, 2019, reported that both red blood cells and their nuclei in geese are larger compared to chickens. The measurement of a bird's RBC ranges from 11-16µm in length and 6-10µm in breadth. Among birds, Rheiformes bear the largest blood cells. Their RBCs stain orange-pink with deep purple-colored nucleus (Gallo et al., 2015). Samour et al., 2010 noticed that the dimensions of peafowl RBCs are 13.74±0.77µm and 6.56±0.65µm, with nuclear dimensions of 5.28±0.45µm and 2.20±0.40µm. The cytoplasm was slightly eosinophilic, with a strongly basophilic nucleus. The size of fully mature erythrocytes of chicken and turkey found in peripheral blood is 12×6µm. The nuclear periphery having densely populated clumps of condensed chromatin surrounds the central loosely arranged chromatin (Khan et al., 2016). Dimensions of erythrocytes decrease with an increase in age as nuclear chromatin

condenses as mentioned by Kramer, 2015 and Khan et al., 2016. Macrocytes, microcytes, and anisocytes reflect changes in the size of erythrocytes. These can be observed in mono-layer blood film and the degree of anisocytosis can be determined by the presence of different sizes of erythrocytes (Campbell, 2012).

Since, erythropoiesis is intravascular or intrasinusoidal, occasional rubricytes or basophilic erythroblasts are found in blood smears of healthy birds (Haile and Chanie, 2014). These are round immature erythrocytes with circular nuclei. They have a very thin ring-like basophilic cytoplasm around the nucleus. Like this, other immature RBCs are metarubricytes or polychromatic erythroblasts. These cells are less circular with basophilic and more abundant cytoplasm than rubricytes in the case of Rhea. The nuclei of such cells are round with partially condensed nuclear chromatin. Young polychromatic erythrocytes were also observed in Rheas. They are slightly oval with a larger non-basophilic cytoplasm and elongated nucleus having very condensed chromatin and a 1/1 Nucleus to Cytoplasmic (N:C) ratio. The presence of immature erythrocytes in the blood smears of birds does not always indicate any disease. Occasionally binucleated erythrocytes or mitotic cells are seen in the blood smear of birds, but if present in large numbers may indicate any neoplastic process, viral infection, or genetic disorder. (Gallo 2015).

Immature erythrocytes are also known as reticulocytes which have a distinct ring of aggregated reticular material (reticulofilamentous substances) circling the nucleus (Khan et al 2016). Chickens and turkeys have a greater number of reticulocytes in peripheral blood compared to mammals. These authors also observed more polychromasia in young birds. Artifacts on slides occur during blood smear preparation and cause hindrances in the correct identification and counting of blood cells. Some of the common artefactual problems include cytoplasmic vacuoles, smudge cells, and stretching cells like a spindle, bi-lobed nuclei, bare nuclei, and erythroplastids (Haile and Chanie, 2014 and Wakenell, 2010).

Round immature erythrocytes are also noticed in emus but with an irregular nuclear periphery. The mature RBCs which differ from typical oval shapes are called poikilocytes, such as schistocytes and dacryocytes. Schistocytes are erythrocyte fragments with two pointed ends found in peripheral blood. These fragments form when RBCs have to cross through defective blood vessels forcibly. Dacryocytes commonly known as teardrop RBCs have the shape of a drop of tear (Dash, 2020). Sometimes, tear-drop erythroplastids or enucleated cells are also found.

According to Tadjalli et al., 2013, in adult male ostriches, the centrally located reticulocyte nucleus contained reticular chromatin without nucleolus. The cytoplasm of erythrocytes was light blue to gray. These were oval-shaped but smaller and with wider nuclei compared to mature erythrocytes.

Like rubricytes, small number of polychromatophilic erythrocytes are sometimes present in avian blood smears. These cells are the last stage of mature erythrocyte development. The cytoplasm of such cells has more ribosomal RNA and appears bluish than mature RBCs. Their chromatin material is denser compared to mature erythrocytes. More than 1-5% polychromatophilic red blood cells, indicates increased erythropoiesis to overcome anemia (Clark et al., 2009).

Hypochromatic RBCs are also occasionally observed. These cells are paler compared to mature erythrocytes and have an area of cytoplasmic pallor covering more than half of the cytoplasm. These cells have vacuoles and their nuclei are pyknotic (Campbell, 2012).

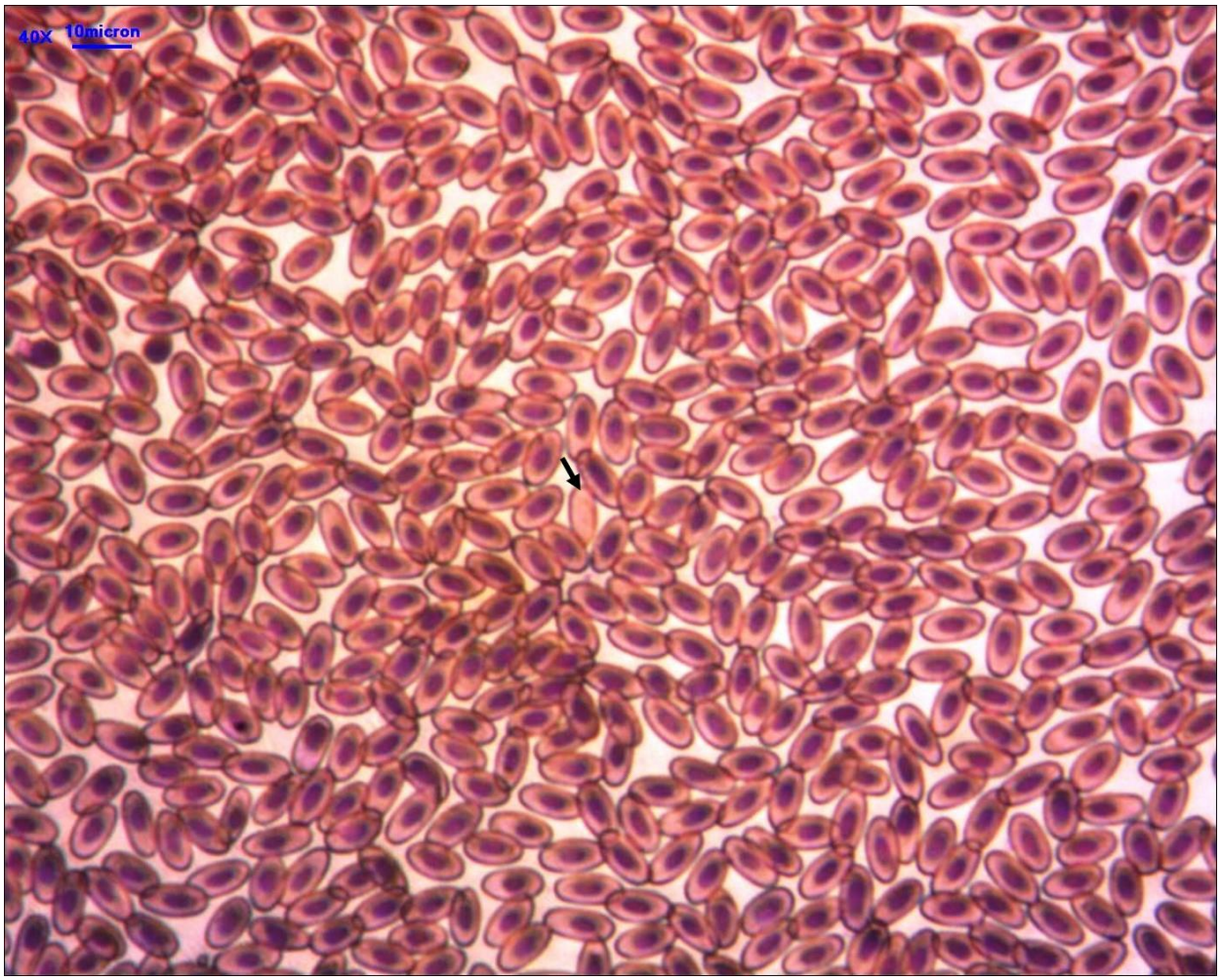

Figure 1: Normal ovoid bird erythrocytes. An erythroplastid (indicated by arrow) at the center.

Lymphocytes

Typical avian lymphocytes are the smallest and most numerous leukocytes and one of the agranulocytes. These circular cells have centrally or eccentrically placed nuclei almost covering the whole cell. The nucleus is also round, non-lobular, purple in color when stained, and has condensed chromatin. The cytoplasm is homogenous, basophilic, forms a narrow halo around the nucleus, and lacks vacuoles. The amount of cytoplasm is less in small lymphocytes and increases as the cell becomes a large lymphocyte. They have a high N:C ratio and measure 6-12µm in diameter (Gallo et al., 2015 and Kramer, 2015). The amount of cytoplasm is lower when compared to monocytes (Narkkong et al., 2011 and Lazăr et al., 2012). Haile and Chanie 2014, also observed cytoplasmic projections or protrusions in small lymphocytes which can easily be confused with thrombocytes. But thrombocytes can be identified by their clear cytoplasm and presence near other cells. Sometimes, reactive lymphocytes are present in the peripheral blood film. Such cells increase in size and the cytoplasm becomes deeply basophilic. These reactive lymphocytes can be confused with rubricytes. However, the latter has a low N:C ratio and is present along with polychromatophilic erythrocytes during the regenerative response to anemia. The presence of reddish (eosinophilic or azurophilic) granules in lymphocytes was also reported. Such rare granules can be different from dust-like granules present in monocytes sometimes (Bounous and Stedman, 2000).

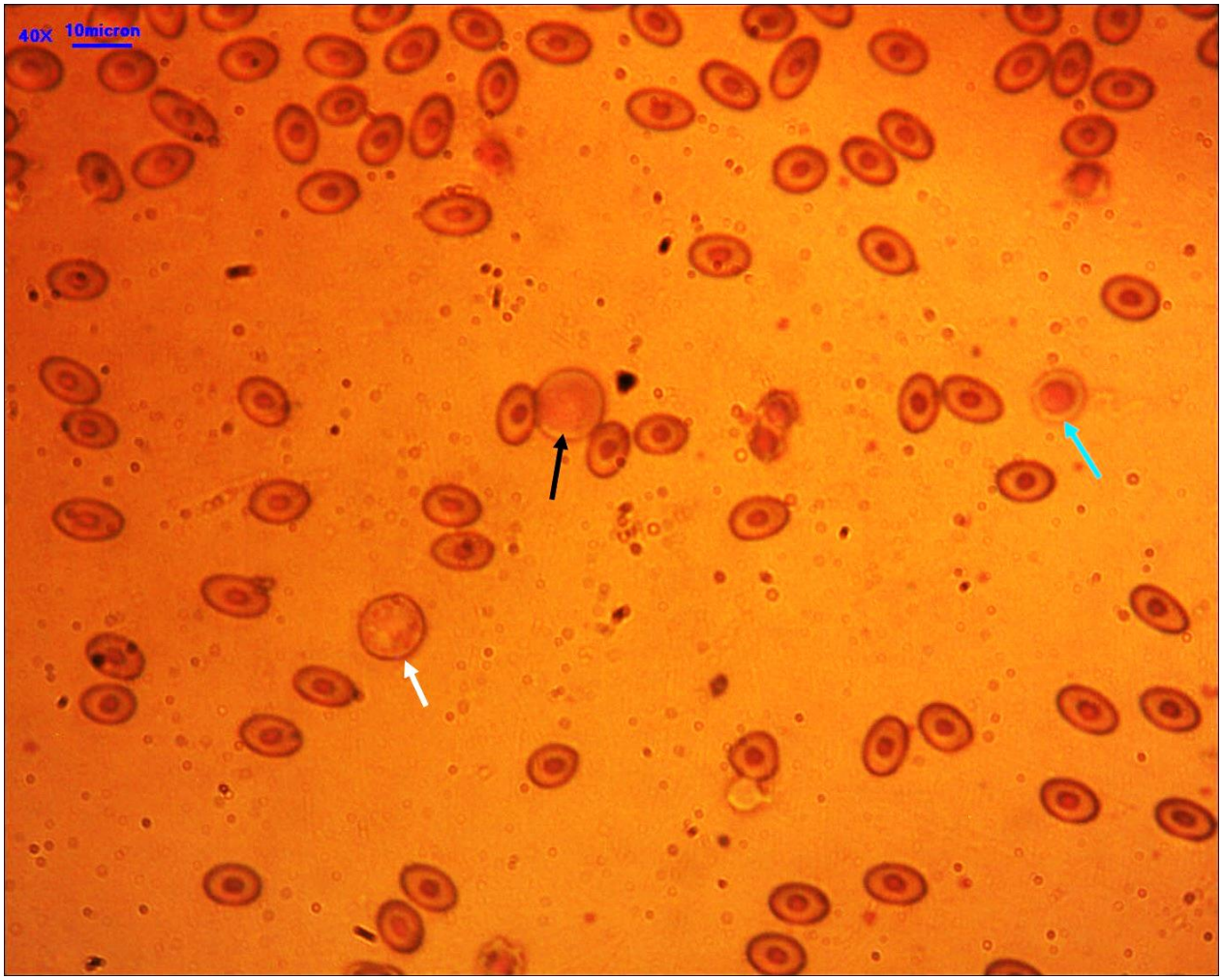

Figure 2: Rubricyte (indicated by black arrow), polychromatic RBC (indicated by blue arrow) and mitotic RBC (indicated by white arrow).

Depending on the size of the lymphocytes, they are small, medium, and large lymphocytes. Khan et al., 2016, observed that small lymphocytes have round but irregular membranes and the nucleus is large and round but placed eccentrically in adult Uttara fowl. The nucleus occupies almost the whole cell and the cytoplasm was present as a thin lining (weak perinuclear zone) around it. Just like small lymphocytes, medium, and large lymphocytes also have large circular nuclei. The amount of cytoplasm gradually increases with the advancement of lymphocyte size. It is more in medium lymphocytes and forms a wide band around the nucleus. The highest amount of cytoplasm is present in large lymphocytes and forms a ring around the nucleus. Lymphocytes are basophilic and among the three types of lymphocytes, the large one is the most bluish. As per Thrall et al., 2004, the nucleus in avian lymphocytes is slightly indented but Deldar, 1998 opined the presence of a round nucleus in small lymphocytes and an indented nucleus in large lymphocytes of birds.

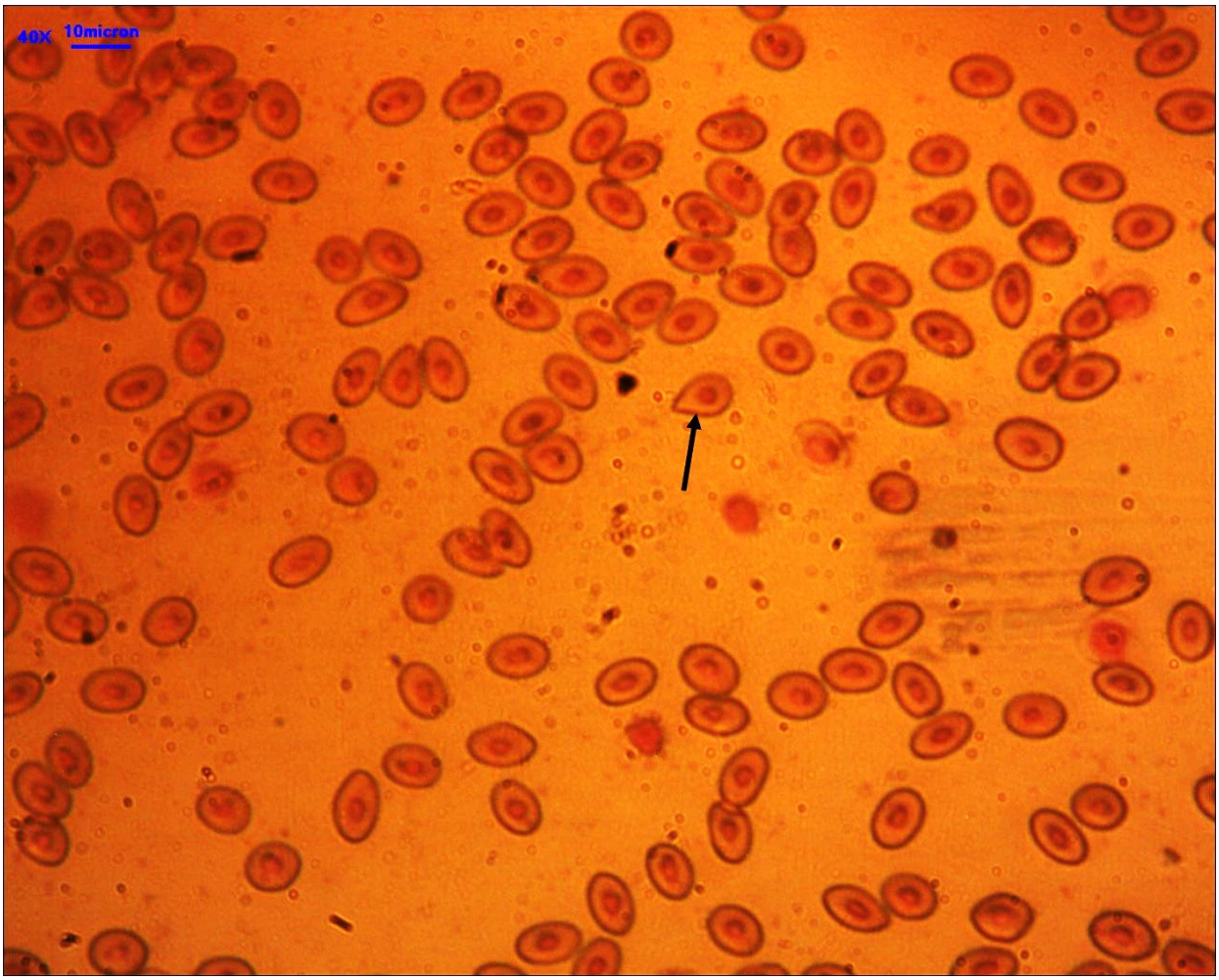

Figure 3: Dacryocyte (indicated by black arrow). This blood smear has lots of artifacts.

The lymphocyte of birds is larger in size compared to other animals. Among birds, aquatic species have the largest lymphocytes, which according to Tripathy and Acharya 2019 may be due to nutritional status, ambient temperature, and the prolonged effect of the stress factor. But as per Kolesnik et al., 2020, neonatal chicken lymphocytes are smaller in size compared to that of mammals. The three types of lymphocytes are not only based on their size but also on their N:C ratio, the structure of nuclear chromatin, the position of the nucleus in the cell, and the color of cytoplasm and nucleus when stained. The nuclear chromatin of small and medium lymphocytes is highly basophilic therefore, their chromatin is called basichromatin and the chromatin surface is heterogenous. But, the nuclear surface of mammalian lymphocytes of the same stage is homogenous. Large lymphocytes have distinguished perikaryon, eccentrically placed nuclei, and comparatively lighter basophilic chromatin. Neonatal chicks have a little amount of cytoplasm in large lymphocytes with centrally positioned large, pycnotic nuclei having compact and highly basophilic chromatin. All lymphocytes, irrespective of size are basophilic but the shades of blue may differ.

Both Khan et al., 2016 and Kolesnik et al., 2020 had worked on chicken but their observations are opposite which may be due to differences in breed and age of the concerned subjects.

The size of lymphocytes in Indian peafowl was 6.52-0.87µm with typical cellular and nuclear details. N:C ratio in this study was estimated to be 3:4. Very little amount of small-sized strongly basophilic granules were present in the cytoplasm. These granules were found in 25% of all lymphocytes observed (Samour et al., 2010).

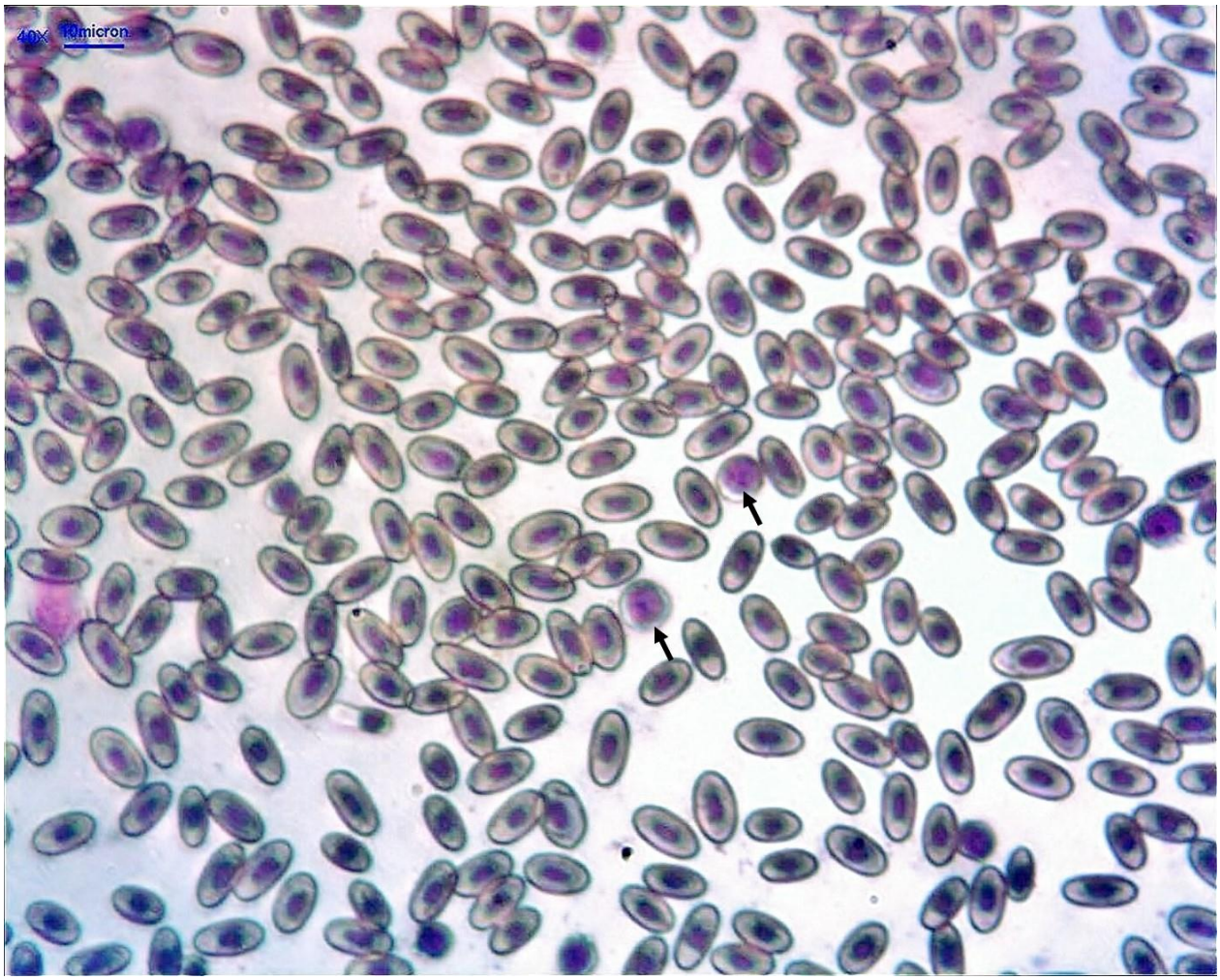

Figure 4: Lymphocytes (indicated by black arrows).

Monocytes

Monocyte, a type of agranulocyte, is the largest leukocyte in the peripheral blood of birds. It has a bluish cytoplasm with a horse-shoe-shaped nucleus. The amount of cytoplasm in monocyte is also more abundant compared to other blood cells. As per Tripathy and Acharya 2019, the cytoplasm of monocyte is more bluish than that of lymphocytes. These cells are part of innate immunity and produce cytokines as an inflammatory response, they can also phagocytose and be defensive against pathogens.

According to Lucas and Jamroz, 1961, the average size of a monocyte is 12µm. Their cytoplasm has small and discrete vacuoles and pleomorphic nuclei with less condensed chromatin than lymphocytes. The shape of a monocyte ranges from circular to oval to irregular shape. The shape of the nucleus differs from oval to various shapes and the cytoplasm is clear and cross-linked (Green and Blue-McLendon, 2000). The nucleus may be positioned centrally or eccentrically. Most of the chromatin is loosely arranged with small areas of condensed chromatin (Kramer, 2015).

Khan et al., 2016 reported that the nucleus of monocytes of Uttara fowl is large, eccentrically placed, kidney or dumbbell-shaped. The cytoplasm had a foamy appearance due to the presence of vacuoles and the cytoplasm is light bluish in color.

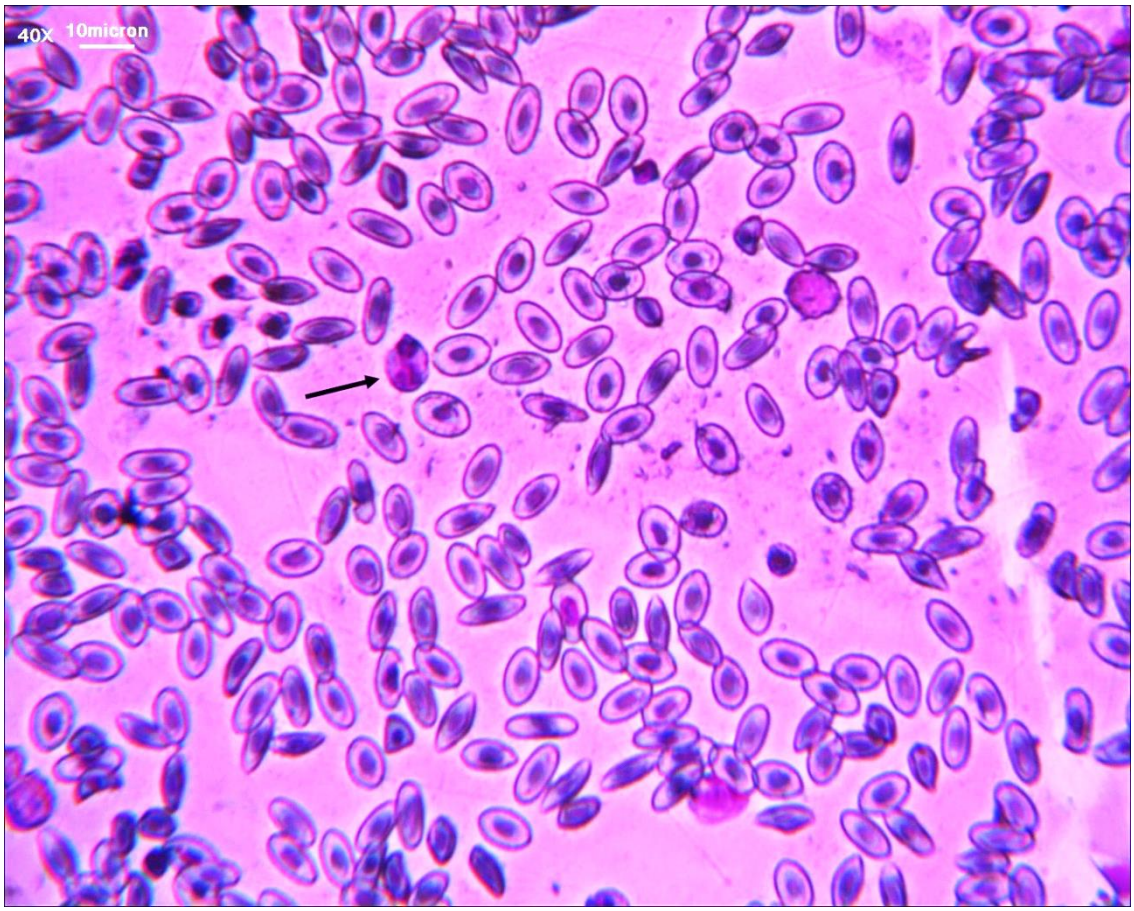

Figure 5: Monocyte with pleomorphic nucleus (indicated by black arrow).

Monocytes are also known as mononuclear and macrophages. They have a peculiar N:C ratio, due to the presence of a large volume of cytoplasm and polymorphic nuclei (nuclei can take any shape). Normally as cells mature, their nuclei reduce in size and thus there is a decrease in N:C ratio. But in monocytes, this ratio remains 3:1 or 4:1 even after maturation. Monocyte contains heterochromatin which forms a reticular network-like pattern. During phagocytosis, with the formation of pseudopodia, they are called macrophages and the shape of these cells becomes irregular (Kolesnik et al 2020). Lazăr et al., 2012 observed fine azurophilic granules in the monocyte cytoplasm of B.U.T 6 turkey. But Narkkong et al., 2011, observed fine dark blue granules in monocytes of the eastern sarus crane.

The monocytes of chicken and turkey measure 14µm in diameter (Haile and Chanie 2014) and have similarities with mammalian counterparts. Due to the pleomorphic nature of the monocyte nucleus, it becomes difficult to differentiate and identify it many times. The number of monocytes should be very less in healthy individuals. The same authors have observed a range of shapes of monocytes in their study, from bean-shape to a very irregular shape with a reticular chromatin pattern.

The monocytes in Indian peafowl were 10.84-0.89µm with slightly round to irregular shapes. Cytoplasm is colorless and has a lace-like network with large irregular kidney to round-shaped nucleus. The size of vacuoles varied from 1.5µm to 3µm and was visible in 50% of cells examined. The N:C ratio was 1:1 (Samour et al., 2010). Tadjalli et al., 2013 did similar observations in adult male ostrich.

Significance of Morphological Studies on Avian Blood Cells

Blood cell morphology can be helpful in detecting the causes of underlying diseases. It can be an important contribution to avian veterinary care. Such studies can provide differential diagnoses of pathological conditions such as the presence of blood parasites (haemo-parasites) and monitoring immune responses to them. By investigating the

morphology of blood cells, the avian health status can be clinically analyzed and thus reliable information on metabolic disorders, nutrient deficiencies, chronic stress status, and pathological aspects can be provided.

Conclusion

Hematology, the study of blood focuses on various blood parameters which can suggest the health status of birds. Among various parameters, the detailed study of blood cells can disclose known and unknown aspects of avian blood. Although all blood cells have their unique function, erythrocytes and agranulocytes play important role in the quick identification of physiological and pathological problems. More studies on these cells with more advanced technology are required in the case of avian subjects as they are vast in numbers and categories.

References

Narkkong, N.A., Aengwanich, W. and Tanomthong, A. 2011. "Morphology and morphometrics of hematological cells from eastern sarus crane, *Grus antigone sharpii*". Comparative Clinical Pathology, No. 20(4): 299-304. https://doi.org/10.1007/s00580-010-0990-9.

Lazăr, R., Boişteanu, P.C., Muntean, C., Apetroaei (Petrescu), C-A. and Ciobanu, M.M. 2012. "Characterisation of the haematological profile of the hybrid B.U.T 6 turkey raised in Romania". Lucrări Ştiinţifice - Seria Zootehnie, No. 58: 254-257. https://www.uaiasi.ro/firaa/Pdf/Pdf_Vol_58/Roxana_Lazar.pdf.

Hartman, F.A. and Lessler, M.A. 1963. "Erythrocyte measurement in birds". The Auk, No. 80(4): 467-473. https://doi.org/10.2307/4082852.

Tripathy, A. and Acharya G. 2019. "A study on effect of habitat on cytomorphometry of poultry birds". International Journal of Scientific Research and Reviews, No. 8(1): 2864-2874. www.ijsrr.org/pdf/2374.pdf.

Gallo, S.S.M., Ederli, N.B., Bôa-Morte, M.O. and Oliveira, F.C.R. 2015. "Hematological, morphological and morphometric characteristics of blood cells from rhea, *Rhea Americana* (Struthioniformes: Rheidae): a standard for Brazilian birds". Brazilian Journal of Biology, No. 75(4): 953-962. https://doi.org/10.1590/1519-6984.03414.

Samour, J., Naldo, J., Rahman, H., and Sakkir, M. 2010. "Hematologic and Plasma Biochemical Reference Values in Indian Pea fowl (*Pavo cristatus*)". Journal of Avian Medicine and Surgery, No. 24(2): 99-106. https://doi.org/10.1647/2008-019.1.

Khan, I.M., Mrigesh, M., Singh, I., Singh, B., Arora, N. and Choudhary, O.P. 2016. "Cytomorphological study on blood cells of Uttara fowl (*Gallus Domesticus*)". Indian Veterinary Journal, No. 93(2): 32-36. DOI:10.14202/vetworld.2016.320-325

Kramer, M. 2015. "Avian blood collection and hematology". World Small Animal Veterinary Association World Congress Proceedings. https://www.vin.com/doc/?id=7259248.

Campbell, TW. 2012. "Hematology of Birds". In Veterinary Hematology and Clinical Chemistry, 2nd edition, edited by Thrall, MA, Weiser, G, Allison, RW and Campbell, TW, 242-243. United Kingdom: Wiley-Blackwell: A John Wiley & Sons, Inc., Publication.

Haile, Y. and Chanie, M. 2014. "Comparative Aspects of the Clinical Hematology of Birds: A Review". British Journal of Poultry Sciences, No. 3(3): 88-95. https://doi.org/10.5829/idosi.bjps.2014.3.3.83263

Wakenell, PS. 2010. "Hematology of Chickens and Turkeys". In Schalm's Veterinary Hematology, 6th Edition, edited by Weiss, DJ, and Wardrop, KJ, 961-962. Iowa, USA: Wiley-Blackwell.

Dash, I. 2020. "Morphological and morphometrical analyses of Blood Cells of Emus (Dromaius novaehollandiae) in Captive condition". Research Journal of Forensic Sciences, No. 8(2): 1-8. www.isca.me/FORENSIC_SCI/Archive/v8/i2/1.ISCA-RJFS-2020-005.pdf.

Tadjalli, M., Nazifi, S., Abbasabadi, B.M. and Majidi, B. 2013. "Histomorphometric study on blood cells in male adult ostrich". Veterinary Research Forum, No. 4(3): 199-203. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4312382/?report=classic.

Clark, P., Boardman, WSJ., and Raidal, SR. 2009. Atlas of Clinical Avian Hematology, 37-40. West Sussex, United Kingdom: Wiley- Blackwell. DOI:10.1016/j.tvjl.2009.11.015

Bounous, DI. and Stedman, NL. 2000. "Normal Avian Hematology: Chicken and Turkey". In: Schalm's Veterinary Hematology, 5th Edition, edited by Feldmen, BF, Zinkl, JG, and Jain, NC, 1147-1154. Lippincott Williams and Wilkins.

Thrall, MA., Baker, DC., Campbell, TW., Fettman, MJ., Lassen, ED., Rebar, A., Weiser, G. and DeNicola, D. 2004. Hematology of Birds. Veterinary Hematology and Clinical Chemistry, 529-553. Lippincott Williams and Willkins. A Wolters Kluwer Company, Philadelphia USA.

Deldar, A. 1998. "Blood and Bone Marrow." In: Text Book of Veterinary Histology, 5th Edition, edited by Dellmann, DH., and Eurell, J., 62-79. William and Wilkins.

Kolesnik, E., Derkho, M., Strizhikov, V., Strizhikova, S., Sereda, T., Gizatullina, F., and Rebezov, M. 2020. "Functional morphology of birds' blood leukocytes". Journal of Experimental Biology and Agricultural Sciences, No. 8(Spl-2-AABAS): S374-S380. http://dx.doi.org/10.18006/2020.8(Spl-2-AABAS).S374.S380.

Lucas, AM, and Jamroz, C. 1961. Atlas of Avian Haematology. Agriculture Monograph 25, pp214. Washington: United States Department of Agriculture.

Green, R.A., and Blue-Mclendon, A., 2000. Ratite Hematology. In: Schalm's Veterinary Hematology, 5th Edition, edited by Feldman BV., Zinkl JG., and Jain NC., 1201-1206. Baltimore: Lippincott Williams & Wilkins.

Conflicts of Interest

The author declares no conflict of interest.