MORPHOLOGIC CHARACTERISTICS OF PERIPHERAL BLOOD CELLS OF MOOSE IN SWEDEN

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ABSTRACT: In order to interpret the morphologic changes of blood cells in diseased moose (*Alces alces*) with any validity, it is first necessary to obtain sufficient reference material to establish normal morphology. Blood was collected from 74 apparently healthy chemically immobilized moose in northern Sweden. Blood smears were prepared, then stained with May-Grünewald Giemsa stain. Morphologic characteristics of the peripheral blood cells were studied using light microscopy, and described. Cattle cells were used for purposes of comparison. Moose neutrophils showed prominent basophilic granules in the cytoplasm and a four to six lobed nucleus. The cytoplasmic granules of the eosinophils were very small. Moose basophils contained fewer cytoplasmic granules, than those of cattle. The morphology of lymphocytes and monocytes was similar to that of cattle. A slight to moderate amount of basophilic stippling and Howell-Jolly bodies was common in erythrocytes from both yearlings and adults. A generally high erythrocyte sedimentation rate was observed, but rouleaux formation was rare in the blood smears.

ALCES VOL. 33 (1997) pp.119-123

Hematology is often used as a tool for monitoring the health and disease status of animals. The morphology of blood cells can contribute important information and is often of more value than absolute cell counts (Hawkey and Dennett 1989). In order to distinguish abnormal cells, a knowledge of the normal cell morphology in healthy animals is essential. Normal hematological data has now been published for a variety of wild animals. However, information concerning the normal blood cell morphology of these species is rarely presented. The most comprehensive sources of information on this topic is Hawkey and Dennett's (1989) Colour Atlas of Comparative Veterinary Hematology. This book includes the morphologic features of different blood cells from many domestic and exotic species, but obviously cannot present each cell of every species.

Since the mid 1980's a new wasting syndrome has been observed in moose in Sweden (Stéen *et al.* 1993, Merza *et al.* 1994). Investigation of this disease has required the accumulation of reference material from clinically healthy moose. The aim of the present study was to describe the morphologic characteristics of the peripheral blood cells in healthy moose.

MATERIALS AND METHODS

During February and March 1993, 74 apparently healthy moose were immobilized in Västerbotten and Norrbotten in northern Sweden. The animals were of both sexes and of varying ages. They were located with a helicopter and immobilized with a combination of etorphine/acepromazine (Large Animal Immobilon vet; C-Vet, Bury St. Edmunds, U.K.) and xylazine (Rompun® vet; Bayer, Leverkusen, Germany). Blood was collected from either the jugular or lateral saphenous vein into sterile EDTA tubes (Vacutainer® System; Becton Dickinson, Meylan Cedex, France). Blood smears were usually prepared and air dried on the day the sample was collected. The slides were stained

with May-Grünewald Giemsa stain. The morphology and size of leukocytes, erythrocytes and platelets was examined by light microscopy at 100 x magnification under oil immersion. To determine the size of cells, blood smears from ten animals were chosen by random. From each blood smear, approximately six cells of each category were measured. New methylene blue was used to detect Heinz bodies and reticulocytes. The staining techniques used are described in more detail in Schalm's Veterinary Hematology (Jain 1986). A "mild occurrence" of Howell-Jolly bodies and basophilic stippling, is defined as one cell per field of vision at 100 x magnification.

RESULTS

Neutrophils

Only mature neutrophils are described, due to a very low occurrence of immature neutrophils. The cell diameter varied considerably, ranging from 10.5-20 µm. The nucleus in mature neutrophils displayed several, commonly four to six, prominent lobes. Filaments were generally seen between the lobes, but pinching-in without true filament formation also occurred. The nuclear chromatin was clumped into dark-staining areas separated by smaller lighter-staining zones. The cytoplasm appeared clear, pale grey and contained numerous very noticeable granules. These granules varied in size and ranged in color from mild to intense purple, or basophilic (Fig. 1).

Eosinophils

The eosinophil varied in size from 12-17.5 µm. The nucleus commonly consisted of two, or sometimes three prominent segments. Simple narrowing of the segments was common, but filaments also occurred. Band shaped nuclei were occasionally seen. The nuclear chromatin formed dark-staining areas separated by lighter-staining regions. The cytoplasm was grey and contained numerous very small, distinct, eosinophilic granules. The granules did not usually cover any part of the nucleus (Fig. 2)

Basophils

The basophils were ranging from 11-16 µm in size and usually appeared dense and darkly staining. The nuclear chromatin showed as dark clumps which varied in size. The cytoplasm was grey and contained prominent basophilic granules of variable shape and size, usually larger than the granules in other leukocytes. These granules commonly covered parts of the nucleus (Fig. 3).

Lymphocytes

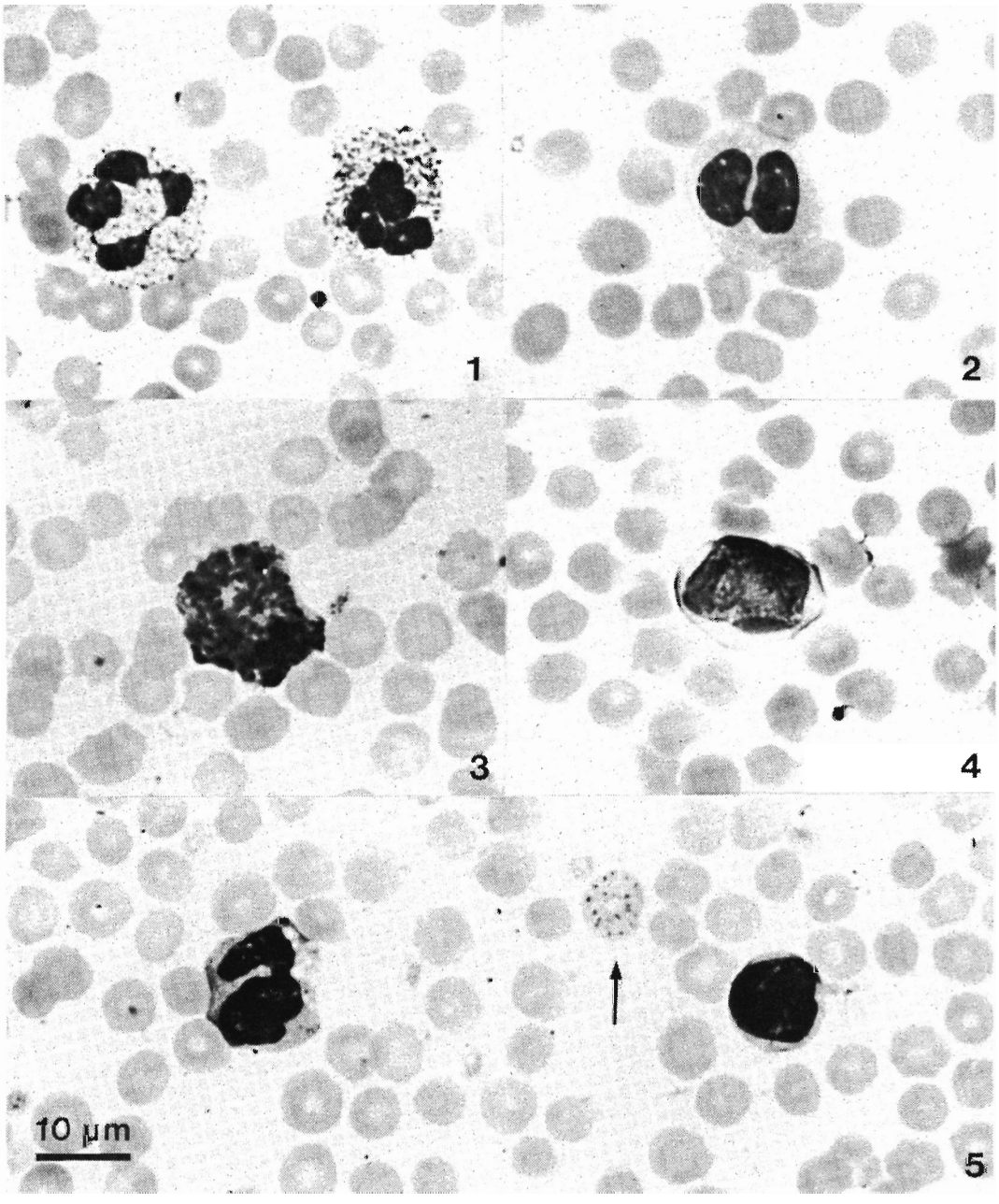
The lymphocytes varied in size from 8-17 µm, the smaller ones being most numerous. Small lymphocytes had a large nucleus to cytoplasmic ratio, and appeared round to oval in shape. The chromatin made the nuclei seem dense and deeply stained. In medium and large lymphocytes, the nuclei were often irregularly round or oval in shape. The chromatin in these larger cells usually gave the nucleus a more homogenous and lighter-stained appearance than in small lymphocytes. The cytoplasm of lymphocytes was clear and pale blue. A few basophilic granules were sometimes seen (Fig. 4 and 5).

Monocytes

The diameter of monocytes were ranging from 12-17.5 µm. The shape of the nucleus was pleomorphic, ranging from irregularly oval and kidney-like, to lobulated. Round nuclei were occasionally observed. The nuclear chromatin showed a mottled pattern. The cytoplasm was greyish blue and usually granulated with small, dust-like, pinkish, indistinct granules. Vacuoles were sometimes seen (Fig. 5).

Erythrocytes

The erythrocytes varied from 5-8.5 µm in size. The cells did not sickle when exposed



Figs. 1 - 5. Morphology of blood cells from clinically normal moose. 1. Two neutrophils with prominent basophilic granules, typical of moose neutrophils. 2. A typical eosinophil with small eosinophilic granules. 3. A basophil with prominent basophilic granules obscuring the form of the nucleus. 4. A large lymphocyte with a lighter-staining nucleus than in small lymphocytes seen in 5. 5. A monocyte with vacuolated cytoplasm and a small lymphocyte. Between the two white cells is an erythrocyte showing basophilic stippling (arrow), a common finding in moose in this study.

to oxygen. A rapid sedimentation rate (ESR) was observed in test tubes, although rouleaux formation in smears was uncommon. Slight anisocytosis was seen in all blood samples. Mild to moderate basophilic stippling (Fig. 5) was detected in 25%, and Howell-Jolly bodies in 12%, of the animals (yearlings and adults). No Heinz bodies were found. Reticulocytes were uncommon.

Platelets

The platelets were usually distributed singly, varying considerably in shape and size (range = 1.5-5.5 μm). The pseudopods were of differing lengths. The cytoplasm was pale grey and contained few to numerous, pale or dark purple granules.

DISCUSSION

Moose neutrophils, eosinophils and basophils were all easy to identify and to distinguish from those of cattle. Neutrophils demonstrate why it is so important to investigate cell morphology in a species rather than generalizing from a related species. First, basophilic, or purple, granules in the cytoplasm of neutrophils were typical for mature neutrophils in moose and should be regarded as normal. Although basophilic granules in neutrophils are reported from some other artiodactyls and chimpanzees (*Pan troglodytes*) (Hawkey and Dennett 1989), healthy domestic animals normally do not show neutrophils with granules in the cytoplasm, unless the cell is exhibiting toxic changes (Jain 1993). Second, neutrophils in moose normally seem to have a greater number of segments, commonly four to six, than those of cattle. Upcott and Hebert (1965) reported that red deer (*Cervus elaphus*) showed neutrophils with two-lobed and very occasionally three-lobed nuclei. More than five lobes is considered to be a pathologic finding in domestic animals. These cells are named pleokaryocytes. Hyper segmented neutrophils have also been found to be related to cellular ageing (Jain 1993).

The small and distinctly reddish cytoplasmic granules of the moose eosinophil make the cell easy to recognise. The granules are smaller than those in cattle. The few basophils observed were dark and dense, but had less granulated cytoplasm. The granules that covered parts of the nucleus made the nuclear form difficult to characterize.

The morphologic characteristics of lymphocytes and monocytes in moose were similar to those in cattle. Small lymphocytes predominate in healthy moose, whereas large lymphocytes dominate in cattle (Jain 1993). Small lymphocytes were easy to characterize, while large ones were easily confused with monocytes. The best way to distinguish between large lymphocytes and monocytes was to identify the small, dust-like, pinkish granules in the cytoplasm of monocytes. Granules often came into sight if the focus of the microscope was changed slightly, but it was not always possible to detect them. The presence of vacuoles in monocytes can also facilitate their identification, however, storage in EDTA can also cause vacuolation of the cytoplasm and disintegration of leukocytes (Jain 1993).

The rapid sedimentation of erythrocytes in moose blood was not measured in a quantifiable manner, such as the Wintrobe method. It was clearly observed in standing test tubes and is mentioned since the knowledge might be of clinical importance. A high ESR is normally connected with erythrocytes forming rolls (rouleaux formation) and/or high levels of fibrinogen (Jain 1993). Rouleaux formation of the moose erythrocytes was, however, not observed. The high ESR in moose is similar to that seen in horses, but much higher than in cattle (Jain 1993). The rapid ESR in moose makes it important to thoroughly mix the blood before removing any for examination.

Variation in size (anisocytosis) is common among erythrocytes in various species

(Jain 1993). Slight anisocytosis was evident in this study and is also described in red deer (Upcott and Hebert 1965).

Basophilic stippling is caused by aggregated ribosomes and appears as dark bluish granules scattered throughout the erythrocytes (Jain 1993). A slight occurrence of basophilic stippling was a common finding in this study. This has been reported as a not unusual finding in some immature artiodactyls (including moose), although it is a pathologic finding in adults (Hawkey and Dennett 1989). In this investigation, basophilic stippling occurred in juvenile and adult animals alike, and apparently can be considered normal. Basophilic stippling can also be associated with disease as seen in some animals with chronic lead poisoning, or as a response to hypochromic anemia (Jain 1993).

Howell-Jolly bodies, small, deep purple nuclear remnants in erythrocytes, were also observed with some frequency in moose of all ages. These are seen normally in some species, but may be of pathologic significance in others (Hawkey and Dennett 1989). A low occurrence of erythrocytes with Howell-Jolly bodies in moose should be considered to be normal in a healthy animal.

"Sickling" is an *in vitro* phenomenon which occurs in normal erythrocytes of many species of deer (Hawkey and Dennett 1989). The sickle shape is formed when the blood is exposed to oxygen at room, or refrigerator, temperature. No sickle-shaped erythrocytes were found in moose blood exposed to oxygen in this study.

This investigation was important in order to gain familiarity with the normal morphologic characteristics of peripheral blood cells in moose. This is necessary so that pathologic abnormalities in the morphology of the blood cells may be recognised and used in disease investigation and health assessment.

ACKNOWLEDGEMENTS

This study was financially supported by the Swedish Environmental Protection Agency and the Karl Erik Önnesjö Foundation. We are grateful to Maria Bergsten, the staff of the Swedish Hunters' Association in Norrbotten and the staff at the Department of Animal Ecology, Swedish University of Agricultural Sciences in Umeå, for collecting blood samples, and to Inger Lilliehöök for valuable comments and Katie Brown for editing the manuscript.

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