Avian Haematology

David N. Phalen DVM, PhD, Dipl. ABVP (Avian)

Wildlife Health and Conservation Centre and Avian,
Reptile and Exotic Animal Hospital, University of Sydney,
415 Werombi Road, PMB 3, Camden, NSW 2570

The haemogram is an indispensable part of the avian practitioner's diagnostic armamentarium. To maximize the information obtained from the haemogram, it is necessary to optimize collection techniques, understand the similarities and the differences between the avian haemogram and that of other species, and to consider the information gathered in light of the circumstances under which the sample was collected.

Anticoagulants

All anticoagulants examined to date produce artifactual changes in bird blood that can, at the minimum, complicate the interpretation of the blood smear and, in the worse case, may invalidate the haemogram entirely. **EDTA is an unsatisfactory anticoagulant for avian blood.** Heparin can be used, but unless the needle used to collect the blood is heparinised, the sample is immediately placed in a heparinised container, and the blood is mixed thoroughly, clumping of white cells is common and clot formation occurs too frequently. Sodium citrate is the best anticoagulant. It has the disadvantage, though, of being a liquid and therefore dilutes the blood sample. This is not a problem if the tube is filled to capacity as the dilution factor for a full tube is known. However, in small birds, collecting enough blood to fill a 3 ml sodium citrate tube is impossible. Instead, a smaller volume can be collected and 10% of the volume of citrate added. Unfortunately, even small errors in measurement will result in fairly significant dilution effects. Blood should be kept on ice until it is analysed. As the length of time increases between sample collection and analysis cell counts become increasingly inaccurate.

Both heparin and sodium citrate produce morphological changes in blood cells. The best way to avoid this problem is to make blood smears in the field or in the clinic with the blood remaining in the needle immediately after the blood is drawn. Rapid drying of the smears by waving them in the air will also improve the cell morphology. The cover slip method of making blood smears may make superior smears then the slide push method. When making blood smears with slides, there are always a large number of smudge cells, most of these are red blood cells, but in some cases it appears that the heterophils may also smudge out. I have found that by using the cover slip technique, smudge cells are far fewer. I have also found that students and veterinarians alike make better slides with the cover slip method. The cover slip method is very easy. A drop of blood is placed on a long cover slip and a second long cover slip is placed on top of it. The two cover slips are then pulled apart. The stained cover slips can then be inverted and either permanently mounted on a regular glass slide, or held in place with a drop of immersion oil.

White Blood Cell Count

If you are currently doing mammalian haematology in your practice, you should have no difficultly doing avian haematology. However, because avian RBCs are not lysed by the routine methods used for mammalian blood, it will be necessary to obtained the Eosinophil Unopette (Bendict-Dickenson) for white blood cell determination. To use this stain, blood is added to the Unopette, the contents are mixed, and

they are added directly to the haemocytometer. The phloxine stain in the Unopette container stains heterophils and eosinophils red. The haemocytometer is left in a humidity chamber for 5 minutes to allow the cells to reach a single plain of focus, and then all the stained cells in the nine large squares on each side of the haemocytometer are counted using 10 X magnification. To calculate the combined heterophil and eosinophil count, 10% is added to the value obtained and this new value is multiplied by 16. To calculate the total white blood cell count (WBC), the absolute combine heterophil and eosinophil count is divided by the combined heterophil and eosinophil fraction of the differential.

For example: If the differential contains 70% heterophils and 5% eosinophils and the two chambers of the haemocytometer had a combined total of 650 stained cells, then:

The total heterophil and eosinophil count would be: (650 + 65) X 16 = 11,740 cells/µl

The total WBC would be: 11,740 / .75 = 15,653 cells/µl

The lymphocyte count and monocyte count would then be calculated from the absolute WBC.

Blood cannot be left in the phloxine reservoir for more than about 15 minutes. Further delays in reading the sample result in nonspecific staining, artificially elevating the WBC count.

Instead of the above method, many people use estimated WBC counts. Values obtained by this method are at best "ball park" estimates. However, much of the time that is all that is needed. To do an estimated count, the number of WBCs are determined for each of 10 high dry (40X) fields. Fields to be scanned should be in the feathered edge where the cells are just touching. The average value per field is calculated and this value is multiplied by 2,000 giving an estimated total white blood cell count.

The Blood Smear

On first glance, the avian blood smear may appear bewildering. However, once the basic features of avian blood cell morphology are understood, principles already familiar to the practitioner can be readily applied.

The Red Blood Cell (RBC). The avian red blood cell is larger than the mammalian cell, is oval instead of round, and is nucleated. Its single nucleus is highly condensed, oval, and centrally placed in the cell. The cytoplasm stains uniformly and is pale pink to red. The average life expectancy of the avian RBC is approximately 30 days. Polychromatophilic RBCs are common, comprising up 1-5% of the RBCs. Decreased haematopoiesis, which commonly occurs during anorexia and chronic disease, results in the development of a mild to moderate anaemia. Juvenile RBCs, normally found in the bone marrow, are seen frequently but in low numbers in most normal smears. Following a significant blood loss the number of immature RBCs seen in circulation may rise dramatically. RBCs are fragile and are often disrupted when the smear is made. In young birds, RBCs tend to be larger, hypochromic and tend to stain unevenly. Young birds will typically have a higher percentage of polychromatophilic RBCs in the circulation.

Thrombocytes. In place of platelets, the bird has circulating thrombocytes. Thrombocytes are smaller than RBCs, but are similarly shaped. Their cytoplasm is clear, often contains 1 or more large clear vacuoles, and may contain 1-4 small red granules. The thrombocyte nucleus contains highly condensed chromatin, is centrally located, and oval. Thrombocytes activate rapidly after blood collection, rounding up, spreading out, and clumping with each other. Activated thrombocytes are commonly mistaken for lymphocytes. Regardless of the state of activation, thrombocytes generally maintain their vacuoles and red granules. One to five thrombocytes per high powered field are expected. If they are already clumped, thrombocyte values are difficult to determine. Thrombocytopenia may be seen following severe blood loss and crushing injuries and has been

reported to occur with leukaemia. Immature thrombocytes are infrequently seen in the circulation unless there has recently been a massive bleed or extensive soft tissue injury.

I have only seen one bird with a thrombocytosis. This bird was chronically bleeding from its ventriculus. An estimate from a blood smear suggested that the total thrombocyte count was in excess of 250,000 thrombocytes per microliter of blood. Normal would be approximately 50,000 thrombocytes per microliter of blood. Radiographically, this bird had soft tissue density of the femurs, a normally air-filled bone. At necropsy, the bone was found to be filled with haematopoeitic cells.

Heterophils. The heterophil is the avian analog to the neutrophil. This cell is generally round and its cytoplasm is packed with brick red, fusiform, slender to thick, granules. These granules are so dense that they may overlap making the cytoplasm appear to be entirely red. The nucleus is segmented. The degree of segmentation is proportionate to the age of the cell. Immature heterophils, bands, have a horseshoe shaped nucleus. In the bone marrow, the maturation sequence of heterophils includes a progression from cells with basophilic round granules to cells with both basophilic and eosinophilic round granules, to cells will only eosinophilic round granules, to band cells which have spindle-shaped granules.

Bands and other immature stages of the heterophil are not found in the blood of normal birds. When a left shift is seen, inflammatory disease is occurring in the bird. The severity of the left shift correlates with the severity of the disease. In situations were heterophils are being used faster than they can be produced, a degenerative left shift will develop. Birds will have a heteropaenia and immature cells (bands, metamyelocytes, myelocytes, and promyelocytes) will be found in the circulation. Occasionally, even myeloblasts will be seen in the circulation. The best way to become familiar with erythroid and myeloid progenitor cells is to look at bone marrow impression smears.

Heterophils exhibit toxic changes in birds with relatively acute severe infectious diseases. Toxic changes are manifested by asynchronous maturation of nucleus and granules. Thus, toxic heterophils may have either a round nucleus and spindle-shaped granules, or a segmented nucleus with immature cytoplasmic granules.

For some unknown reason, heterophils may degranulate in some preparations. When this occurs, the cytoplasm is left filled with clear vacuoles and scattered red granules (central bodies). The degree of degranulation can vary from a few scattered cells to the majority of heterophils. Degranulation is commonly seen in birds of prey, especially owls. It may not be clinically significant.

Total heterophil counts are generally less than 10,000 cells per microliter. Although, in nestling birds and ratite birds, the counts can be much higher. Stress can significantly impact the heterophil count. In most species of birds the heterophil count will increase with stress and the lymphocyte count will decline. In a stress response, the heterophils will be mature and bands and more immature cells will not be seen. In contrast, stress will induce a heteropaenia and lymphocytosis in some species of birds. This type of stress response is less common.

Eosinophils. Eosinophils are easily distinguished from heterophils. Although they are the same size and have a similar nucleus, their cytoplasmic granules are round and a lighter shade of red. Eosinophils are found in most avian blood smears in concentrations of less than 1,000 cells/µl. Raptors typically have higher eosinophil counts that range normally up to 2,000 cells/µl.

Lymphocytes and monocytes. Lymphocytes are the second most common leukocyte in the circulation. They can vary in size from that of a rounded up thrombocyte to the size of an average monocyte. When they are small they are round, have little or no distinguishable cytoplasm and a dense round nucleus. Larger lymphocytes have more cytoplasm and may be round, but more often

are quadrilateral with cytoplasmic margins abutting on adjacent cells. Occasionally, lymphocytes may contain a few red or even basophilic granules. Their cytoplasm is generally faintly blue and wispy. In severe inflammatory disease, lymphocytes may be reactive. These cells have an increased amount of cytoplasm with an increased basophilia. Nuclear chromatin is also generally less clumped. Lymphocytes, especially small lymphocytes, will have blebs of their marginal cytoplasm.

The avian monocyte and the mammalian monocyte are essentially identical. They tend to be larger than lymphocytes and are generally round. The nucleus may be round, indented, or even nearly bilobate. Chromatin is less condensed than that of the lymphocyte, but nucleoli and clumps of chromatin are present. The cytoplasm is finely stippled with both basophilic and eosinophilic stippling. The cytoplasm may also appear to contain numerous small clear vacuoles.

Several fields of each blood smear should be examined before the differential count is made. Slide preparation and staining technique will result in variability requiring that the observer get a "feel" for each slide and the morphology of the thrombocytes, lymphocytes and monocytes. Even with the above stated criteria, it will sometimes be impossible to separate all the lymphocytes from all the monocytes. Therefore, most differentials will contain up to 5% unidentified cells.

The absolute monocyte count and monocyte morphology are important aides in the diagnosis of chronic granulomatous inflammatory disease. The absolute monocyte count should not exceed 1,000 cells per µl in a heathy bird. Increases above 1,500 cells/µl are highly suggestive of diseases such as aspergillosis, avian tuberculosis, psittacosis, subacute to chronic egg yolk peritonitis, abscesses, perforating ventricular foreign bodies, and other chronic diseases. In diseases characterized by severe granulomatous changes, e. g., aspergillosis, monocytes in the circulation will often take on the characteristics of a macrophage. These cells are larger than the average monocyte and have a greater volume of cytoplasm. Often, the cytoplasm of these cells is lobulated or blebbed around the margin. The nucleus is also enlarged with less chromatin clumping and at least one prominent nucleoli.

Packed Cell Volume (PCV). The PCV in most birds ranges from 45-55%. Lower PCVs are expected in nestlings. Anaemia is common in birds that are in poor condition or that are anorexic. The average avian RBC is thought to survive for only approximately 30 days. So, the PCV can drop rapidly if RBC production stops. Haemolytic anaemia is rare, but is associated with lead poisoning in Amazon parrots. A spontaneous case of immune-mediated haemolytic anaemia has also been observed in a conure and a Senegal parrot. Blood loss is replaced rapidly in birds.

Estimated Total Solids (TS). Total solids, often called total protein, is determined by the refractometer. Values in birds in general (30.0-40.0 g/l) are considerably lower than those of mammals. Total solids values in adult ratites and chickens are higher than those reported in other avian species ranging up to 5.0 mg/dl. TS values are affected by the amount of albumin, gamma globulins, and lipoproteins in the serum or plasma. High TS levels may indicate chronic inflammation, dehydration, or lipaemia. Low TS levels are generally associated with debilitated animals