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INTRODUCTION

The interpretation of blood samples from birds poses several large challenges including:

- difficulties in collection of adequate volume of blood for analysis
- a dearth of available information of haematological characteristics in health and in response to disease and notably
- the vast number of species of which we are only beginning to catalogue the individual haematological idiosyncrasies.

In most situations appropriate formal reference intervals are not available to compare with the patient's haematological data. In these situations, the interpretation of the morphology of haematological cells can provide valuable insight into the bird's haematological response to disease. To adequately recognise any deviation from the normal cell morphology, a good quality sample of blood must be collected, a good quality blood film prepared and stained and a good quality microscope used to examine the blood film.

This paper reviews the typical morphology of avian erythrocytes and heterophils and the changes in morphology that may occur in response to disease.

AVIAN ERYTHROCYTES IN HEALTH AND IN RESPONSE TO DISEASE

The erythrocytes of birds, when examined in Romanowsky stained blood films by light microscopy, are ovoid with a centrally located ovoid nucleus and evenly-coloured eosinophilic cytoplasm (Fig 1.). The 'ovoid' shape varies between species with some species having noticeably rounded cells whereas other species have a narrower, elongated shape. The nucleus is ovoid and composed of darkly basophilic, coarsely clumped chromatin. When examined by transmission electron microscopy, mature erythrocytes are ovoid with a smooth, mostly flat surface not distended by the nucleus in several species examined (Clark et al. 2009) (Fig 2.). *Reticulocytes* are erythrocytes that when stained, by the incubation of living cells with a 'supravital' stain, such as new methylene blue, exhibit granular aggregations of RNA ('reticulum'). When stained in this manner, some residual cytoplasmic RNA will be evident in most avian erythrocytes. As a consequence, the term 'reticulocyte' should be reserved for cells with aggregated 'reticulum' that forms a distinct (but incomplete) ring around the nucleus. The number of these cells typically correlates well with the number of *polychromatophilic erythrocytes* in a Romanowsky stained blood film (Johns et al., 2008).

Polychromatophilic erythrocytes exhibit a uniformly more basophilic cytoplasm due to the presence of residual RNA. Both reticulocytes and polychromatophilic erythrocytes can be considered to represent the penultimate stage of erythroid development. Less mature stages of erythroid development may be observed in the blood of birds. Most commonly encountered are *rubricytes*. Rubricytes are typically smaller and more rounded than mature erythrocytes. Their nucleus is typically round and is composed of coarsely clumped chromatin that is less dense than chromatin of mature erythrocytes. Rubricytes have a nuclear to cytoplasmic ratio that is greater than the nuclear to cytoplasmic ratio of mature erythrocytes, with a small to moderate rim of markedly basophilic cytoplasm.

***Anaemia*, the decrease in the circulating mass of erythrocytes, may result from causes that may be broadly classed as 'haemorrhagic' (loss of erythrocytes), 'haemolytic' (destruction of erythrocytes) or 'hypoproliferative' (decreased erythropoiesis). Erythrocytes with variant morphology may be evident, depending on the cause of the anaemia and the haematological response. Anaemia may be due to a lack of erythrocytes but those present exhibit typical size (normocytic) and haemoglobin content (normochromatic). Alternately, if erythropoiesis is increased in response to the anaemia, an increased proportion of (morphologically distinct) immature erythroid cells may be evident in the peripheral blood. In these cases, examination of a blood film reveals increased numbers of polychromatophilic erythrocytes and reticulocytes (Fig 3.). Typically increased erythropoiesis and release of cells into the peripheral blood occurs more rapidly in birds than in mammals. A number of experimental and clinical examples of haemorrhagic anaemia affecting birds have been reported. Experimental removal of 30% of estimated blood volume in Japanese quail induced peak reticulocytes at 48 hours and recovery of erythrocyte numbers by 72 hours after phlebotomy (Gildersleeve et al. 1985, Schindler et al. 1987). Similarly, for pigeons with experimental blood loss (15-30% of estimated total blood volume), the birds recovered their PCV to about 90% of initial values within 168 hours (Finnegan et al. 1997).**

AVIAN LEUKOCYTES IN HEALTH AND IN RESPONSE TO DISEASE

Five types of leukocytes are encountered in the blood of birds, namely: *heterophils*, *eosinophils*, *basophils*, *lymphocytes* and *monocytes*. As heterophils, eosinophils and basophils all possess distinct cytoplasmic granules they may be collectively referred to as *granulocytes*. Furthermore, as the predominant cytoplasmic granules of heterophils and eosinophils both exhibit affinity for acidic stains (such as eosin) they may be referred to as *acidophils*. Lymphocytes and monocytes may be collectively referred to as *mononuclear cells.* In this discussion, only heterophil morphology will be considered in detail.

In many species of bird, *heterophils* are typically the most commonly encountered granulocyte and often the most commonly encountered leukocyte in the peripheral blood of birds. When examined in Romanowsky stained blood films by light microscopy, heterophils are typically irregularly round leukocytes with a lobed nucleus, basophilic nucleus and prominent acidophilic cytoplasmic granules (Fig 4.). The nucleus commonly has two to three lobes and, although basophilic, is often dark at the

periphery and fades to a pale aqua-blue colour more centrally. The cell's granules are typically evenly distributed at moderate to high density and often obscure most of the cytoplasm (which is palely basophilic when apparent) and some of the nucleus. In some heterophils, the granules may exhibit a '*central granular body*', these are a round to ovoid, pale or refractile structure located in the mid-section of the granule. In some instances, the central granular body may be more prominent than the surrounding matrix of the granule.

The morphology of 'typical' heterophils may be altered in response to inflammation. These changes may occur as a result of either the release of leukocytes from sites of haematopoiesis before they are mature or by the direct actions of toxins upon the cells in the peripheral blood.

Atypical heterophil morphology may be manifested in the nucleus, cytoplasm or both (Figs 5-7.). Usually, atypia of the nucleus is indicated by decreased segmentation, and consequently decreased number of nuclear lobes. The nuclear structure is often difficult to assess when the cell contains a typical number of cytoplasmic granules, as the granules commonly obscure part of the nucleus. Consequently, changes in the complexity of the nucleus are most easily assessed when there is a concomitant decrease in the density of cytoplasmic granules. Direct effects of toxins on the nucleus of leukocytes may result in karyolysis or karyorrhexis. These must be carefully distinguished from cells where the nucleus has been artifactually lysed during the production of the blood film or due to ageing of the blood sample. In the author's experience, artifactual causes of lysis are far more commonly encountered than true pathological effects on the cells in the peripheral blood.

Atypical heterophils may exhibit morphological abnormalities that encompass changes in the cytoplasm and the granules it contains. As previously described, typical heterophils contain many elongated, fusiform granules that typically obscure the cytoplasm and partially obscure the nucleus. The cytoplasmic granules of heterophils may change in shape, color and number (density) in response to inflammation. Typically, with mild inflammation, the granules are less pointed but maintain typical colour. In more significant inflammation, the granules become round, larger and more basophilic than typical granules. The typical number of granules is usually maintained with mild inflammation. In cases of more significant inflammation, the number of granules within cells is decreased and agranulated heterophils or those that possess only one to several granules may be observed.

The decreased number of granules serves to make the cytoplasm more visible and provides greater opportunity to assess the characteristics of the cytoplasm. The cytoplasm may exhibit an increased basophilia, due to the presence of increased numbers of ribosomes. The cytoplasm may also exhibit a 'foamy' or vacuolated appearance due to the direct action of toxins on the cells. However, these morphological changes must be differentiated from a similar appearance that may occur due to delayed processing of a sample of blood.

The morphological characteristics and the proportion of the cells that exhibit these characteristics vary with the severity of the inflammation. 'Mild' changes typically include: mildly decreased

numbers of granules, slightly rounded granules and increased basophilia of the cytoplasm. 'Moderate' changes include: a greater decrease in the density of granules and rounded granules. Severe changes include: round granules, basophilic granules, large granules, 'foamy'/vacuolated cytoplasm and karyolysis. The number of abnormal heterophils may be graded as 'few' (5 to 10 percent), 'moderate' (11 to 30 percent) and 'marked' (greater than 30 percent) (Campbell 2004). Examples of leukocytes exhibiting atypical morphology, in response to disease, have been reported for a range of bird species (Hawkey & Dennett 1989, Campbell 2004, Clark & Raidal 2009, Clark et al. 2009). However, morphological atypia of leukocytes may not be present in all birds with inflammation. For example, in a study of two species of black cockatoos with inflammation or traumatic injury, only 3/21 (14%) of birds exhibited heterophils with morphological changes (Jaensch & Clark 2004).

The aetiology inciting the inflammation, the duration of the inflammation, the magnitude of the inflammation, the species' haematological characteristics, the individual's haematological characteristics and numerous other factors may influence the observed haematological response to disease. These responses are largely yet to be documented for most species of birds and most diseases.

CONCLUSION

Careful examination of a blood film can reveal morphological changes in haematological cells that can indicate a response to disease. This can provide important information when changes in the concentration of haematological cells cannot be discerned, either due to insufficient of blood for analysis or when appropriate reference intervals are not available for comparison.

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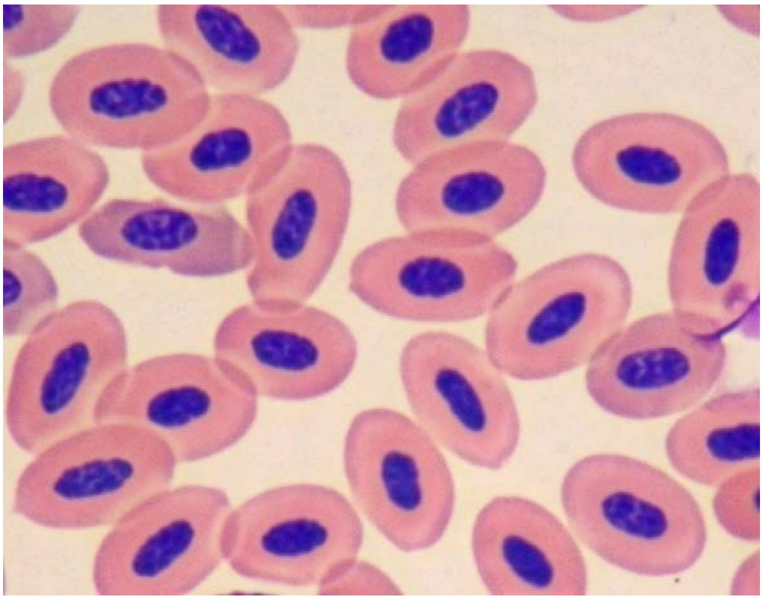

Figure 1 Blood from a blue-breasted quail showing typical avian erythrocytes. (Modified Wright's stain)

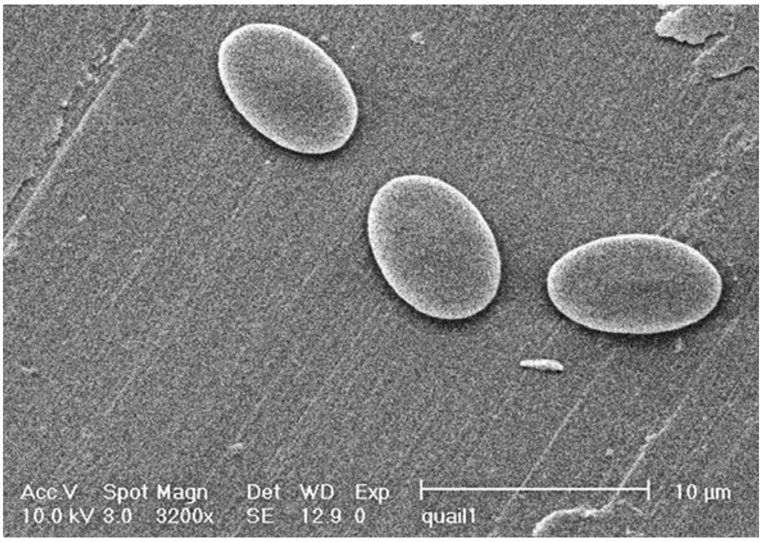

Figure 2 **Blood from a blue-breasted quail showing scanning electron microscopic appearance of avian erythrocytes.**

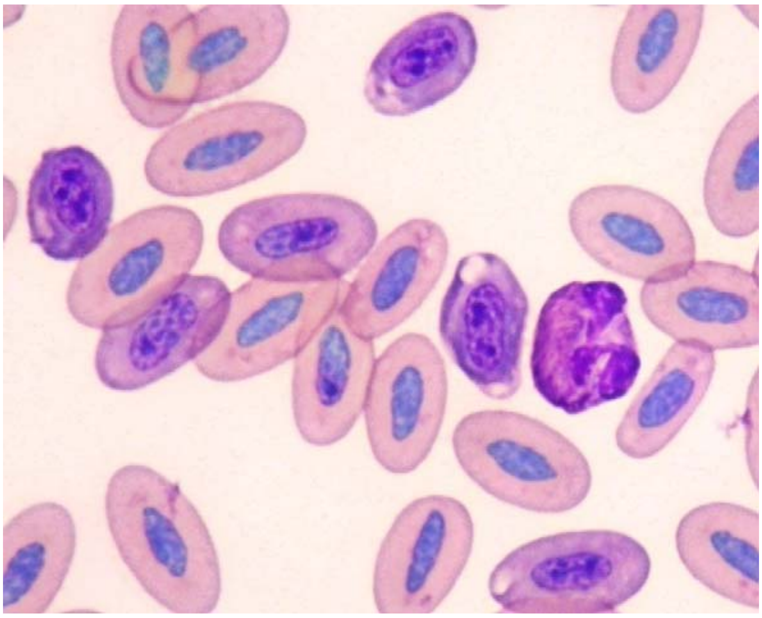

Figure 3 **Blood from a black shouldered kite, following vehicle trauma and subsequent blood loss, showing increased numbers of polychromatophilic erythrocytes indicative of increased erythropoiesis. The bird had a PCV of 0.20 L/L and 19% polychromatophilic erythrocytes and 2% rubricytes (Diff Quik stain)**

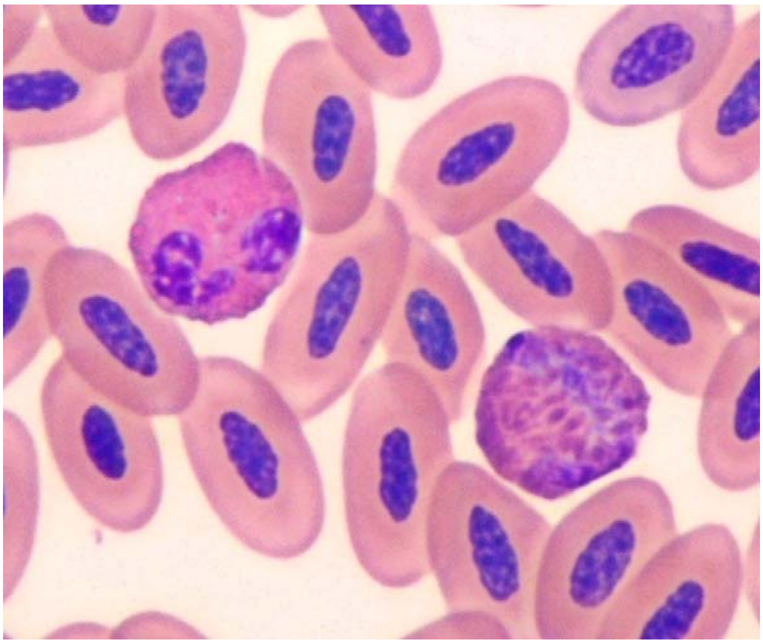

Figure 4 **Blood from a wedge-tailed eagle, illustrating a heterophil with typical morphology (left) and an eosinophil (right). (Modified Wright's stain)**

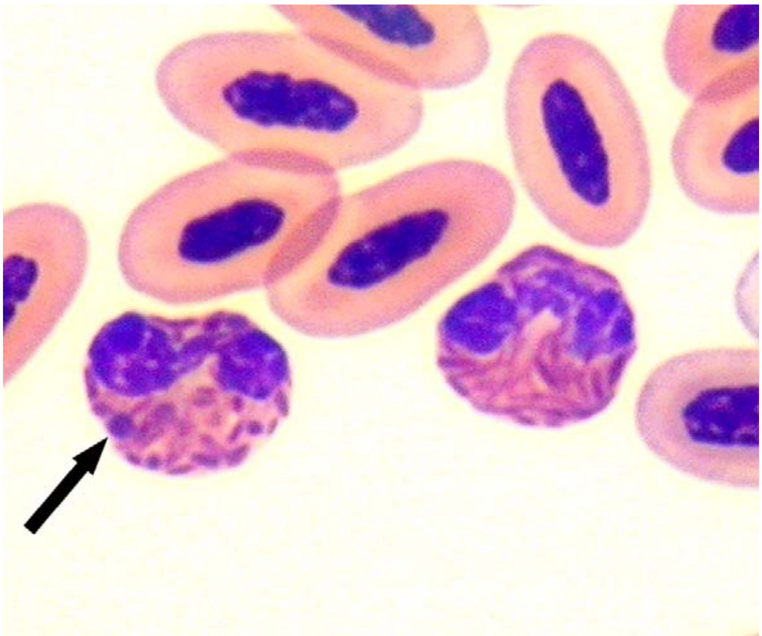

Figure 5 **Blood from a Port Lincoln parrot, following vehicle trauma, illustrating a typical heterophil (right) and a heterophil with atypical granulation (arrow). (Modified Wright's stain)**

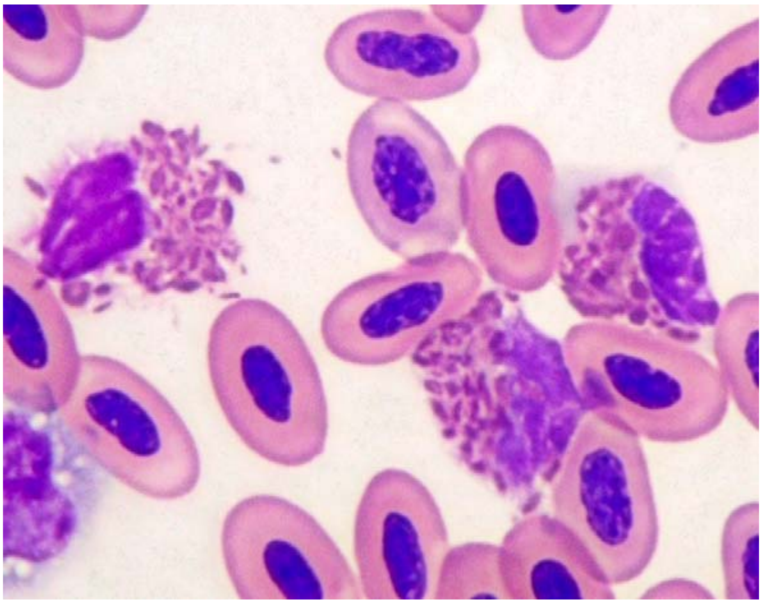

Figure 6 **Blood from a Major Mitchell cockatoo showing three heterophils (one is disrupted) that contain atypical granules and have increased cytoplasmic basophilia. (Modified Wright's stain)**

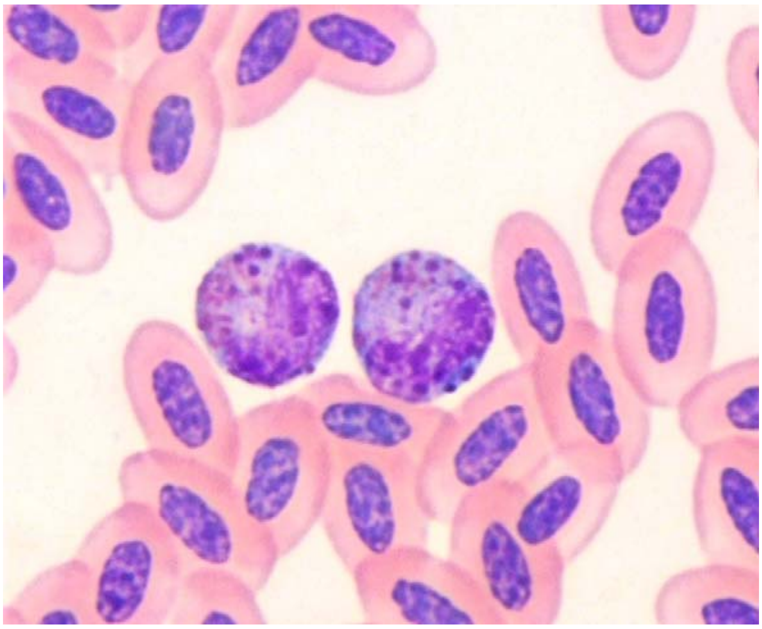

Figure 7 **Blood from an Australian kestrel with heterophilic meningo-encephalitis and bacteraemia. Two immature heterophils exhibiting decreased granulation, atypical granule morphology, increased cytoplasmic basophilia and a hyposegmented nucleus are shown. (Modified Wright's stain)**