B. Hematological

Deborah J. Monks
Brisbane Bird and Exotics Veterinary Service
Cnr. Kessels Rd. and Springfield St.
Macgregor, QLD 4109, Australia

Neil A. Forbes
Great Western Referrals,
Unit 10 Berkshire House, Country Park Business Park
Shrivenham Rd., Swindon, Wiltshire, SN1 2NR, United Kingdom

INTRODUCTION

Disease diagnosis and assessment of therapeutic efficacy often relies on hematological analysis (Howlett 2000, Cooper 2002). Rehabilitation centers can use hematological changes to help detect sub-clinical disease, in pre-release assessment of individuals, and as a prognostic indicator for new admissions. The health of raptor populations can be monitored similarly. Nutritional status, disease, or food supply differences among populations or immune suppression due to various stressors all can be detected using hematology (van Wyk et al. 1998, Cooper 2002).

Because raptors are at the top of many food chains, their health can reflect the health of entire ecosystems (Cooper 2002). Hematological alterations can indicate changing habitat quality and food availability or may imply exposure to pollutants or toxins (Hoffman et al. 1985, Mauro 1987, Bowerman et al. 2000, Seiser et al. 2000). Habitat loss and fragmentation have resulted in increased exposure of some raptor populations to parasites, which can alter host-parasite balances. Increased parasite pathogenicity may be implied by hematological alterations (Loye and Carroll 1995).

Recently, research has focused on determining the reference ranges that distinguish among species of raptors. A number of published references provide parameters by sex and age (Rehder et al. 1982, Ferrer et al. 1987, van Wyck et al. 1998, Bowerman et al. 2000). Computerized databases also are available, including ISIS (www.ISIS.org) and LYNX (Bennett et al. 1991).

The discussion of biochemical parameters is beyond the scope of this chapter. Several comprehensive references provide additional details in this area (Campbell 1994, Joseph 1999, Fudge 2000, Cooper 2002).

Sampling

Physiological variables affecting hematological testing. Blood sampling should be performed as soon as possible after capture and prior to other procedures, as the stress of capture and restraint can alter the leucogram (Wingfield and Farner 1982, Sockman and Schwabl 2001) and result in leucocytosis, heterophilia, lymphocytosis, or lymphopenia (Fudge 2000). Parga et al. (2001) advocated the use of the heterophil/lymphocyte ratio, rather than the absolute number of heterophils or lymphocytes, as a more sensitive indicator of stress in raptors, although the actual ratio may vary among species. Leucocyte numbers also may be altered with concurrent diseases (Howlett 2000, Parga et al. 2001).

Researchers should be aware that other physiological factors might affect hematological parameters. The following variables should be considered when plan-

ning hematological testing, so that efforts can be made to minimize their impact: (1) erythrocyte production may decrease with increasing ambient temperatures and may vary with season (Hunter and Powers 1980, Rehder et al. 1982); (2) hematocrit may be increased by high androgen levels and decreased by high estrogen levels; (3) molt decreases hematocrit in both sexes (Sturkie 1976, Rehder et al. 1982); (4) up until fledging, hematocrit and hemoglobin levels increase with age (Rehder et al. 1982, Bowerman et al. 2000); (5) some studies have reported differences in hematocrit between sexes, whereas others have found no correlation (Sturkie 1976, Rehder et al. 1982, Dawson and Bortolotti 1997); (6) Rehder and Bird (1983) demonstrated diurnal variation in hematocrit and red-blood-cell (RBC) count of Red-tailed Hawks (*Buteo jamaicensis*); and (7) certain sedative and anesthetic drugs also can cause leucogram or hemogram changes (Mauro 1987, Fudge 2000). All of this leads us to recommend consistency in sampling.

Venipuncture procedure. Birds that weigh less than 500 g usually are sampled with a 25-gauge hypodermic needle and a 1- or 2-ml syringe, whereas a 23-gauge hypodermic needle is best used for birds of more than 500 g (Cooper 2002). Veins of very small birds can be nicked with a scalpel blade and blood collected in a capillary tube (Dawson and Bortolotti 1997). Butterfly catheters may lessen the effect of bird movements during sampling (Cooper 2002). Smaller-gauge needles increase the risk of hemolysis. The use of larger needles increases the risk of hematomas (Fudge 2000). Excessive negative pressure may collapse veins (Jennings 1996). Adding anticoagulant to the syringe prior to venipuncture may dilute the blood sample, although some authors advocate this if clotting of samples is a problem (Rehder et al. 1982, Cooper 2002). Because of inconsistent results, we do not recommend sampling from talon clipping (Campbell 1994). Regardless of technique, the phlebotomy site must be prepared aseptically to prevent bacterial contamination (Fudge 2000).

Avian blood volume ranges from 6 to 12% of body mass and no more than 5–10% of the total blood volume should be removed. This equates to approximately 0.5–1% of total body mass (Campbell 1988, Fudge 2000). Smaller volumes should be removed from unhealthy or stressed birds (Cooper 2002).

When repeatedly sampling the same bird, allow for sufficient time for erythrocyte replenishment between sampling (Mauro 1987). The average life span of an avian erythrocyte is 28 to 45 days (Rodnan et al. 1957). That said, American Kestrels (*Falco sparverius*) bled at 10% of blood volume weekly for 20 weeks showed no decrease in hematocrit (Rehder et al. 1982).

Inappropriate restraint can result in blood-vessel laceration and prolonged bleeding. Hematoma formation may considerably increase the total blood volume removed. To reduce this risk, pressure should be applied to the phlebotomy site for at least 1 to 2 minutes after venipuncture, and the bird should not be released until hemostasis is complete.

Normal venipuncture sites. The jugular vein is the largest accessible vein, although hematoma formation there can be a problem in inexperienced hands (see below). The right jugular generally is larger than the left and also has an overlying apteria (featherless area). A popular alternative is the basilic vein, which crosses the elbow ventrally. If the bird is struggling vigorously, it can be difficult to sample, and wing trauma (including fractures) can occur. It is not always easy to find in smaller raptors. Large hematomas can develop after iatrogenic tissue trauma or insufficient post-sampling pressure. A third location for blood sampling is the median metatarsal vein, which is found proximal to the tarsometatarsal joint. There is less chance of hematoma formation in this vein due to the anatomy of the surrounding soft tissue (Fudge 2000).

After 25 years of blood-sampling American Kestrels at the Avian Science and Conservation Centre at McGill University in Montreal, the large jugular vein has become the preferred sampling site. With one person holding the bird's head stable and in an appropriate position to expose the vein, hematomas are rarely encountered using this method (I. Ritchie and D. M. Bird, pers. comm.).

Sample preparation. One or two blood smears should be made at the time of collection using non-anticoagulated blood. Avian blood cells are fragile and rough smear techniques can result in large numbers of unidentifiable (smudge) cells (Jennings 1996, Fudge 2000). If protected from moisture, air-dried, unfixed blood smears may last up to 72 hours (Howlett 2000).

Human pediatric blood tubes, which are available commercially, are quite suitable for raptor blood. The tubes are available as ethylenediaminetetraacetic acid (EDTA), heparin, and plain-gel, and when filled to the line, provide the correct sample to anticoagulant ratio. Hemolysis can be reduced by precise sampling and removing the needle from the syringe prior to filling the sample pots with blood (Fudge 2000). Heparinized capillary tubes may be capped with plasticine and stored.

Choice of anticoagulant. The laboratory where the analyses will be performed should be contacted prior to sample collection for preferred anticoagulant, storage, and other processing information.

Blood for hematological analysis usually is collected into EDTA. Note that erythrocytes may lyze within 24 to 48 hours of exposure to EDTA. This is particularly marked in some non-raptors (Campbell 1994). Heparin may affect the affinity of blood cells to Romanowksy stains and cause clumping of leucocytes and thrombocytes (Jennings 1996, Howlett 2000). Fudge (2000) found that citrated blood provided better cell integrity for automated analysis.

Sample Storage and Processing

Time from sampling to processing should be as short as practical, and samples that are not analyzed immediately should be kept cool (approximately 4°C). One person should do the blood smear staining and interpretation to minimize variability. Wright's, Giemsa, and modified Wright's-Giemsa stains all provide good cell morphology, although new staining techniques also seem to do well (Campbell 1988, Fudge 2000, Samour et al. 2001, Cooper 2002, Kass et al. 2002).

Factors affecting analysis. Sample clotting can occur due to slow sample collection, tissue trauma, inadequate sample mixing and overfilling of anticoagulant sample pots (Jennings 1996). Hemolysis and lipemia can alter a number of hematological and biochemical parameters, including total protein levels (Joseph 1999, Cooper 2002). Inaccurate cell identification can occur with blood smears made from old or anticoagulated blood, exposed to formalin fumes or stained with expired stains (Fudge 2000). Failure to detect hemoparasites can result from poor-quality smearing or staining techniques, operator inexperience, or from the use of poor-quality microscopes (Cooper 2002).

Relevant international agreements, including CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), and country and local laws must be considered before transporting samples internationally (Cooper 2002).

Hematological Parameters

Listing "normal" hematological values for each species is beyond the scope of this chapter. Researchers should consult relevant peer-reviewed publications and databases for specific information. General information can be found in Samour (2000), Cooper (2002), and Redig (2003).

Total plasma protein. Although sometimes considered a biochemical parameter, analysis of total plasma protein (TPP) is required for complete interpretation of the erythron, especially in instances of anemia. Protein electrophoresis and fibrinogen determination also may be performed.

The erythron. Evaluation of the erythron involves determining hematocrit or packed cell volume (Hct or PCV - l/l), hemoglobin (Hb - g/l) and the RBC count (x 10^{12}/l) followed by calculation of mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). For additional information consult Howlett (2000) and Fudge (2000).

The accepted hematocrit range for raptors is 35–55 l/l (0.35–0.55) (Fudge 2000, Cooper 2002). Lower values have been obtained from apparently healthy birds (Rehder et al. 1982). Abnormalities must be interpreted in conjunction with TPP and fibrinogen levels (see Table 1). A reticulocyte count of 5–10% is considered physiologically normal.

Anemia can be characterized as regenerative or non-regenerative, based on the numbers of reticulocytes. Some hematologists maintain that there is no sat-

Table 1. Changes in the Erythron. (Based on Joseph [1999] and Fudge [2000].)

Condition	PCV	TPP	Fibrinogen	TPP:Fibrinogen
Dehydration	Increased	Increased	Increased	>5
Polycythemia	Increased	Normal	Normal	1.5–5.0
Anemia	Decreased	Normal	Normal or increased	Depends on cause
Infection or Inflammation	Normal	Normal	Increased	<1.5

isfactory method of obtaining an objective reticulocyte count in raptors; instead they rely on the subjective analysis of stained blood smears, noting the degree of polychromasia and the numbers of rubricytes, prorubricytes, and rubriblasts, if present (M. Hart, pers. comm.). Others are comfortable using a vital stain such as new methylene blue, or Wright's stain, to preferentially stain reticulocytes (Fudge 2000). In the presence of anemia, a reticulocyte count of <5% indicates a poor regenerative response, whereas a count of >10% indicates a good regenerative response (Cooper 2002). Polychromasia greater than 1–5% also can indicate an appropriate regenerative response. Anemia can be classified according to etiology or erythrocyte morphology (see Tables 2 and 3). Concurrent dehydration may mask the signs of anemia.

Hematocrit and TPP will decrease with chronic undernourishment (Ferrer et al. 1987, Cooper 2002). Unfortunately, the severity and duration of food deprivation required to cause these changes are uncertain. In Common Buzzards (*B. buteo*) starved for 13 days, these parameters changed only when feeding was resumed (Garcia-Rodriguez et al. 1987). Conversely, American Kestrels typically die after five days of starvation (Shapiro and Weathers 1981). Dawson and Bortolotti (1997) found that hematocrit was not accurate in predicting nestling survival in American Kestrels. Body size, species ecology and developmental stage also influence an individual's ability to withstand sub-optimal nutrition.

The leucogram. Detailed anatomy and function of leucocytes is not discussed here. The reader should con-

Table 2. Classification of anemia according to erythrocyte morphology. (Based on Fudge [2000].)

	Type of anemia		
	Normocytic normochromic	Hypochromic microcytic	Hypochromic macrocytic
PCV	Decreased	Decreased	Decreased
MCHC	Normal	Decreased	Decreased
MCV	Normal	Decreased	Increased
Polychromasia	None to slight	Increased	Increased
Anisocytosis	None to slight	Normal to increased	Normal to increased
Possible causes	Generally non-regenerative, reduced RBC production	Iron deficiency, chronic blood loss, chronic disease	Acute blood loss, early stages of lead toxicity

Table 3. Classification of anemia according to etiology. (Based on Campbell [1994, 2000], Fudge [2000], and Howlett [2000].)

Insufficient erythrocyte production	Acute or chronic blood loss	Increased erythrocyte destruction
Malnutrition	Blood-sucking ectoparasites	Hemoparasitism
Chronic disease including mycobacteriosis and aspergillosis	Gastrointestinal parasitism	Bacterial septicemia
Chemicals (lead and aflatoxicosis)	Trauma	Acute aflatoxicosis
Iron and folic acid deficiencies	Rupture of organs or neoplasms	Toxemia
Some neoplasms		

sult Campbell (1988, 1994) and Fudge (2000) for additional information.

Although reference ranges should be established for individual species, it is generally true that vultures and eagles tend to have higher white blood cell (e.g., WBC x 10^9/l) counts than hawks, falcons and owls (N. Forbes, pers. comm.). Both the total and the differential leucocyte count should be obtained. The differential leucocyte count should be expressed both as an absolute count and as a percentage. Consult species-specific reference ranges for normal values. As a guide, in most owl species, the lymphocyte percentage ranges from 40–70%, while in most other raptors, the heterophil is the most common cell (Joseph 2000, Cooper 2002). It is believed that the eosinophil differential can range from 10 to 35% in healthy raptors (Joseph 2000). Conversely, Samour et al. (1996) found that eosinophils were closely associated with parasitism and were not present in such proportions in "normal" individuals. Table 4 lists some leucocyte abnormalities and potential etiologies.

Hemoparasites. Blood parasites are found in many raptors, with incidences varying geographically and among parasite and host species (Joseph 1999). Hemoparasites can cause increased TPP levels, leucocytosis, anemia or death (Garvin et al. 2003, Redig 2003). Table 5 lists hemoparasites in raptors and details regarding pathogenicity, as well as vectors and diagnoses. Pierce (1989) provides a color reference to hemoparasites.

Table 4. Changes in the leucogram of raptors. (Based on Campbell [1994, 2000], Fudge [2000], and Howlett [2000].)

Leucogram changes	Potential etiologies
Leucocytosis	Bacterial infections, including mycobacteriosis, stress, trauma, toxicity, fungal infections including aspergillosis, leukemia
Leucopenia	Overwhelming bacterial infection causing depletion of bone marrow, viremia, depression of bone marrow
Heterophilia	Bacterial infections, including mycobacteriosis, stress, fungal infections including aspergillosis, toxemia
Herteropenia	Overwhelming bacterial infection, viremia, bone marrow suppression, deficiency diseases
Toxic heterophil changes (cytoplasmic basophilia, vacuolization and degranulation, karyorhexis, karyolysis)	Septicemia, viraemia, toxemia, severe infection
Monocytosis	Infection, including mycobacteriosis and aspergillosis, chronic disease, tissue necrosis
Lymphocytosis	Some infectious and metabolic disorders, some neoplasms
Lymphopenia	Stress, uremia, immune suppression, some neoplasms, viremia
Reactive lymphocytes	Infection, including salmonellosis and aspergillosis
Eosinophilia	Parasitism, including hemoparasites, tissue damage, hypersensitivity (questionable)
Eosinopenia	Corticosteroids, stress
Basophilia	Tissue damage, parasites (inconsistent), hypersensitivity (questionable), chronic disease
Fibrinogen (increased)	Infection, inflammation, hemorrhage
Fibrinogen (decreased)	Liver failure
Thrombocytosis	Rebound response to hemorrhage, response to excessive thrombocyte demand (including phagocytosis)
Thrombocytopenia	Excessive peripheral demand or depression of production (e.g., severe septicemia)

Table 5. Hemoparasites of raptors. Based on Cooper (2002), Gutierrez (1989), Joseph (1999), Lacina and Bird (2000), Redig (2003), Remple (2003), Samour and Peirce (1996), Samour and Silvanose (2000).

Species	Site of infection and incidence	Extent pathogenic	Vector and transmission	Diagnosis
Leukocytozoon spp. (Hemosporidia)	Peripheral RBC and WBC — relatively common in a number of species, seasonal incidence	Generally non-pathogenic. May cause illness and occasional deaths due to anemia in young, debilitated or heavily infested birds	Simuliid black flies	Blood smears — non-pigmented gametocytes in RBC cytoplasm. Occasionally found in muscle, heart, spleen, kidney and liver tissues on histology
Hemoproteus spp. (Hemosporidia)	Peripheral RBC — more common in Strigiforms	Generally non-pathogenic. May cause illness and occasional deaths due to anemia in young, debilitated or heavily infested birds	Hippoboscid flies and Culicoides midges	Blood smears — pigmented gametocytes occupying >50% RBC cytoplasm
Plasmodium spp. (Hemosporidia — 34 spp.)	RBC, WBC, thrombocytes and reticulo-endothelial cells. Disease reported in falcons, especially Gyrfalcons (*Falco rusticolus*) and gyr-hybrids	Pathogenicity varies. Clinical signs: anemia, thrombosis, dyspnea, acute death	Culicine, Aedes occasionally Anopheles mosquitoes	Blood smears — pigmented gametocytes, trophozoites and schizonts in RBC, WBC and thrombocytes. May displace nucleus from central position. Unfixed spleen and liver sections
Microfilaria	Free in plasma — sporadic reports in variety of species	Uncertain	Uncertain	Blood smears
Babesia spp. (piroplasm)	Peripheral RBC — a few reports only in Prairie Falcon (*Falco mexicanus*), Saker Falcon (*F. cherrug*), Barn Owl (*Tyto alba*), Bearded Vulture (*Gypaetus barbatus*)	Pathogenicity controversial. Poor performance, possible death	Ticks, including *Ornithodorus concanensis*	Blood smears
Atoxoplasma spp. (coccidia)	Mononuclear leucocytes — reported in Spotted Owl (*Strix occidentalis*)	Uncommonly reported	Ingestion of sporulated oocysts	Reddish intracytoplasmic inclusions indenting leucocyte nucleus
Trypanosomes (flagellated protozoa)	Free in plasma — reported in a variety of species	Not known to be pathogenic	Blood-sucking arthropods	Blood smears. Examination of buffy coat

Management Considerations

Hematological parameters respond to physiological or environmental alterations within hours to weeks. Determining these parameters is easy and inexpensive and can indicate perturbation of the individual, or the population and, in some instances, the ecosystem. If abnormalities are identified, more specific tests (including biochemical, serological and toxicological analysis and polymerase chain reaction [PCR]) should be performed.

The correct interpretation of hematological values requires well-established "normals." Unfortunately, there are gaps in understanding here. Many species have poorly defined "normal" ranges and information regarding basic physiological changes accompanying undernourishment is lacking for many raptors. It also should be noted that databases, published reference ranges, and laboratory reference ranges may have been obtained by different methods and from different numbers of animals in various clinical states and, therefore, may not be directly comparable.

CONCLUSIONS

Although raptor hematology is now a crucial part of clinical veterinary medicine, its use as a management tool in wild populations remains limited. Application to population and conservation medicine has been hampered to date by a scarcity of "normal" values, incorporating age, sex and physiological variables. As more research is conducted, the use of hematological techniques will increase.

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