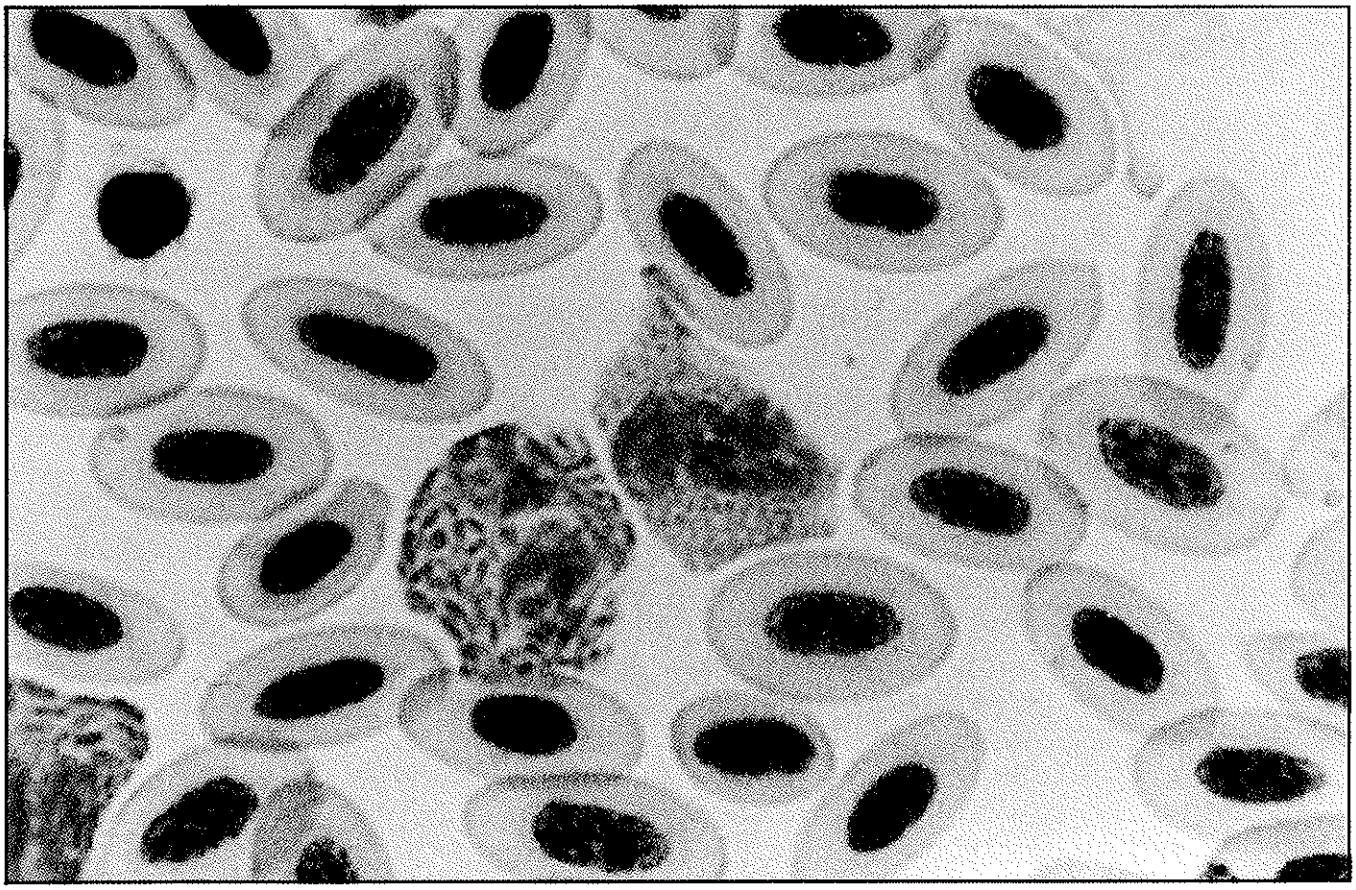
Fall 1992 Vol. 15, No.3

WILDLIFE JOURNAL

Journal of the International Wildlife Rehabilitation Council



Special Topics Issue: Avian Hematology

Wildlife Rehabilitation: A Perpetual Learning Process

Lake Tahoe Horizon Casino/Resort Lake Tahoe, Nevada

October 14-17th, 1993

Start planning now to attend our 16th Annual Conference! We are working up an exciting program for our members that will provide a balanced mix of papers, workshops, roundtables and field trips. Our hosts, Lake Tahoe Wildlife Rescue, have a wildlife care facility nestled in the Sierra Nevada mountains that you won't want to miss. We have an incredible room rate of $74 per night from the Lake Tahoe Horizon Resort/Casino! For hotel reservations and information call (702) 588-6211. Imagine gaming at world class casinos, dancing at *The Cabaret* (night club) or seeing top entertainers at the *Show Room*, playing at the video room, or hot tub, swimming or golfing at the Edgewood Golf course, boating, riding the Heavenly Valley Tram or taking a hike along Lake Tahoe or along the Tahoe Rim Trail! Join us for morning bird walks or a cruise on the M.S. Dixie, a Mississippi-style paddle boat. We may even go out at night looking for mountain beavers and owls! Come and spend a long weekend with the best in wildlife rehabilitation!

As has become our custom, we will be offering skills seminars in advance of the conference for serious rehabilitators wanting to hone their skills. Depending on facilities, we plan to offer both basic and advanced topics (Fluid Therapy, Wound Management, Necropsy) this year.

The program committee has gathered speakers from far and near with the latest wildlife rehabilitation papers, workshops, and roundtables. Join Katherine McKeever for an evening discovering the sex life of owls at our ice breaker. We will have paper sessions on wildlife care administration, veterinary care, education, and as always papers on birds, reptiles, and mammals! Our hands-on workshops, with their advance registration, are always top notch. This year we are planning workshops on handling reptiles, avian hematology, making raptor hoods, basic necropsies and more! Our roundtable discussions this year will tackle topics on blood transfusions, research priorities in wildlife rehabilitation, ethics, and more! Look for our conferencewith the completed program in June. Mark your calendar now!

The airport that services Lake Tahoe is the Reno Cannon International Airport. From there take the Tahoe Express Shuttle (14 non-stops daily) to the Lake. For reservations and current price information call them at (800) 446-6128.

The office is open from 9 a.m. to 4 p.m. Monday-Friday and can be reached at (707) 864-1761 (fax # 864-3106).

WILDLIFE JOURNAL

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Cover Photo: An eosinophil (right) and heterophil (left) with rod-shaped granules.

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Editorial . . .

Hematology: A Window to the Inside View of Avian Health

As a veterinarian, I have many tools (radiographs, biopsy, necropsy, ECG's, etc). available to assess the health or illness of an animal patient. However, when dealing with the avian patient, *none are more important than hematology.* In appropriate cases, the serum chemistries can also serve a cornerstone role. These types of tests can be taken from all but the smallest birds (for example, hummingbirds are too small for serum chemistries), can be obtained from sick birds and are accessible to most rehabilitators through their veterinarian. In fact, wildlife rehabilitators can be taught hematology to varying degrees, depending on their volume, interest and available equipment.

Presented in this special topics issue are articles designed to assist the wildlife health professional take good samples and perform basic hematological tests. *All rehabilitators* need to know how to get a blood sample, do PCV's, total solids, and make good smears. These tests give essential information about the current health status of a bird in rehabilitative care and they are very "do able." The more we learn about caring for emaciation, the more we realize that basic hematology combined with total solids are essential for success in these types of cases.

With the information we know in the '90's, the days when birds were released based *strictly* on how they looked and acted need to stay in the '80's. Quite frankly, these visual parameters have been shown to be ineffective predictors of post-release survival. Not all the proof of this has been published yet, but I have seen much of it and it is on its way to press in one form or another.

There are several articles that did not make it into this issue. One article on seabird hematology that will be a summary of previous work is still winding its way through review for a major scientific journal. There are also two major normal reference range studies underway for raptors, one specific to accipiters and the other to western buteos, strigiformes, and falcons, for which the information is not ready yet. This issue will lay some basic foundations on which we can build on as the information becomes available for our use.

We know that we serve a variety of wildlife care health providers—from the at home rehabilitator to the avian veterinarian. We have tried to provide some learning bridges with glossary information for those who are on a learning curve. IWRC also routinely provides "hands-on" skills seminars for hematology at our annual conferences for those wanting to get more supervised practice. These are all important skills to learn and we are here to help you do it.

-Jan White, DVM-

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Sample Collection for Hematology Testing

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Introduction

Knowing proper sample collection techniques will enhance the ability of the wildlife care professional to efficiently find answers to pressing diagnostic questions. Sample collection techniques used by the Consolidated Veterinary Diagnostic Laboratory are discussed in this article. While labs may vary in their ability to handle various samples, the procedures described below are standard in hematology. Always check with the laboratory to which the samples will be submitted for their specific procedures.

Preparation for Collection

The preservative of choice for most hematology tests is EDTA, which is in a lavender top tube (LTT). The following tubes are not acceptable: heparin (green tube) causes clumping of the white cells and platelets; citrate (blue tube) does not preserve red blood cell (RBC) or white blood cell (WBC) morphology and there's an inappropriate dilution due to the amount of anticoagulant present; potassium oxalate (grey tube) causes shrinkage or swelling (depending on the type of oxalate) of the RBCs and can produce artifacts on the WBCs.

A 2cc lavender top tube (full) contains enough specimen to perform a complete red blood cell count (CBC), platelet count and reticulocyte count in a lab such as CVD that is set up to run avian samples. For canines, if a microfilaria test is requested the 3cc tube should be used. Always note the expiration date on the tubes before you use them. *Do not use expired tubes.* The preservative may not properly keep the blood from coagulating or keep the cells intact until they reach the lab. Use glass slides and slide holders. It is advisable to make fresh smears (called client smears) at the time the blood is drawn for reasons listed below.

Collection

If drawing blood for multiple tubes, always add specimen to the tubes with anticoagulant first. Always gently mix (invert the tube 6-8 times) the specimen *immediately* after adding it to the tube. Tubes with anticoagulant should always be full. When the lavender tube is not completely full, accuracy of the results may be questionable due to EDTA dilution. Excess EDTA is known to cause shrinkage of the RBCs. This can result in a falsely decreased mean corpuscular volume (MCV) and falsely decreased hematocrit. Also when a short sample is submitted it may not be possible to recheck abnormal results.

If the draw was difficult it is possible to have hemolysis and/or clots present. If this is readily apparent, the sample should be redrawn if possible. Hemolysis will falsely decrease the RBC, hematocrit, and affect the calculated parameters mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Clots in a sample will yield poor cell distribution which will affect all parameters in the CBC, reticulocyte count and platelet count. Sometimes micro-clots and platelet clumps are present that are not visible to the naked eye. These usually result from a difficult draw or improper mixing of the sample.

If a syringe with a large gauge (small bore) needle is used to draw the sample it is better to take the cap off the tube to put the blood in the tube. If the vacuum is not released from the tube it may pull the cells through the needle too quickly causing them to lyse. This can be crucial on small mammals.

One should always try to make a fresh blood smear and let it air dry. Do not fix it chemically or by heating. *Always label the smears and the tube(s)* with the species, animal identification number or case number and the date.

In cases where it is not possible to obtain a good specimen, a WBC estimate and differential can be done from a good client smear.

Post Collection and Transport

Samples should be stored refrigerated at 2° - 8°C (41°-46°F) until they're transported to the laboratory. Samples exposed to heat or cold for prolonged periods of time are subject to cell lysis and/or degeneration. ***Never freeze or transport on dry ice any specimen that has red blood cells in it.*** When specimens are properly stored, CBC parameters are usually stable for a minimum of 24 hours. If there will be a delay in getting the specimen to the lab and smears were not made at the time the blood was drawn, smears should be made from the LTT.

A good sample is essentialin order to evaluate cell toxicity or reactivity, RBC morphology, and platelets and intracellular organisms such as FIA (feline infectious anemia), ehrlichia (canine and equine), babesia (feline), eperythrozoonosis (llamas) anaplasma (bovine) or avian parasites.

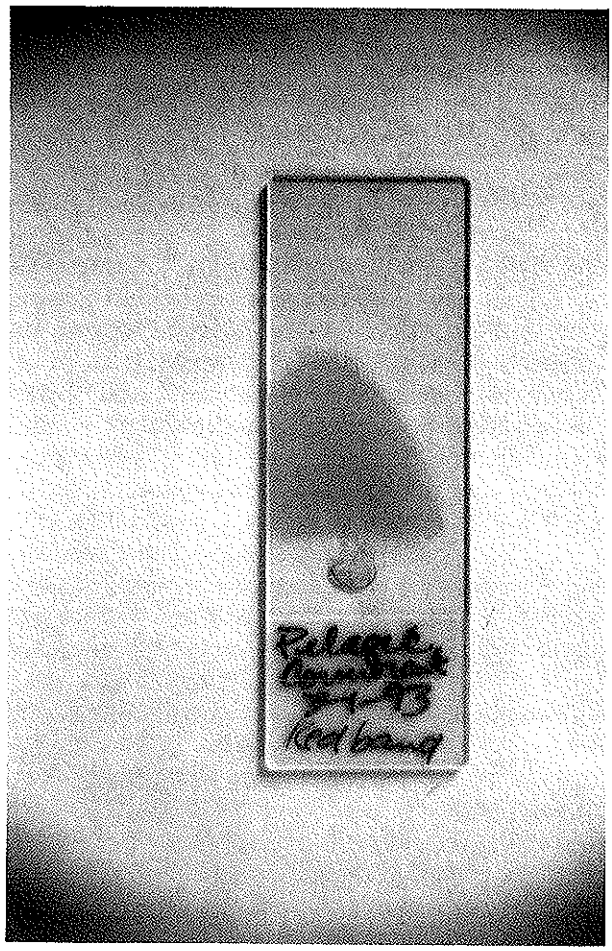
Whenever there are problems with a sample, some tests parameters my not be valid. When their exists a potential for test results to be less than accurate, it is our policy to include a statement on the final report which alerts the client that the test parameters may not be valid. Specimens with clots, short samples, RBC clumping, autoagglutination, presence of hemolysis or lipemia are examples of "problem samples."

Glossary

anaplasma — Blood parasite in cattle and wild ruminants; affects RBC's
autoagglutination — Spontaneous clumping or agglutination of RBC's in drawn blood
babesia — Blood parasites found in cattle, sheep, horses, dogs, and other vertebrates; transmitted by tick; destroys RBC's
CBC — Complete blood count; normally consists of PCV (packed cell volume), plasma protein concentration, total WBC count, WBC differential count, RBC morphology, platelet count; also, hemoglobin concentration, mean corpuscular volume, estimate of platelet concentration
ehrlichia — A blood parasite of dogs; affects WBC's; transmitted by ticks
eperythrozoonosis — A blood parasite of swine, ruminants that affects RBC's
FIA — Feline Infectious Anemia; an acute or chronic anemia of cats
hematocrit — Packed cell volume (PCV); percentage of blood volume taken up by RBC's
hemolysis — Destruction of RBC's
lipemia — Abnormal amount of fat in the blood
lyse — To destroy blood cells
mean corpuscular hemoglobin (MCH) — The measure of hemoglobin content of RBC's
mean corpuscular hemoglobin concentration (MCHC) — The measure of hemoglobin concentration in the average red blood cell
mean corpuscular volume (MCV) — Measure of the volume of red cells
microfilaria — First-stage larvae of heartworm, observed in blood samples
platelet — Small round or oval cell found in the blood of vertebrates; concerned with clotting; a thrombocyte
reticulocyte — Immature red blood cell

Conclusion

A good sample can become meaningless if it is improperly handled or transported. With many wild animals, second samples are difficult to obtain. Therefore, utilizing good sampling and handling techniques is crucial to get valid test results. The collection techniques described in this article are designed to reduce poor results by recommending proper ways to collect and submit blood samples.



A good smear with a "rainbow" effect.
(See text on page 5-6 for details.)

Simple Things That Make A Difference: Good Avian Blood Films Make Good Results

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Introduction

It is important to submit a blood slide (smear) whether or not a lavender blood tube is submitted for analysis. Both smears that are made at the time the blood is drawn and smears made later at the laboratory are important for different reasons. This article focuses on tips on making good smears and how results are affected by poor quality slides.

Making Good Blood Films

The WBC estimate and differential are performed by evaluating several fields of evenly distributed cells. It is imperative that the smear be made immediately after blood collection as avian blood clots quickly. When making smears from a toenail clip wipe away the first drop of blood (it can contain excess platelets and tissue fluid). If smears are made following a venipuncture it helps to remove the needle before putting a drop of blood on the coverslip (this avoids squeezing the fragile avian cells through the needle a second time). Make 2-4 coverslips. Since avian cells are so fragile it is important to use a "light hand" when making the smear. Coverslips that are 22mm x 30mm x 1.5mm or 22mm x 22mm x 1.5mm thick work better than full size glass slides. The best way to make the smear is to place a small drop of blood on the short end of the coverslip. Place another coverslip over that one and gently but quickly pull the two away from each other. A good smear has a "thumbprint, feather edge" appearance which results in a nice monolayer of cells where RBCs appear side by side with small spaces between them. Allow them to air dry. Do not chemically fix the smears.

The Consolidated Veterinary Diagnostics (CVD) laboratory advises its clients to always send a freshly made "client" slide even if they are submitting fresh blood. The reason is that blood of some species of birds do not preserve as well as others in EDTA or heparin. The cells stay intact for actual WBC and RBC counts but over time morphological changes may occur that mimic true toxicity or reactivity. With the client slide available the technologist can accurately evaluate these parameters and obtain good quality results.

Types of Poor Quality Smears

1. Thick Smear - can be caused by too much blood used and/or blood not spread over a large enough area of the coverslip. This results in a smear that is not a monolayer of cells. Cells are usually crushed very close together or overlapping. The dried smear appears opaque when held up to the light. It is very difficult to evaluate.

Parameters Affected: cells do not take up stain evenly. With cells close together it is difficult to see the cytoplasm of the heterophils and evaluate toxicity. Lymphs may stain darker, giving the false impression of reactivity. The WBC estimate will be falsely increased. Since many avian parasites are found in the RBCs it will be difficult to scan the cells when they are so close or overlapping. The platelet estimate may be inaccurate due to clumped platelets.

2. "Pushed Smear" - results when too much force is used to make the smear and the white blood cells are concentrated at the edges of the smear and not evenly distributed in the body of the slide.

Parameters Affected: cells can appear

stretched and distorted. The WBC estimate and differential will be inaccurate due to poor cell distribution. An increased number of smudge cells may be present due to WBC and RBC breakage.

3. Thin Smear - too little blood used to make the smear. Cells are scattered far apart on the slide.

Parameters Affected: platelets may appear artificially decreased. WBC estimate will be falsely decreased. Differential may be inaccurate due to poor cell distribution. With fewer RBCs to scan, avians with only rare parasites may be missed. WBCs may appear toxic because they're stretched and distorted.

In the presence of certain disease states, severe anemia (manifested as a thin smear) or dehydration (manifested as a thick smear), it may be difficult to make good quality slides. It is important in such cases to submit several smears for evaluation.

Practice Making Smears Makes Better Smears

Practice is the key to making smears that are "just right." Take extra blood from a normal bird when first developing these skills and make a hundred or so smears. Place them upward toward the light. Look for a "rainbow" effect of various color or hues along the feathered edges of the smear. While not foolproof, this will be an indicator of a decent smear thickness.

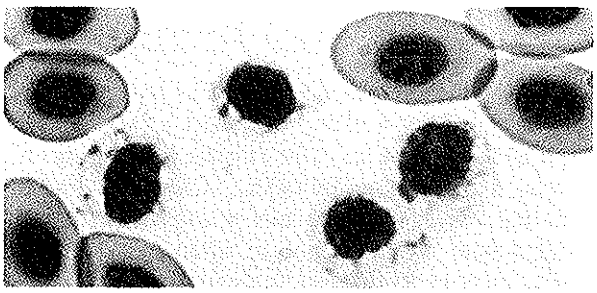
Safe Transport of Coverslips

Do not attach coverslips to anything with scotch tape. It is nearly impossible to remove them without breaking. The best way to transport is to wrap them in a piece of tissue paper, place them in a flat cardboard or plastic slide holder and tape the cover closed. Try to avoid using the plastic "tube-type" slide holders as the tiny coverslips often get stuck in the bottom. If shipping the slides with whole blood or serum samples requiring ice packs, make sure that the ice packs do not come in contact with the tissue covered blood films.

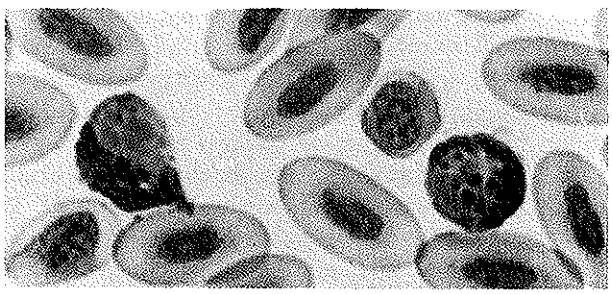
Conclusion

Using these tips, avian blood films can be made well and yield high quality results. Wildlife rehabilitators, like veterinarians, depend on laboratory technologists to give them high quality results. They can also enable technologists to do so by submitting high quality smears.

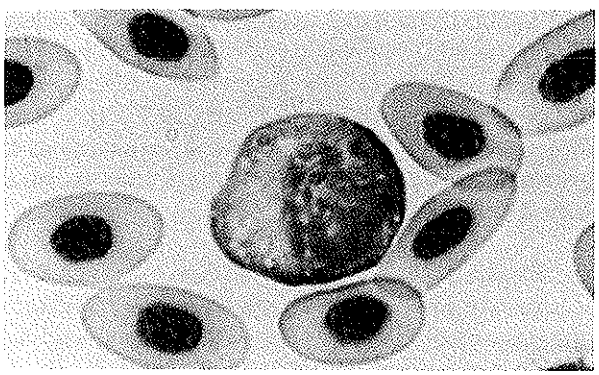
Avian Blood Cell Types



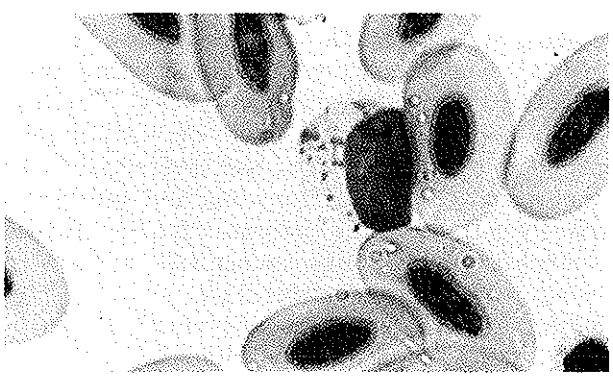
Four thrombocytes



Two basophils. Note large dark granules.



Note the "moth-eaten" look of the cytoplasm of this monocyte.



Note the small granules in the cytoplasm of this lymphocyte.

Avian Hematology: An Overview of Specimen Processing, Sample Evaluation and Interpretation

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Introduction

To ensure that an animal's health can be properly evaluated, all laboratory samples must be processed accurately and efficiently. To optimize sample information, blood collection and specimen processing will be addressed. The effects of anticoagulants, dilution errors and lipemia on these samples will be discussed along with recommendations to increase blood stability. The avian response to disease can be quite variable and dramatic. This is often reflected in their cellular response to disease. A complete understanding of the cellular components of avian blood and how they respond to disease situations is of utmost importance.

Cellular Identification

Erythrocytes

The avian erythrocyte is a nucleated red blood cell, oval in shape, with a centrally placed oval nucleus. The cytoplasm stains a red-orange with the nuclear chromatin tightly packed and a bluish purple in color. Erythroid development in birds takes place within the venous sinuses (of the bone marrow) and is kept separate from the extravascular myeloid production (WBC's). This is in contrast to mammals where red and white cell production take place in the extravascular bone marrow stroma. The young or immature RBC is called a reticulocyte (polychromatophilic erythrocyte). Normally 5-15% circulating reticulocytes are found in the peripheral blood. These cells are oval shaped with a round to oval nucleus. The cytoplasm stains pale blue and the nucleus usually shows chromatin clumping. It is suggested that only those RBC with five or more reticulum clumps be counted as reticulocytes. This is because both basophilic erythroblasts and mature erythrocytes may contain clumping reticulum. A developing erythrocyte stage known as the rubricyte may be present in the peripheral blood, during responses to a regenerative anemia. These cells are round with a strongly basophilic cytoplasm. There is a small N:C ratio and the nuclear chromatin is coarse and blue to black in color. As the cell matures the cytoplasm turns more eosinophilic and assumes the oval shape.

Thrombocytes

These are nucleated cells which function similarly to the mammalian platelets. They do not develop from megakaryocytes, but have a blast stage like the other blood cells. The typical thrombocyte is smaller than the RBC, slightly oval, with a round to oval nucleus. The cytoplasm stains a pale blue to purple and contains a small number of granules that stain a pink to reddish purple. As the cell ages, it becomes more rounded with just a narrow rim of cytoplasm around the nucleus. These older thrombocytes may often be confused with lymphocytes. A differentiating characteristic is that the lymphocyte nucleus is larger and stains less basophilic. The thrombocyte is also more fragile and may disintegrate easier.

Heterophils

The heterophil in birds is equivalent to the mammalian neutrophil, with its main function to defend the body against pathogenic organisms. Heterophils are typically round with a bilobular nucleus and cytoplasm containing granules. The nuclear stain is extremely variable and may often be incompletely colored with a Wright's stain. Heterophil granules are usually rod shaped and stain a pinkish red. The diversity of stain reaction

which these granules show accounts for the heterophil name compared to the neutrophil which stains quite easily with neutral dyes. The granules are often in various stages of dissolution. As they break down they give the relatively clear cytoplasm a pink color. In the early stage of granulocytopoiesis, precursor rods will appear round and stain a deep magenta-orange, and be fewer in number than in the mature cell.

Eosinophils

This cell is identical in function to the mammalian eosinophil. Increases are usually associated with hypersensitivity reactions such as allergy or parasitism. The eosinophil is a round cell with a lobed nucleus that stains blue and reveals details of chromatin patterns. The cytoplasm stains a pale blue and contains large round granules faint pink to lavender-red in color, depending on the species. There can be so many granules that the cell appears to have a "blanket of color" without being able to identify each specific granule. These granules are impervious to aqueous solutions and do not dissolve.

Lymphocytes

As in the mammalian blood, these cells are essential components of the immune defense system. In our pet birds under normal conditions, the lymphocyte is usually the most numerous white blood cell found in the peripheral blood. The difficulty in differentiating lymphocytes from monocytes or even thrombocytes comes when evaluating different stages of disease. Under normal physiologic conditions the lymphocyte is smaller than the heterophil and round. The nucleus is round, eccentrically or centrally placed, has dense clumps of chromatin and occasionally bisected. The cytoplasm is pale blue and may contain dark blue or reddish granules. As the body responds to disease there are marked changes in the size and formation of the lymphocytes.

Large lymphocytes: This type of cell is most difficult to distinguish from the monocyte. This cell has a large nucleus which is rounded or slightly indented. The cytoplasm is pale blue and often vacuolated. Dark (azurophilic) cytoplasmic granules or cytoplasmic blebbing (pseudopodism) may be present.

Small lymphocytes: These cells are often the size of thrombocytes. There is dense nuclear chromatin and very little cytoplasm which is a weak basophilic color. Any cytoplasmic blebbing may help to differentiate these cells. At times the cytoplasm may become deeply basophilic, almost resembling a rubricyte (immature form) of a plasma cell. The key is the nuclear pattern which is similar to a normal lymphocyte. This type of cell is often called a reactive lymphocyte.

Monocytes

Monocytes are considered to be a second line of cellular defense. It is generally agreed that the blood monocyte will become a macrophage. With the Wright's stain, the cytoplasm is blue-grey with a ground glass appearance. The nucleus can be variable in shape (classically a kidney bean shape) centrally or eccentrically placed, or forming a bridge across the middle of the cell.

Basophils

This cell tends to be the most easily recognized leukocyte in the peripheral blood. The nucleus is round and centrally placed. The granules, which are very rich in histamine, have a strong affinity for the basic dyes and stain a blue-violet to black. The cytoplasm is usually clear but at times may appear blue if there has been extensive washing out of granules. The exact function of the basophil is still under controversy. Some feel that the basophil is related to the tissue mast cell, while others propose the cell is an immature circulating mast cell behaving like the monocyte-macrophage relationship.

Cellular Response to Disease

Depending on the type of disease situation that is present, the avian response can be quite dramatic. It is important for the clinician to be able to evaluate the peripheral blood response in order to establish therapy and prognosis.

Anemia alone is not a disease but a reflection of some other disease process. It is important to classify the anemia as regenerative or nonregenerative.

Regenerative anemias may be a result of acute or chronic blood loss, intravascular or extravascular. Blood or gastrointestinal parasitism, bacterial infections, toxicities such as lead or oil (Heinz body anemias) may all be etiologies of anemia. There is usually a decline in the Hct, Hb and RBC parameters within a few hours of blood loss. The maximum decline and recovery phase will vary with the magnitude of loss and the species involved. The response seen is initially normocytic and normochromic but gradually changes to a macrocytic normochromic picture. This is due to the active erythropoiesis and the larger size of the immature cells. It is not uncom-

mon to find a higher percent (>50%) reticulocytes in the peripheral blood. Often a dramatic anisocytosis and poikilocytosis is present. The recovery phase will not return to normal until the cause of anemia is removed.

Non-regenerative anemias are the result of erythrocyte maturational defects or a decrease in the bone marrow production of the RBC. Few or no reticulocytes are seen in the peripheral blood. The mature RBCs often give the appearance of being hypochromic and are in different stages of degeneration. Viral infections, iron deficiency anemias, nutritional deficiencies, certain antibiotics and pesticides can be inciting causes to stimulate this type of anemia.

Leukocytosis may or may not be present with bacterial infections. A shift of the heterophil-lymphocyte ratio may be all that is seen. At times a bacterial infection can cause a leukocytosis with a regenerative or degenerative left shift. The heterophils may be toxic or ruptured. The presence of a monocytosis with the leukocytosis often suggests the possibility of a chlamydial, aspergillosis, or tuberculosis infection. In certain chronic conditions epitheloid cells may be present. These cells are formed from macrophages which form from monocytes. They are sometimes seen in sheets in the peripheral blood. Epitheloid cells can also divide to form new immature macrophages and are considered to be secretory.

Specimen Collection and Processing

Blood Collection and Preparation

Several techniques are available depending on the size and physical condition of the bird and the amount of blood required. The total blood volume (in mililiters) of a bird is 6 to 13 per cent (species variation) of the body weight (in grams). Ten per cent of the blood volume (in milliliters) can be taken safely from a healthy bird, reducing this amount in a sick bird.

Toenail clipping: little risk of blood loss, easily accessible, requires minimal restraint. Always clean the nail thoroughly before clipping to avoid skewed uric acid levels due to dried urate contamination of the nail. Milking the nail to increase blood flow will lead to inaccurate cell counts or contamination of the blood with tissue juices.

Venous puncture: a sample can be obtained much quicker than with a toenail clip. However, more restraint is required and the possibility of hematoma formation exists. Again, clean the area with alcohol before drawing blood.

Skin prick: a useful method for very small birds. Very important to cleanse the skin properly before sampling. A 25 or 27 gauge needle pricks the skin directly over the medial metatarsal vein. Blood is then collected directly from the skin.

Venous sites:

Medial Metatarsal Vein: runs superficially along the medial tarsometatarsus.

Jugular Vein: found in the jugular furrow adjacent to the cervical vertebrae. The right is often larger than the left.

Ulnaris Vein: also known as the wing vein. Very prone to hematoma formation.

Blood Smear Preparation

The avian erythrocyte is a very delicate cell. The two-slide push or wedge smear techniques often used for mammalian blood will rupture the avian erythrocyte resulting in smudged cells. This technique also results in margination of the granulocytes and monocytes at the edges and tail of the smear. I have found the coverslip or coverslip/slide method produces consistent results, with little damage to the avian erythrocyte. A small drop of blood is placed in the center of a coverslip. A second coverslip is then placed gently on top of the first at an angle. As the blood fans out between the two coverslips, they are pulled gently apart with a sliding motion and quickly air dried.

Anticoagulants

There are two main anticoagulants used in avian blood collection. EDTA (ethylenediaminetetraacetic acid) is the best choice for routine hemotologic procedures. EDTA has preserving power for 6-12 hours and preserves the cellular elements better than heparin. Three types of EDTA tubes are available.

1. Microtainers coated with EDTA (Becton-Dickinson) - difficult to use due to premature clotting.
2. Microtainers with EDTA coated beads (Becton-Dickinson) - work well but require more blood.
3. 2 ml EDTA glass tubes with the excess EDTA removed - the most effective, no need to worry about continuous flow or a large sample size.

Whatever microtainer is used, there must be complete mixing or clotting may occur. Excess EDTA may cause shrinkage of the erythocytes

resulting in an erroneously low packed cell volume (PCV) and may cause degeneration of the white blood cells. Blood collected into EDTA may not be used for chemistry evaluations. Blood smears are best made directly with fresh blood. If EDTA blood is used, the smears should be made within 15 minutes after obtaining the sample.

Heparin is the anticoagulant that lines the standard microhematocrit tube (Scientific Products 1.2mm x .75mm) often used by avian practitioners. The reasoning is twofold. The blood may not clot as quickly in this tube vs a plain hematocrit tube. Many practitioners make their blood smears from this heparinized blood. This blood can later be used for chemistry evaluation. Several factors need to be considered when heparinized blood is used.

1. Heparin may cause clumping of the leukocytes.
2. Heparin may interfere with the stainability of the leukocytes.
3. Heparin will not prevent clotting longer than 8 hours (often minutes in the heparinized microhematocrit tubes).
4. Heparin may elevate the inorganic phosphorous level or interfere with cholinesterase values.

Hemolysis

This is often a result of how the blood is collected. Only when the concentration of hemoglobin exceeds 0.02g/dl does the serum appear grossly hemolyzed. The following facts need to be addressed.

1. Icteric serum may hide the appearance of a hemolyzed sample.
2. Serum lipase values may be dramatically lowered.
3. Urea Nitrogen may be falsely elevated.
4. Inorganic phosphate increases with a hemolyzed sample, as does serum potassium and alkaline phosphatase.

Lipemia

Post-prandial lipemia is not a concern in our avian patients. However, certain disease states (liver) and seasonal influences (egg production) may result in a lipemic sample. If the colorimetric method to determine some blood values is used, lipemia elevates the optical density and falsely elevates the total protein, hemoglobin and amylase. Lipemia also interferes with the staining capabilities of the blood smear.

WBC Counts

There is a direct and indirect method to obtain a WBC count. ***Indirect***: The WBC *estimate* is a white blood cell count based on the ratio of white blood cells to red blood cells in the peripheral blood. Because this is a ratio, the WBC count must be corrected for a low PCV or Hct. The formula for the estimation is to count all the white blood cells in 10 high dry fields, divide by the number of fields counted and multiply by two thousand. This gives the average number of leukocytes per cubic millimeter of blood. It is important that a monolayer blood film area of the slide is evaluated. The leukocyte count must be adjusted for a low PCV. The formula for this is:

$$\frac{\text{Observed PCV}}{\text{Normal PCV}} \times \text{WBC} = \text{Corrected WBC}$$

The *eosinophil Unopette*, although more time consuming is a more reliable method for counting WBCs. The Unopette method used to count mammalian WBC cannot be used. The dilution fluid ruptures all non-nucleated cells allowing only nucleated cells to be counted. In avian blood all cells are nucleated. The Eosinophil Unopette diluent, phloxine, stains the heterophils and eosinophils. The lymphocytes, monocytes and basophils must be accounted for indirectly. The formulation for calculation is:

$$\text{Total WBC} = \frac{\text{Total heterophils + eosinophils}}{\%\ \text{heterophils + eosinophils}} \times 100$$

Direct: A toluidine blue stain is used to perform a direct WBC count. The toluidine blue comes as a powder, manufactured by Polysciences Inc. This powder which is stable indefinitely is made into a 0.1% solution with saline. This stock solution maintains its effectiveness for six months. The standard Red Cell Unopette™ (Becton-Dickinson) is used to provide further dilution of the toluidine to a 0.01% concentration. Blood is submitted in EDTA or heparin and a hemocytometer is required for the count.

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Avian Technician's Hematology Guide

PCV	*Increase*	1. dehydration 2. abnormal myeloid production	*Decrease*	1. regenerative anemia polychromasia, macrocytosis anisocytosis, reticulocytosis, > 10% 2. nonregenerative anemia, depression anemia secondary to disease states, normocytic normochromic reticulocytes < 5% (species vary) 3. lead toxicity, RBC ballooning "D-shaped" cells Chromatin clumping
TP	*Increase*	1. dehydration 2. chronic inflammatory disease	*Decrease*	1. liver disease 2. parasites 3. blood loss anemia with a low PCV
	Normal	1. with a decreased PCV, depression anemia or hemolytic anemia		
WBC	*Increase*	1. slightly (rarely >25,000) bacterial: psittacosis hemoparasites 2. great(>25,000) granulomatous infection fungal disease psittacosis	*Decrease*	1. viral 2. severe chronic infection 3. overwhelming septicemia 4. toxic chemicals
Thrombocytes		1. last cell line to become reactive 2. reactivity with any disease 3. phagocytic properties		
Heterophils (+/- toxic)	*Increase*	1. bacterial 2. granulomatous 3. psittacosis 4. leukocytozoan	*Decrease*	1. neoplasia 2. overwhelming septicemia
Lymphocytes (reactive +/-)	*Increase* (+/- pyknotic)	1. viral 2. hemoparasites 3. abnormal lymphoid production (lymphoid leukosis or neoplasia)	*Decrease*	1. bacterial 2. granulomatous disease 3. psittacosis 4. lead toxicity
Monocytes	*Increase*	1. chronic bacterial infection 2. granulomatous disease 3. psittacosis 4. parasites	*Decrease*	1. ?
Eosinophils	*Increase*	1. parasites 2. allergic reaction 3. marked tissue damage	*Decrease*	1. ? Significance not known
Basophils	*Increase*	1. respiratory tract disease bacterial diseases: psittacosis granulomatous disease 2. parasites 3. lead toxicity	*Decrease*	1. ? Significance not known

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Glossary

alkaline phosphatase — Enzyme present in almost all tissue, particularly in bone and liver/gall bladder system
amylase — Enzyme derived from pancreas; function is to break down starches and glycogen
anisocytosis — Variation in size of RBC's (red blood cells)
azurophilic — Staining pink with Wright's stain
basophilic — Staining various intensities of blue with Wright's stain
bilobed — Having two small lobes
blast — Immature stage in cellular development
blebbing — pseudopodism
cholinesterase — An enzyme
chromatin — Deeply staining substance in the nucleus of a cell that contains DNA (genetic material)
colorimetric — Pertaining to the use of an instrument for determining the color intensity of a substance or fluid
eosinophil — White blood cell containing prominent granules that stain red to pink with Wright's stain
eosinophilic — Capable of staining various degrees of red with Wright's stain
epitheloid — Resembling epithelium (layer of cells forming the epidermis of the skin and the surface layer of mucous and serous membranes); a transformed macrphage
erythroblast — Immature, nucleated red blood cell
erythroid — Concerning the red blood cells
erythropoiesis — Formation of red blood cells
etiologies — Causes of disease
extravascular — Outside of a vessel
granulocytes — Cells that develop in the bone marrow and contain cytoplasmic granules; neutrophils, eosinophils, basophils
granulomatous — Containing granular tumors or growths
granulopoiesis — Formation of granulocytes
Hb — Hemoglobin
Hct — another term for the PCV (packed cell volume)
Heinz body — Refractile inclusions causing bulging of RBC's
hematoma — Swelling caused by a break in a blood vessel
hemocytometer — Instrument used to count blood cells
hemolysis — Destruction of red blood cells
histamine — Substance in the body released from injured cells
hypochromic — Containing reduced amounts of hemoglobin; decreased staining intensity, pale or washed-out appearance
icteric — Yellowish
left shift — Increased numbers of immature neutrophils
leukocyte — White blood cells (WBC)
leukocytosis — Increased numbers of WBC's
leukocytozoan — Parasite of avian RBC's
lipase — An enzyme that breaks down fats
lipemia — Fat in the blood
macrocytic — Pertaining to an abnormally large RBC
macrophage — Cell having the ability to ingest microorganisms, cells, foreign particles
margination — Accumulation
mast cell — Cell containing heparin and histamine; important in cellular defense mechanisms including blood coagulation
megakaryocytes — Giant cells occuring in bone marrow, presumed to generate platelets
monocytosis — Excessive number of monocytes in the blood
myeloid — Resembling cells produced in the bone marrow
neoplasia — Development of abnormal tissue formations; tumor or growth
neutrophil — Mature granulocytic WBC's that are phagocytic
normochromic — Possessing normal color and hemoglobin content
normocytic — Having average sized RBC's
packed cell volume (PCV) — Percentage of whole blood composed of red blood cells
peripheral blood — Blood that circulates around the extremities
phagocytic — Having the ability to ingest and destroy cells, foreign bodies
plasma cell — Ovoid cells with eccentrically placed nuclei; produce antibodies
poikilocytosis — Presence of irregularly shaped RBC's
polychromasia — Variation in hemoglobin content
polychromatophilic — Stainable with more than one kind of stain
post-prandial — Following a meal
pyknotic — Thickness, shrinkage of cells through degeneration
RBC — Red blood cell
reactive lymphocyte — Antigenically stimulated lymphocytes; immunocytes
regenerative anemia — Increased RBC production in blood and marrow
reticulocytes — Young, large RBC's
reticulum — Masses of chromatin
rubricyte — Immature red blood cell
secretory — Pertaining to secretion
septicemia — Presence of pathogenic bacteria in the blood
stroma — Foundation-supporting tissues of an organ
tarsometatarsus — Bone of the lower shank of birds, containing fused tarsal and metatarsal bones
total protein — Measurement of protein in plasma
urea nitrogen — Urea concentration of serum or plasma; called blood urea nitrogen (BUN); renal function indicator
vacuolated — Having air- or fluid-filled spaces in cell cytoplasm
venous sinuses — Channels of the vena cava
Wright's stain — Stain used to study blood cells and reveal malarial parasites

A Literature Review of Selected Hematologic Parameters of Raptors

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Overview

A recent review of the literature supports the fact that available hematology and chemistry values for various species of raptors is limited. When interpreting the results of the blood studies conducted on these birds, one must document the method of determination as this will effect the values presented. The total protein will vary depending on whether a refractometer, autoanalyzer or protein electrophoresis was used to determine its value. The packed cell volume (PCV), red blood cell (RBC) count and erythrocyte indexes were fairly consistent among studies. The white blood cell (WBC) count had a wide range of values reported and the author questions the method of determination and health of some of the raptors included in the normal values reported.

The results from some of the raptor studies lumped various species of Strigiformes and Falconiformes together regardless of species, age or sex. The following tables reflect this literature search on a selected number of raptor species. This information should be used only as a general guideline realizing that age, sex, air temperature, molt, reproduction, exercise, body weight and time of day will affect some of these values.

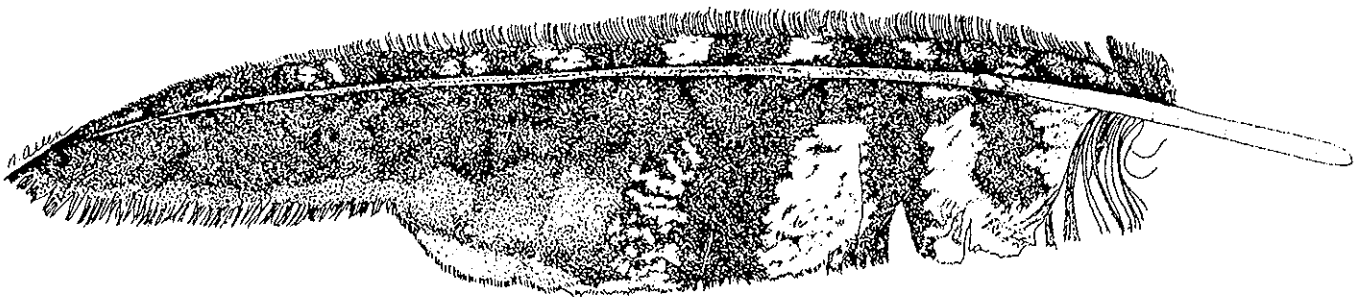


Table I

Selected Raptor Hematologic Parameters [1,2,3,5,6,7,8,9]

SPECIES	PCV %	RED BLOOD CELLS (million/mm^3)	WHITE BLOOD CELLS (thousands/per mm^3)	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)
American Kestrel *(Falco sparverius)*	35-47	2.9 - 3.3	6-12	2.5-4.0	
Golden Eagle *(Aquila chrysaetos)*	36-45	2.0-3.2	8-22	2.5 - 3.9	1.1-1.7
Bald Eagle *(Haliaeetus leucocephalus)*	34-45	2.27-2.90	8-17	3.0-4.1	1.1-1.6
Red Tailed Hawk *(Buteo jamaicensis)*	34-44	2.19-2.76	6-20	3.5-4.5	1.4-2.8
Harris' Hawk *(Parabuteo unicinctus)*	37-41	2.2 - 2.5	10-15	3.5-4.8	1.2-2.3
Barn Owl *(Tyto alba)*	42-47	2.2-2.5	13-21	3.9-4.6	
Common Screech Owl *(Otus asio)*	41-49	2.5-3.7	7-13	3.5-4.0	

Table II

Raptor Values by Group [2,4,5]

Category	PCV %	RBC (million/mm^3)	WBC (thousands/mm^3)	Total Protein (g/dl)
Raptors (in General)	39-44	1.8-4.4	15-24	2.3-5.4
Strigiformes	37-45	2.1-3.2	6-25	3.5-4.9
Eagles	34.5-48.5	2.0-3.4	10.7-27	3.6-5.0
Hawks/Falcons	37-46	2.4-3.6	8-26	3.6-5.0
Vultures	38-48	2.1-3.7	6.3-21.3	3.3-4.3

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Announcements

Members' Forum

Starting with our next issue of the *Wildlife Journal,* we will include a question and answer column. This column will be an opportunity to learn what is on your mind and present some direct answers to issues that are of most concern to you. We encourage all members to submit questions on animal care, ethics or whatever pertains to wildlife rehabilitation.

While all signed letters will be answered, only a limited number will fit our column space. All letters must be signed and include a self-addressed, stamped envelope for response.

Letters will be published anonymously if requested. If you need a quick reply, please include your phone number and we will call you collect. Letters are to be sent to the IWRC office Attn: Members' Forum Editor, 4437 Central Place, Suite B-4, Suisun, CA 94585. We are all excited about the addition of our new column. Let's hear from you!

Members' Forum Editor
Marge Gibson

Jobline Information

We keep on file current job announcements. If you have a jobavailable or are looking for one, don't forget to call us at IWRC (707) 864-1761 from 9 am to 4 pm (PST). When we have enough jobs at one time, we do put out a job line. We will list internships as well. (See page 16 for current listings.)

Used Rat Cages For Sale

The bottoms are 13 1/2" x 11 1/2" x 7" made of opaque plastic with a mesh net top. The top fits tightly and dips to hold food. A glass water jar comes with each cage. Price $6.00 plus postage and handling. For information call Jim Fitzpatrick at Carpenter Nature Center (612) 437-4359 (CST).

Rehabilitation Survey Available

Cornell University has released the final report from their ***Survey of New York State Wildlife Rehabilitators.*** This is part of a three phase study to identify ways to develop and maintain a relationship with rehabilitators that enhances the Bureau of Wildlife's ability to provide wildlife-related benefits to the people of New York.

Copies of this report are available from Mr. Bill Siemer, 124 Fernow Hall, Dept. of Natural Resources, Cornell University, Ithaca, NY 14853 (phone: 607-255-8192) for $4.50.

IWRC Jobline Information

The IWRC Jobline is a service to our membership. If your organization has a job opening or an internship available, send us a description of the job and its qualifications, and we will list it in our *Wildlife Journal*, which is published quarterly.

Howl Wildlife Center

Rehabilitation Manager: Oversee day to day operation of wildlife center. Duties include animal care, maintenance, supervision of staff, and various managerial tasks. Qualifications: Minimum of two years full-time rehabilitation experience with a variety of species, supervisory experience, four year degree or equivalent experience. Salary DOE.

Shift Supervisor: Responsible for daily care of wildlife patients, carry out wildlife treatment plans, maintain facilities, supervise volunteers and interns, assist veterinarian, maintain records. Qualifications: Minimum three months full-time rehabilitation experience. Salary DOE.

Seasonal Wildlife Care Staff: (Full-time, April-September) Assist Shift supervisor with wildlife care and maintenance. Qualifications: Some rehabilitation experience, either as staff or steady volunteer, required. Salary DOE.

Wildlife Care Internships: (12 weeks) Interns will learn various aspects of wildlife rehabilitation. Qualifications: Wildlife sciences and veterinary medicine students preferred. Stipend $200/month & housing provided.

For complete job descriptions and applications (specify position requested), contact:

Jeanne Wasserman
HOWL Wildlife Center,
Box 1037
Lynnwood, WA 98046
(206) 743-1884.

Wildlife Care Center

Wildlife Rehabilitation Manager: Minimum two years zoo or wildlife rehabilitation experience with variety of species. Duties include managing day to day operations, assisting the veterinarian, and supervising staff. Salary $18-20 k + benefits.

Veterinary Technician: Minimum two years zoo or wildlife experience with a variety of species. Avian experience a plus. Salary commensurate with experience.

Send resume / cover letter to:

P. Rich
The Wildlife Care Center
1061 W. Oakland Blvd.
Fort Lauderdale, Fl 33311
(305) 565-7699.

**Full-time positions, salaries commensurate with experience and qualifications. These positions are available immediately. Interested parties should send resume/references and salary requirements to : *June Curran, Wildlife Care Center, 1061 W. Oakland Blvd., Ft. Lauderdale, Fl. 33311, 305-565-7699.*

****Executive Director:*** Results-oriented *Leader* to direct a high volume rehabilitation/ education facility. Must have demonstrated experience in administration, planning, communications, community involvement and budgeting.

****Veterinarian:*** Responsible for all medical decisions and treatment regarding the health of wildlife patients. Some wildlife medical training and/or experience preferred. Must work closely with the managing director/rehabilitator.

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