Avian Haematology

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Avian haematology varies from mammalian haematology in the appearance of some cells, the ease of staining and counting, and a shorter life span which results in greater lability of cell numbers.

The classification and functions of avian blood cells closely follows the mammalian cell lines. The major differences in appearance are as follows:

- erythrocytes are oval and nucleated;
- platelet equivalents are present as whole cells rather than fragments and these cells, known as thrombocytes, are also oval and nucleated; and
- the predominant (neutrophil equivalent) polymorph contains much more strongly eosinophilic rod-shaped granules than the neutrophilic granules of mammals, and is known as a heterophil.

These variations have implications for practical assessment of avian blood. White cells cannot be counted by red cell lysis and nuclear counting as used in mammals, as both erythrocytes and thrombocytes would be included in the count. However, the strong staining of the relatively numerous heterophils allows indirect counting methods which would not be practical in mammals.

Cell numbers are more influenced by environmental factors than in mammals, but otherwise show similar responses to infection and other insults.

Another variation is the greater frequency of abnormalities due to neoplastic cells and parasites.

NORMAL CELL MORPHOLOGY AND RESPONSES (Romanowsky stains)

ERYTHROCYTES

The typical mature avian erythrocyte is an oval cell with an oval centrally placed nucleus and orange pink cytoplasm of uniform texture. The nuclear chromatin pattern varies with the age of the cell, being leptochromatic (fine unclumped chromatin strands) in recently mature erythrocytes, becoming more pachychromatic (densely clumped) as the cell ages.

The average chicken erythrocyte is approximately 11µ in length and 6.6µ in width, which is typical of the majority of birds. There is variation both within and between species, however, with the largest erythrocytes occurring in the Osprey (length 16.5µ, width 10.0µ), and the smallest recorded in the Carolina chickadee with a length of 6.0µ.

The mean corpuscular haemoglobin concentration is slightly lower in birds than mammals, but when adjusted for the nuclear volume, the concentration is similar or even higher.

The life span of avian erythrocytes is shorter than that for most mammals, and this has been attributed to the higher avian body temperature and metabolic rate. For chicken erythrocytes life span is 28-35 days; for ducks 42 days; pigeons 35-45 days; and quail 33-35 days. Erythrocyte life span for domestic mammals varies between 60 and 160 days.
Perhaps as a consequence of the shorter life span, developmental stages of erythrocytes are common in the circulating blood of birds, and if present are not considered pathologic, though the numbers will increase dramatically with regenerative anaemia. Polychromatic erythrocytes usually make up 1-5% of the erythrocytes of normal mature chickens, but have been recorded as high as 20% in both chickens and ducks. In psittacine birds up to 10% reticulocytes is considered normal. Indeed if reticulocyte stains are used in birds, cytoplasmic RNA clumps, which give these cells their colour, are seen in most erythrocytes, and it is suggested that only those erythrocytes with a complete reticulum ring around the nucleus should be counted as reticulocytes.

Factors Influencing PCV

Species variation in PCV shows a rough negative correlation between size and PCV. However, as PCV is highly influenced by environmental factors there is considerable within species variation.

Most aviary birds have a PCV of 45-55% with slightly higher values in very small birds (e.g. the canary PVC may be up to 60%), and lower values in larger birds (e.g. as low as 40% in cockatoos). For chickens the mean PCV for females and immature birds is around 30%, though adult males may have a PCV OF 40%. Ducks range from approximately 33-40% PCV. For aviary birds a suggested guideline for anaemia is below 35%, though this obviously is not applicable to poultry.

As well as species, PCV is influenced by age, sex, season, time of day, environment, starvation, and hormonal influences, as well as disease.

There is a gradual increase in PCV with age, though this is more marked in males than females.

A diurnal rhythm is shown with the highest PCV at midnight. This is also more marked in males.

In hens the lowest erythrocyte counts and PCVs were found at seasons of highest reproductive activity, and sitting hens had lower PCV than laying hens (also lower thrombocyte and white cell counts). Estrogens decrease erythrocyte count, while androgens and thyroxine have an erythropoietic effect.

Environmental factors such as cages versus floor runs will also have an effect with caged layers being found to have higher PCVs than hens on a run or floor system (though they had lower heterophil counts which might be expected from lower pathogen exposure).

Starvation, hypoxia and hypothermia all lead to haemoconcentration and an increased PCV, which is a factor to consider in assessing sick anorexic birds. Starvation for 48 hours increased the haematocrit of chickens by 30%. Hypoxia, especially in smaller birds, may lead to PCVs of up to 80%.

Causes of Anaemia

Anaemia from blood loss may occur with traumatic haemorrhage; with haemorrhage as a result of organ lesions such as ulcerated neoplasms; with gastrointestinal haemorrhage as a result of shock; with ectoparasites or gastrointestinal parasites and with coagulopathies.

Haemolytic anaemia may result from infection by certain blood parasites, from aflatoxicosis, toxic chemicals such as petroleum, and from some toxic plants (possibly with Heinz bodies).

Anaemia from erythropoietic depression occurs with almost any infection, possibly due to the short life span and greater turnover of erythrocytes. Chronic infections tend to produce nonregenerative anaemia. Erythropoiesis is also depressed by nutritional deficiencies such as iron and folic acid; by toxins such as chloramphenicol, lead and aflatoxins; by chronic renal disease such as in mammals; and by the avian leukosis complex.

Abnormal Erythrocytes

A number of artifactual and sporadic variations are illustrated by Lucas and Jamroz. Abnormal erythrocytes may also be present.

Spherical forms with oval nuclei may be seen in anaemia as the nucleus and stroma differentiate ahead of changes in cell shape. This shape may also be found in first generation erythrocytes of the embryo, where once again the maturation is accelerated.

The number of circulating immature erythrocytes may be markedly increased in regenerative anaemias. Polychromatic erythrocytes may be very common without adverse prognosis.

Overmature small erythrocytes with pycnotic nuclei may be seen in splenic disease.

THROMBOCYTES

The typical avian thrombocyte has been described as a cell slightly smaller than an erythrocyte, elongated with rounded ends, but not having the regular oval contour of the erythrocyte. The thrombocyte nucleus also has a slight oval shape, but is not as elongated as that of the erythrocyte. The cytoplasm is typically clear and may contain one or more red granules or vacuoles at the poles.

Mature chicken thrombocytes vary in size from 7-10µ long by 4-5.5µ wide. Larger thrombocytes are found in some species of ducks, turkeys, pheasants, pigeon, dove, owl, cuckoo and several others, but the cells are readily recognisable in all avian species and indeed in reptiles, amphibians and fishes.

There are occasional variations. The specific granules of the turkey thrombocytes were usually in vacuoles. In the pigeon, the thrombocyte cytoplasm takes up more stain and the cells are rounder as well as being larger so they are more likely to be confused with small lymphocytes.

Less mature forms containing a larger nucleus to cytoplasm ratio, more cytoplasmic basophilia, less vacuolation and less chromatin clumping may appear in blood. The earliest seen are about the middle of the developmental process.

Thrombocyte appearance may be altered by disintegration on exposure to air, often showing loss of oval shape and granules and nuclear pycnosis.
A more genuine variation is cells described as "reactive" thrombocytes which are rougher in outline with frothy, sometimes pinkish cytoplasm and possibly more red granules. These are typically associated with disease states.

Thrombocyte Numbers and Lifespan

There are no readily available figures on lifespan, but if similar to amphibians may be as long as 5 months if not undergoing prior functional disintegration.

Avian thrombocyte counts are typically around 20-30,000/µl of blood, which is about 1 thrombocyte per 75 red cells or about the same number as total leucocytes in poultry, and considerably more than the total leucocyte count in many species of small birds. Thus errors of classification on differential smears can be significant. Thrombocytes may clump heavily before smears are made, reducing the apparent number, but normally one should expect to see 1 or 2 thrombocytes per average monolayer oil immersion field.

Thrombocytopenia may occur in severe septicaemias, diffuse intravascular coagulation, and leukaemias. Prolonged clotting time without necessarily thrombocytopenia can occur with severe liver disease, and with vitamin K responsive haemorrhagic disorders, especially in psittacines.

Thrombocyte Function

Avian thrombocytes are involved in blood clotting, and also have a phagocytic function, but are not directly involved in the initiation of blood clotting as are mammalian platelets.

Avian thrombocytes contain large quantities of 5-hydroxytryptamine (serotonin) and acid-phosphatase, but little thromboplastin which is the essential platelet derived factor in the mammalian intrinsic (or blood initiated) clotting mechanism. It is not surprising therefore, that avian plasma is also deficient in the other, liver derived factors of the intrinsic coagulation pathway including factor XII (Hageman factor) which initiates this pathway, and factors V and VII. (The latter is the factor most depressed by warfarin, which means that birds are much less susceptible to this chemical than mammals).

The initiation of avian blood coagulation therefore depends on the extrinsic clotting pathway which involves the release of tissue thromboplastin by damaged tissue, including blood tissue. (Though avian thrombocytes contain little thromboplastin and do not utilise the intrinsic pathway, studies have shown that avian blood samples generate about a quarter as much thromboplastin as human blood samples, though this is believed to be of the tissue type).

Both the intrinsic and extrinsic pathways lead to the formation of activated factor X which initiates the common pathway leading to the conversion of fibrinogen to soluble and then insoluble fibrin monomers.

Avian thrombocytes do have an essential role to play in blood clotting. Clots are essentially composed of degenerate thrombocytes or platelets on a fibrin stroma, plus other entrapped cells. The formation of an effective clot involves the interaction of fibrin generation and a viscous metamorphosis of platelets or thrombocytes, though the actual mechanism is not fully understood. Certainly, degenerate thrombocyte form the major component of chicken hemostatic vascular plugs.

Avian thrombocytes have been shown to aggregate in vivo and in vitro to the site of ADP release, which in the case of blood clots could well be derived from trapped or damaged erythrocytes which contain high concentrations of ADP. Clumped thrombocytes appear to form pseudopodia, as can be readily seen in blood films exposed to air. In mammals fibrin has been shown to attach to these pseudopodia initiating platelet proteins with contractile properties to polymerize and form definite microfilaments within the platelets.

These proteins are actin\myosin like, and the combined effect of their contraction and the fibrin attachment is to retract the clot and release thrombocyte products into the plasma. These products include ADP, which aggregates more platelets, and the vasoactive serotonin which in vivo aids hemostasis by vasoconstriction.

Thrombocytes do have other functions including phagocytosis. Phagocytosis has been demonstrated for thrombocytes of a wide range of animals, including frogs and the thrombocyte analogous "thigmocytes" of crustaceans, and has been proposed as possible function for mammalian platelets, particularly in regard to small solid particles.

The chicken thrombocyte has been demonstrated to phagocytose both small particles and bacteria, and to concentrate them in acid phosphatase-rich perinuclear lysosomes where the bacteria undergo degradation. Carlson et al (1968) showed that with phagocytosis, and possible under general adverse conditions, these thrombocytes showed an increase in cytoplasmic vesicles, rough endoplasmic reticulum and other cytoplasmic organelles, as well as extrusion of the red granules and assumption of cytoplasmic projections.

This would explain the rounder, rougher and more vesiculated appearance of "reactive" thrombocytes mentioned earlier as typical of diseased states.

They also demonstrated that the ultrastructure of the typical red staining granules was of electron dense membranous vesiculated bodies typical of secondary lysosomes or "residual bodies", which would also explain their appearance only late in thrombocyte maturation, and their increased number in reactive cells.

LEUCOCYTES

Avian leucocytes are very similar to their mammalian counterparts, and respond in a similar way.

Total leucocyte counts may be quite high in poultry, but are usually in the order of 5-11,00/µl in most pet species. In chickens the total leucocyte counts maybe 30,000/µl in immature and female birds, but only about half this in males. There is considerable variation with species, strain, and physiological state. Young birds generally have lower leucocyte counts than adults.

Mononuclear Leucocytes

Lymphocytes and monocytes in all species of wild and domestic birds, appeared very similar to their mammalian equivalents. The only consistent difference is that in mammals the "azurophil" granules of both lymphocytes and monocytes appear similar, while in birds the granules seen occasionally in lymphocytes stain intensely and are termed magenta bodies, whereas the azurophil granules of the monocyte are small, stain rather faintly, and usually have an orange colour. However, the lymphocyte granules may also occasionally have an orange tint, depending on granule size and staining method.

Lymphocytes are often the most common cell in avian blood, varying from about 20-75% of total leucocytes. Both small (up to 8µ in diameter) and medium (8-10µ) are normally seen, with the predominant size varying with individual birds and physiological state. As in mammals, large lymphocytes (diameter more than 10µ) usually occur only in tissues. The cytoplasm is usually scant and often homogeneous. Also as in mammals, cytoplasmic magenta bodies are not common, and usually occur only under antigenic stimulation.

Lymphopaenia occurs with viraemia, with some chemical toxins and with folic acid deficiency. Lymphopaenia accompanied by heterophilia occurs in riboflavin and B1 deficiency, and with any cause of glucocorticoid excess. Lymphocytosis suggests chronic viral infections, possible immune mediated diseases, or lymphoid leukemia.

Monocytes are generally larger than circulating lymphocytes, with a larger cytoplasm to nucleus ratio. It has been estimated that the size range of 25% of chicken lymphocyte nuclei overlaps with 22% of monocytes nuclei, whereas with total cell area, only 15% of lymphocytes overlap with 7% of monocytes. The average monocyte diameter is about 12µ. As in mammals, the cytoplasm has a "ground glass" appearance. The small azurophil granules may be numerous and usually stain orange. A "hof" or pale area is often visible and may also stain orange. Monocytes generally do not represent more than 5% of leucocytes in healthy birds. Monocytosis is observed in chronic infections (mycobacteria, chlamydia) especially granulomatous lesions.

HETEROPHILS

The heterophil of birds and reptiles is the equivalent to the neutrophil in mammals. It is generally a relatively small cell, being 4-9µ in diameter in the chicken. As in mammals, the mature heterophil generally shows more nuclear lobulation than eosinophils or basophils.

The specific inclusions for heterophils are long fusiform rods, pointed at each end, which take the eosin stain brilliantly. These rods contain a central granule, which varies in staining with species and technique, sometimes being visible, others being seen as a clear vacuole. For general purposes the central granule is not readily noticed in most species on routine counts, but it may be useful to define heterophils and eosinophils in unfamiliar species.

Anyone studying avian blood would recognise that the heterophil granule is soluble in water, even after methanol fixation. Prolonged contact with water, as with the longer bulk staining method for Wright's stain, leaches out the heterophil rods leaving the bare central granules. This often leads to confusion with eosinophils. However, the dissolved rods usually give the cytoplasm an orange or pinkish colour which distinguishes it from the clear blue cytoplasm of eosinophils. Other useful distinguishing features are the greater nuclear lobulation of heterophils and often recognisable clear granular clefts or unstained granules. Also, the heterophil may show a remarkable resistance to nuclear staining. This artifact is seen also in the heterophils of reptiles, and is not seen with eosinophils.
Species variation in heterophil appearance is slight, though the granule size may vary both within and between species.

Variations in number are much greater. Indeed heterophil variations probably contribute most to the almost legendary variability of avian blood counts. Part of this variability is no doubt due to the very strong diurnal rhythm of heterophils. Pigeons showed rises of up to 140% in heterophil counts taken in the afternoon, compared to morning counts. Nevertheless, heterophil counts may show a four-fold range in normal chickens (3-12,000) with more variation within than between groups of birds. However, it is usually possible to detect heterophilia in the case of bacterial infections, often accompanies by monocytosis. Overwhelming infections may result in leucopaenia.

EOSINOPHILS

The eosinophil is not of a similar size, with generally a readily stained bilobed nucleus, clear pale blue cytoplasm, and found refractile granules which are not leached by aqueous stains. There is more variation in eosinophil granule size than in the heterophil, but less variation in number. The eosinophil granules are large in the turkey, pheasant, some ducks and the dove. The Canada goose and the owl have small granules. Passerine eosinophils are generally like the chicken. Some finches and the robin have very fine refractile granules.

There is no close correlation between avian eosinophil levels and parasitism. They are often increased with marked tissue damage. Many eosinophils are found in the ovary.

The youngest eosinophils that have been observed in the peripheral blood are mesomyelocytes.

BASOPHILS

Avian basophils closely resemble mammalian basophils both in light microscopy and ultrastructure. The granules are round, strongly basophilic, and again water soluble. When degranulated, they give a pink/purple tinge to the normally clear cytoplasm.

HAEMATOPOIESIS

Blood cell replacement is achieved through a series of divisions and maturation steps initiated by the division of stem cells committed to one cell line. These committed stem cells have been shown to arise from a pluripotent stem cell which is called a colony forming unit (CFU), has the appearance of a small lymphocyte, and may circulate in blood which allows it to reseed all blood cell lines to artificially depleted haemopoeitic sites.

Colonies of specific blood cell lines develop in response to specific inducer substances acting on committed stem cells, leading to the production of larger metabolically more active blast cells which are committed to the development cascade.

Erythropoietin is the inducer that influences the committed erythrocyte stem cell to produce erythroblasts. Erythropoietin also stimulates the mitotic rate in the later erythroid stages, and stimulates an enzyme (aminolevulinic acid synthetase) necessary for the synthesis of haem. Erythropoietin is produced in the liver by the interaction of a plasma -globulin with an erythropoietic factor produced as a response to anoxia. In mammals this latter factor is normally produced by the kidney, though it can also be produced by other organs, possibly the liver. The site of avian erythropoietic factor is not certain. Avian erythropoietin is not active in mammals, and vice versa.

Once initiated, erythrocyte development takes about 3-5 days, involving four mitoses to produce 16 erythrocytes.

Granulocyte development typically involves five mitoses over about the same period. There are a number of factors influencing granulopoiesis, including a macrophage derived Colony Stimulating Factor which acts in a similar way to erythropoietin, and factors from both macrophages and neutrophils which limit this activity.

There are also factors which regulate the release of various cell types into the blood, particularly for the less numerous granulocytes for which the non-circulating reserves are very large.

ERYTHROPOIESIS

The stages of the avian erythrocyte series are erythroblast; early, mid and late polychromatic erythrocytes; reticulocytes; and mature erythrocytes. The blast cells of all of the cell lines are similar,

though there are small differences. Blast cells are large cells with a small amount of basophilic cytoplasm. The erythroblast has a very prominent nucleolus, which is a feature of protein producing cells. It also has slightly more cytoplasm than others, and this is an intense blue sometimes with discernible unstained mitochondrial clefts.

Changes with maturation include:-

1. Loss of a visible nucleolus. This is prominent in the blast cell, still visible but less prominent in the early polychromatic erythrocyte, and usually absent thereafter.

2. Gradual increase in chromatin clumping.

3. Reduced RNA content and increased haemoglobin. The high RNA content for cell synthesis gives an intense basophilia to the early polychromatic cells, while the mid polychromatic cells have a slight basophilia to slight eosinophilia, and the late stage has a greyish to moderate eosinophilic cytoplasm as the proportions of RNA and haemoglobin change. The mature cell has a uniform reddish colour. RNA remnants are still present in the reticulocyte, but this may not be distinguishable without special stains.

4. Assumption of an oval shape. The cell outline may be slightly oval by the mid-polychromatic stage. The nucleus usually elongates in the late polychromatic stage. If development is accelerated, as in anaemia or in the embryo, the round shape may remain.

MONONUCLEAR DEVELOPMENT

Developmental stages of these cells are rarely recognised or studied routinely. Monoblasts are not distinguishable from other blast forms, but developmental stages showing a gradual loss of cytoplasmic basophilia, the development of the azurophil granules which vary in number in mature cells, and sometimes the development of a "hof" are recognised.

Lymphocyte differentiation is more a subject for the lymphoid system, though sometimes large blast forms are seen, especially in the lymphoid neoplasias. Mention has already been made to the maturation of thrombocytes. The maturation starts with thromboblasts that are similar to the other blast cells, but with a nucleolus that may be obscured by heavy chromatin clumping. Cell elongation and cytoplasmic vacuolation occur quite early, but magenta coloured granules only appear in some of the later cells.

GRANULOPOIESIS

The immature granulocyte stages are divided into a fairly typical granuloblast, a slightly more recognisable metagranuloblast, a promyelocyte stage with non-specific granules, and a myelocyte stage where specific granules are recognised in each cell type. The myelocyte stage may be further divided into mesomyelocytes, containing less than half the mature complement of granules, and a metamyelocyte stage with more than half the number of granules, but still some immature features.

The granuloblast is similar to other blast cells, but no nucleolus is visible.

The metagranuloblast contains cytoplasmic vacuoles and an eccentric large nucleus. The vacuoles of the heterophil series are of approximately equal size. Those of the eosinophil are said to be more sharply defined. In the basophil metagranuloblast both vacuoles and a few magenta granules are present.

The promyelocyte varies in size, but is generally smaller. The nucleus, which is still round, may be obscured partly by granules, especially in the neutrophil line. Heterophil progranulocytes contain characteristic magenta bodies and rings which are gradually mixed pale orange spheres which later mature into the specific rods and granules. The eosinophil has no true promyelocyte stage as the specific granules appear at this stage. The cytoplasm is already a clear blue. The basophil progranulocyte contains a few magenta bodies which rarely form rings, but no orange spheres.

The nucleus is still round in the early or meso-myelocyte stage, but by the late or meta-myelocyte stage is becoming bean shaped in the heterophil and eosinophil and round but slightly eccentric in the basophil. Magenta rings and granules may still be present in the heterophil, as well as specific granules. The mature granules of the basophil myelocyte are already water soluble.

EMBRYONIC haemopoiesis AND CHANGES AT HATCHING

Both the site and the appearance of haemopoietic cells varies between the embryo and the adult. This is well studied in the chicken, but also occurs in mammals where the embryonic erythrocytes are nucleated. In both the chicken and the mammals primary erythropoiesis occurs initially in the yolk sac. These cells are followed by definitive erythrocytes that are produced predominantly in the bone marrow.

As the red cells change from primitive to definitive, the types of globin genes transcribed and globin peptide chain produced changes. These two cell types are both present at hatching and can be separated by cell fractioning techniques because of these biochemical differences. The primitive cells are progressively replaced by definitive red cells usually by about 5-8 days post hatching.

The earliest or primary erythrocytes develop rapidly, often with disturbed mitosis and without the maturation of cell shape seen in the adult so that they are round and usually more basophilic than definitive cells, though enucleate and lobulated forms are also seen.

The first erythroblasts appear before 24 hours incubation, and the early cells circulate as soon as vessels are established, completing their development within the blood. Polychromatic but functional forms are recognisable by about 65-70 hours of incubation.

Thrombocytes are another early circulating cell. The blast forms seen within the first day or so are virtually all erythroblasts. By about day 4 there is a wave of blasts which are predominantly thromboblasts, which are functional in the embryo even at the blast stage. As these cells mature they remain rounder and more primitive than the adult forms. By about day 6 the predominant blast is that of the definitive erythrocytes.

Granulocytes may be seen sporadically in the blood during the last week of embryonic life, and occasionally during the second week. However, the presence of large numbers should suggest infection in the embryo. Large numbers of granulocytes, particularly heterophils, are produced in the embryo, but are normally stored and released into the blood only on hatching. The number of organs which produce and store these cells is quite large, including the spleen, the bursa, and organs such as the pancreas which are not normally hemopoietic. The granuloblast wave occurs at about day 8. In the embryo bone marrow, both granulocytes and erythrocytes are produced in approximately equal numbers, while in the spleen the granulocytes predominate.

After hatching, the stored heterophils are released into the blood mostly by about 12 hours. This is followed in the spleen by a wave of lymphocyte and monocyte development, often preceded by smaller waves of eosinophil and basophil development. The lymphocytes and monocytes are released into the blood from about day 4, when the monocyte ratio may be quite high. Between about day 4 and day 8 the leucocytes assume adult proportions.

TUMOUR CELLS IN BLOOD

This is to be covered in more detail in other sections. Briefly, conditions in which circulating tumour cells are likely are Marek's disease, and the leukaemias of the leukosis/sarcoma retrovirus group.

In Marek's disease leukaemia may be present, though this is certainly not a consistent feature. Often there is only a mild leucosis, though a lymphoid leukaemia characterised by increased large lymphocytes and lymphoblasts is sometimes seen. The leukaemic cells are predominantly T cells. Anaemia is also seen with Marek's disease, possibly through destruction of haemopoeitic tissue.

The leucosis/sarcoma virus group contains strains which stimulate solid tumours of various tissues,and strains which stimulate leukaemia of lymphoid, myeloid, or erythroid cell lines. Many strains produce an anaemia independent of neoplasia.

Lymphoid tumours are more often solid. They are characterised by a uniform population of lymphoblasts of the B cell line.

Erythroblastosis is a leukaemic form characterised by large numbers of intravascular erythroblasts which proliferate rapidly but fail to mature. Maturation may reach the polychromatic stage, especially early in the disease or during remission, but often only erythroblasts are seen. The latter resemble the normal erythroblast, having a large round nucleus, fine chromatin, one or two well stained nucleoli, and a relatively large amount of basophilic cytoplasm. A perinuclear halo may be seen. In natural infections thrombocytes and myeloid cells may also be increased an immature.

Erythroblastosis with concurrent anaemia is often difficult to differentiate from anaemia resulting from non-neoplastic causes. Usually many more blasts than polychromatic erythrocytes are seen in erythroblastosis though.

Myeloblastosis involves spectacular leukaemia of up to 2 million myeloblasts/cmm. They may be 75% of all blood cells. They are large cells with only slightly basophilic clear cytoplasm and a large nucleus with one or two nucleoli which, like the normal myeloblast, do not stain prominently. Often promyelocytes and myelocytes are also present, with granules readily identifying the cells. Secondary anaemia with associated polychromatic erythrocytes may also occur.

Myelocytomatosis is a similar condition, in which the myeloid cells are much better differentiated. However, this is generally, though not exclusively, an aleukaemic solid cell tumour. The cells contain large numbers of usually spherical acidophilic granules, but a large vesicular nucleus, usually with a distinct nucleolus which is not seen in comparable normal cells.

PARASITES OF BLOOD

The most important avian blood parasites in Australia belong to the family Plasmodiidae, which includes Leukocytozoon, Haemoproteus and Plasmodium (malaria). All have similar life cycles, with transmission and sporogony in insects, and schizogony and gametogony in avian cells.

Leucocytozoon species, of which there are about 70 all occur in birds except one in a Brazilian lizard. The life cycle involves schizogony in tissues, especially RE cells and gametogony in erythrocytes or leucocytes. They are of economic significance in poultry in Northern American, and have been reported in at least 19 species of native birds, especially in recent years in the Currawong. The clinical signs are usually referable to the asexual tissue stage, which often involves splenomegaly, but in the Currawong involves the muscles.

Plasmodium, or avian malaria, is characterised by pigment in erythrocytes, schizogony in blood, gametes in mature erythrocytes, and transmission by mosquitos. At least 35 species are described, some world-wide in distribution. Has been reported in Australia in sparrows, starlings, silver gulls, grey falcons, black swans and wild duck, as well as poultry. Infections may be clinically important, especially if stressed, and human anti-malarian drugs have a high success rate, as most were developed using bird Plasmodium.

Haemoproteus species are widespread in Australian birds, with reports in 54 species including poultry, passerines, and psittacines. Infections are usually inapparent, but depression, anaemia and death with splenomegaly and hepatomegaly may occur. Schizogony occurs only in endothelial cells of visceral organs, and gametes are seen only within circulating erythrocytes, where they frequently form a regular C shape round the nucleus. Transmission is by flies or midges.

Other avian blood parasites include avian piroplasmosis (Aegyptionella spp.), an important disease in many species of birds in the Mediterranean, Asia and Africa, which has not been reported in Australia; trypanosomes which have been found in Australia, but which have not been reported to cause disease; and Lankasterella which has been seen in several species and occasionally causes death in sparrows and canaries. The latter is suspected of being an intermediate stage of Isospora species. The piroplasms occur in erythrocytes, are transmitted by the tick Argas persicus, and may cause severe anaemia. Microphilaria of subcutaneous filarial nematodes may also be seen.

Bacteria can be seen in blood with any septicaemia, but blood smears are used for diagnosis generally only with spirochaetes.

TECHNIQUES FOR AVIAN HAEMATOLOGY

A number of methods have been published for avian haematology, which gives an indication of the difficulties encountered in getting good results. These difficulties arise, firstly, because the nuclei of erythrocytes and thrombocytes prevents leucocyte estimation by nuclear counts as done in both manual and automated counting systems which count particles after cytoplasmic lysis. Secondly, as already indicated, some cells stain poorly and have water soluble granules.

LEUCOCYTE COUNTS

I have given two avian counting solutions. Initially, leucocytes numbers were estimated indirectly by comparing the ratio of erythrocytes and leucocytes. The 1931 Wiseman solution is a semi-direct method that is still used. This uses phloxine (an eosin-related stain) to stain the eosinophilic granules of heterophils and eosinophils, in a buffered, acidified formalin fixative which allows prolonged storage. Blood is diluted 1:100 or 1:200 in tube or red cell pipette, and stained for 1-3 hours in a refrigerator or moist chamber before remixing for counting the red stained cells in a standard hemocytometer chamber. The total leucocytes are calculated from the number and percentage of heterophils plus eosinophils.

$$\text{Calculations: Total eosinophilic cells} = \frac{\text{Count/chamber} \times 200}{9}$$

$$\text{Total leucocytes} = \frac{\text{total eosinophilic cells}}{\%\ \text{heterophils + eosinophils}}$$

The main disadvantage is the prolonged staining time. Commercially available eosinophil Unipettes using phloxine B achieve staining in 5 minutes.

Staining is much faster (one minute) with the Natt and Herrick 1952 method, which utilises methyl violet to stain the nuclei, the cytoplasm of the erythrocytes and mononuclear cells, and the polymorph granules. This allows differentiation of all the major cell types, facilitating not only direct leucocyte counts, but also erythrocyte and thrombocyte counts in the same chamber. Experience suggests that lymphocytes and degenerate thrombocytes may be difficult to tell apart. A buffered formalin solution is again used.

Solutions**:**

Wisemans solution: Mix 20 mg phloxine; 5 ml formalin; and 95 ml Ringer's solution and add 0.5 ml of 0.1N HCL/100 ml to give a pH of 5.7.

Natt and Herrick's solution: Dissolve in order the following chemicals in 1000 ml distilled water:

NaCl	3.88 g
Na_2SO_4	2.50 g
$Na_2HPO_4.12H_2O$	2.91 g
KH_2PO_4	0.25 g
37% formalin	7.50 ml
Methyl Violet 2B	0.10 g

Filter after standing overnight. Draw whole blood to the 0.5 mark of a red cell-diluting pipette, and draw the Natt and Herrick's solution to the 101 mark. Leukocytes stain a dark blue and erythrocytes have a transparent cytoplasm and pale-staining nucleus.

STAINED SMEARS

All the standard Wright's Giemsa and May-Grunwald's Giemsa methods can be used but the following points should be noted.

1. Heterophil and basophil granules are still susceptible to water leaching even after methanol fixation, so longer bulk staining methods should be avoided. Increased concentrations of Wright's stain have been recommended to reduce staining time.

2. Staining varies with pH. This is usually optimal with slightly acid pH, so use distilled water or slightly acidified tap water. Buffers can be used, but those prepared for routine mammalian smears are usually too alkaline to stain the red granules of the heterophils.

3. Very aged solutions stain poorly. For Wright's it is suggested that this is due to the progressive oxidising action of the alkali present. Personal experience suggests that very old batches will give good stains under optimal conditions, but are much more sensitive to errors in pH and slide preparation. Indeed, severe pH artifacts could not be induced in recently prepared solutions. Addition of a small amount of Giemsa stain (.33g/l) and glycerol (30 ml/l) to the Wright's stain (3g/l methanol) is claimed to stabilise the solution. Mix in a brown bottle for 30 minutes, incubate for 3 days at 37^{o}C, shaking twice daily, filter and use for at least two years. (Shen and Patterson, 1983). The formula for Wright's buffer is as follows:

Dibasic sodium phosphate (Na_2HPO_4)	3.80 g
Monobasic potassium phosphate (KH_2PO_4)	5.47 g
Distilled water	1000 ml

An air-dried smear is stained with Wright's stain for 1-3 minutes. An equal amount of Wright's buffer is added and gently mixed until a metallic-green sheen forms on the surface. Allow to stand for 5-6 minutes. Gently rinse with water and allow to dry.

4. Poor slide preparation reduces quality of staining. Old slides stain too blue, with gradual loss of cell detail. Slides dried too slowly give contracted cells which are not readily differentiated. All other slide faults give a similar appearance of fading nuclei, degranulation, and increased smear cells which are probably mainly erythrocytes. Causes include heat drying; sweating in summer humidity with delay in fixation; too long in anticoagulant, especially heparin; and partial clotting.

   For good slides it is preferable to make slides directly from the vein or needle tip at collection. Two drops are sufficient for a blood count on a small bird: one for the slide, and one for filling the diluting pipette. The latter can be rinsed with EDTA to prevent clotting, blowing out the excess.

   Another common error is slides that are too thick. There may be some excuse for this with pheasants, where the blood viscosity is reported to be high.

5. Quick dipstick stains can be used, but poor granule staining will result. Users must be prepared to use other characteristics to classify polymorphs.

   Bone marrow examination is best done on smears prepared from bone marrow gently suspended in serum.

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