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# Cytology of Inflammation

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The inflammatory response of birds can be classified as either heterophilic, eosinophilic (rarely reported as they may be difficult to detect with routine staining), mixed cell, or macrophagic (histiocytic) depending upon the predominant cell type. Inflammatory cells arrive at the lesion by active migration in response to various chemotactic factors, and the type of inflammatory response present may suggest a possible aetiology and pathogenesis.

**Heterophilic Inflammation of Birds**

Inflammation occurs whenever chemotactic factors for inflammatory cells are released. The most common causes are microbes and their toxins, physical and chemical trauma, death of cells from circulatory insufficiency, and immune reactions. The inflammatory process begins with the acute phase. Acute inflammation is initiated by the action of vasoactive amines, cytokines, and cell breakdown products released by the damaged tissue in the microcirculation of the affected tissue. These chemicals and products cause capillary dilatation, increased blood flow into the area, and increased lumen diameter of the capillaries, which allows the large serum proteins (such as fibrinogen, complement, and immunoglobulins normally confined within circulation) to exude into the tissues. Leukocytes actively migrate into the affected tissue. The degree of the leukocyte migration depends on the stimulus, which is particularly marked in certain bacterial infections. The cells that leave the blood include heterophils, eosinophils, monocytes, lymphocytes, and thrombocytes.

Heterophils are typically the first cells to arrive at the scene where they destroy ingested organisms. Chemicals released from dying heterophils causes lysis of surrounding tissues and recruitment of other heterophils to form an abscess (a focal accumulation of heterophils, tissue fluids, fibrin, and necrotic tissue). Monocytes in circulation in conjunction with local tissue histiocytes (macrophages which have multiplied at the site of inflammation) appear quickly and begin to phagocytize tissue debris and infectious agents as the inflammation becomes established. Monocytes and macrophages, stimulated by the cell-mediated immune response, join the heterophils to create a mixed cell inflammation, the most common cellular inflammatory response seen in birds. They can develop into epithelioid and multinucleated giant cells. As the inflammatory process continues and becomes chronic, granulomas may develop as the macrophages form into layers that resemble epithelium and this is the reason for the term “epithelioid cells.” As the lesion matures, fibroblasts proliferate and begin to lay down collagen. These proliferating fibroblasts appear large compared to the small densely staining fibroblasts of normal fibrous tissue. Lymphocytes appear within the stroma and participate in the cell-mediated immune response. Fusion of macrophages into giant cells occurs in association with material that is not readily digested by macrophages. The results of acute inflammation may be complete resolution, development of an exudative or necrotic lesion with continuation of the inflammatory response, or progression to chronic inflammation.

Heterophilic inflammation of birds is represented by a predominance of heterophils (greater than 80 percent of the inflammatory cells) in the cytologic sample (Figure 1). Heterophil granules in cytological specimens tend to lose their normal rod-shaped appearance and either appear more rounded or degranulated as demonstrated by Campbell and Grant (2010).

Heterophilic inflammation is classified by the presence or absence of degeneration of these granulocytes (Figures 2 and 3). Infectious agents produce toxins and induce cytokine activation resulting in cell chemotaxis and degenerative changes in the heterophils. According to Baker and Lumsden (2000), some aerobic bacteria and fungi cause degenerative cell changes, whereas many anaerobic bacteria and mycoplasma initiate chemotaxis, but do not affect the morphology of the heterophils. The nuclear features of degenerate heterophils include swelling, karyorrhexis, and karyolysis. A swollen nucleus appears larger than normal. Karyorrhexis or rupture of the nuclear membrane and fragmentation of the nuclear chromatin indicates the end stage of cell death and is represented by multiple pyknotic nuclear segments (representing nuclear fragmentation) in the center of the cell. Therefore,

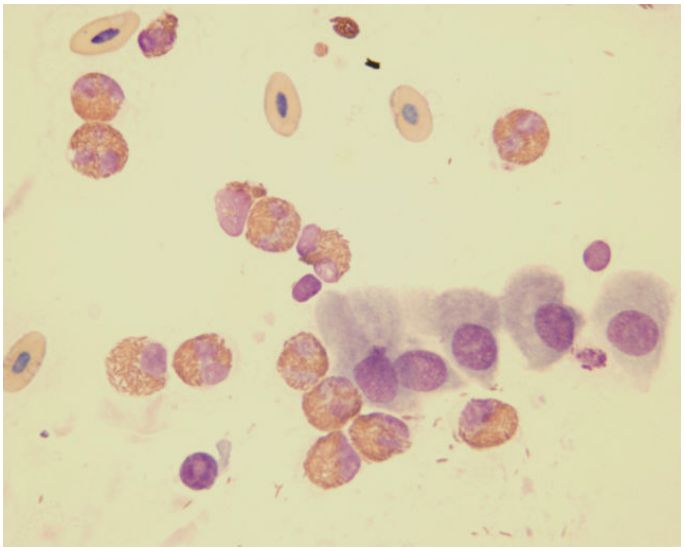

**Figure 1**: Heterophilic inflammation in a conjunctival scraping of an Emu (*Dromaius novaehollandiae*), Wright-Giemsa stain (1000X).

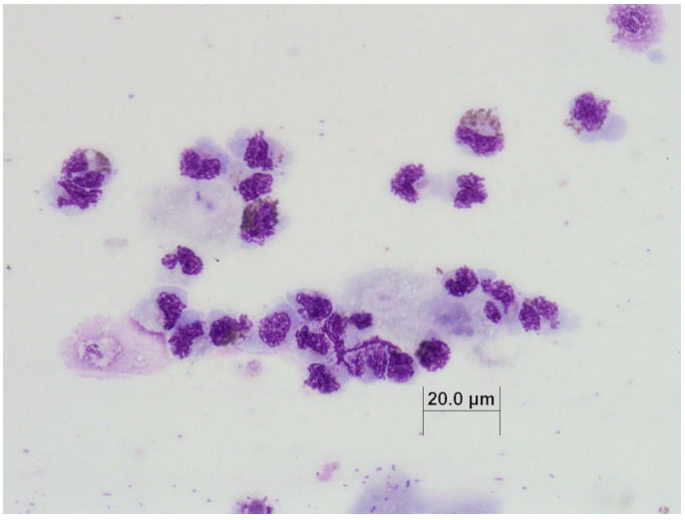


**Figure 2**: Degenerate heterophils in a swab sample of the oral cavity of a Screech owl (*Megascops asio*), Wright-Giemsa stain (1000X).

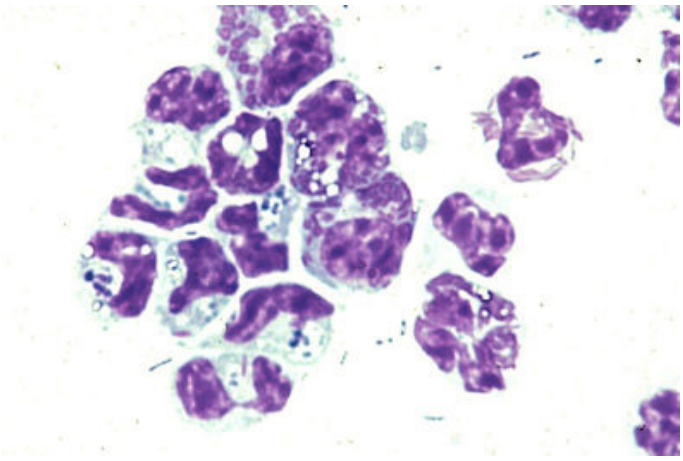

**Figure 3**: Septic inflammation with degenerate heterophils (peritonitis) in an aspirate of the coelom of a cockatoo (*Cacatua alba*), Diff Quik stain (1000X).

karyorrhexis is seen as multiple dark, dense, round structures that were once the cell nucleus. Karyolysis occurs as the nucleic acids are hydrolyzed, and the nucleus loses its basophilia and appears swollen with poorly defined homogenous pink chromatin with Romanowsky stains. The cytoplasmic features of degenerate heterophils include increased basophilia, vacuolization, and varying degrees of degranulation. As reported by Perman et al. 1979) in describing degenerate mammalian neutrophils, degenerate heterophils represent rapid cell death and suggest the presence of toxins, such as bacterial toxins, in the microenvironment.

Nuclear pyknosis indicates a slow progressive degeneration of a cell in a nontoxic environment and may represent the natural aging of the cell. Pyknosis is characterized by nuclear shrinkage causing the chromatin to become dense and deeply basophilic. Pyknotic nuclei appear as a single round mass with an intact nuclear membrane or if involving cells with lobed nuclei, such as heterophils, there will be multiple small round masses.

Heterophilic inflammation usually indicates an acute phase of the inflammatory response in birds. According to Klasing (1991), the acute inflammatory response of birds begins with inflammatory cells migrating and adhering to endothelial cells in the post-capillary venules near the site of the invading agent or cell damage if caused by a noninfectious etiology. Carlson and Allen (1969) and Nair (1973) found that heterophils appear in large numbers in dilated blood vessels in the area of inflammation within 30 minutes of injections of noninfectious and infectious agents. Maxwell and Robertson (1995) discovered that basophils also appear within hours of injury. Avian thrombocytes also participate in the early inflammatory response where they actively engage in phagocytosis of foreign materials and bacteria according to Awadhiya et al. (1980 and 1981) and Maxwell and Robertson (1998). When heterophils arrive at the site of inflammation, they become activated, leading to phagocytosis of the invading agent. The ingested agent initiates a cascade of cellular events from the release of granule-derived hydrolytic enzymes, oxygen free radicals, and other chemotactic factors to the general disintegration of the cell. This cellular degeneration is a characteristic feature of inflammation with loose, intact granules and their remnants being accompanied by the gradual increase in phagocytic macrophage activity as reported by Maxwell and Robertson (1998). Following continued accumulation in the tissues, degranulating heterophils become unrecognizable and necrotic within the center of the lesion. Montali (1988) demonstrated that in about seven days, with macrophage involvement, the characteristic heterophilic granuloma develops. Apparently, the necrotic center of heterophilic inflammatory lesions produces necrotoxins that are chemotactic to macrophages and a granuloma quickly develops. Therefore, granuloma formation in birds may be in response to necrotic tissue rather than an infectious organism. Giant cell formation is a common occurrence in avian inflammatory lesions because the necrotic tissue stimulates a foreign-body-like reaction. Thus, unlike mammalian giant cell formation, the presence of giant cells in avian inflammatory lesions does not necessarily

suggest chronicity.

Heterophilic inflammation in birds is associated with a suppurative inflammatory process that is often associated with infectious agents, especially when these leukocytes are degenerate. Close examination of the heterophils for infectious agents should be made when this type of inflammation is present. Septic lesions caused by pathogenic bacteria are often responsible for heterophilic inflammation: however, fungal agents may also cause heterophilic granulomas associated with degenerate and non-degenerate heterophils in lower vertebrates as reported by Toplon, et al. (2012). Heterophilic inflammation can also be associated with noninfectious agents, such as foreign material.

**Septic Inflammation**

Septic inflammation is indicated by the presence of intracellular bacteria that have been phagocytized by leukocytes often appear within vacuoles called phagosomes, which are membrane-bound vesicles formed by invagination of the cell membrane (Figure 3). Most bacteria stain blue with Romanowsky stains.

**Mixed Cell Inflammation**

When there is an influx of macrophages and lymphocytes into the inflammatory lesion a mixed cell inflammation (pyogranulomatous) is created (Figure 4). A pyogranulomatous response consists of a mixture of heterophils, epithelioid macrophages and multinucleated giant cells. A mixed cell inflammation indicates an established, active inflammatory lesion (originally known as chronic active inflammation). Mixed cell inflammation is typically represented by a predominance of heterophils (greater than 50 percent of the inflammatory cells) with an increased number of mononuclear leukocytes. Heterophils in mixed-cell inflammatory lesions are usually normal and nondegenerate in appearance. Lymphocytes and plasma cells are often associated with acute heterophilic granulomas, whereas the presence of epithelioid cells (macrophages that contain no vacuoles or phagocytized material) and connective tissue cells (i.e., fibroblasts) suggest chronic granulomas. Frequently, the epithelial and mesenchymal cells adjacent to inflammatory lesions proliferate resulting in the presence of these cell type demonstrating features of tissue hyperplasia. This type of inflammatory response is commonly observed with a variety of infectious (i.e., bacterial and fungal) and noninfectious (i.e., traumatic and foreign body) aetiologies.

Chronic inflammatory lesions, such as bacterial abscesses, may contain a large amount of noncellular, amorphous debris in the center of the lesion. Material obtained from this area will be poorly cellular, and the few cells that are present are often degenerate and difficult to evaluate. Active lesions will reveal an inflammatory response if the sample is obtained from the margin of the lesion. Septic lesions may reveal bacterial phagocytosis in heterophils or macrophages. Fibroblasts proliferate in mature lesions to lay down collagen.

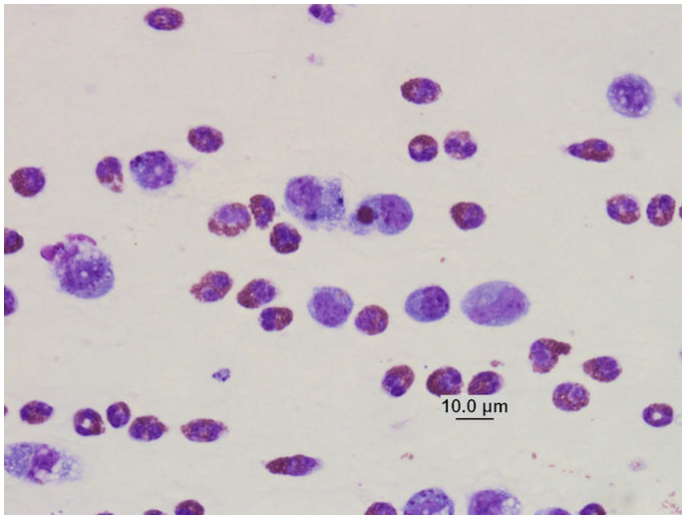


**Figure 4**: Mixed cell inflammation from a sinus aspirate of an Amazon parrot *(Amazona auropalliata*), Wright-Giemsa stain (1000X).

Lymphocytic and plasmacytic infiltration is indicated by increased numbers of lymphocytes and plasma cells in the cellular response as reported by Raskin (2001a). Conditions that are often associated with this type of cellular response include early viral infections, immune-mediated disorders, and chronic inflammation. According to Nunoya et al. (1997), infection with *Mycoplasma* spp. is often associated with lymphoplasmacytic inflammation. The lymphocyte population is composed of small and intermediate-sized mature lymphocytes and plasma cells. Plasma cells are large, round to oval lymphocytes with an abundant, deeply basophilic cytoplasm. The nucleus is eccentrically located and appears mature. A prominent Golgi apparatus is found adjacent to the nucleus. According to Cazzini et al. (2013), plasma cells distended by round, clear to light blue structures (Russell bodies) are often referred to as Mott cells. The nucleus of the plasma cell is round and contains dense, coarse chromatin and no nucleoli.

Because of the rapid influx of macrophages (within a few hours) and lymphocytes into inflammatory lesions, mixed cell inflammation (pyogranulomatous) is the most common type of inflammation seen in birds.

**Macrophagic (Histiocytic or Granulomatous) Inflammation**

Qureshi (1998) and Park et al. (2017) maintain that macrophages are important in antigen presentation, production and secretion of cytokines, phagocytosis, and bacterial killing by the production of reactive oxygen radicals and nitrogen intermediates. Macrophagic (histiocytic or granulomatous) inflammation consists primarily of macrophages with multinucleated giant cells and lesser numbers of other inflammatory cells (Figure 5). This type of inflammatory response does not always exfoliate

well and may be of low cellularity. According to Raskin (2001a), macrophagic inflammation in mammals is suggestive of chronic inflammation and is often seen with foreign body reactions and mycobacterial infections. This likely to be true in birds as well according to Cowan et al. (2014). However, macrophagic inflammation may also have a different pathogenesis than heterophilic and mixed cell inflammation in birds and is indicated by a predominance of macrophages (greater than 50 percent of the inflammatory cells) in the cytological sample. Large activated macrophages resembling epithelial cells (epithelioid macrophages) that later develop into multinucleated giant cells, apparently responding to necrotic tissue, are a feature of this type of inflammation as demonstrated by Campbell and Grant (2010). Burkhard et al. (2001) indicated that epithelioid cells often appear as clusters of macrophages with blue-gray to pink cytoplasm, and distinct cytoplasmic margins.

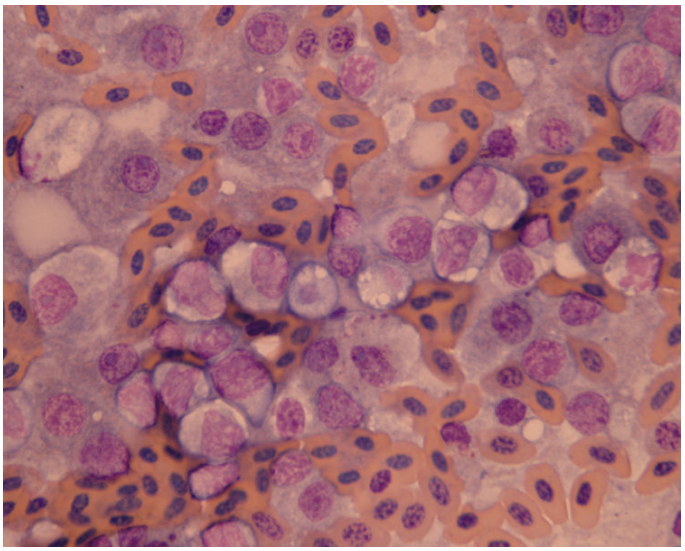

**Figure 5**: Macrophagic inflammation in an imprint of a liver biopsy of a partridge (*Alectoris chukar*), Wright-Giemsa stain (1000X).

**Eosinophilic Inflammation**

In mammals, an increased number of eosinophils (10% of the inflammatory cells or greater) in the inflammatory response is indicative of an eosinophilic inflammation. Eosinophils phagocytize antibody/antigen complexes and therefore may have a role in maintaining homeostasis during infection. They are particularly numerous when antigens are continually being released, as in parasitic disease. Thus, eosinophilic inflammation is often associated with hypersensitivity or allergic reactions, parasitic infestation, mast cell tumors, and eosinophilic granulomas or paraneoplastic responses in mammals. Eosinophilic inflammation associated with an allergic response (type I hypersensitivity reaction) may also demonstrate increased numbers of mast cells, lymphocytes, and plasma cells.

Eosinophilic inflammation is rare in birds. This may be due to the difficulty in differentiating eosinophils from heterophils in cytologic samples using routine cytologic stains or to avian eosinophils behaving differently from mammalian eosinophils (Figure 6). Maxwell (1987) and Chad and Eyre (1978) provided evidence that avian eosinophils do not act as modulators of immediate hypersensitivity reactions as they do in mammals, but they may participate in delayed hypersensitivity reactions.

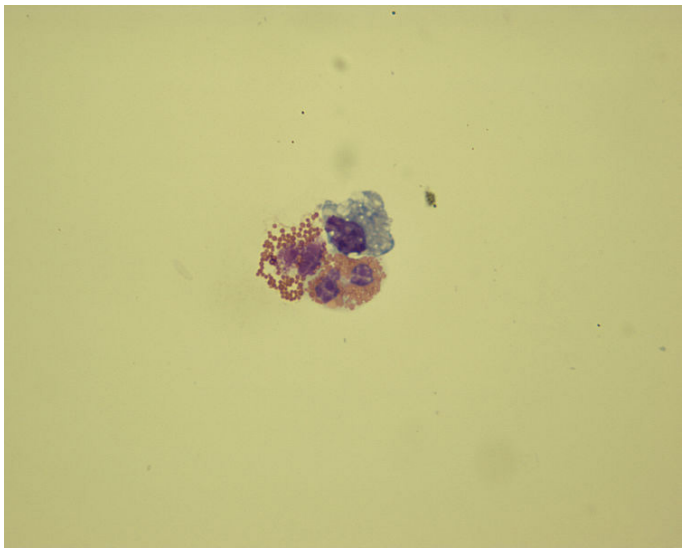

**Figure 6**: An eosinophil, ruptured heterophils, and a macrophage in a concentrated tracheal wash sample from an eagle *(Haliaeetus leucocephalus*), Wright-Giemsa stain (1000X).

**Inflammatory Lesions of the Alimentary Tract**

Inflammatory lesions involving the upper alimentary tract (i.e., stomatitis, esophagitis, and ingluvitis) can be caused by traumatic injury, foreign bodies, chemical irritation (e.g., silver nitrate used for hemostasis when trimming beaks of birds) and infectious agents. The cytology reveals an increased number of inflammatory cells and a variable number of squamous epithelial cells. An increased amount of background debris and lightly basophilic proteinaceous material may be present as well. Green (1992) maintained that basal cells are present in inflammatory lesions whenever ulceration of the epithelium is present. Septic lesions reveal inflammatory cells (heterophilic or mixed cell inflammation) containing intracytoplasmic bacteria (either primary or secondary pathogens), and may contain degenerate leukocytes (primarily heterophils).

Chronic inflammatory lesions may reveal many epithelial cells with a high nucleus to cytoplasmic ratio, multiple prominent nucleoli, and abnormal chromatin patterns. These cells represent a reactive epithelial response to the inflammation and should not be confused with a neoplastic condition. In such cases, it is important to correlate the clinical and the cytological findings to arrive at a reasonable interpretation of the cellular response. When deeply ulcerated lesions or chronic inflammatory lesions are present, fibroblasts may be present in the cytology specimen. Fibroblasts appear as spindle-shaped cells with single, oval to elongated nuclei (occasional multinucleation occurs during rapid proliferation).

Cooper (1978); Heindenreich (1995); and Boydell and Forbes (1996) maintain that caseous lesions in the oropharynx of birds can be associated with bacterial infection, trichomoniasis, hypovitaminosis A, capillariasis, candidiasis, and viral infections. Cytology can be useful in the differentiation of these diseases (Figures 7 and 8).

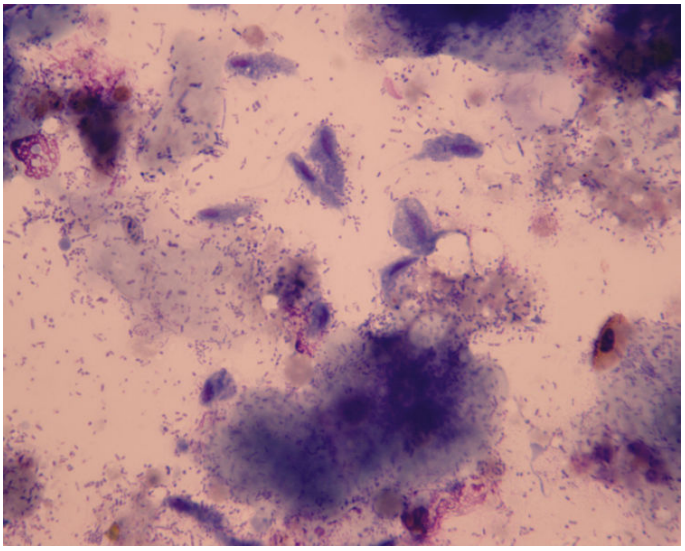

**Figure 7**: Numerous piriform-shaped protozoa resembling *Trichomonas* sp in a swab sample of the oral cavity of a Screech owl (*Megascops asio*), Wright-Giemsa stain (1000X). These are best confirmed on a wet mount preparation.

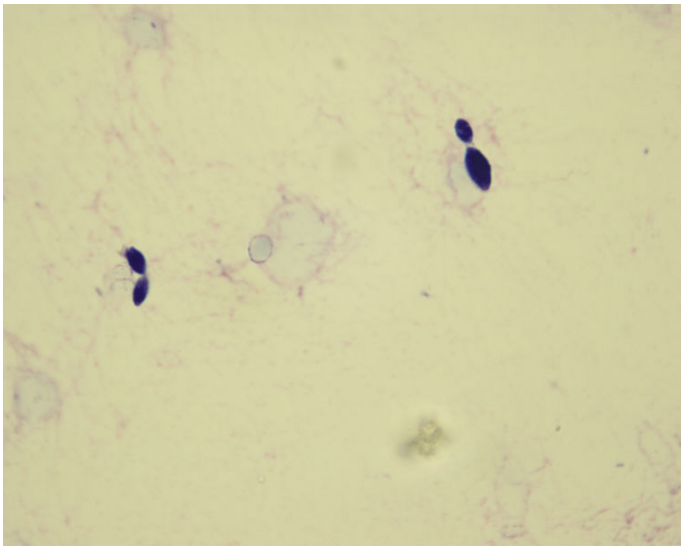

**Figure 8**: Narrowly-based budding yeast resembling *Candida albicans* in an oral swab from an African Grey parrot *( Psittacus erithacus*),Wright-Giemsa stain (1000X).

Ingluvitis (inflammation of the avian crop) is a common disorder of companion birds. Clinical signs of this disorder include delayed emptying time of the ingluvies, regurgitation, and weight loss. Birds with a peracute bacterial ingluvitis ("sour crop") or candidiasis are typically nestlings being hand-raised. The disorder is characterized by an acidic crop fluid (normal crop pH is 4.5-6.7) with a foul-smelling fermenting odour. Cytological samples of this condition contain few inflammatory cells and many bacteria represented by one morphological type, which is considered abnormal and indicates a need for bacterial culture (Figure 9). This may represent an acute bacterial infection where inflammatory cells have not had time to respond or the inflammatory response has been overwhelmed. A predominance of Gram-negative bacilli present in a smear from the upper alimentary tract, such as the crop, of noncarnivorous birds should be considered abnormal, whereas they are commonly present in carnivorous birds.

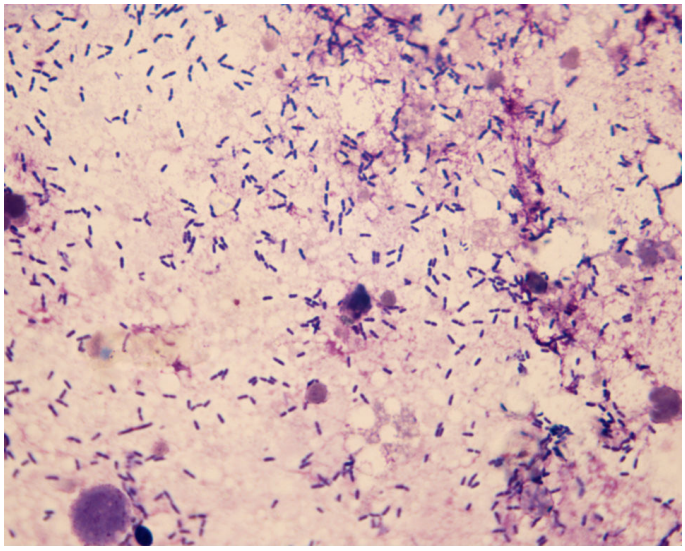

**Figure 9**: Numerous bacteria represented by one morphologic type in a crop aspirate of a cockatiel (*Nymphicus hollandicus*) with acute septic ingluvitis, Wright-Giemsa stain (1000X).

Septic lesions of the ingluvies (septic ingluvitis) reveal inflammatory cells (heterophilic or mixed cell inflammation) exhibiting bacterial phagocytosis. The heterophils may appear degenerate.

Cytologic diagnosis of mycotic infections, such as candidiasis and zygomycete fungi, of the proventriculus and ventriculus of birds is made by identification of fungal elements (narrowly based budding yeast and hyphae) in a gastric wash specimen.

Part of the evaluation of feces is the evaluation of the flora. A mixed population of bacteria is expected. Therefore, a predominance of one type of bacteria (uniform population) is suggestive of bacterial overgrowth and indicates a need for bacterial culture as this may represent an acute bacterial infection before the inflammatory response or the inflammatory response has been overwhelmed.

Inflammatory cells found on cloacal and fecal swab samples from birds are considered abnormal and reflect an inflammatory lesion involving the cloaca, lower intestinal tract, urinary tract, or reproductive tract. Ulcerative lesions may reveal basal epithelial cells and numerous erythrocytes with the inflammatory cells. Granulomatous lesions are typified by numerous macrophages with occasional multinucleated giant cells, plasma cells, and heterophils. Septic inflammation is indicated by leukocytic phagocytosis of bacteria.

**Inflammation of the Respiratory Tract**

The presence of inflammatory cells in the cytology sample supports the diagnosis of respiratory tract inflammation, and the type of inflammation present may reveal the aetiology. For example, a sample containing mixed cell (pyogranulomatous) inflammation with evidence of foreign material would support the diagnosis of a respiratory foreign body. Primary and secondary bacterial infections are indicated by heterophilic (suppurative) or mixed cell inflammation with evidence of bacterial phagocytosis.

Along with the inflammatory response, severe infections of the respiratory tract may reveal degenerate ciliated respiratory epithelial cells (Figure 10). These cells exhibit losses of cilia, cytoplasmic vacuolation, cell lysis, and karyolysis. Septic lesions are confirmed by the presence of leukocyte phagocytosis of bacteria. Ulcerative lesions may show an increase in erythrocytes and cellular debris.

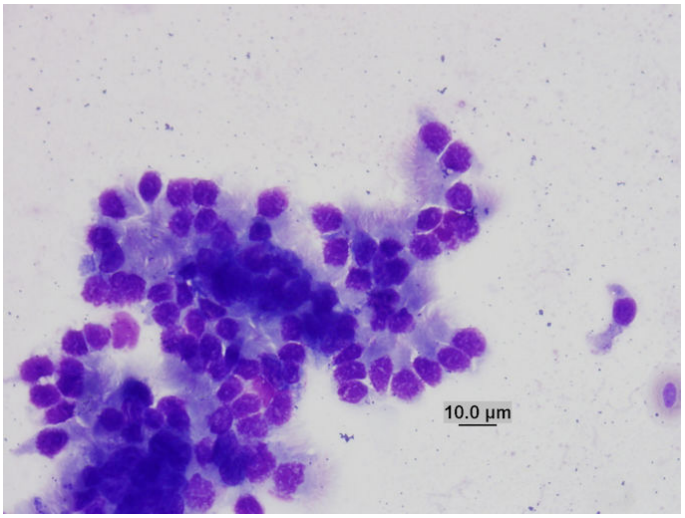


**Figure 10**: Ciliated respiratory epithelial cells in a tracheal wash from a macaw (*Ara ararauna*), Wright-Giemsa stain (1000X).

The cytology of sinusitis is represented by an increase in inflammatory cells in the sinus aspirate smear. The number and type of inflammatory cells depend on the nature of the infectious agent, severity of the inflammation, and duration of the disease. A moderate to marked amount of background debris, and the causative agent may be present as well. Septic sinusitis is indicated by the presence of a relatively monomorphic population of bacteria, and intracytoplasmic bacteria within leukocytes (typically heterophils). Septic sinusitis may not reveal the microorganisms on the cytology specimen; however, a positive culture of an uncontaminated sample would support the diagnosis. The presence of bacterial cocci that tend to form chains is suggestive of a *Streptococcus* sinusitis. The *Mycoplasma* organisms appear as fine to aggregated basophilic bodies within epithelial cells or macrophages.

Tracheal wash samples of birds that exhibit a large number of heterophils and macrophages, increased numbers of goblet cells, and increased mucin in the background indicate a tracheobronchitis or pneumonia. Heterophils are more predominant in acute lesions. When chronic inflammation is present, increased numbers of macrophages, lymphocytes, and plasma cells are present. Macrophages associated with chronic inflammation of lower respiratory tract are often large and multinucleated. Heterophilic inflammation in birds is associated with a suppurative inflammatory process that is often associated with infectious agents, especially when these leukocytes are degenerate. Close examination of the heterophils for infectious agents should be made when this type of inflammation is present. Heterophilic inflammation can also be associated with noninfectious agents, such as inhalation of foreign material (e.g., smoke inhalation), in some cases. Degenerate ciliated respiratory epithelial cells (i.e., loss of cilia, cytoplasmic vacuolation, cell lysis, and karyolysis) may be seen with severe infections. Fragmented ciliated respiratory epithelial cells associated with a mononuclear leukocytic inflammatory response are suggestive of a viral aetiology. Septic lesions are confirmed by the presence of leukocyte phagocytosis of bacteria.

A foreign body reaction or aspiration tracheobronchitis associated with the lower respiratory tract may reveal large reactive macrophages that form giant cells containing phagocytized foreign material. Macrophagic inflammation is common in certain avian diseases, such as mycobacterial and C*hlamydophila* infections. Montali et al. (1976); Pond and Rush (1981); and Sandford et al. (1994) have found that multinucleated giant cells are typically present in granulomatous lesions in inflammatory diseases such as tuberculosis. Areas of macrophagic inflammation and heterophilic inflammation can occur together as macrophages respond to necrotic materials. Therefore, depending upon where the sample is obtained from the inflammatory lesion, a macrophagic inflammatory response may predominate the cytology.

Tully et al. (1995) reported that macrophagic inflammation associated with the tracheobronchial tract and lungs is often associated with fungal, foreign body, or mycobacterial lesions. The macrophages present may appear as reactive multinucleated giant cells, or as epithelioid cells. According to Burkhard et al. (2001), epithelioid cells appear as clusters of macrophages with blue-gray to pink cytoplasm, and distinct cytoplasmic margins.

Mycotic infections are indicated by the presence of mixed-cell or macrophagic inflammation and fungal elements. Macrophages, heterophils, lymphocytes, and plasma cells are typically part of the cellular response to infections. Fungal hyphae may stain poorly in some specimens and appear as large negative-staining structures among the inflammatory cells (Figure 11).

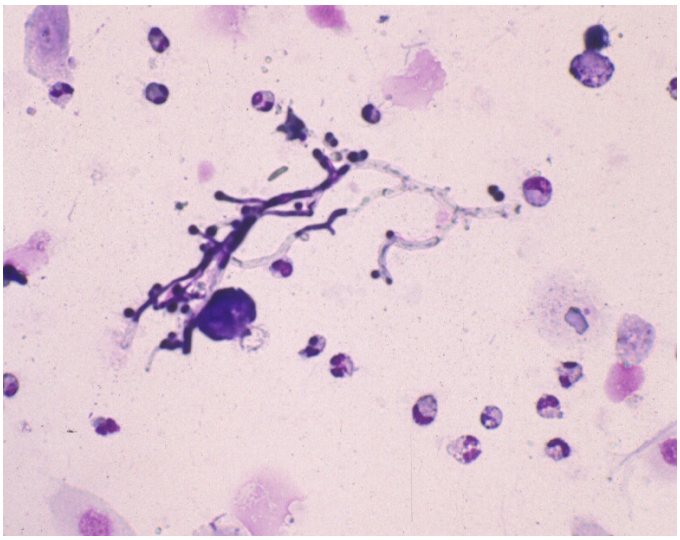

**Figure 11**: Septate branching fungal hyphae is a tracheal wash from an African Grey parrot *(Psittacus timneh*) with aspergillosis, Diff Quik stain, (1000X).

**Inflammation of the Skin**

Lesions involving the skin and subcutis can be classified using the basic categories of cytodiagnosis: inflammatory, hyperplasic, and neoplastic. Inflammatory lesions can have an infectious or a noninfectious aetiology. Suppurative inflammation of the skin and subcutaneous tissue is common because bacterial infections of the skin and subcutaneous usually result in this type of inflammation where intracellular bacteria are often easy to find and the heterophils often appear degenerate. Thus, bacterial infections are characterized cytologically by numerous degenerate heterophils, cellular debris, and bacteria. Septic inflammation is indicated by the presence of intracellular bacteria. The appearance of the bacteria may suggest the aetiology; however, bacterial culture is necessary for a definitive diagnosis.

Baker and Lumsden (2000) commented that granulomas in the skin are represented cytologically by the presence of a macrophagic inflammation with epithelioid cells (epithelial macrophages) and giant cells. Granulomas are often caused by noninfectious agents or infectious agents.

Noninfectious inflammatory lesions of the skin and subcutaneous tissues include foreign body reaction, xanthogranulomatosis (xanthomatosis), feather follicle cysts, and haematoma. Foreign bodies in the skin or subcutis cause mixed cell inflammation or macrophagic inflammation with multinucleated giant cells. The macrophages present in the cytology sample may contain phagocytized material. A secondary septic inflammation may also be present.

Cutaneous xanthogranulomatosis or xanthomatosis is a granulomatous inflammation of the skin of birds (Figure 12). According to Raskin (2001b), cutaneous xanthomatosis results from the accumulation of excess lipid material (cholesterol and triglycerides) and an associated macrophage-rich inflammation in the skin. Monks et al. (2006) proposed the term, xanthogranulomatosis, to replace the term, xanthomatosis, currently used to describe the syndromes in avian species in which intracytoplasmic lipid is found within tissue macrophages. The lesions occur as discrete yellow nodules or plaques, or may appear as a diffuse thickening of the skin. The lesions can be locally invasive.

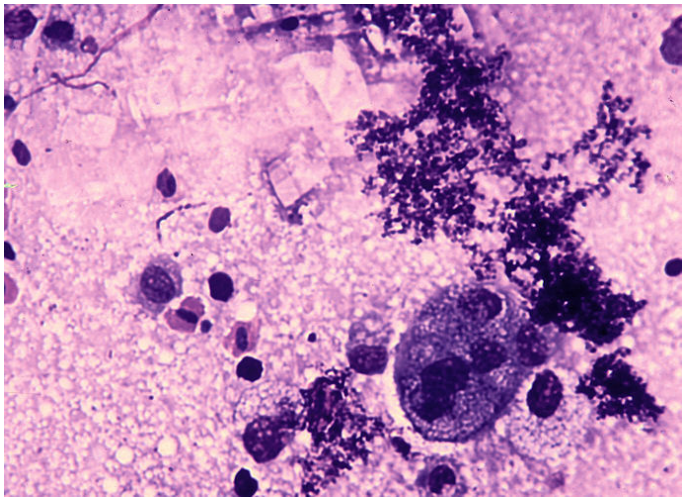

**Figure 12**: Macrophagic inflammation with a multinucleated giant cell, cholesterol crystals (angular clear crystals), and stain precipitate (dark purple granular material) associated with cutaneous xanthomatosis in a Budgerigar (*Melopsittacus undulatus*), Diff Quik stain, (1000X). Image from Campbell T. 2015. *Exotic Animal Hematology and Cytology*, 4th edition, Ames, IA, Wiley Blackwell, p. 255.

Xanthogranulomatosis is common in budgerigars (*Melopsittacus undulatus*), but may occur in other birds as well. Petrak and Gilmore (1982) and Quesenberry et al. (1997) report that xanthomatous skin lesions in birds occur primarily on the wings, dorsal cervical area, the back, sternum, ventral abdomen, and uropygial area. The affected skin is often raised, thickened, highly vascularized, friable, and devoid of feathers. Cutaneous xanthomas tend to develop in areas where physical trauma, local pressure, bleeding, or inflammation has occurred or is occurring.

Although the exact aetiology and pathogenesis of xanthomatosis (xanthogranulomatosis) is poorly understood, Kuriyama et al. (1991) and Bennett and Harrison (1994) maintain that a high-fat diet, a disorder of lipid metabolism, metabolic conditions causing hypercholesterolemia, necrosis of the epithelium and prior hemorrhage in the lesion may be underlying factors in the development of this disorder. Exposure to toxic fat-soluble substances (such as chlorinated hydrocarbons) that accumulate in the tissues inducing the inflammatory characteristics of xanthomatosis (xanthogranulomatosis) has also been proposed by Peckham (1955); Turrel et al. (1987); and Raynor et al. (1999) as a possible. Xanthogranulomatosis have also been associated with the skin overlying cysts and tumors, such as lipomas, osteosarcomas, and lymphoid leukosis lesions. Cutaneous xanthogranulomatosis may also develop as a sequela following feather cyst removal in psittacine birds where a significant amount of

cutaneous hemorrhage may occur.

Cytological features of xanthogranulomatosis include numerous highly vacuolated (foamy) macrophages, multinucleated giant cells, and cholesterol crystals. The background of the cytologic sample is often heavy with round clear areas that resemble fat droplets. Cholesterol crystals appear as angular, variable shaped, negative-staining (clear), notched plates, which often appear stacked in the noncellular background. These crystals will often dissolve in the alcohol fixatives of some stains and subsequently appear as a negative image in the cytology sample. The foamy macrophages (highly vacuolated) will stain positive with lipid stains.

The cytologic findings of xanthomatosis (xanthogranulomatosis) reflect what is observed on histology. Histological characteristic of cutaneous xanthogranulomas have features of both xanthomas and granulomas with numerous highly vacuolated, lipid-laden macrophages (called foam or xanthoma cells), cholesterol clefts, fibroblasts, and multinucleated giant cells according to Raynor et al. (1999).

**Inflammation of Synovial Joints**

Inflammatory joint disease can be caused by infectious agents such as bacteria, fungi, parasites, mycoplasma, and viruses, or by noninfectious etiologies such as gout and immune-mediated disease. Madani and Dorrestein (2012) reported that microfilaria are occasionally found in the joint fluid of arthritic birds living in the wild or recently captured from the wild. Cytology of inflammatory joint disease reveals an increase in heterophils, abnormal color and clarity, and reduced viscosity and mucin quality. The background of smears made from synovial fluid with poor mucin quality lacks the granular appearance of normal joint fluid. The presence of multinucleated osteoclasts or spindle-shaped fibroblasts indicates erosion of the articular cartilage, exposing underlying bone, or erosion into the fibrous layer of the articular capsule.

Septic arthritis is indicated by a high cell count and the presence of high numbers of heterophils. Extracellular bacteria are suggestive of bacterial involvement, and bacterial phagocytosis is considered diagnostic for septic joint disease. Bacteria often are not inherently obvious on the slide; however, the presence of degenerative neutrophils/ heterophils is suggestive of a septic lesion.

Whitehear et al. (2004) and Van Wettere et al. (2012) reported that *Mycoplasma* spp–induced arthritis and tenosynovitis is well documented in birds, especially in the galliformes where infection with *Mycoplasma synoviae* or M. *gallisepticum* causes articular and tendon lesions in populations of chickens and turkeys. Cytological examination of the synovial fluid of affected joints typically reveals a heterophilic (heterophilic arthritis) or mixed cell inflammation with no apparent aetiologic agent. The cell count of the fluid can be high (i.e. 42,000 to 55,000 leukocytes/µl) according to Van Wettere et al. (2012).

Traumatic injury to the joint (traumatic arthritis) may reveal an increase in the number of inflammatory leukocytes, primarily heterophils in cases of acute trauma. Depending upon the degree of joint injury, synoviocytes and mesenchymal cells representing exfoliation of cells from the underlying cartilage may be present (Figure 13). Cartilage fragments may also be found. These are represented by the presence of amorphous material with variable staining characteristics on the Romanowsky-stained smear. The mucin clot test and viscosity of synovial fluid associated with acute traumatic arthritis is typically normal as long as haemarthrosis is not involved.

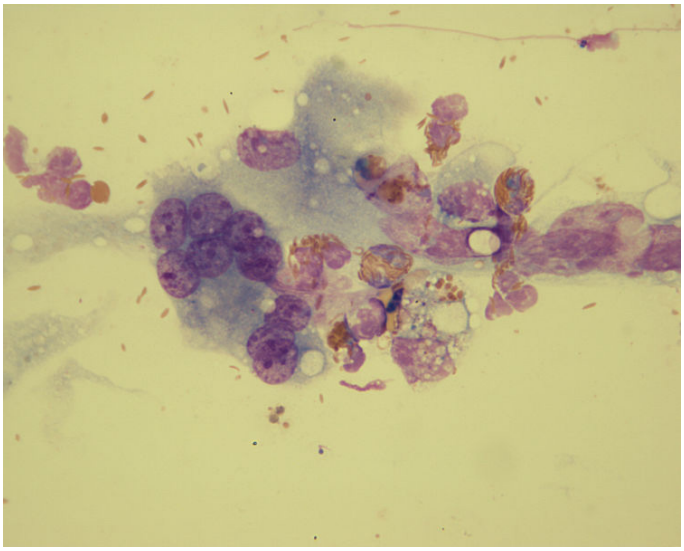

**Figure 13**: Mixed cell inflammation with a multinucleated giant cell (likely an osteoclast) in the joint fluid of a Gyrfalcon (*Falco rusticolus*), Wright-Giemsa stain (1000X).

Articular gout is a common disorder of companion birds and is often diagnosed by the gross appearance of the affected joint. Cream-colored to yellow deposits (tophi) in the joint can be seen through the thin skin of birds in advanced cases. These urate deposits occasionally bulge out through the overlying skin.

Early gout lesions may reveal a joint that is distended with a creamy, gritty fluid resembling an exudative effusion. The presence of needle-shaped crystals (monosodium urate) in the synovial fluid sample is diagnostic for gout (Figure 14). The urate crystals are birefringent under polarized light, which aids in their identification. Large numbers of inflammatory cells (usually mixed cell inflammation) are usually present as well. The viscosity of gouty synovial fluid is usually low, and the mucin clot test often provides poor results.

Joints affected by immune-mediated disease exhibit increased numbers of nondegenerate heterophils. Increased numbers of lymphocytes and plasma cells may also be seen. However, an easily recognized immune mediated cause of joint disease in birds is rare.

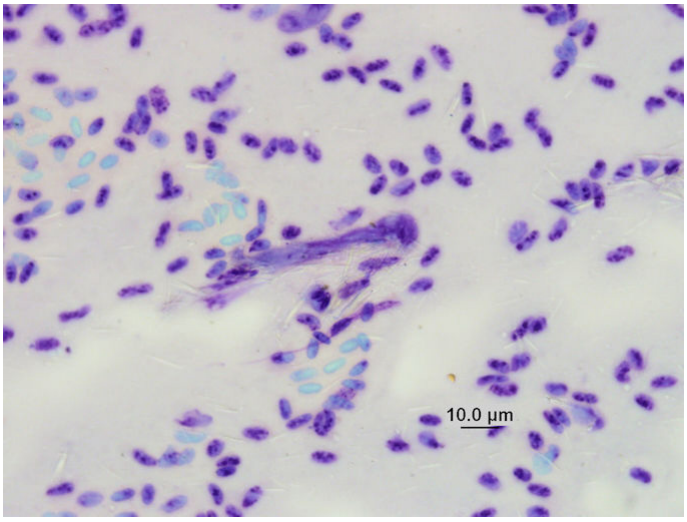


**Figure 14**: Needle-shaped crystals representing uric acid crystals in the joint fluid of a cockatiel (*Nymphicus hollandicus*) with gout. Wright-Giemsa stain (1000X).

Degenerative joint disease is represented by synovial fluid with mild increases in cell numbers compared to the marked increases observed with inflammatory joint disease. The cells in synovial fluid from a degenerative joint are represented primarily by mononuclear cells (greater than 90% of the cells), such as macrophages, lymphocytes, and synovial cells. Degernes et al. (2011) report that degenerative joint disease is common in captive waterfowl. Joint aspirates associated with degenerative joint disease often reveal synoviocytes and mesenchymal cells representing exfoliation of cells from the underlying cartilage may be present. Cartilage fragments may also be found. These are represented by the presence of amorphous material with variable staining characteristics on the Romanowsky-stained smear. The viscosity and mucin clot tests may be normal or reduced with degenerative joint disease.

**Inflammation of the Liver**

Hepatitis (inflammatory responses involving the liver) is indicated by the presence of numerous leukocytes in the cytology specimen. Suppurative inflammation, heterophilic inflammation, is suggested when high numbers of mature heterophils are observed relative to the red blood cell numbers. The presence of heterophils associated with aggregates of hepatocytes provides further cytologic support of suppurative inflammation. Heterophilic inflammation in birds may be differentiated from hepatic heterophil granulocytopoiesis by the presence of a predominance of mature heterophils and an absence of immature, developing heterophils. Septic inflammation is indicated by the presence of bacteria within leukocytes. Extracellular bacteria must be differentiated from "cadaver" bacteria or bacterial contamination of the sample when the sample is obtained during necropsy.

Mixed cell or macrophagic inflammation of the liver can be associated with mycotic, parasitic, or mycobacterial infections (Figure 5). Granulomatous lesions reveal macrophages that are often vacuolated and multinucleated giant cells.

**Ophthalmic Inflammation**

Conjunctivitis is a common cause for sampling the conjunctiva for cytologic examination and is recognized by the presence of many inflammatory cells. Heterophilic inflammation of the conjunctiva can be caused by bacteria, viruses, or noninfectious agents, such as trauma, foreign bodies, and environmental irritants (Figure 1).

Degenerate heterophils are often associated with bacterial infections. Bacterial conjunctivitis is the most commonly diagnosed cause of conjunctivitis in birds, and is frequently associated with upper respiratory tract infections as reported by Abrams et al. (2005). Numerous bacteria may be associated with this condition. Common isolates include *Pseudomonas* spp., *Staphylococcus* spp., *Pasteurella* spp., *Citrobacter* spp., *Escherichia coli*, *Klebsiella* spp., and *Haemophilus* spp. Devriese et al. (1988) and Yamamoto, (1991) maintain that *Haemophilus* conjunctivitis is often associated with systemic disease in birds. Hood (1978) and Pocknell et al. (1996) found that *Mycobacterium avium* has been associated with conjunctival granulomas of the nictitating membrane of birds, especially ratites.

The mucopurulent discharge associated with a bacterial conjunctivitis in mammals is not typically seen in birds owing to the differences in the activities of mammalian neutrophils and heterophils of birds. Instead, bacterial infections in birds tend to be more caseous. Septic conjunctivitis is confirmed cytologically by the presence of phagocytosis of the bacterial pathogens. The epithelial cells in septic lesions may show degenerative changes such as cytoplasmic vacuolation, karyolysis, or karyorrhexis.

*Chlamydia* organisms may be seen on cytologic specimens from conjunctival scrapings. They appear as intracytoplasmic inclusion bodies in epithelial cells (Figure 15).

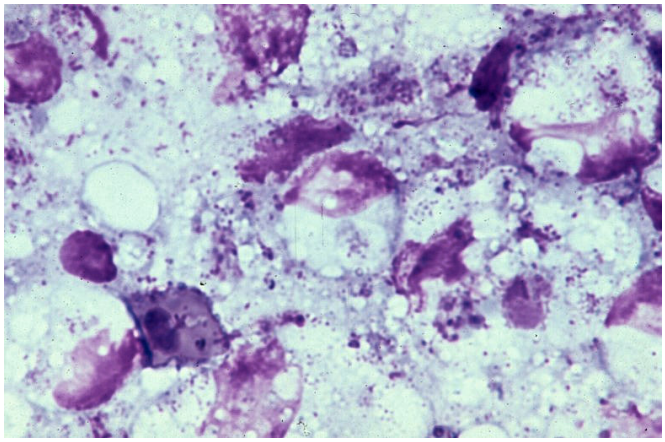

**Figure 15**: *Chlamydia* inclusions in the cytoplasm of an epithelial cell. Wright-Giemsa stain (1000X).

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