

**C**ytology is designed to be a rapid, inexpensive “in-house” diagnostic procedure, and the use of cytodagnosis should be easily within the realm of any veterinary clinician. The basic cytodagnosis of inflammation, tissue hyperplasia, malignant neoplasia and normal cellularity are easily differentiated from each other (see Figures 10.10, 10.11). One who is well versed in mammalian cytodagnosis should have little trouble in the interpretation of avian samples. The goal is to achieve a quick presumptive or definitive diagnosis during the patient’s initial visit to the veterinary clinic in an effort to provide an immediate and specific treatment plan. Cytology can then be used to monitor the success of therapy by evaluating changes in microbial and cell populations within or on the host. Cytology should be considered as a part of the minimum database in birds with discharges, masses or swellings. Cytologic evaluation of tissue imprints and fluids collected during a postmortem examination can be used to develop a presumptive diagnosis that can guide disease management decisions within the flock until a definitive diagnosis is provided by culture, DNA probe or histopathology. Cytological samples are of greatest value if they are collected fresh and immediately processed for evaluation. To obtain a cytologic sample and send it to an outside laboratory defeats the purpose and usefulness of cytology. By cytologically examining antemortem and postmortem samples, the clinician can compare the information that is derived from cytology, radiographs, CBC, serum chemistries and histopathology. This will serve to improve understanding of the pathogenesis and cellular effects of a disease process.

CHAPTER

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10

CYTOLOGY

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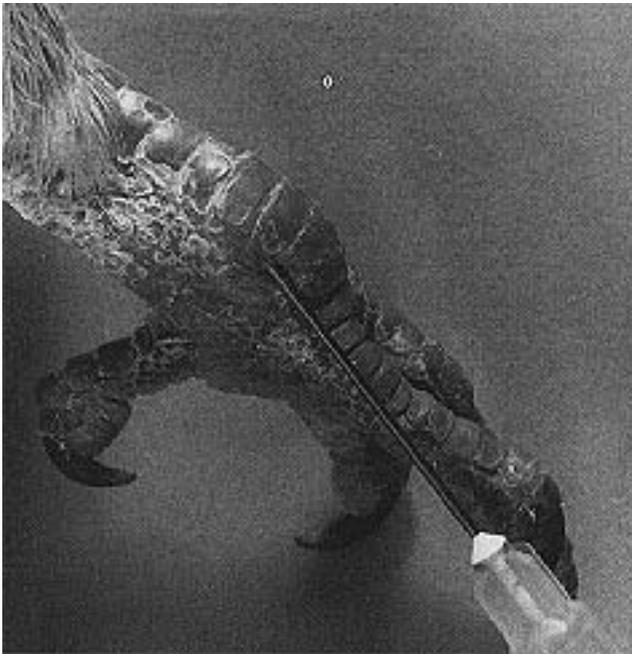
Terry W. Campbell

## Sample Collection

A variety of sample collection methods can be used to obtain samples for cytologic examination.<sup>4</sup> The method of choice depends upon the location and nature of the material being sampled. Cytologic sample collection methods can be divided into two broad categories: aspiration and contact smears.

### Sample Collection by Aspiration

Fine-needle aspiration biopsy is a simple, inexpensive procedure for obtaining material for cytologic examination (Figure 10.1). Using an alcohol swab, the skin overlying the biopsy site is cleansed and allowed to dry. Excessive application of alcohol should be avoided. A hypodermic needle (eg, 22 ga, one-inch needle) attached to a syringe (12 ml or larger) is inserted into the tissue to be sampled. A full vacuum is applied to the syringe using the syringe

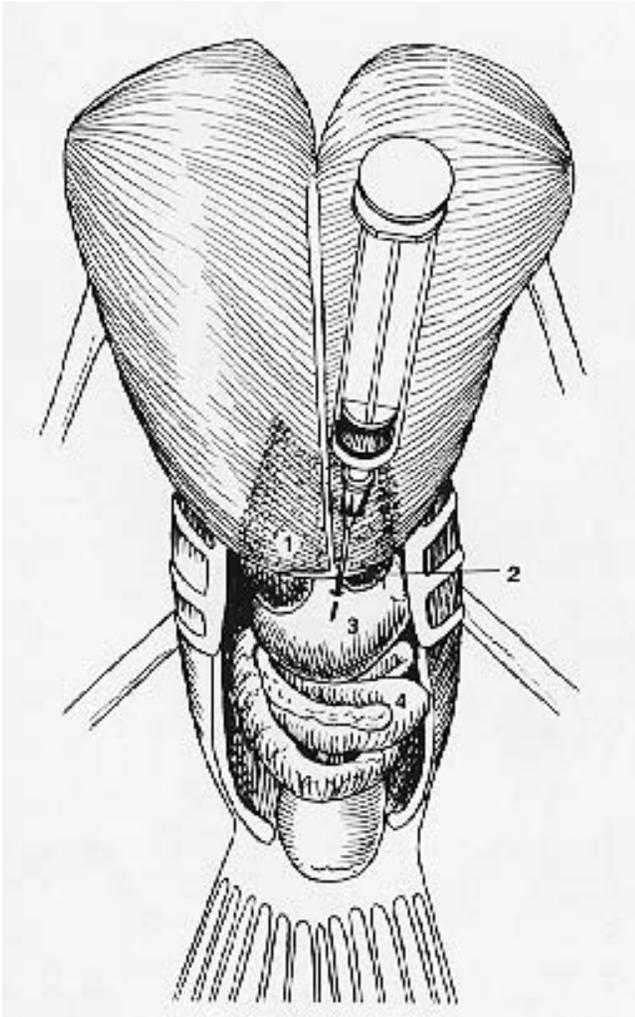


**FIG 10.1** A mature pigeon hen was presented for lameness, an unwillingness to fly and depression. The hen had been incubating eggs, and it was uncertain how long she had been clinically symptomatic. Several joints were swollen and firm. The elbow and ankle joints were severely affected. The masses in areas where the skin was thin appeared grossly as small, white-to-yellow nodules. Cytologic examination of a fine-needle aspirate from the mass revealed numerous crystalline structures suggestive of urate crystals. Articular gout is common in birds that become dehydrated or that have primary or secondary renal disease.

plunger. The needle is moved at different angles in the tissue without releasing the vacuum. It is important to release the vacuum before withdrawing the needle from the tissue, because the aim of the procedure is to obtain a small amount of sample in the lumen of the needle only, not in the syringe itself. Once the needle has been withdrawn from the tissue, it is detached from the syringe and the syringe is filled with air. The needle is reattached to the syringe, and with the point of the needle lying against the slide surface, the air within the syringe is used to force the sample onto a glass microscope slide. A second glass microscope slide placed on top of the first allows the sample to spread between the two glass surfaces when the slides are pulled horizontally apart. Two specimens for cytologic examination are thus created. This technique is often referred to as the “squash preparation technique” because the sample is compressed between the two slide surfaces.

Abdominocentesis is an aspiration biopsy procedure used to collect cytologic samples from birds with abdominal fluid accumulation. The abdominal space is small in normal birds and contains little fluid. Because the abdominal air sacs occupy a large portion of the abdomen, it is difficult to enter the peritoneal cavity of normal birds. However, as peritoneal fluids accumulate, the air sacs are compressed laterally, increasing the size of the peritoneal cavity and making it easier to sample. Abdominocentesis begins with a surgical preparation of the site along the ventral midline just distal to the point of the keel. The needle (21 to 25 ga, one-inch) is attached to a syringe and is directed through the body wall at the midline, pointing toward the right side of the abdomen to avoid the ventriculus, which lies to the left of the midline (Figure 10.2). The abdominal fluid is aspirated into the syringe and prepared for cytologic examination, either by making a direct smear as one would prepare a blood film or by using a concentration method.

The goal of abdominocentesis is to collect fluid from the abdominal cavity for diagnostic purposes. If the abdomen is distended with a soft-shelled egg, ovarian cyst, dislocated bowel loops or an abdominal mass, the fluid may not be collected during abdominocentesis. The material that is collected (eg, gut contents, egg yolk, cells from a mass) should be evaluated with respect to its potential source. Interestingly, some avian species (macaws) will produce *small* quantities of fluid in response to egg-related peritonitis, while others (cockatiels) will produce voluminous fluids.

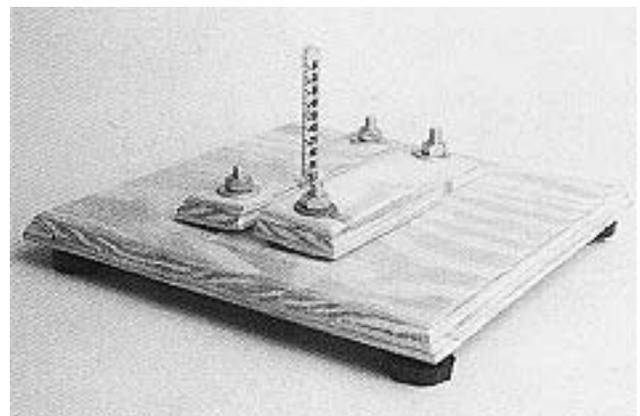


**FIG 10.2** For abdominocentesis, the needle is attached to a syringe and is directed through the body wall at the midline, pointing toward the right side of the abdomen. 1) Caudal edge of sternum 2) liver 3) ventriculus and 4) intestines.

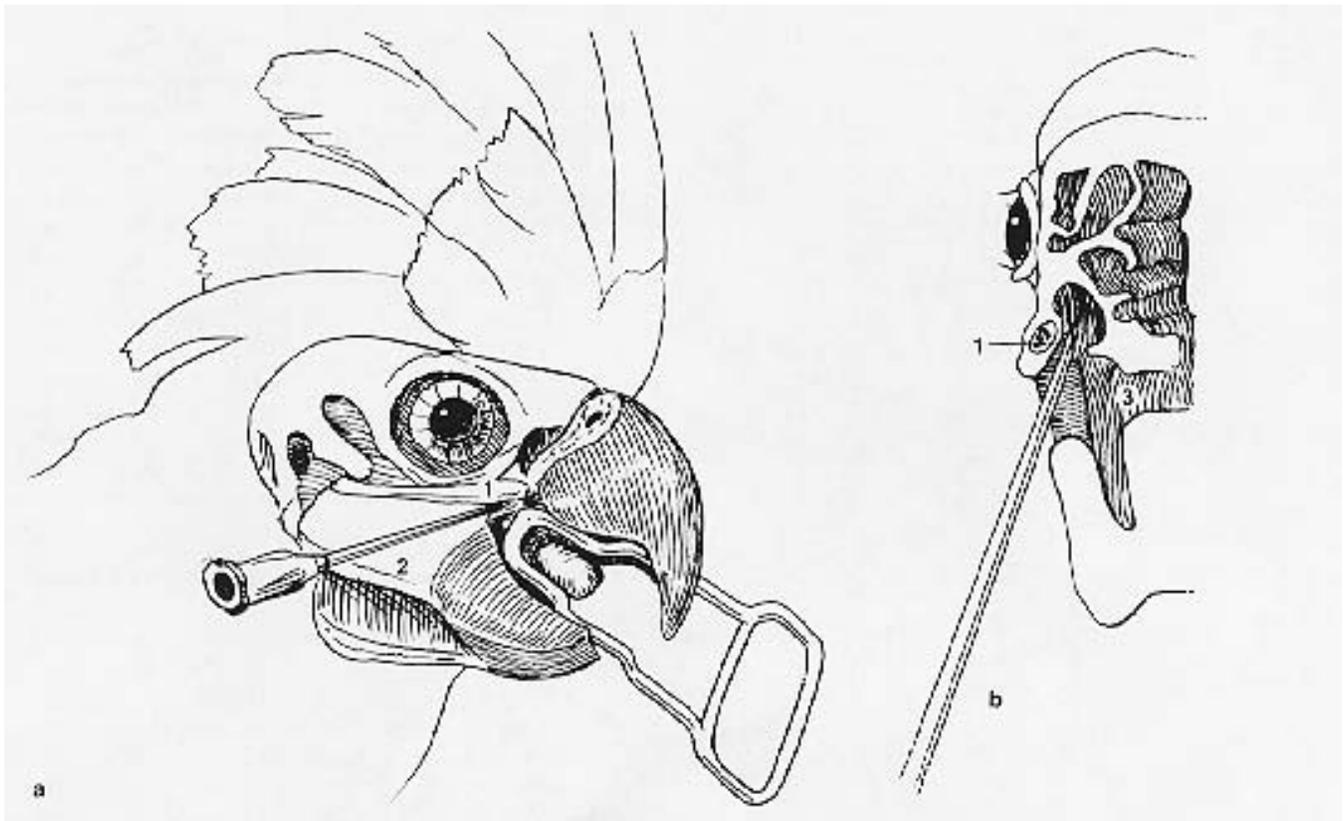
Fluid samples having low cellularity require a concentration procedure for easier examination of the cells. A variety of techniques can be used to concentrate cells on microscope slides. A simple method is to marginate the cells on a smear made by the conventional wedge technique used for making blood films. A drop of the fluid sample is placed on a microscope slide and spread slowly using a spreader slide. Just prior to reaching the end of the smear, the spreader slide is quickly backed slightly into the advancing smear, just before lifting it from the surface of the slide containing the smear. This should produce a slide with the margined cells concentrated at the end of the film.

Cells can be concentrated by centrifugation in a manner similar to that used in mammalian urinalysis procedures. The fluid is placed in a plastic test tube and centrifuged at 600 G (gravity) for ten minutes. Unlike urine sediments, cytologic sediments from poorly cellular fluids do not have a visible button or pellet at the bottom of a spun tube. Therefore, the concentrated cells are usually obtained by aspirating the fluid at the bottom of the tube into a pipette or syringe. The sample is then placed onto a microscope slide and a smear is made in the manner described for concentrating cells in a smear. Special cytocentrifuge equipment<sup>a</sup> is available for concentrating cells on microscope slides while absorbing the fluid onto filter paper. This equipment is expensive and not practical for the average veterinary laboratory.

Because centrifugation distorts the appearance of the cells, a cell concentration method that utilizes gravity provides a concentrated sample with normal appearing cells. A simple, inexpensive sedimentation device can be made for use in the veterinary laboratory. This device consists of a base to support the slide and a clamping mechanism to hold the fluid column onto the microscope slide (Figure 10.3). The column that holds the fluid is made from a one millimeter tuberculin syringe barrel with the tip removed. The base of the syringe barrel allows for the syringe to be held in place by a clamp (usually made of wood). A piece of filter paper (eg, Whatman #2) is cut to the dimensions of the microscope slide and a standard 2 mm paper hole punch is used to create a hole in the center of the filter paper. The filter paper is placed on top of the slide, and the base of the tuberculin syringe barrel is placed on top of the filter paper with the opening of the syringe superimposed over the hole in



**FIG 10.3** Centrifugation can distort the appearance of cells that are intended for cytologic evaluation. A simple device that uses gravity to concentrate cells provides cytologic samples of better quality than centrifugation (courtesy of Terry Campbell).



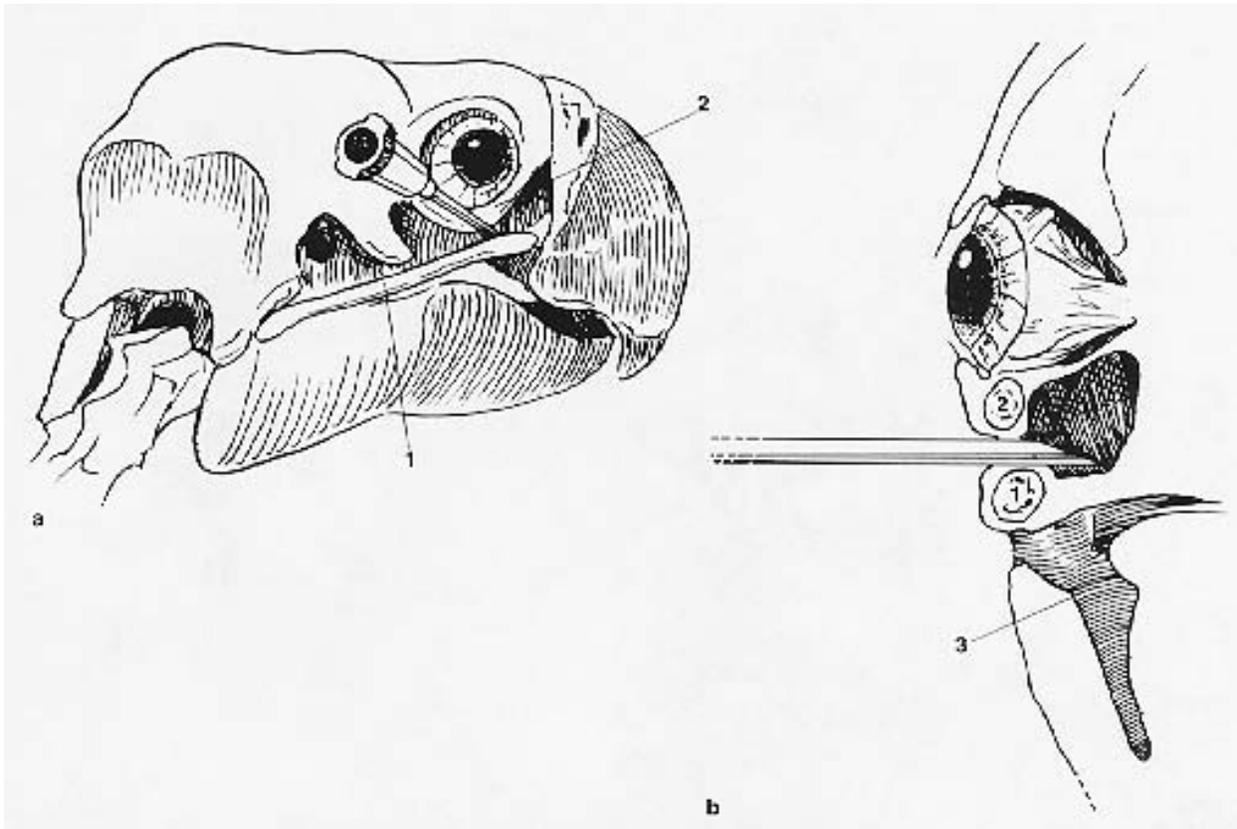
**FIG 10.4** Aspiration of the infraorbital diverticulum of the infraorbital sinus in psittacine birds can be performed by **a**) passing a needle through the fleshy skin at the commissure of the mouth and directing it toward a point midway between the eye and external nares, **b**) keeping parallel with the side of the head and passing under the zygomatic arch. 1) Zygomatic arch 2) mandible 3) oral cavity.

the filter paper. The clamp is used to secure the column to the slide. A small amount of fluid (eg, 0.2 to 0.5 ml) is placed into the syringe column. When allowed to stand undisturbed, the fluid is drawn by gravity and absorbed into the filter paper. The cells in the fluid fall onto the surface of the slide where they adhere. Once the fluid has drained from the column, the apparatus is disassembled and the slide is allowed to air dry. After staining, the cells can be found concentrated in the two millimeter circle created by the filter paper and column.

Cytologic evaluation of the ingluvies (crop) can be performed from samples obtained by aspiration. This is indicated in birds showing clinical signs of regurgitation, vomiting, delayed emptying of the crop or other crop disorders. A crop aspirate is obtained by inserting a sterile plastic, metal or rubber feeding tube through the mouth and esophagus into the ingluvies (see Figure 15.6). The tube should pass freely and not be forced into the crop. Passage of the tube is facilitated by extending the head and neck to straighten the esophagus. The crop content is gently

aspirated into the tube using a syringe attached to the free end. Excessive vacuum should be avoided to prevent damage to the crop mucosa. In cases where material cannot be aspirated for examination, a wash sample can be obtained by infusing a small amount of sterile isotonic saline into the crop and aspirating the fluid back into the tube and syringe.

Aspiration of the infraorbital sinus of birds suffering from sinusitis can provide diagnostic material for culture and cytologic examination. One technique of sinus aspiration in psittacine birds samples the large sinus between the eye and the external nares (Figure 10.4). With the head and body properly restrained, a needle (eg, 22 ga one-inch) is passed through the fleshy skin at the commissure of the mouth. The needle is directed toward a point midway between the eye and external nares, keeping parallel with the side of the head. The needle passes under the zygomatic bone, which lies between the lower corner of the rhinotheca (upper beak) and the ear. Often the passage of the needle is improved by keeping the bird's mouth open with an oral speculum. Once the



**FIG 10.5** The infraorbital diverticulum immediately beneath the eye can be approached by passing a needle **a**) over the zygomatic arch, with **b**) the needle directed rostrally. This procedure requires some practice and complete restraint to prevent damage to the globe. 1) Zygomatic arch 2) suborbital arch of prefrontal and temporal bone 3) oral cavity.

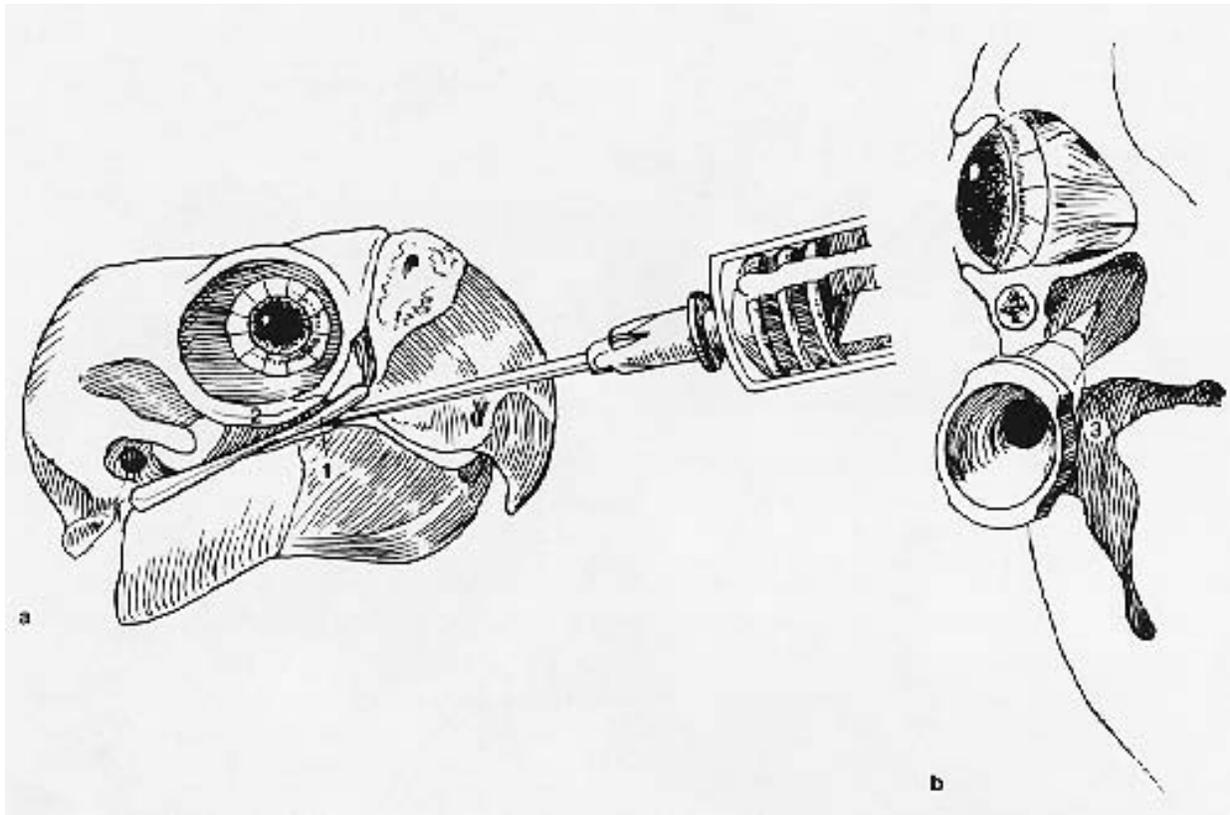
needle has entered the sinus, the sinus contents can be aspirated. A caudally misdirected needle could result in penetration of the ocular orbit; however, more commonly, a misdirected needle results in penetration of the surrounding muscles, causing peripheral blood contamination of the sample. It is important to note that in some species (eg, some passerine birds), the sinuses may not communicate with each other as they do in psittacine birds. Therefore, a bilateral sinusitis may require bilateral aspirations. (*Ed note: If a routine sinus flush does not produce an adequate sample, the anesthetized bird may be held with the head parallel to the floor and the affected sinus down. The sinus is flushed from underneath with the needle directed up; see Chapter 22*).

A second site of sinus aspiration is the small sinus immediately below the eye. This sinus usually yields a smaller sample volume than the previously described sinus. This sinus can be entered directly by inserting the aspiration needle at a perpendicular angle through the skin just below the eye (Figure 10.5). It can also be approached from a rostral direc-

tion by entering through the commissure of the mouth, directing the needle under the zygomatic bone and ending in the sinus cavity below the eye (Figure 10.6).

Collection of synovial fluid by arthrocentesis is another example of sample collection by aspiration. After the skin over the joint has been prepared as for surgery, a needle (22 ga or smaller) attached to a syringe is used to aspirate a small amount of fluid by direct penetration of the joint space. The cytologic sample is prepared by making direct smears using the "squash preparation technique."

Wash samples are aspiration techniques in which a small amount of sterile isotonic saline is infused into an area and immediately re-aspirated in an effort to collect a cytologic sample from locations that may be difficult to sample or that provide a poorly cellular field. Tracheal washes are commonly performed in birds suspected of having respiratory disease of the trachea, syrinx and bronchi. Depending on the patient, this procedure can be performed with or with-



**FIG 10.6** Aspiration of the suborbital diverticulum of the infraorbital sinus can be accomplished by **a)** entering through the commissure of the mouth, directing the needle under the zygomatic bone and **b)** ending in the sinus cavity below the eye. 1) Zygomatic arch 2) suborbital arch 3) oral cavity.

out general anesthesia. A soft, smooth-tipped, sterile plastic or rubber tube or catheter small enough to pass through the trachea is inserted through the open glottis taking care not to contaminate the tip in the oral cavity. The tube is passed to the level of the thoracic inlet near the syringe. An oral speculum should be used in birds (eg, large psittacine birds) capable of biting off the tube. The animal is held parallel to the floor, and sterile saline (0.5 to 2 ml/kg body weight) is quickly infused into the trachea and immediately re-aspirated to complete the wash sample. Similar wash techniques can be used to collect cytologic samples from the air sacs, ingluvies and infraorbital sinus.

### Contact Smears

Cytologic samples can also be obtained by direct contact between the tissue being sampled and the microscope slide. Often referred to as contact smears, these samples are used to evaluate postmortem tissues or antemortem tissue biopsies. Imprints of solid tissues should be made from freshly cut surfaces that

have been blotted with a clean paper towel to remove the excess fluid and blood. It is best to lay the slide against the tissue surface using the weight of the slide to make the imprint. If the tissue is brought to the slide, too much force is used and the resulting specimen is too thick for evaluation.

Contact smears made from tissues that exfoliate poorly (eg, connective tissue) may require traumatic exfoliation to improve the cellularity. One method of improving cellular exfoliation is to scrape the tissue to be sampled with a scalpel blade and to make the contact smear from either the scraped surface or the material remaining on the scalpel blade. Using a drop of oil on the scalpel blade may improve the ability to detect mites but will interfere with staining for cytologic evaluation. Imprints should be made from biopsy of internal organs (eg, liver, spleen and kidney) using the impression technique.

Scrapings are commonly performed to collect cells from the palpebral conjunctiva, cornea, oral cavity or tissues that normally yield poorly cellular samples. A metal or plastic spatula is used to gently scrape these

tissues, and the exfoliated cells are transferred to a microscope slide.

Cytologic samples can also be obtained using a sterile swab.<sup>b</sup> Once the sample has been collected, the swab is gently rolled across the surface of a clean microscope slide, using light pressure in order to avoid cell damage. The swab should be rolled in one direction only and not rolled back and forth across the smear to prevent the creation of an excessively thick smear. Cytologic samples of internal tissues can be obtained using endoscopic equipment. Samples can be obtained either from the tip of the endoscope or by using brushes or biopsy forceps. The sample is applied directly to a microscope slide.

## Evaluation of the Cytologic Sample

Tables 10.1 to 10.3 describe the use of stains most commonly available for cytology.

### Classification of Cells and Cellular Responses

The cells observed in the cytologic sample can be classified as either hemic, epithelial, mesenchymal or nervous tissue cells.<sup>13</sup> Hemic cells are those cells found in the blood and the hematopoietic tissues (see Chapter 9). It is extremely important to recognize

TABLE 10.2 Results of Cytologic Staining

	STAIN USE	RESULTS
<b>Acid-fast stain</b>	Mycobacterium . . . . .	red
	Other bacteria . . . . .	blue
	Leukocytes . . . . .	blue
	Cellular debris . . . . .	blue
<b>Giemsa stain</b>	Cell nuclei . . . . .	reddish purple
	Chlamydial elementary bodies . . . . .	purple
	initial bodies . . . . .	blue
	Mycoplasma . . . . .	pink or purple
<b>Gram's stain</b>	Gram-positive bacteria . . . . .	violet
	Gram-negative bacteria . . . . .	red
	Eukaryotic cells (except yeast) . . . . .	red
	Yeast . . . . .	deep violet
<b>Modified Gimenez stain</b>	Chlamydial elementary bodies . . . . .	red
	initial bodies . . . . .	blue
	Heterophil granules . . . . .	red
	Eosinophil granules . . . . .	red
	Mycoplasma . . . . .	like chlamydia
<b>New methylene blue stain</b>	Granulocytes . . . . .	purple nuclei, pale blue cytoplasm
	Erythrocytes . . . . .	purple nuclei, distinct cytoplasmic border, cytoplasm greenish blue
	Heterophil granules . . . . .	not stained
	Eosinophil granules . . . . .	not stained
	Fibrin . . . . .	not stained
<b>Stamp stain</b>	Chlamydia, rickettsia . . . . .	bright red
	Cocci tissue, other organisms . . . . .	green
<b>Sudan III stain</b>	Fat globules . . . . .	red-orange
	Cell nuclei . . . . .	blue
	Cell cytoplasm . . . . .	green
<b>Wright's stain</b>	Blood cells(see hematology)	

TABLE 10.1 Cytologic Stains Commonly Used in Avian Practice

#### 1. Romanowsky stains (Wright's and Wright-Giemsa)

These stains are commonly used for peripheral blood films and routine cytology. They require air-dried smears. Commercially prepared quick stains are available to simplify the staining procedure. These stains can be used to prepare a permanently stained slide.

#### 2. New Methylene Blue stain

This is a routine cytologic stain used as a wet preparation on dried smears. It does not provide a permanent stain. It is useful in the demonstration of fibrin, lipid droplets, fungal hyphae and other structures that stain poorly with alcohol-based stains.

#### 3. Acid-fast stain

This specific stain is used to demonstrate acid-fast positive organisms, such as *Mycobacterium* sp. Acid-fast positive organisms stain red, whereas other bacteria stain blue. This stain is not used to evaluate cells.

#### 4. Gram's stain

This is a microbiologic stain used primarily for the classification of bacteria grown on culture media. Gram-positive organisms stain deep violet, whereas gram-negative organisms stain red. Because of the nature of material on most cytologic preparations, it is difficult to

achieve uniformity of staining on the smear. This stain is not used to evaluate cells.

#### 5. Macchiavello's stain

This stain is used to identify chlamydia and mycoplasma inclusions. Chlamydia elementary bodies (0.2 - 0.3  $\mu$ ) stain red, whereas the initial bodies (0.9-1.0  $\mu$ ) stain blue. Mycoplasma colonies resemble chlamydia. Other particles in some smears may stain red and make the interpretation of the smear difficult. This stain is not used to evaluate cells.

#### 6. Gimenez stain

This stain is used to identify chlamydia inclusions which stain red against a blue-green cellular background. There is less interference with non-chlamydia particles staining red with this stain as compared to Macchiavello's stain. This stain is not used to evaluate cells.

#### 7. Stamp stain

This stain is used to detect chlamydia and rickettsia, which appear as small, bright red, "cocci" intracytoplasmic inclusions.

#### 8. Giemsa stain

This stain is used to identify chlamydia and mycoplasma.

hemic cells because these cells can be either important features of the cellular response or common contaminants of the cytologic sample.

Epithelial cells typically exfoliate easily and are found in clusters or sheets.<sup>13</sup> Epithelial cells vary in shape depending upon their origin. They can be oval, cuboidal, columnar or polygonal (eg, squamous epithelial cells). Epithelial cells typically have an abundant cytoplasm, small round-to-oval nuclei and distinct cytoplasmic margins. Cells from secretory epithelium may contain cytoplasmic granules or vacuoles.

Mesenchymal cells tend to exfoliate poorly and normally occur as single cells. These cells vary in shape and usually have indistinct cytoplasmic margins. The fibroblast is the most frequently encountered cell of this group. Fibroblasts are typically spindle-shaped with small nuclei that usually follow the shape of the cell. The cytoplasm has indistinct margins. Fibroblasts usually exfoliate as single cells rather than in sheets or clusters.

Nervous tissue cells are rare in cytologic specimens.<sup>13</sup> They may be seen as deeply basophilic, stellate cells with cytoplasmic projections.

During the cytologic examination, an assessment of the cells is made by identifying the majority of the cell types, the morphology of the cells and character of the noncellular background. The goal of cytology is to identify the cellular message and classify the cell response into one of the basic cytodiagnostic groups. These groups include inflammation, tissue hyperplasia or benign neoplasia, malignant neoplasia and normal cellularity.<sup>14</sup>

### Inflammation

A cytodiagnosis of inflammation is made when an increased number of inflammatory cells is detected in the cytologic sample. The inflammatory cells of birds are heterophils, lymphocytes, plasma cells and macrophages (Figure 10.7). Peripheral blood heterophils and lymphocytes have been described in the hematology chapter. It should be emphasized that heterophils found in tissues and fluids other than peripheral blood may not appear the same as those found in hemic tissue. Heterophils found in inflammatory lesions often degranulate and may resemble mammalian neutrophils. Depending upon the microenvironment, they may appear degenerate. Plasma cells are large, oval lymphocytes with an



**FIG 10.7** Cytology is an effective technique for differentiating between masses caused by infectious agents and those caused by neoplasia. In this goose, several fine-needle aspirates from a soft tissue mass associated with a humeral fracture revealed numerous degenerating heterophils and macrophages containing phagocytized bacteria suggestive of osteomyelitis. There were no pleomorphic cells, abnormal nuclei or mitotic figures suggestive of a neoplasm. Because neoplastic cells were not demonstrated, the client chose surgical removal of the humerus, which was uneventful. The presence of feather follicles (arrows) visible on the displaced antebrachium should not be confused with intralesional gas production.

abundant, deeply basophilic cytoplasm; an eccentric, mature nucleus; and a prominent perinuclear halo (Golgi). Macrophages are large cells with an abundant cytoplasm that may contain small granules, vacuoles or foreign material. Macrophages and their nuclei vary in shape and can coalesce into multinucleated giant cells.

Eosinophils may be included in the list of inflammatory cells; however, eosinophilic inflammation is either extremely rare in birds or difficult to detect based on routine cytologic methods. Heterophils and eosinophils may be difficult to differentiate in cytologic samples using the standard Romanowsky stains. Eosinophils of domestic fowl stain peroxidase-positive and heterophils stain peroxidase-negative with the benzidine or p-phenylenediamine methods.<sup>9,11</sup> A suspected eosinophilic inflammatory response may be confirmed by peroxidase staining; however, one must keep in mind that cytochemical staining may vary among avian species. Avian eosinophils may not behave in the same manner as mammalian eosinophils.<sup>2,7,8,10</sup> Because these cells were given the same name, there is an implied similar function, but the function of avian eosinophils is currently unknown.<sup>11</sup>

The inflammatory response is classified as either heterophilic, mixed-cell or macrophagic inflammation based upon the types of inflammatory cells present.

Heterophilic inflammation is represented by a predominance of heterophils (greater than 70 percent of the inflammatory cells) in the cellular response. Heterophilic inflammation indicates an acute inflammatory response in birds.<sup>11</sup> It is important to examine the heterophils closely for signs of degeneration or phagocytized material.

Degenerate heterophils indicate a toxic microenvironment, usually caused by microbial toxins. Degenerative changes in heterophils include increased cytoplasmic basophilia, vacuolation, degranulation and nuclear karyolysis. If bacterial phagocytosis can be demonstrated, the cytodagnosis of septic heterophilic inflammation can be made. If only extracellular bacteria are found, it cannot be determined that there is a bacterial etiology since the extracellular bacteria may represent either normal flora (depending upon the location of the inflammation) or contaminants of the sample.

Because macrophages migrate quickly (within a few hours of onset) into inflammatory lesions, mixed-cell inflammation is the most commonly found inflammatory response in birds.<sup>11</sup> Mixed-cell inflammation is represented by the presence of heterophils and mononuclear leukocytes (eg, macrophages, plasma cells and lymphocytes). Heterophils represent at least 50 percent of the inflammatory cells in mixed-cell inflammatory responses. Mixed-cell inflammation usu-

ally represents an established, active inflammation. The heterophils in this type of inflammation are usually nondegenerate, suggesting a microenvironment free of microbial toxins even though there may be a bacterial etiology.

Macrophagic inflammation is indicated by the predominance of macrophages (greater than 50 percent) in the inflammatory response. This type of inflammation does not necessarily imply chronicity, but may be suggestive of a number of etiologies (eg, intracellular pathogens). Macrophagic inflammation is common to certain avian diseases. These include avian tuberculosis, chlamydiosis, foreign body reaction, mycotic infections and cutaneous xanthomatosis. Multinucleated giant cell formation is often associated with macrophagic inflammation. Giant cells can appear within hours of the onset of some inflammatory responses and, unlike in mammals, their presence does not imply chronic inflammation.<sup>1,5,12</sup>

### ■ Tissue Hyperplasia or Benign Neoplasia

Tissue hyperplasia resulting from cellular injury or chronic stimulation is difficult to differentiate from benign neoplasia based upon cytology. Cells from hyperplastic tissue appear mature and do not exhibit much pleomorphism. They may appear immature by exhibiting increased cytoplasmic basophilia owing to the increased RNA activity within the cell.<sup>14</sup> Proliferating cells may also exhibit an increase in mitotic figures; however, the nuclear features do not show immaturity. Examples of tissue hyperplasia, frequently seen in birds, include the fibrous and epithelial cell proliferation adjacent to chronic inflammatory lesions, thyroid hyperplasia (especially in budgerigars) and squamous hyperplasia secondary to hypovitaminosis A. A common benign neoplasm of birds is the lipoma, especially in budgerigars (see Color 25).

### ■ Malignant Neoplasia

Cells obtained from malignant neoplasms show varying degrees of pleomorphism. The severity of the malignancy increases with the greater degree of pleomorphism. The appearance of the cell nucleus can provide important clues to the detection of a malignant neoplasm.<sup>14</sup> Increased nuclear size, which is reflected by an increased nucleus to cytoplasm (N:C) ratio, is suggestive of an abnormal cell. Nuclear anisocytosis (variation in size) and pleomorphism (variable nuclear shapes) are features of malignant cells. Multinucleation can also be a feature of malignancy.

The nuclear chromatin may also be abnormal in malignant cells. Coarse, hyperchromatic chromatin is suggestive of neoplasia. Other nuclear features of malignant cells include abnormal nucleoli (very large or multiple, such as greater than five), irregular nuclear margins, abnormal or increased mitotic figures and abnormal lobation, especially in cells that normally do not have lobed nuclei.

Cytoplasmic features of malignant cells include increased basophilia, abnormal vacuolation or inclusions, decreased volume, variation in cell margins and variability in the staining.<sup>14</sup> Abnormal cytoplasmic inclusions may include satellite nuclei (small nuclear fragments) and phagocytized cells.

Once a decision has been made for the cytodiagnosis of malignant neoplasia, an attempt to classify the neoplasm should be made. The four basic classifications of malignant neoplasms based upon cytologic features include carcinomas, sarcomas, discrete-cell neoplasia and poorly differentiated neoplasia.<sup>14</sup> Carcinomas are malignancies of the epithelial cells; therefore, the abnormal cells in the sample have features of epithelial cells. Adenocarcinomas are frequently seen in birds, especially ovarian adenocarcinomas. Cytologic evidence of adenocarcinomas includes epithelial cells that tend to form giant cells, have cytoplasmic secretory vacuoles and tend to occur in aggregates (eg, balls, rosettes or loose groupings). Sarcomas are malignancies of mesenchymal cells and therefore tend to exfoliate cells poorly. Fibrosarcomas are the most frequently encountered sarcomas of birds (see Color 25). Cells from fibrosarcomas are abnormal-appearing fibroblasts, which are spindle-shaped cells that typically exfoliate as single cells. Abnormal fibroblasts show increased cellular size and N:C ratios, nuclear and cellular pleomorphism and exfoliation when compared with normal fibrous tissue. Other mesenchymal cell neoplasms such as chondromas, chondrosarcomas and osteogenic sarcomas may produce a heavy eosinophilic background material (chondroid or osteoid) that can be seen on the microscope sample.

A common discrete or round cell neoplasm of birds is lymphoid neoplasia (see Color 25). The abnormal lymphocytes found in this type of neoplasm exfoliate extremely well. Cellular features of malignant lymphocytic tissue include a marked increase in the number of lymphoblasts, nuclear and cellular pleomorphism, increase in cytoplasmic basophilia and mitotic figures, and abnormal or multiple nucleoli.

Poorly differentiated neoplasms produce cells having features of malignant neoplasia; however, the cells are difficult to classify as carcinomas or sarcomas. In such cases, a cytodiagnosis of a poorly differentiated neoplasm is made.

Circumstantial evidence for a malignant neoplasm without the demonstration of abnormal cells is seen in older birds (eg, female budgerigars) with a spontaneous hemoperitoneum and no history of trauma. This is suggestive of an ulcerated neoplasm leading to abdominal hemorrhage. Ovarian adenocarcinomas of budgerigars and cockatiels often present in this manner. Evidence for malignancy may also be obtained by the demonstration of ectopic cells in unusual anatomic areas. An example of this would be the presence of a large number of cells other than hepatocytes and hemic cells in a cytologic sample of the liver. This is suggestive of a metastatic lesion, even if the cells do not have features of malignant neoplasia.

### ■ Mixed Cellular Response

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Occasionally, a mixed-cellular response may be seen, especially in areas of ulcerated neoplasms. A cytologic sample obtained from an ulcerated neoplasm may reveal features of malignant neoplasia as well as inflammation or hemorrhagic effusion.

## Cytology of Commonly Sampled Fluids and Tissues

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### ■ Abdominal Fluids

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Birds presented with abdominal distention may have an abnormal accumulation of fluid within the peritoneal cavity that may be detected by palpation or radiology. Cytologic evaluation of this fluid is often the main technique for establishing a presumptive or definitive diagnosis.

Abdominal effusions can be classified based upon cellularity, types of cells present, protein content, specific gravity and gross appearance. Abdominal fluids are classified as transudates, modified transudates, exudates, hemorrhage and malignant effusion.<sup>14</sup> Transudates are odorless, transparent fluids characterized by a low cellularity (total cell counts

usually less than  $1000 /\text{mm}^3$ ), a specific gravity less than 1.020 and a total protein less than 3.0 g/dl. Transudates are typically colorless or have a straw color resembling diluted serum. Transudative effusions do not clot. These poorly cellular fluids contain primarily macrophages and occasional mesothelial cells. Transudates occur as a result of oncotic pressure changes or other circulatory disturbances. The same causes for abdominal transudative effusions in mammals most likely occur in birds. These include hepatic cirrhosis, cardiac insufficiency and hypoproteinemia.

Modified transudates resemble transudative effusions; however, they have an increased cellularity (total cell counts usually less than  $5000 /\text{mm}^3$  but greater than  $1000 /\text{mm}^3$ ). The mononuclear leukocytes predominate in this type of effusion with occasional mesothelial cells and rare heterophils. The mesothelial cells usually appear reactive. Reactive mesothelial cells tend to be round or oval with increased cytoplasmic basophilia (Color 10.1). The cell margins often have a scalloped or villus-like appearance. The nuclei have coarse chromatin and prominent nucleoli. Multinucleation, cytoplasmic vacuolation and mitotic activity are often associated with reactive mesothelial cells. Proliferation of mesothelial cells results in the exfoliation of mesothelial cell aggregates that appear as cellular sheets, balls or rosettes (Color 10.2). Care should be taken not to mistake these cells for malignant neoplasia. Modified transudates result from hydrostatic pressure changes or irritation of long-standing transudative effusions. Transudative and modified transudative effusions are commonly found in the abdominal cavity of mynah birds suffering from hemochromatosis.

Exudative effusions are characterized by high cellularity (total cell counts usually greater than  $5000 /\text{mm}^3$ ), a specific gravity greater than 1.020 and a protein content greater than 3.0 g/dl. The majority of the cells found in exudative effusions are inflammatory cells (Color 10.3). Acute exudative effusions demonstrate primarily a heterophilic inflammatory response; however, macrophages quickly move into the fluid, creating a mixed-cell inflammatory response within a few hours of onset. Lymphocytes and plasma cells are often seen in long-standing exudative effusions. Exudative effusions vary in color and turbidity. They are frequently viscous, have a foul odor and tend to clot. Abdominal lesions often associated with exudative effusions include septic peritonitis, egg-related peritonitis and abdominal malignancies.

Hemorrhagic effusions are identified by the presence of erythrocytic phagocytosis in the fluid sample (Color 10.4). Without demonstration of erythrophagocytosis, one cannot differentiate hemorrhage from peripheral blood contamination of the sample. If thrombocytes are present, the sample was most likely contaminated with peripheral blood during the sampling procedure. Thrombocytes disappear rapidly in hemorrhagic effusions. Proof of erythrophagocytosis is made by the detection of macrophages that have phagocytized erythrocytes (suggestive of recent hemorrhage), or that contain iron pigment or hemosiderin crystals resulting from erythrocyte degradation (implying a duration greater than 48 hours). Iron pigment appears as gray to blue-black pigment in the cytoplasm of macrophages using Wright's stain. Hemosiderin appears as diamond-shaped, golden crystals within the macrophage cytoplasm.

Malignant effusions have features of either exudative or hemorrhagic effusions, but contain cells compatible with malignant neoplasia (Color 10.5, 10.6). Abdominal effusions caused by neoplasms are the result of blockage of blood or lymphatic vessels. Cystadenocarcinomas of the ovary of older female birds are a common cause of malignant effusions. These effusions can resemble hemorrhagic or exudative effusions that contain epithelial cells with features of malignant neoplasia. These cells often form cellular aggregates of balls or rosettes and have cytoplasmic secretory vacuolation.

Urate peritonitis is a rare effusion that can occur in the abdomen of birds when urinary fluids leak into the abdominal cavity. The cytology of the acute lesion is poorly cellular but contains a marked number of sodium and potassium urate crystals. These crystals are the same ones found in the urate portion of the bird's droppings. Urate crystals are spherical (2 to 8  $\mu\text{m}$ ) and have a spoke-wheel appearance. They are also birefringent under polarized light. The milky appearance of this type of abdominal effusion resembles that of the urate portion of avian droppings. If the bird survives this condition long enough, inflammatory cells will migrate into the fluid.

### ■ Cytology of the Alimentary Tract

The oral cavity, esophagus, ingluvies (crop) and cloaca are often sampled for cytologic examination. Lesions in the oral cavity may have different etiologies but similar gross appearance. Therefore, sampling of oral lesions for cytologic examination is a quick and simple procedure for differentiation of these etiolo-

gies. The differential diagnoses for common oral lesions include septic stomatitis, candidiasis, trichomoniasis and squamous cell hyperplasia. The normal cytology of the oral cavity shows occasional squamous epithelial cells, varying amounts of background debris and extracellular bacteria represented by a variety of morphologic types (Color 10.7). Bacteria associated with the surface of squamous epithelial cells are considered part of the normal flora. *Alysiella filiformis*, a gram-negative bacteria common to the upper alimentary tract of birds, occurs as small coccobacilli in pairs forming ribbon-like chains, and is often associated with squamous epithelial cells<sup>6</sup> (see Color 10.7 for Diff-Quik stain).

Smears made from a bacterial abscess reveal either a heterophilic or mixed-cell inflammation with bacterial phagocytosis (Color 10.8, 10.9). Heterophils may appear degenerate if bacterial toxins are present. Squamous epithelial cells are usually present. An increase in the amount of background debris and bacteria is also common.

Cytologic evidence for candidiasis is the presence of numerous narrowly based budding yeast (Color 10.10). *Candida* yeast are typically oval and often stain deeply basophilic with the Romanowsky stains. Occasionally they stain poorly, however, and may appear as “ghosts” in the cytologic specimen. *Candida* sp. can be a normal inhabitant of the upper alimentary tract of birds and may average as few as one per high power field (40x). The cytodagnosis of candidiasis is made when the yeast increase in numbers. Because these organisms can be part of the normal flora of the upper alimentary tract of birds, low numbers of the yeast do not usually elicit an inflammatory response. However, an inflammatory response often occurs when the infection has involved the mucosa indicating the condition has become more serious. The presence of hyphae formation also indicates a potential lethal infection and suggests a systemic involvement by the yeast (Color 10.11, 10.15).

Trichomoniasis is best diagnosed by observing the movement of the piriform flagellate protozoa in a wet mount preparation. However, it is important to recognize these organisms in a stained cytologic sample if wet mount preparations are not part of the cytologic routine or trichomoniasis is not suspected. Trichomonads appear as basophilic, piriform cells with flagella on Wright's stained smears (Color 10.12, 10.13). These cells vary in staining intensity from poorly stained to deeply basophilic. The cell nucleus usually stains more eosinophilic than most cell nu-

clei. An eosinophilic axostyle can often be seen as a straight line running from the nucleus to the opposite pole of the cell. Eosinophilic flagella at the nuclear end and an undulating membrane on one side of the cell are usually present. Because trichomonas protozoa are not considered part of the normal flora and fauna of the alimentary tract of birds, an inflammatory response is usually found associated with trichomoniasis lesions. Much debris and extracellular bacteria are usually present. The gross appearance of trichomoniasis can vary from ulcerations to the accumulation of large amounts of necrotic debris, depending on the host (species)-parasite relationship.

The gross appearance of lesions caused by squamous hyperplasia and metaplasia from hypovitaminosis A can resemble lesions caused by bacteria, yeast and protozoa; however, the cytology has a very different appearance. Normally, squamous epithelial cells exfoliate as single cells or small groups following gentle scraping of the oral cavity. However, lesions resulting from squamous cell hyperplasia produce smears containing large numbers of cornified squamous epithelial cells that exfoliate in large sheets or aggregates. In the early stages of this condition, there is little background debris. Therefore, the cytology resembles that of the vaginal cytology of a dog in estrus. It is equally important in the diagnosis of squamous cell hyperplasia to note what is not present in the cytologic specimen. One does not see inflammatory cells (at least in acute lesions), yeast or protozoa. Squamous hyperplasia often occurs in the tissue surrounding the choanal slit in the roof of the mouth. As this lesion becomes increasingly chronic, secondary bacterial infections often occur, creating a septic inflammatory response associated with the squamous cell hyperplasia on the cytologic sample.

Cytologic evaluation of the esophagus and ingluvies (Color 10.14) is indicated in birds with clinical signs of regurgitation, vomiting, delayed crop emptying or other suspected esophageal and crop disorders. The normal cytology reveals occasional squamous epithelial cells and a variable amount of background debris and extracellular bacteria (represented by a variety of morphologic types). A rare yeast is accepted as normal. It should be emphasized that some foods (eg, monkey biscuits) fed to birds may contain yeast as a source of supplemental B vitamins. In these birds, there may be a high number of nonbudding yeast in a cytologic sample (see Color 8). In addition, the crop will have a normal pH and no other cytologic abnormalities. A sample of the food can be stained to confirm the source of the nonbudding yeast.

The same lesions and cytodiagnoses described for the oral cavity also apply to the normal cytologies of the esophagus and crop. Another cytologic indication of a disorder involving the esophagus and crop is the presence of many bacteria represented by one morphologic type (as compared to the normal variety of types), even though there is no apparent inflammatory response (Color 10.15). This condition is typical of a peracute ingluvitis, and the disorder is often referred to as “sour crop.” It is indicative of a peracute bacterial infection, and an inflammatory response has either not been established or the response has been overwhelmed and the degenerate heterophils cannot be recognized in the background debris. The pH is often greater than 7, whereas normal crop pH is 6.5 to 7. *Capillaria* ova may be detected in cytologic samples from the esophagus or crop of some birds with capillariasis. These ova are double operculated and may not stain (see Chapter 36).

Examination of the cloacal cytology is indicated whenever a disorder of the lower intestinal tract, reproductive tract, urinary tract or cloaca is suspected. The normal cytology of the cloaca reveals a few epithelial cells (noncornified squamous or columnar), extracellular bacteria (variety of morphologic types), background debris and urate crystals. Abnormal findings would include the presence of inflammatory cells, large numbers of yeast and a uniform population of bacteria. Because the cloaca is a common opening to the intestinal tract, urinary tract and reproductive tract, cells found in cloacal samples may have originated from any of these systems or the cloacal tissue. Therefore, if inflammatory cells are found, for example, one cannot determine which system is involved based upon cytologic findings alone.

The use of a speculum and a swab or tube may allow collection of cytologic samples at the cloacal opening of the intestinal tract, urinary tract or reproductive tract. Uterine samples may be obtained through the cervix, especially in hens that have recently laid eggs. Abnormal post-parturient hens (usually showing uterine inflammation) may require flushing of the uterus with lactated Ringer’s solution until the inflammatory cells disappear from the wash fluid. Cytology of the lower intestinal tract is usually poorly cellular with occasional epithelial cells, background debris and a variety of extracellular bacteria. Special stains may be required for the detection of pathogens, such as *Mycobacterium* and *Giardia* spp (see Table 10.1).

The normal fluid excreted from the urinary tract of birds is a poorly cellular, cream-colored, thick, mucoid semisolid (see Color 8). The cytology reveals a marked amount of sodium and potassium urate crystals. Abnormal urinary fluid is watery and may contain cellular elements such as inflammatory cells and cellular casts.

### ■ Cytology of the Respiratory Tract

The normal cytology of the nasal and infraorbital sinuses of birds reveals occasional noncornified squamous epithelial cells and low numbers of extracellular bacteria with little background debris. The normal cytology of tracheal wash samples consists of a few ciliated respiratory epithelial cells and goblet cells (Color 10.16, 10.17). An occasional squamous epithelial cell may be found. These cells may represent cellular contamination from the oral cavity if the end of the tube is not passed directly into the glottis or they may originate from the syrinx, which contains bistratified squamous epithelium in some birds. Ciliated respiratory epithelial cells are columnar or prismatic in shape and have an eccentric nucleus at the small pole of the cell. Eosinophilic cilia are located at the opposite, larger pole of the cell. Goblet cells are columnar cells with eccentric nuclei. They lack cilia but contain eosinophilic cytoplasmic granules and vacuoles.

Cytologic evidence for periorbital sinusitis is provided by the presence of inflammatory cells in the aspirate. Lesions with a bacterial etiology are indicated by a septic, heterophilic or mixed-cell inflammation. Mycotic lesions often reveal either a mixed-cell or macrophagic inflammation with the presence of fungal elements, such as yeast, hyphae or spores. Sinus infections associated with chlamydia often reveal a mixed-cell or macrophagic inflammation (Color 10.18). Chlamydial inclusions appear as small, blue-to-purple spherules, often in dense clusters, within the cytoplasm of macrophages when stained with Wright’s stain. Chlamydial stains, such as Gimenez or Macchiavello’s stains, may be used to aid in the detection of chlamydia (see Color 10.33). The chlamydial inclusions appear red, and the host cells appear blue-green with Gimenez stain. The chlamydial elementary bodies stain red, and the larger initial bodies stain blue with Macchiavello’s stain (see Color 10.34).

A septic tracheobronchitis is identified from a tracheal wash sample by the presence of inflammatory cells showing bacterial phagocytosis. An endoscope is

excellent for collecting cytologic samples from the trachea of psittacine birds the size of an Amazon parrot or larger. In severe cases, the ciliated respiratory epithelial cells appear degenerate. Degenerate respiratory epithelial cells show loss of cilia, cytoplasmic vacuolation and karyolysis. Degeneration and fragmentation of the ciliated respiratory epithelial cells in association with a macrophagic and lymphocytic inflammation are suggestive of a viral etiology. Inflammation of the trachea and bronchi usually results in an increase in goblet cells and mucin formation, which causes an increased thickness to the noncellular background.

Mycotic lesions involving the trachea, syrinx and bronchi may reveal fungal elements on the tracheal wash samples. Aspergillosis is a common fungal pathogen of the avian respiratory tract. Aspergillosis is characterized by thick, septate hyphae that branch at 45° angles (Color 10.19, 10.20). Occasionally conidiophores can be seen. Other fungal lesions, such as phycomycosis, may reveal nonseptate, branching hyphae (Figure 10.8). Mycotic lesions usually reveal a mixed-cell or macrophagic inflammation. Aspiration of foreign material also results in a macrophagic inflammation. A mixed-cell inflammation generally occurs when secondary bacterial pathogens become involved.

The cytologic evaluation of the lower respiratory tract (lungs and air sacs) is made from either biopsy samples, endoscopy impressions or imprints from necropsy specimens. Imprints of avian lung tissue have an alveolar-like appearance microscopically. The walls of these alveolar-like structures may reveal abnormal cytologic findings of inflammatory cells and etiologic agents such as yeast or fungi (Color 10.19, 10.20). Lung tissue is highly vascularized and imprints usually contain a marked number of erythrocytes.

Normal air sac samples are poorly cellular with the presence of a few noncornified epithelial cells. Bacterial infections show the typical septic inflammatory patterns. Chlamydial and mycotic lesions demonstrate mixed-cell or macrophagic inflammation with the presence of chlamydial inclusions or fungal elements, respectively.

Neoplastic lesions of the respiratory tract of birds are rare; however, they can occur. Cytologic evidence for malignant neoplasia is the presence of cells showing features of malignant cells. A secondary inflammatory response is often associated with malignant lesions.



**FIG 10.8** A mature Blue and Gold Macaw hen was presented for progressive dyspnea and weight loss of two weeks' duration. Radiographs indicated a diffuse soft tissue density in the right caudal thoracic air sac (arrows). Endoscopy indicated a white thickening of the air sac. Impression smears of endoscopically guided biopsies revealed branching fungal hyphae characteristic of aspergillosis. Abnormal clinical pathology findings were limited to marked leukocytosis (WBC=35,000). An agar gel diffusion test was considered positive for *Aspergillus* sp. antibodies.

### Cytology of the Skin

Bacterial infections involving the skin are usually associated with a heterophilic or mixed-cell inflammation. Cytology of skin samples typically contains squamous epithelial cells, debris and extracellular bacteria. Therefore, bacterial phagocytosis must be demonstrated to detect a septic inflammatory lesion.

Foreign bodies typically create a macrophagic inflammatory response with multinucleated giant cell formation. If a secondary bacterial infection has been established, lesions caused by foreign bodies may show a mixed-cell inflammatory response.

Cutaneous xanthomatosis is a unique condition of birds caused by an excessive accumulation of lipids in the skin (see Color 25). A macrophagic inflammatory response with multinucleated giant cells and cholesterol crystals is observed on the cytologic specimen (Color 10.21, 10.22). Cholesterol crystals appear as angular, translucent crystals that vary in size and shape. They often appear stacked upon each other. Skin affected with xanthomatosis is thickened, yellow and friable. It is often found in areas where previous hemorrhage (eg, feather cysts and skin trauma) or pressure from underlying tumors (eg, lipomas) has occurred (see Color 25).

Subcutaneous lipomas produce a cytologic specimen that appears “greasy” on the unstained slide. The cytology reveals numerous lipocytes, which vary in size (Color 10.23). Avian lipocytes often have large cytoplasmic vacuoles in association with clusters of small vacuoles. The vacuoles tend to be round. The cell nucleus appears pyknotic and pushed to one edge of the cell, often appearing as if pushed beyond the cell margin. The background material in slides from lipomas resembles the cytoplasm of the lipocytes and contains numerous fat droplets. These clear, round, fat droplets usually partially dissolve in the alcohol-based stains (eg, Wright’s stain) but are easily seen in the water-soluble stains such as new methylene blue. Special fat stains such as Sudan IV can be used to demonstrate the fat droplets.

The cytology of feather cysts varies, depending upon the chronicity of the lesion (see Color 24). Early stages of feather cyst development reveal a marked number of red blood cells in the sample. Often erythrophagocytosis can be found. As the lesion becomes more chronic and caseous exudation develops, the cytology resembles that of mixed-cell inflammation with a marked amount of background debris and occasional multinucleated giant cell formation. Feather fragments may also be found.

Cutaneous and subcutaneous malignant neoplasms are rare in birds, but can be detected on cytologic examination. Lymphoid neoplasia produces a highly cellular sample of immature lymphocytes (Color 10.24). These lymphoblasts and prolymphocytes are large, round cells that exfoliate as single cells. They have large nuclei with fine chromatin and multiple or large prominent nucleoli. The cytoplasm stains basophilic. Bizarre-appearing lymphocytes and mitotic figures may also be present. The background of lymphoid tissue, such as lymphoid neoplasms, typically contains small, irregular, blue cytoplasmic frag-

ments. Finding these fragments may be helpful in the cytologic identification of lymphoid tissue.

Cutaneous melanomas have also been found in birds. Poorly differentiated melanomas reveal mesenchymal cells that contain few cytoplasmic melanin granules. The gross appearance of the involved skin shows dark pigmentation. The malignant cells usually exfoliate as single cells, and the background may contain melanin granules from ruptured cells. The round melanin granules vary from black to dark brown to golden in color.

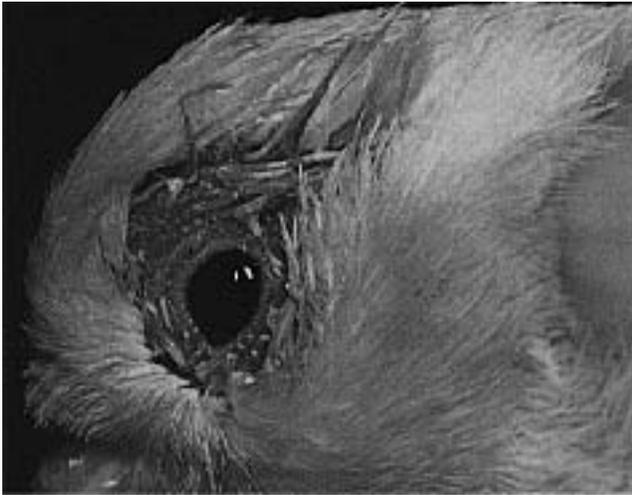
Avian poxvirus lesions reveal clusters of squamous epithelial cells that contain large cytoplasmic vacuoles (Color 10.25). The large cytoplasmic vacuoles found in the affected squamous cell push the cell nucleus to the cell margin. These vacuoles represent the ballooning degeneration of the squamous epithelium typical of pox lesions. These cytoplasmic vacuoles often contain small, pale eosinophilic inclusions with oil immersion examination of Wright’s stained smears. A secondary septic inflammatory response is often associated with ulcerated pox lesions.

### ■ Cytology of the Cornea and Conjunctiva

Normal conjunctival scrapings provide poorly cellular samples with little background material. The cells normally found are epithelial cells that may contain intracytoplasmic pigment granules. The normal cytology of the cornea is also poorly cellular and consists of occasional noncornified squamous epithelial cells. Inflammatory lesions involving the cornea and conjunctiva reveal inflammatory cells and increased numbers of exfoliated epithelial cells. The epithelial cells often demonstrate degenerative changes, such as cytoplasmic vacuolation, karyolysis or karyorrhexis. Chronic inflammatory lesions may show an increase in the number of epithelial cells that contain pigment granules. Chronic lesions may also reveal the presence of cornified squamous epithelial cells that are not normally found in the conjunctiva or cornea (Figure 10.9).

### ■ Cytology of Synovial Fluid

The amount of fluid in synovial joints of most birds is normally too small for sampling; however, an abnormal accumulation of joint fluid may provide enough sample for evaluation. Normal synovial fluid is poorly cellular. The cells are mononuclear cells, representing either synovial lining cells or mononuclear leukocytes. The background of normal synovial fluid



**FIG 10.9** An adult canary was presented with bilateral epiphora and mild conjunctivitis. Cytologic evaluation of conjunctiva scrapings may have been helpful in determining an etiology for this bird's problems. This bird responded to treatment with tylosin (courtesy of Michael Murray).

cytology consists of a heavy, granular, eosinophilic substance representing the mucin in the fluid.

An increase in the inflammatory cells and change in the color, clarity, and viscosity of the fluid is indicative of inflammatory joint lesions (see Figure 12.77). There may be a decrease in the granular eosinophilic background material, suggesting a decrease in mucin content. Erosion of the articular cartilage may result in the presence of multinucleated osteoclasts in the synovial fluid. Spindle-shaped fibroblasts suggest erosion into the fibrous layer of the articular capsule. Septic joint lesions may demonstrate bacterial phagocytosis by leukocytes (primarily heterophils). An increase in the number of inflammatory cells, especially heterophils, is also seen with traumatic arthritis. The presence of erythrocytes and erythrophagocytosis is supportive of a cytodiagnosis of hemarthrosis.

Articular gout produces a cream-to-yellow-colored deposit in affected joints (see Color 21). The cytology of this material reveals numerous, needle-shaped crystals (monosodium urate) (Color 10.26). These crystals are birefringent under polarized light. They occasionally stain eosinophilic with Wright's stain. Inflammatory cells are often present and the mucin content is often reduced, as reflected in the reduction in the amount of eosinophilic granular background.

## ■ Cytology of Internal Organs

The liver, kidney and spleen are occasionally sampled by biopsy to achieve an antemortem diagnosis in birds. Cytologic evaluation should also be performed whenever lesions involving these organs are found on postmortem examinations.

Birds typically do not have lymph nodes as found in mammals. Avian lymphoid tissue appears as lymphoid aggregates in the walls of the intestines, internal organs (especially the spleen and liver) and skin. The cloacal bursa of young birds is a sac-like lymphoid nodule found in the dorsal wall of the proctodeum of the cloaca (see Figure 5.6). The cytology of normal lymphoid tissue shows a predominance of small mature lymphocytes (greater than 90 percent of the lymphoid cells) (Color 10.27). The larger prolymphocytes, lymphoblasts and plasma cells normally occur in low numbers. Reactive lymphoid tissue demonstrates an increase in the number of immature lymphocytes (prolymphocytes and lymphoblast) and plasma cells (Color 10.28). Reactivity of the lymphoid tissue is suggestive of antigenic stimulation of the immune system. Lymphoid hyperplasia causes an increase in the lymphoid tissue mass; however, the cytology appears normal with the exception of a slight increase in the number of prolymphocytes. Lymphoid neoplasia produces a marked increase in the number of immature lymphocytes, especially lymphoblasts, in the cytologic specimen. The neoplastic cells may show varying degrees of cellular features of malignant neoplasia. There is usually an increase in the number of mitotic figures in samples obtained from lymphoid neoplasia.

Cytologic samples of the liver are usually highly cellular with a predominance of hepatocytes, erythrocytes and free nuclei. Depending upon the location of sampling, there may be numerous lymphocytes present. Hepatocytes are large epithelial cells that occur in sheets or clusters or as single cells. Normal hepatic cytology reveals uniform-appearing hepatocytes. These cells have an abundant, basophilic, finely granular cytoplasm and a round-to-oval, slightly eccentric nucleus. Binucleation is occasionally seen. Hepatocytes are easily ruptured during slide preparation; therefore, the background of hepatic tissue resembles that of the hepatocyte cytoplasm, and many free nuclei are commonly seen. Normal hematopoiesis is occasionally found because the liver is a common location for ectopic hematopoiesis. Also, macrophages containing iron pigment (hemosiderin) are occasionally seen.

Inflammatory lesions of the liver reveal numerous mature heterophils and an increase in the number of macrophages and plasma cells (Color 10.29). It is important not to confuse normal ectopic granulopoiesis with heterophilic inflammation. If developing stages of the heterophils can be found, the cytology is representative of granulocytopoiesis (see Chapter 9). If the heterophils are mature cells, however, then the cytology indicates inflammation. The hepatocytes may demonstrate degenerative changes in the presence of hepatic inflammation.

Avian tuberculosis produces a macrophagic inflammatory response in the liver (see Color 20). The cytology reveals numerous macrophages and multinucleated giant cells. When stained with Romanowsky stain, the background of the smear contains numerous large bacterial rods that do not stain. Likewise, macrophages may contain numerous bacterial rods that do not stain (Color 10.30). Because mycobacterium have a waxy cell wall, they do not stain with routine cytology stains. Therefore, an acid-fast stain is required to demonstrate the tubercle bacilli, which stain red (Color 10.31). However, the presence of a macrophagic inflammation with multinucleated giant cells and “ghost-like” bacterial rods provides a presumptive diagnosis for tuberculosis.

Avian chlamydiosis often results in a mixed-cell or macrophagic inflammation in the spleen or liver with a marked increase in the number of plasma cells (Color 10.28). Small, blue-to-purple, intracytoplasmic inclusions suggestive of chlamydial elementary and initial bodies may be seen in macrophages (Color 10.32). Confirmation of chlamydial inclusions is aided by special stains (eg, Gimenez or Macchiavello's).

Hepatic lipidosis produces cytologic specimens that appear “greasy” on gross examination. The stained smears reveal enlarged hepatocytes that contain round, cytoplasmic vacuoles (Color 10.35). The background material also contains these round vacuoles suggestive of lipid material.

Primary neoplasm of the liver reveals hepatocytes showing features of malignant neoplasia. Affected cells are usually pleomorphic with deep, cytoplasmic basophilia and immature-appearing nuclei (eg, smooth nuclear chromatin and multiple or large prominent nucleoli). Ectopic cells that show features of malignant neoplasia may also be found and are indicative of a metastatic lesion in the liver.

Occasionally, parasites may be found on splenic hepatic imprints (Color 10.36). Those commonly seen are schizogony of *Haemoproteus* and *Leukocytozoon*, sporozoites of *Atoxoplasma* and microfilaria.

Normally, cytology of the spleen shows a marked number of erythrocytes and lymphocytes, reflecting the cytology of a lymphoid tissue. Macrophages are also present and occasionally contain iron pigment from erythrophagocytosis of senescent red cells. Excessive splenic iron pigment is seen in birds with hemolytic anemia owing to increased red cell degradation by the spleen (Color 10.37). Chlamydial infections often cause a marked increase in the number of splenic plasma cells. Macrophages often demonstrate intracytoplasmic chlamydial inclusions. Developmental stages of blood parasites may also be found in splenic samples (see Color 9). Systemic bacterial or fungal infections may result in an increase in the number of inflammatory cells, especially mature heterophils, in the spleen. Often, the etiologic agent can be found either within the leukocytes or in the noncellular background.

The normal kidney produces a highly cellular sample that contains numerous epithelial cells with an abundant, slightly basophilic cytoplasm and slightly eccentric, round-to-oval nuclei. Numerous erythrocytes and free cell nuclei are usually present. Urate crystals are also common. Abnormal cytology includes an increase in the number of inflammatory cells or the presence of cells having features of neoplasia. Epithelial cells from renal adenomas show increased cytoplasmic basophilia, slight pleomorphism and occasional mitotic figures. Renal adenocarcinomas produce epithelial cells having features of malignant neoplasia. Nephroblastomas (embryonal nephroma) produce poorly differentiated epithelial and mesenchymal cells. The cuboidal epithelial cells are associated with spindle-shaped cells of the fibrous stroma, and the background may contain a heavy, eosinophilic substance. This background material is suggestive of a cellular attempt to produce a matrix (eg, chondroid or osteoid).

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■ **Products Mentioned in the Text**

- a. Cytospin, Shandon Southern Instruments, Sewickley, PA
- b. Calgiswab, Inolex, Glenwood, IL

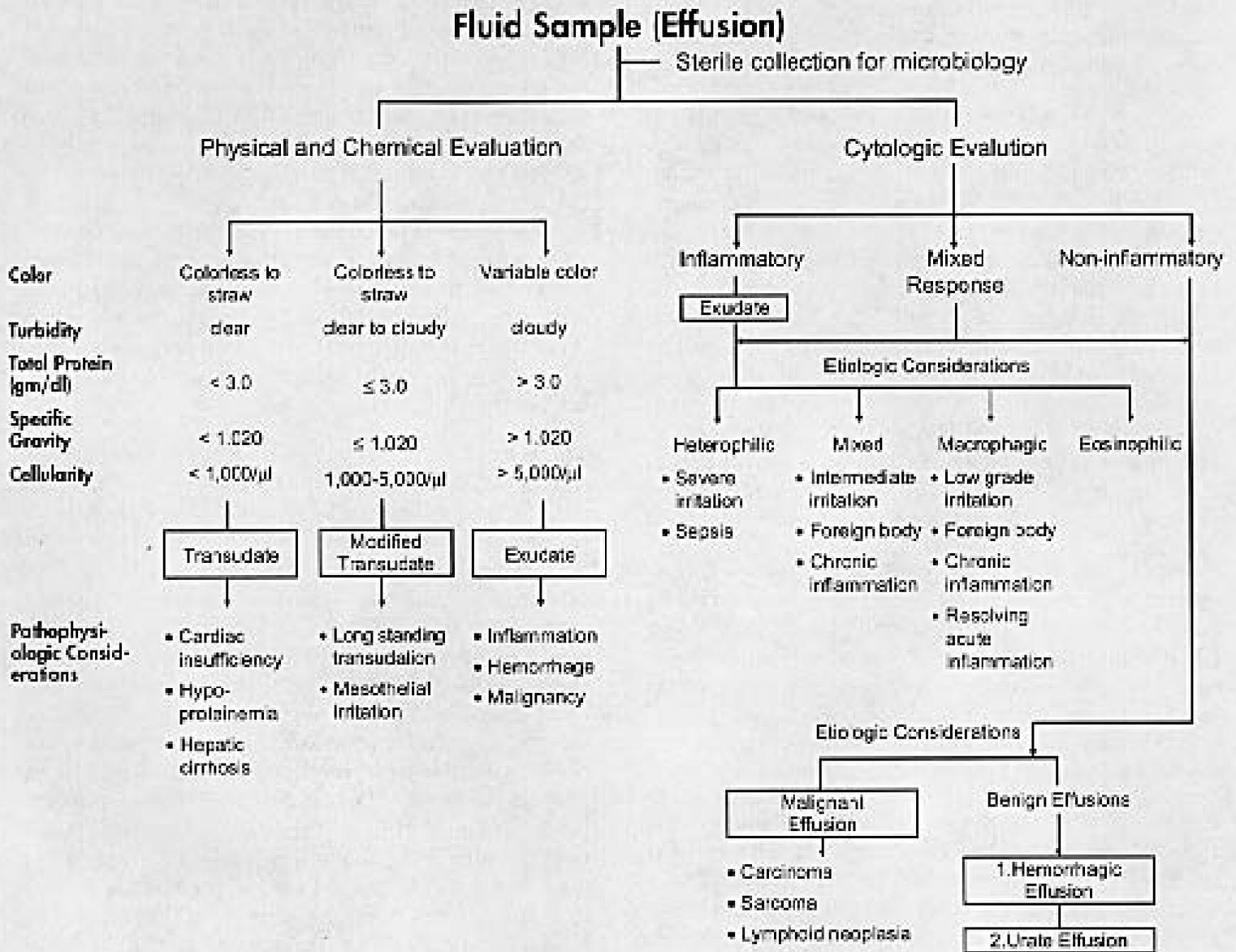


FIG 10.10 Flow chart for characterization of fluids

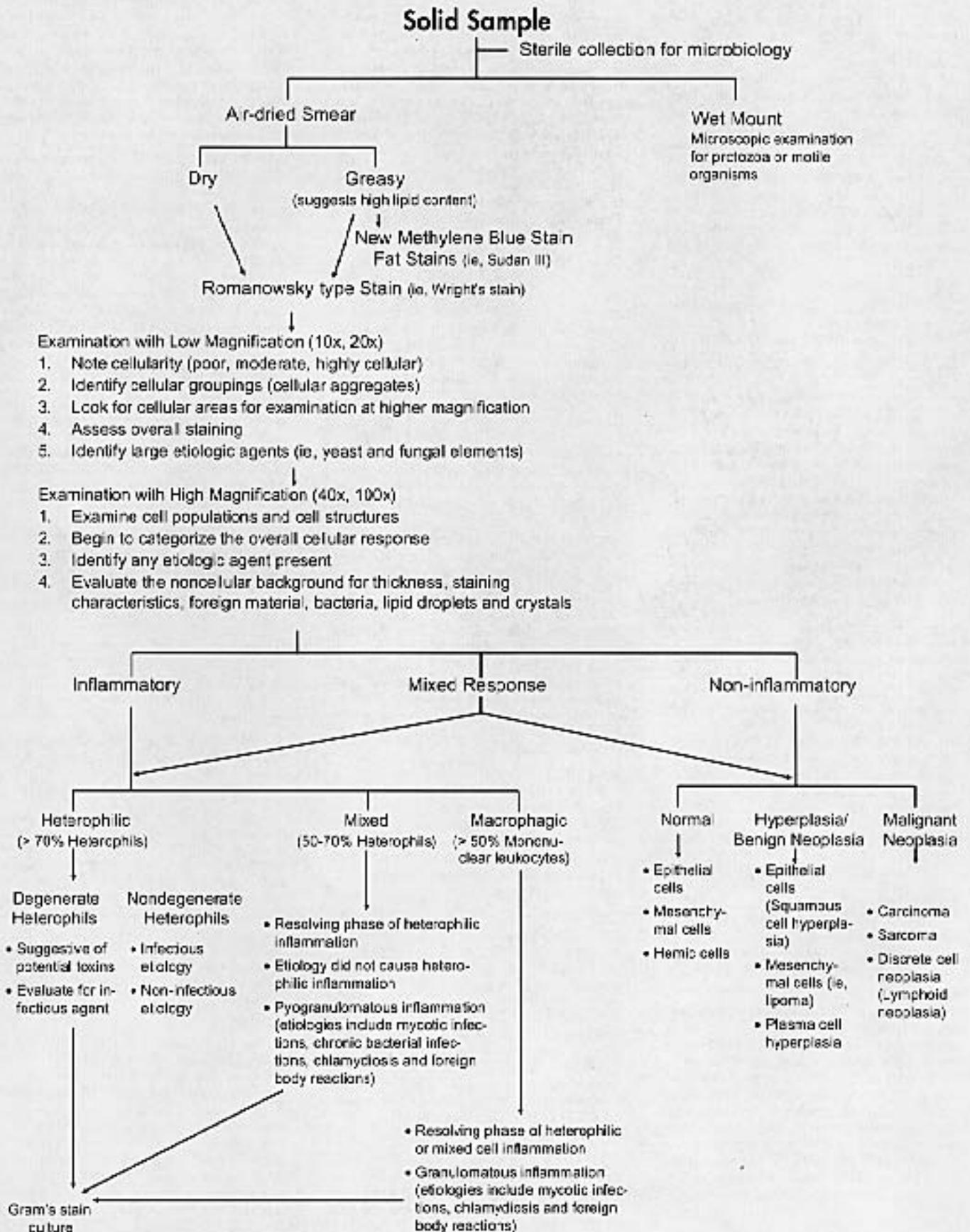


FIG 10.11 Cytologic evaluation of tissue samples

Unless indicated otherwise, cytology photographs are provided courtesy of Terry W. Campbell.

**Color 10.1**

An adult mynah bird was presented with a marked dyspnea at rest and abdominal enlargement. Abdominal palpation suggested fluid within the abdomen. An abdominocentesis was performed. The fluid was pale yellow and slightly cloudy. The specific gravity was 1.025. Fluid was prepared by a cytospin preparation and the smear was stained with Diff-Quik stain. Cytology was compatible with a modified transudate. Illustrated are a reactive mesothelial cell and erythrocytes.

**Color 10.2**

Shown are a cluster of reactive mesothelial cells, macrophages, erythrocytes and one heterophil from the mynah bird described in Color 10.1.

**Color 10.3**

An adult female budgerigar was presented with fluid distension of the abdomen. An abdominocentesis was performed, and a direct smear was made of the fluid and stained with Diff-Quik stain. The fluid appeared thick, red, slightly greasy and contained flocculent material. The photograph demonstrates numerous foaming macrophages, erythrocytes and blue amorphous material in the non-cellular background. A mixed cell or macrophagic inflammation associated with amorphous material is often seen with egg-related peritonitis.

**Color 10.4**

A six-year-old, 37 g female budgerigar was presented with a complaint of abdominal enlargement and dyspnea. There was no history of egg laying. An abdominocentesis was performed, the fluid was prepared with a cytospin preparation and the smear was stained with Diff-Quik stain. The fluid sample was pale orange and slightly cloudy. The specific gravity was 1.032. The macrophage shown demonstrates erythrophagocytosis, indicative of a hemoperitoneum.

**Color 10.5**

A second area of the preparation from the budgerigar in Color 10.4 shows numerous erythrocytes and a large epithelial cell with basophilic cytoplasm, a large nucleus with smooth chromatin and a large nucleolus. Note the numerous epithelial cells with features of malignant neoplasia. Necropsy revealed an ovarian cystadenocarcinoma.

**Color 10.6**

A ten-year-old female cockatiel was presented with a complaint of a large abdomen and dyspnea. The physical examination indicated ascites. An abdominocentesis was performed and a direct smear of the fluid was made and stained with Diff-Quik stain. The fluid was dark yellow and cloudy. The specific gravity was 1.036. Shown is a highly cellular sample with aggregates of pleomorphic cells with abundant vacuolated or basophilic cytoplasm. Necropsy revealed an ovarian cystadenocarcinoma.

**Color 10.7**

An adult female African Grey Parrot weighing 480 g was presented for a pre-purchase examination. A small area of depigmentation was found in the oral cavity adjacent to the choanal slit. A scraping of the depigmented area was made. The smear was stained with Diff-Quik stain. The smear was characterized by low cellularity with an occasional squamous epithelial cell and a variety of extracellular bacteria. The large, ribbon-like bacteria associated with the squamous cells is *Alysiella filiformis*. These cytologic findings are considered normal for the oral cavity.

**Color 10.8**

A hand-raised crow was presented with a two-day history of anorexia. The physical examination revealed caseous material in the oral cavity. A scraping of the material in the oral cavity was made, and the smear was stained with Wright's stain. Numerous nondegenerate heterophils are seen, indicating a heterophilic inflammation.

**Color 10.9**

An adult Red-tailed Hawk was presented with a healed, malaligned fracture of the right radius and ulna. Examination of the oral cavity revealed multiple, raised, white foci just caudal to the choanal slit. A scraping of the lesion was made, and the smear was stained with Wright's stain. The smear shows a marked number of extracellular bacteria and degenerate heterophils, suggesting a severe septic inflammation.

**Color 10.10**

A hand-fed, three-week-old, 68 g cockatiel was presented with delayed emptying of the ingluvies during the past 36 hours. An aspirate of the ingluvies was stained with Diff-Quik stain. Cytology revealed numerous, narrowly based, budding yeast and a marked amount of background debris indicative of candidiasis.

**Color 10.11**

A three-week-old Eclectus Parrot was presented with weight loss and delayed emptying of the crop. A crop aspirate was taken and a smear was stained with Wright's stain. Narrowly based budding yeast and hyphae formation are seen, indicative of severe candidiasis.

**Color 10.12**

A 723 g adult Barred Owl was presented in an emaciated, weak condition. Physical examination revealed multiple ulcerations in the oral cavity. A scraping of the oral lesions was made, and the smear was stained with Diff-Quik stain. The smear shows numerous pale and dark-staining piriform flagellate protozoa with eosinophilic nuclei and flagella (arrow). There is a moderate amount of background debris, free nuclei and bacteria. A few erythrocytes are present. The cytology is indicative of trichomoniasis.

**Color 10.13**

An adult male budgerigar was presented with chronic regurgitation and weight loss. The bird was thin, and regurgitated mate-

rial was present on the feathers of the head and face. A crop aspirate was performed, and the dried smear was stained with Wright's stain. A typical oil immersion field shows numerous piriform flagellate protozoa with eosinophilic nuclei, flagella, undulating membrane and axostyle (arrow). Leukocytes are also present. The cytology indicates severe trichomoniasis.

**Color 10.14**

A six-week-old Military Macaw chick was presented with a history of inadequate growth. A routine blood profile revealed no abnormalities. A crop aspirate was performed and a typical oil immersion field of this cytologic preparation is shown. The sample was poorly cellular and contained a slight to moderate amount of background debris. Bacteria represented by a variety of morphologic types were seen in the background. The cytology is considered normal for the ingluvies.

**Color 10.15**

A four-week-old, hand-raised cockatiel was presented with frequent regurgitation of a fluid with a fermented odor. A crop aspirate was performed for cytologic examination and a smear was stained with Wright's stain. The smear demonstrates a typical oil immersion field showing a uniform population of bacterial rods and yeasts beginning to form hyphae. No inflammatory cells are seen. A cytodagnosis of peracute septic ingluvitis and candidiasis was made, and the bird was successfully treated with antibiotics and a systemic antifungal medication.

**Color 10.16**

Ciliated respiratory epithelial cells (arrow) in a lung imprint stained with Wright's stain from an African Grey Parrot.

**Color 10.17**

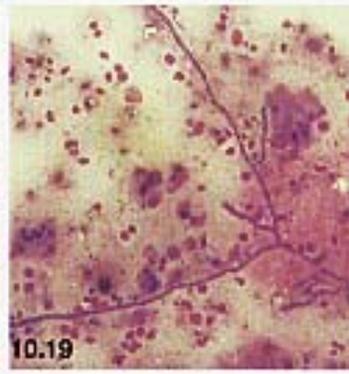
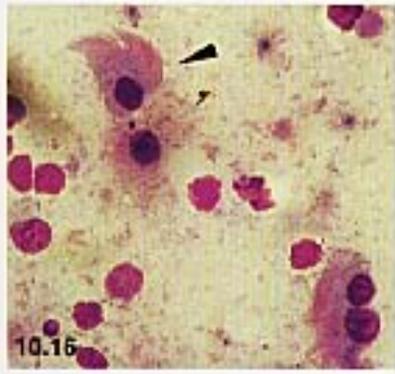
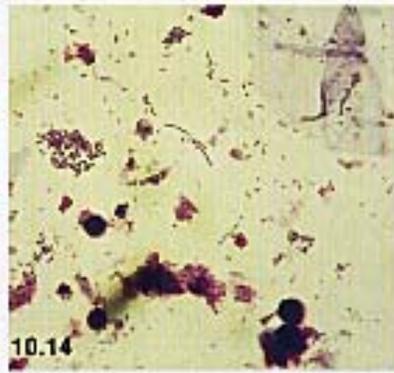
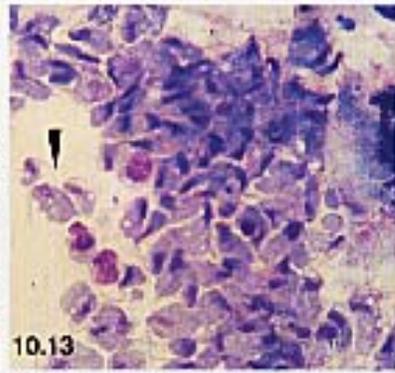
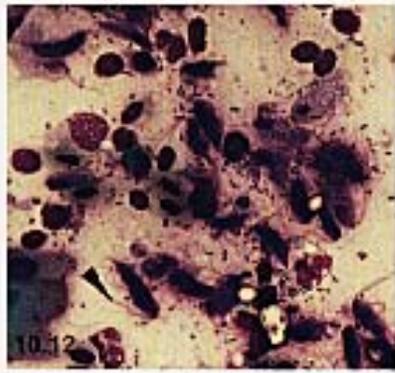
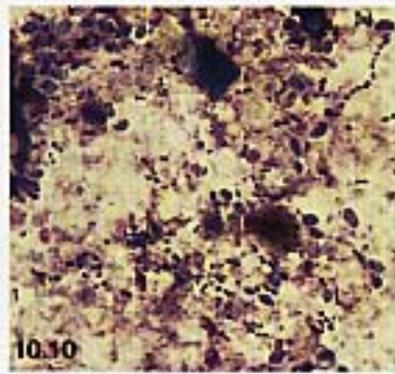
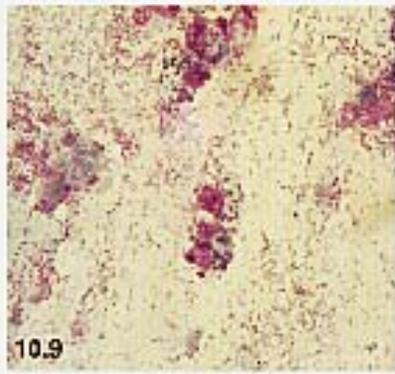
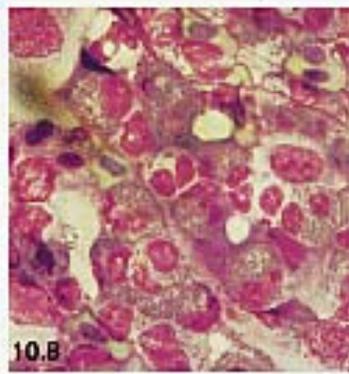
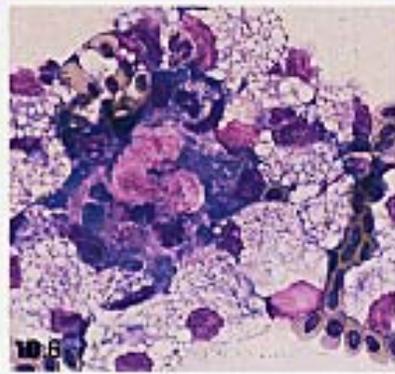
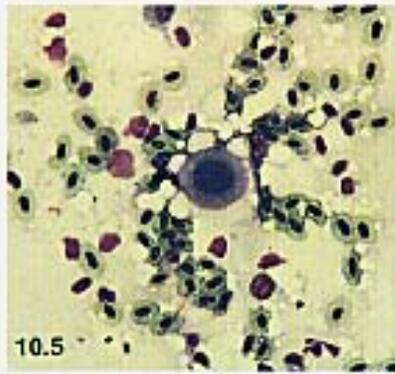
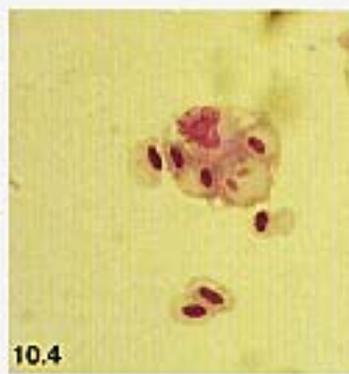
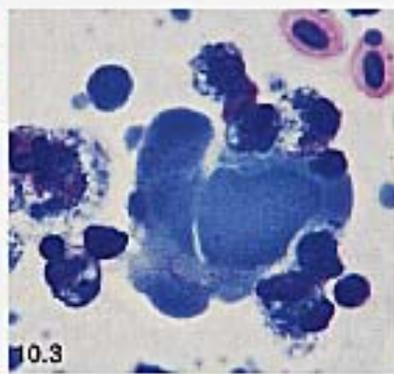
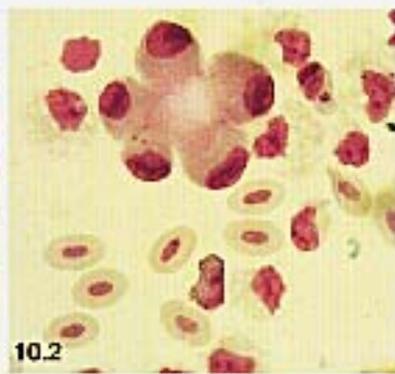
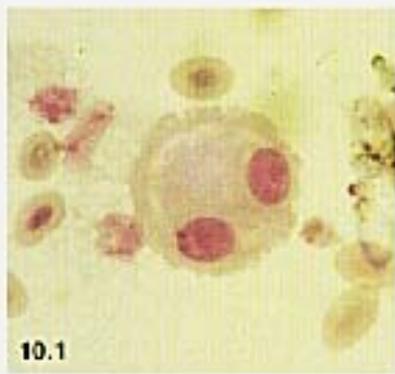
Goblet cells in a tracheal wash sample stained with Diff-Quik stain from a Night Hawk.

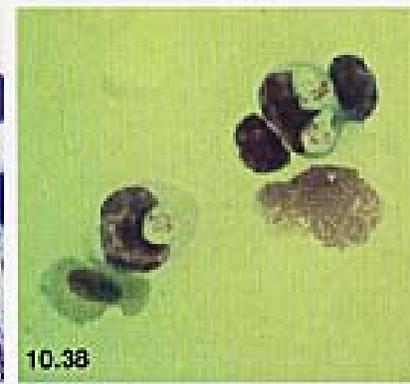
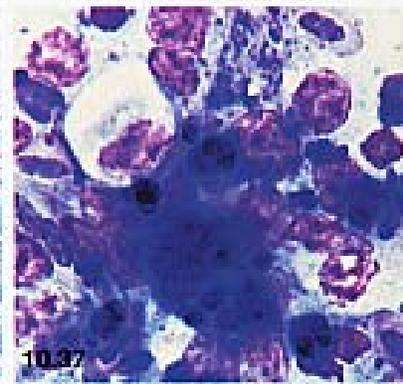
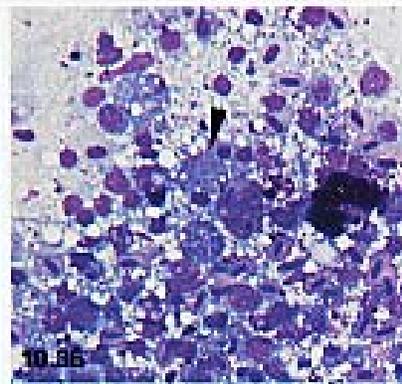
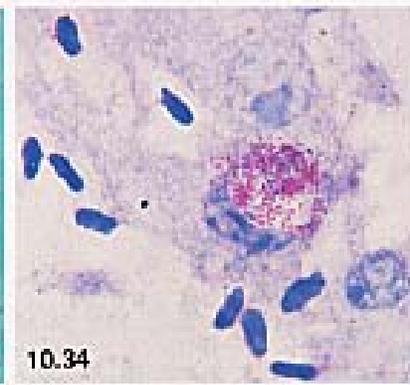
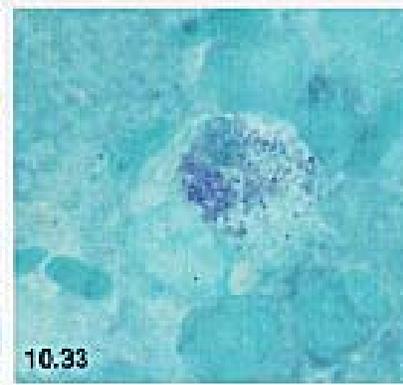
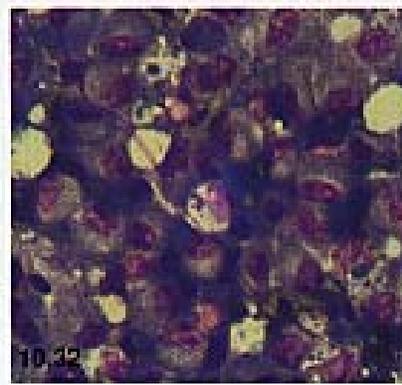
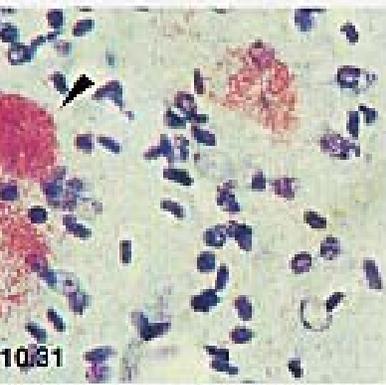
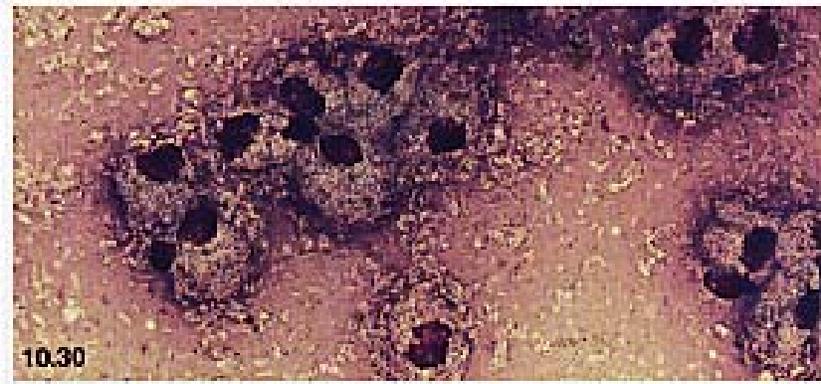
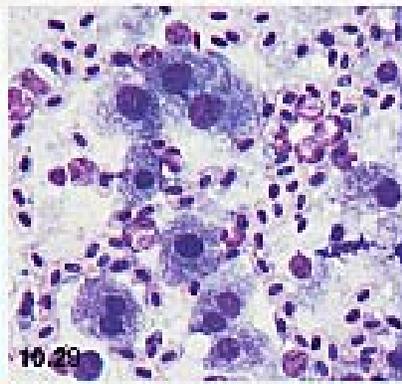
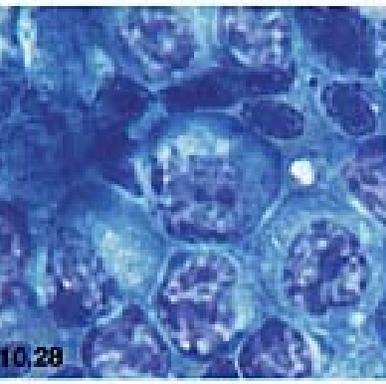
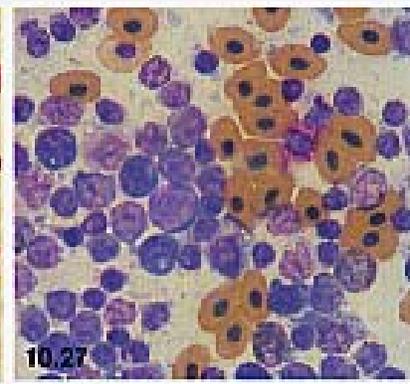
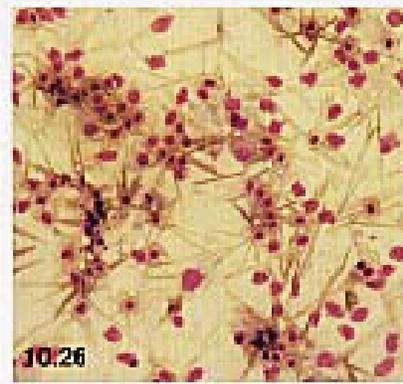
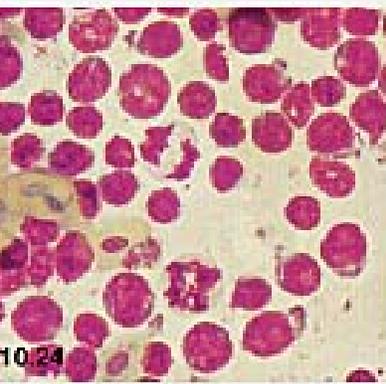
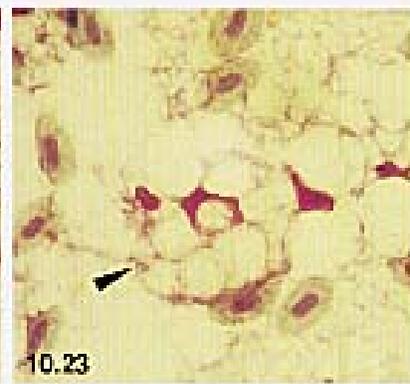
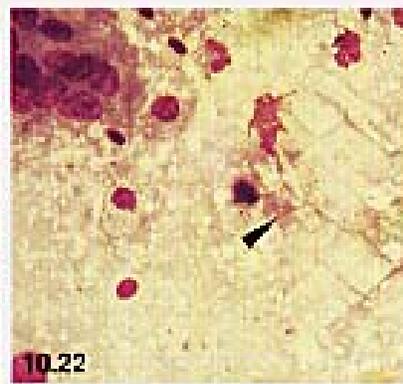
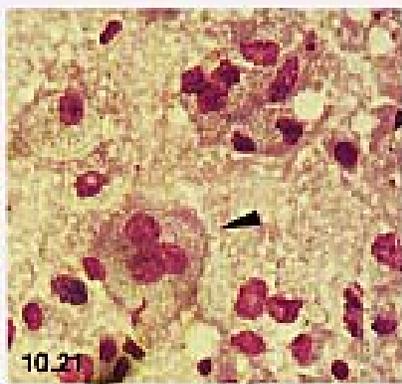
**Color 10.18**

An adult, 28 g, male Scarlet-chested Parakeet was presented with a history of sinus infection. A sinus aspirate was performed and the smear was stained with Diff-Quik stain. Shown is a mixed cell inflammation with degenerate heterophils. The heavy eosinophilic background suggests high protein content, most likely representing inflammatory proteins associated with a sinusitis. Although no etiologic agent can be seen, a bacterial or chlamydial etiology is suspected.

**Color 10.19**

A Blue-fronted Amazon Parrot was found dead. The only pathology noted on gross necropsy was a tan discoloration on the caudolateral margin of the left lung representing one-fourth of the lung mass. An imprint of the lesion was made, and the smear was stained with Wright's stain. High dry magnification was used to demonstrate a mixed cell inflammation and septate branching hyphae. A cytodagnosis of aspergillosis was made.





**Color 10.20**

An adult, 520 g African Grey Parrot on an all-seed diet was presented because it no longer grew, a typical behavior when approached. A tracheal wash sample was collected, and a smear was prepared by a cytospin preparation and stained with Diff-Quik stain. A highly cellular sample containing numerous erythrocytes is illustrated. There are multinucleated giant cells and septate fungal hyphae, indicative of a mycotic infection involving the respiratory tract.

**Color 10.21**

An adult, 1342 g Green-winged Macaw was presented with a complaint of feather loss on the distal end of the right wing. A feather cyst had been removed from this area three months earlier. Physical examination revealed thickened, yellow, friable skin on the dorsal aspect of the right metacarpus. A contact smear of the excisional biopsy of the abnormal skin was obtained and stained with Diff-Quik stain. Multinucleated giant cells and macrophages (arrow) on a heavy granular background are demonstrated.

**Color 10.22**

Multinucleated giant cells and cholesterol crystals (arrow) from the bird described in Color 10.21. These findings are compatible with xanthomatosis, which typically reveals a macrophagic inflammation with multinucleated giant cell formation.

**Color 10.23**

The physical examination of a 30 g, adult, male budgerigar (fed an all-seed diet) revealed a large, firm, subcutaneous mass overlying the keel. A fine-needle aspiration biopsy of the mass was made, and a smear was stained with Diff-Quik stain. Typical avian lipocytes (arrow) are shown. The cytology is compatible with a lipoma.

**Color 10.24**

A 23-year-old, 320 g Spectacled Amazon Parrot was presented with a marked swelling around the right eye and feather loss on the head. A fine-needle aspiration biopsy of the mass on the head was performed, and the smear was stained with Diff-Quik stain. The highly cellular sample shown contains numerous lymphocytes. The majority of the lymphocytes are large, immature and frequently show mytotic activity. These findings are indicative of lymphoid neoplasia.

**Color 10.25**

An adult, 112 g Ring Dove was presented in a morbid condition and died soon after the physical examination. The bird was housed in an outdoor aviary, and was presented for marked depression and multiple, raised, irregular cutaneous lesions on the head, legs and feet. A fine-needle aspiration biopsy of a raised lesion near the right eye was made, and the smear was stained with Diff-Quik stain. Shown are epithelial cells with large cytoplasmic vacuoles (arrow) typical of avian poxvirus lesions.

**Color 10.26**

An adult, 160 g Nanday Conure was presented with a swollen left tibiotarsal and

tarsometatarsal joint and left leg lameness. Arthrocentesis of the affected joint was performed and the smear was stained with Diff-Quik stain. The sample contains numerous free nuclei, possibly from ruptured erythrocytes, and needle-like crystals. The cytology is compatible with articular gout.

**Color 10.27**

An imprint of a normal spleen from a King Penguin that was euthanatized because of a severe skeletal deformity. Note the predominance of small-to-medium, mature lymphocytes, one lymphoblast and two plasma cells.

**Color 10.28**

A splenic imprint from the Yellow-naped Amazon Parrot described in Color 10.32-10.34 shows a marked increase in plasma cells, indicative of reactive lymphoid tissue.

**Color 10.29**

A four-year-old male budgerigar was presented for bilateral leg paralysis. Whole body radiographs revealed a large mass in the area of the kidneys, and a presumptive diagnosis of renal neoplasia was made. At the owner's request, the bird was euthanatized. Necropsy revealed a large, locally invasive mass that appeared to involve both kidneys. A histologic diagnosis of nephroblastoma was made. Necropsy also revealed a slight discoloration of the liver, which appeared pale. The imprint of the liver shown here reveals normal-appearing hep-atocytes and erythrocytes. There is also an increased number of mature heterophils present, suggesting a mild heterophilic inflammation and hepatitis. Histology confirmed the hepatitis; however, no etiology could be determined.

**Color 10.30**

An adult, female, 370 g Blue-fronted Amazon Parrot was presented for necropsy. The bird had shown no previous signs of illness. Necropsy revealed multiple, raised lesions throughout the liver parenchyma. The lesions were varied in size and tended to be round. No other gross lesions were seen. A contact smear of the liver was made, and the smear was stained with Diff-Quik stain. Shown is a typical oil immersion field demonstrating numerous macrophages and bacterial rods in the background that did not stain.

**Color 10.31**

Acid-fast-positive reaction (arrow) of the bacteria in the Blue-fronted Amazon Parrot described in Color 10.30, supportive of a diagnosis of avian tuberculosis.

**Color 10.32**

An adult, 270 g Yellow-naped Amazon Parrot was presented in critical condition with a history of anorexia, weight loss, frequent regurgitation and loose droppings with green urates. Whole body radiographs revealed hepatomegaly and splenomegaly. The blood profile indicated marked leukocytosis, toxic heterophilia and marked elevations in serum AST. The bird died within one hour of presentation. Necropsy revealed a marked reduction of the pectoral

muscle mass, sinusitis, cloudy air sacs, hep-atomegaly and splenomegaly. An imprint of the enlarged spleen was made, and the smear was stained with Diff-Quik stain. A marked number of plasma cells was noted in the splenic imprint (see Color 10.28). Also shown is a macrophage that contains small, intracytoplasmic inclusions suggestive of chlamydia.

**Color 10.33**

Chlamydial inclusions stained with Gimenez stain.

**Color 10.34**

Chlamydial inclusions stained with Macchiavello's stain.

**Color 10.35**

An obese, five-year-old, 125 g female cockatiel was presented for marked lethargy and dyspnea. Whole body radiographs revealed hepatomegaly. The blood profile revealed a lipemic serum sample with a normal CBC and chemistry profile. A biopsy of the liver was performed and the smear was stained with Diff-Quik stain. Shown is the typical appearance of the hepatocyte, which was enlarged and contained numerous vacuoles. The background contained round, fat droplets. The cytology is compatible with hepatic lipodosis.

**Color 10.36**

A severely debilitated, adult, male American Kestrel was presented with an open fracture of the right proximal humerus. The peripheral blood smear revealed a marked number of *Haemoproteus* gametocytes. The bird died 24 hours after presentation and an imprint of the spleen was made and stained with Diff-Quik stain. Round *Haemoproteus* schizonts (arrow) were found throughout the splenic imprint as shown here. There is also a large amount of dark-blue iron pigment present.

**Color 10.37**

An adult African Grey Parrot housed in a pet store was presented with a history of lethargy and anorexia. A blood profile revealed a moderate degenerative anemia (PCV=21%). The bird died within six hours of presentation. Gross necropsy revealed a slightly enlarged spleen. A splenic imprint was made and stained with Diff-Quik stain. Illustrated is a typical oil immersion field of the splenic imprint showing a marked amount of dark-blue iron pigment. The large amount of iron pigment was also seen histologically. This finding is suggestive of a hemolytic anemia; however, the etiology could not be found.

**Color 10.38**

Impression smear from the spleen of a mynah bird. Wright's stain was used to demonstrate *Atoxoplasma* sp. in macrophages. Note that the organism is causing indentation of the nucleus of the infected macrophages (courtesy of Carol Partington).

**TABLE 10.3 Staining Procedures****Acid-Fast Stain**

1. Air-dry then gently heat fix
2. Cover with carbol fuchsin
3. Steam over water bath (3 to 5 min.)
4. Rinse with tap water
5. Decolorize with acid alcohol until most red color is removed
6. Rinse twice in tap water
7. Cover with methylene blue stain (1 min.)
8. Gently rinse with tap water (air dry)

**Gram's Stain**

1. Air dry and gently heat fix slide
2. Cover with crystal violet (1 min.)
3. Gently rinse in tap water
4. Cover with Gram's iodine (1 min.)
5. Gently rinse in tap water
6. Decolorize with 95% ethyl alcohol (15 to 30 sec.)
7. Gently rinse in tap water
8. Cover with safranin (1 min.)
9. Gently rinse in tap water (air dry)

**Macchiavello's Stain**

1. Air dry then heat fix
2. Cover with basic fuchsin (5 min.)
3. Quickly rinse in tap water
4. Dip in citric acid one to ten times (1 to 3 sec.)
5. Rinse in tap water
6. Cover with methylene blue (20 to 30 sec.)
7. Rinse in tap water (air dry)

**Modified Gimenez Stain**

1. Air dry then heat fix
2. Cover with carbol fuchsin (1 to 2 min.)
3. Rinse in tap water
4. Cover with malachite green (6 to 9 sec.)
5. Rinse in tap water
6. Recover with malachite green (6 to 9 sec.)
7. Rinse with tap water (air dry)

**Sudan III Stain**

1. Apply stain to wet or dry smear
2. Apply coverslip

**New Methylene Blue Stain**

1. Completely air dry or use as a wet mount
2. Apply small drop of stain
3. Add coverslip

**Stamp Stain**

1. Air dry smear then heat fix
2. Cover for 10 min. with carbolated fuchsin as used for Gram's stain diluted 1:4 with water
3. Rinse with tap water
4. Differentiate in 0.5% H<sub>2</sub>SO<sub>4</sub> until the preparation looks gray; time according to thickness of the smear
5. Counterstain with 5% malachite green or methylene blue (15 sec.)
6. Rinse with tap water (air dry)

**Wright's Stain**

1. Air dry slide
2. Flood with Wright's stain (stand 1 to 3 min.)
3. Add equal amount of Wright's buffer
4. Gently mix by blowing until a metallic green sheen is formed
5. Allow to stand twice as long as step two (2 to 6 min.)
6. Rinse with tap water (air dry)

**Diff-Quik Stain**

1. Air dry slide
2. Dip in fixative five times (1 sec. each)
3. Dip in solution one to five times (1 sec. each)
4. Dip in solution two to five times (1 sec. each)
5. Rinse in distilled water (air dry)

**Giemsa Stain**

1. Air dry slide
2. Fix in methyl or ethyl alcohol (2 to 7 min.)
3. Air dry
4. Immerse in Giemsa stain (15 to 40 min.)
5. Rinse in tap water (air dry)

**References and Suggested Reading**

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