CHAPTER

14

NECROPSY EXAMINATION

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Necropsy examination of deceased patients should be an integral part of avian clinical medicine. Necropsy examination often is performed to determine the cause of an unexpected death. However, a thorough and systematic postmortem examination also may be used to confirm a clinical diagnosis, identify the etiology of a disease process, explain apparent unresponsiveness to treatment or reveal unrecognized disease processes. Integration of necropsy findings with clinical signs and laboratory data ultimately will enhance the clinician's understanding of disease processes and sharpen clinical diagnostic skills. In addition, necropsy will confirm radiographic interpretations and reinforce applied anatomy, which enhances surgical skills.

Necropsy examination is a relatively straightforward procedure that should follow a written protocol, thereby minimizing the possibility of overlooking important lesions. This chapter emphasizes the necropsy of psittacine and passerine birds; anatomic variations of other avian species such as ratites may be found by consulting appropriate chapters in this textbook and published articles in the veterinary literature.[3]

Maximum necropsy information can be obtained only by following a systematic approach and using ancillary support services as needed to establish a definitive diagnosis. Ancillary support services include histopathology, clinical pathology, microbiology, parasitology and toxicology.

NECROPSY RECORD

Date: _____ Postmortem interval: _____ Case number: _____

Owner: _____ Doctor: _____

Bird's name: _____ Identification: _____

Species: _____ Gender: _____ Age: _____ Weight: _____

Preservation of body: _____ Exposure to other birds: _____ Other bird deaths: _____

Clinical signs prior to death: _____

1. Body condition		18. Adrenal	
2. Oral cavity		19. Esophagus	
3. Integument		20. Crop	
4. Eyes		21. Proventriculus	
5. Ears		22. Intestine	
6. Nares		23. Cloaca	
7. Infraorbital sinus		24. Pancreas	
8. Air sacs		25. Genital	
9. Lungs		26. Subcutaneous	
10. Trachea		27. Musculoskeletal	
11. Pleura		28. Liver	
12. Peritoneum		29. Spleen	
13. Circulatory		30. Heart	
14. Kidney		31. Thymus	
15. Ureter		32. Bursa	
16. Thyroid		33. Bone marrow	
17. Pituitary		34. Nervous system	

NGL = no gross lesions, ABN = abnormal, NE = not examined

Describe abnormalities _____

Disposal arrangements: _____

ANCILLARY DIAGNOSTICS

	Laboratory name	Samples submitted	Shipment method
☐ Histopathology			
☐ Toxicology			
☐ Cytology			
☐ Parasitology			
☐ Microbiology			
☐ Virology			
☐ Other			

Preparing for the Necropsy

Several excellent sources of information, in addition to this textbook, are available to help the clinician verify questionable anatomic structures, identify gross lesions and form a differential diagnosis.[6,12,14-17,22] The clinician should recognize certain limitations of the gross necropsy procedure. While recognition and interpretation of gross lesions may allow construction of a differential diagnosis as to the cause of death, few gross lesions are pathognomonic. Therefore, various ancillary services usually are required to determine the cause of death. Furthermore, communication of clinical, laboratory and necropsy findings to the pathologist will vastly improve interpretation of the tissues and histopathologic evaluation. A close working relationship with a veterinary pathologist who is interested in avian diseases is a definite asset. Lastly, the quality of the final diagnosis is directly proportional to the quality of the specimens submitted and the information provided with them.

Medical Precautions

When performing avian necropsies, the health and well being of the veterinarian and staff members should be considered. Zoonotic diseases of special concern include chlamydiosis, mycobacteriosis, salmonellosis and campylobacteriosis.[5,7,21,25,26,30] Therefore, appropriate protective measures such as surgical masks, eye protection, gloves and disinfectants are recommended. Wetting the carcass with soapy water or disinfectant solutions decreases the possibility of aerosol exposure to potential pathogens and irritating feathers or dander.[30,33] Ventilation hoods or downdraft necropsy tables provide an ideal environment for pathogen containment during avian necropsies; however, such equipment is seldom available in a private practice setting.

Equipment and Supplies

The equipment necessary to perform an avian necropsy will depend on body size, which may vary from a few grams for a Bumblebee Hummingbird to several hundred kilograms for an ostrich.[30] In the case of a small hummingbird, a dissection tray or board, ophthalmic instruments and a magnifying loupe or dissecting microscope may be helpful. With large ratites, rib shears and a Stryker saw will be required.

The body size of most birds encountered in practice will range from a finch to a duck. An assortment of instruments including scissors, poultry shears, scalpels, rongeurs, thumb forceps and hemostats will aid in tissue incision, dissection and specimen procurement. Such instruments should be dedicated for necropsy use only and be thoroughly cleaned and disinfected (eg, glutaraldehyde, phenol, gas, steam) after each use to maintain good functional integrity and prevent carryover of pathogens that could adversely influence future necropsy results. Furthermore, instruments that are sterilized in chemical disinfectants should be rinsed thoroughly before use to avoid killing pathogens in tissues intended for culture.

Ancillary equipment may include sterile swabs[a] and sealable plastic bags[b] to obtain microbiologic and parasitologic specimens; sterile collection tubes for blood, serum or body cavity fluids; and glass slides, stains and a microscope to examine cytologic and blood smear specimens. A camera, macro lens system, flash unit and copy stand can provide photographic documentation of unusual lesions.

The routine fixative for collection of tissue specimens for histologic examination is neutral-buffered 10% formalin solution. Buffering is important to prevent artifacts in the tissues, which can interfere with microscopic examination. Some formalin solution recipes, such as Carson's fixative, provide excellent tissue preservation for both routine histopathology and electron microscopy (see Table 14.2).[27] For more detailed information on sample procurement, refer to the section entitled "Specimen Collection for Ancillary Testing."

Lastly, a printed necropsy form (Figure 14.1) should be available to record important observations. Indelible marking pens should be used to legibly identify all specimen containers concerning patient identification and origin of the specimen(s).

Euthanasia

Euthanasia may be preferred to natural death to alleviate patient suffering. Acceptable methods of euthanasia include carbon dioxide or anesthetic gas

◁ **FIG 14.1** Use of a standard necropsy form (opposite) ensures that all organ systems are examined and important findings are documented.

administration, intravenous barbiturate administration (jugular vein or cerebral sinus) or anesthetic gas administration followed by exsanguination.[29] Of these various techniques, carbon dioxide administration is used least frequently because of excessive terminal motor activity. Anesthetic gas administration is beneficial because blood specimens may be obtained prior to death.

The clinician must realize that the method of euthanasia may have a bearing on gross and microscopic changes observed in necropsy tissues. For example, carbon dioxide-induced hypoxia may result in terminal involuntary motor activity with subsequent bruising, often noted at the base of the skull and misinterpreted as head trauma. Intravenous injection of caustic solutions may result in erythrolysis, edema and coagulative tissue changes, especially within the lungs.

Handling the Carcass Prior to Necropsy

Occasionally, a variable period of time will elapse between the point of death and performance of the necropsy. Examples include the unexpected death of a patient outside of regular clinic hours, delay in obtaining permission for necropsy from the owner or shipment of the carcass to a laboratory for necropsy examination. Unless precautions are taken to minimize autolysis, decomposition of the carcass will limit or preclude the benefits of histopathologic or gross examination of the carcass or various lesions, tissues and organ systems.

Rapid autolysis of avian carcasses is the result of a normally high body temperature (approximately 40°C in adults), body conservation of heat by insulating feathers, and use of incubators, heating pads or heating lamps to increase environmental temperatures of neonates and ill patients. Autolysis may be retarded by soaking the carcass thoroughly in cool soapy water, placing it in a thin plastic bag and storing the body under refrigeration before performing a necropsy or shipping the body to the diagnostic laboratory on ice. When shipping the carcass to a diagnostic laboratory, next-day courier service should be employed to minimize the delay of regular mail service.[6]

A carcass intended for necropsy *should not be frozen.* Placing a carcass directly on ice or dry ice during shipping also may result in freezing of the entire carcass or that portion in contact with the ice. Freezing induces artifactual changes, such as cell lysis and destruction of tissue architecture, which occur as a result of formation and thawing of ice crystals, and may render the tissue nondiagnostic histologically.

The Necropsy Examination

The necropsy examination should begin with a thorough review of the signalment, physical findings, medical history and pertinent laboratory data. An organized, standard necropsy technique is essential for a thorough necropsy examination without overlooking important lesions or organ systems. Because many more mistakes are made from lack of observation than lack of knowledge, a written necropsy protocol should be followed.

External Examination of the Carcass

Carcass identification should be verified by visual inspection based upon signalment (age, species and color) as well as leg band, tattoo or microchip implant data. Leg band numbers and other identifying marks should be recorded on the necropsy form. Palpation of the carcass may reveal fractures; swellings involving subcutaneous air sacs; masses of the skin, subcutis or underlying tissues; or physical deformities. An evaluation of general body condition also should be made, and body weight recorded. A prominent keel may indicate weight loss.

The integument including skin, mucocutaneous junctions, plumage, beak and nails should be examined carefully. Avian skin is generally thin and transparent, in contrast to that of mammals (see Color 24). Accumulations of scales and crusts on legs, feet and cere may indicate bacterial, viral, fungal or parasitic infections. Scabs or swellings involving the skin or mucous membranes may indicate neoplasia, bacterial granulomas or viral-induced lesions (see Color 25). Loss, deformity or color alteration of feathers or fracture of blood feathers could be the result of viral, bacterial, fungal or parasitic infection, as well as trauma or nutritional disease.[19] In birds such as cockatoos and African Grey Parrots, the presence or absence of powder on the beak, legs, feet and nails will provide information concerning proper function of powderdown feathers. Mites should be identified microscopically if present.

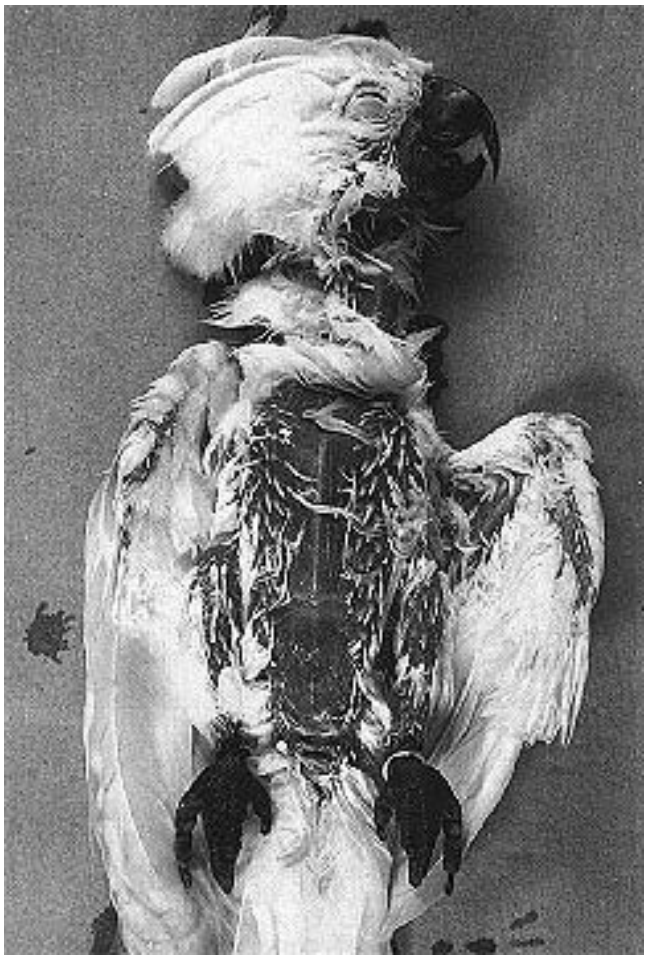


FIG 14.2 Following external examination of the carcass, the plumage has been dampened with soapy water to prevent aerosolization of feather debris and potential pathogens.

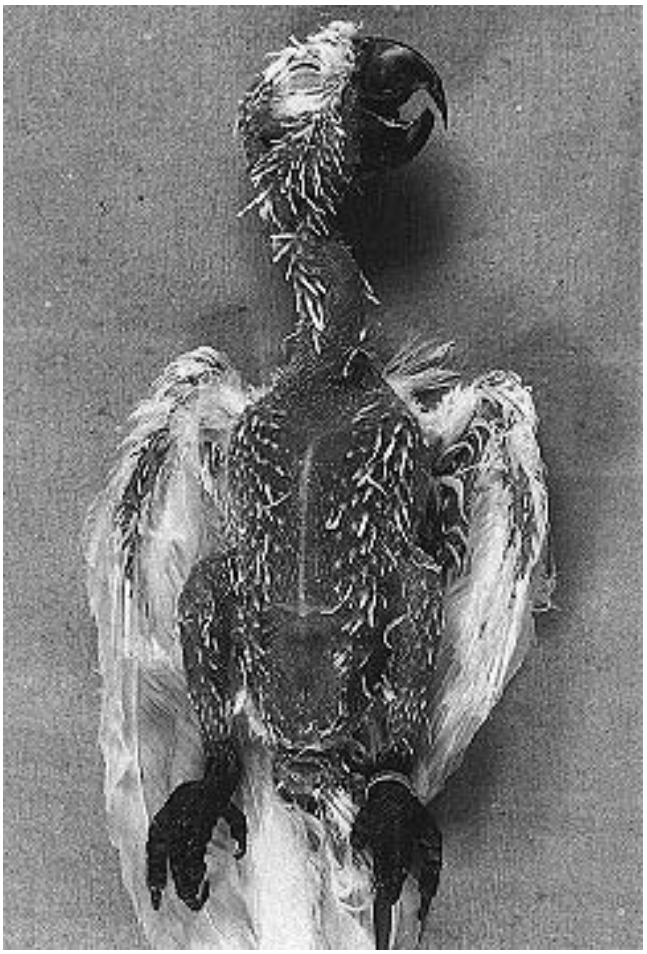


FIG 14.3 The feathers have been removed from the head, neck, ventral thorax and abdomen and legs; the bird is placed in dorsal recumbency.

The beak and nails should be examined for deformities, fissures, fractures or delaminations. Beak pathology could result in difficult prehension of food and subsequent malnutrition. Nail pathology could result in lameness.

All body orifices (eyes, external auditory meatus, nares, oral cavity and vent) should be examined for discharges, masses, foreign bodies, ulcers and plaques. Ocular discharge may be seen with chlamydiosis; bacterial, viral and parasitic infections; or mycoplasmosis. Periocular scabs and masses and oral plaques may be seen in poxvirus infections. Oral plaques alone may be caused by bacterial, viral and parasitic infections as well as by burns, trauma and vitamin A deficiency (see Color 8). Palatine and glossal (tip of the tongue) necrosis may be observed in some birds with psittacine beak and feather disease. Soiling of the vent may indicate enteric disease or cloacal dysfunction. Furthermore, yellow to green discoloration of urates may suggest hepatic or enteric disease (see Color 8). In Amazon parrots, cloacal masses may represent papillomas, which are frequently accompanied by cloacal prolapse. While the external auditory meatus should always be examined, aural pathology is rare, especially in parrots.

At this time, swabs of the choanal slit and vent may be taken for microbiological culture if desired. After cursory external inspection, the feathers may be wetted with soapy water to reduce feather dust and debris (Figure 14.2). The feathers subsequently may be removed to reveal subtle cutaneous pathology such as wounds or hemorrhages. Plucking feathers from the ventral cervical, thoracic and abdominal areas also facilitates further dissection and will avoid obscuring internal lesions (Figure 14.3).

Necropsy Examination

Color 14.1
The majority of the viscera have been removed from the carcass. The liver (l), spleen (s), proventriculus (p), ventriculus (v), duodenal loop (d) and pancreas (arrow) are visible.

Color 14.2
A 25-year-old Scarlet Macaw was presented for egg retention of three days' duration. The egg was surgically removed. The patient became depressed, anorectic and began to regurgitate two weeks postsurgery. Radiographs indicated dilated bowel loops suggestive of an intestinal obstruction. Exploratory laparotomy indicated a fibrous constriction of the ileum. A side-by-side anastomosis was performed, but the bird died postsurgically. Shown are the pancreas (p), inflamed serosal surface of the duodenum (d) and mesenteric hemorrhage (arrow) of the anastomosis.

Color 14.3
Gastrointestinal obstruction and peritonitis in a pheasant with proliferative typhlitis secondary to a *Heterakis isolonche* infection.

Color 14.4
Glistening, transparent membranes typical of normal air sacs in an Umbrella Cockatoo; left caudal thoracic air sac (arrow) and left abdominal air sac (open arrow). The heart (h), proventriculus (p), ventriculus (v) and right liver lobe (l) can also be visualized. Note the position of the reflection of the caudal thoracic air sac from the surface of the liver lobe (see Color 14.18).

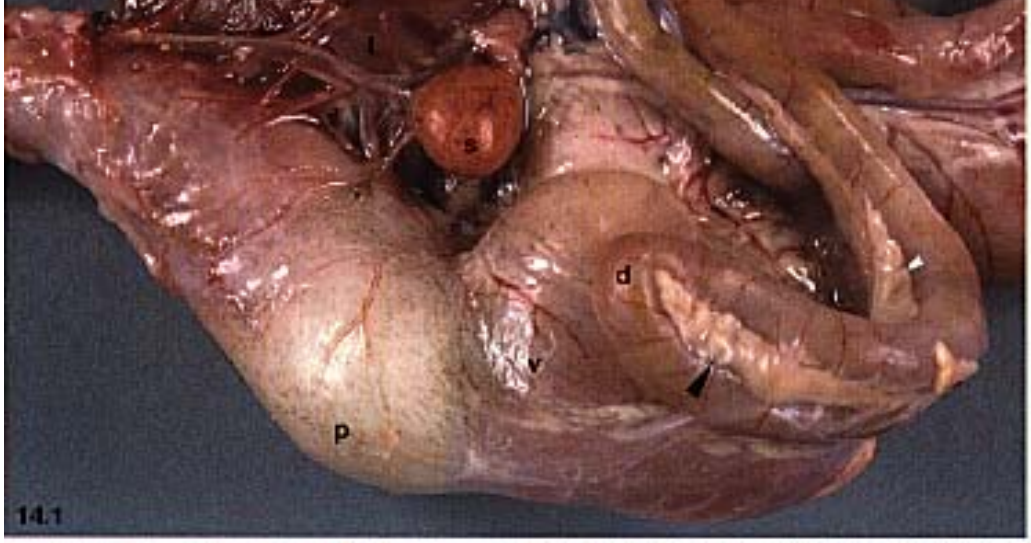
Color 14.5
The pancreas (p) lies between the descending (dd) and ascending (ad) loops of the duodenum. In some species the pancreas is divided into three lobes: the dorsal lobe (arrow), the ventral lobe (open arrow) and the splenic lobe (see Color 14.6), which can be identified only from a dorsal view.

Color 14.6
Distended bile duct (open arrow) in an anorectic cockatoo. Some birds have gall bladders while others do not. In species that do not have gall bladders, bile may accumulate in the right bile duct and appear as though a gall bladder is present. From this dorsal view, the splenic head (arrow) of the pancreas and lateral edge of the liver (l) can also be identified.

Color 14.7
a) An enlarged, hemorrhagic spleen caused by *Pasteurella multocida* in a Common Black Bird (courtesy of R. Korbel). **b)** An enlarged spleen with multifocal granulomas caused by *Yersinia tuberculosis* in a toucan (courtesy of R. Korbel).

Color 14.8
Splenomegaly is a common finding in many bacterial and viral infections. In this case, the enlarged mottled spleen (s) was from a neonatal Blue and Gold Macaw that died from avian polyomavirus. Proventriculus (p), isthmus (i) and ventriculus (v).

Color 14.9
The thoracoabdominal viscera can be visualized by removing the sternum. The right lung (lu), both liver lobes (l), proventriculus (p), ventriculus (v), descending duodenum (dd), ascending duodenum (ad), pancreas (arrow) and colon (c) can be visualized from this view. Note the thin hepatic peritoneal membrane attached to the lobes of the liver.



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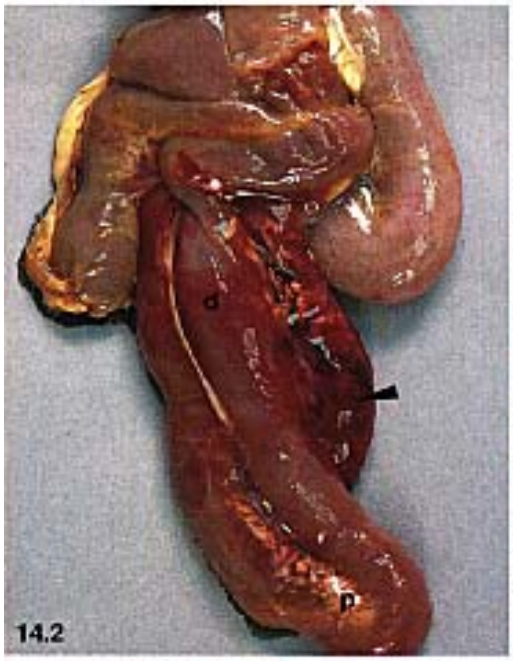

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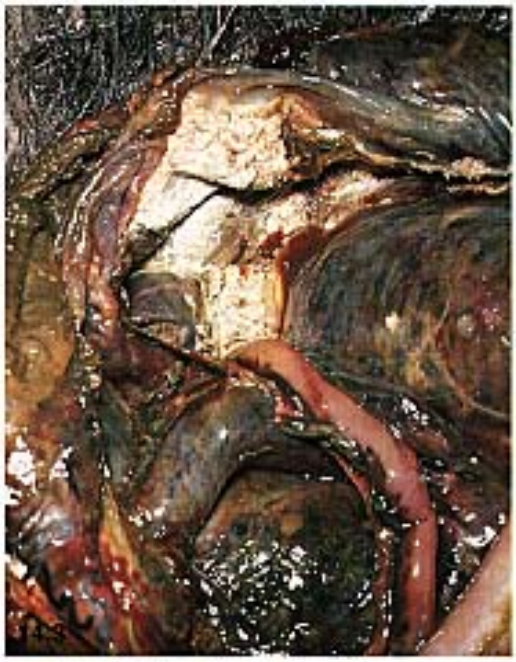

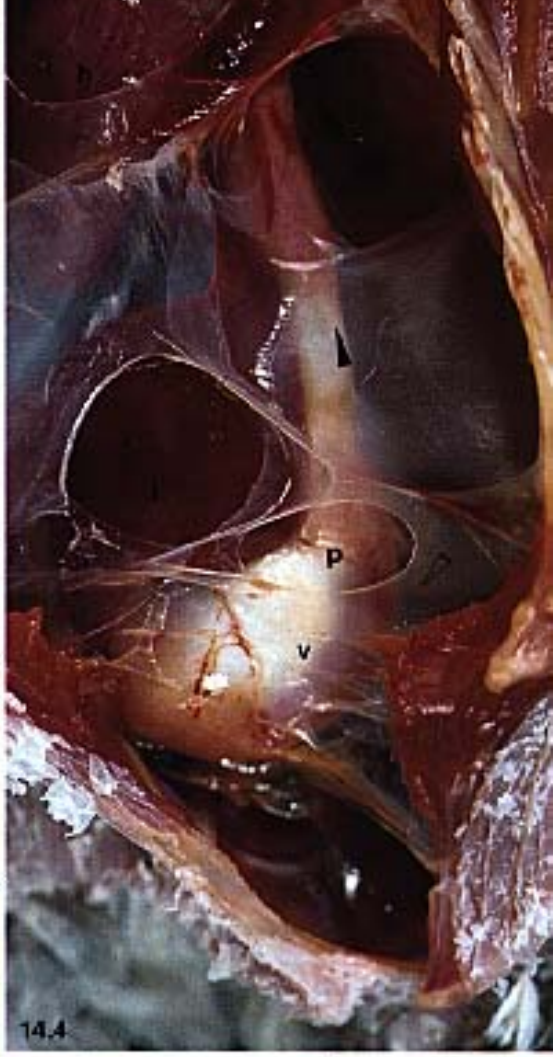

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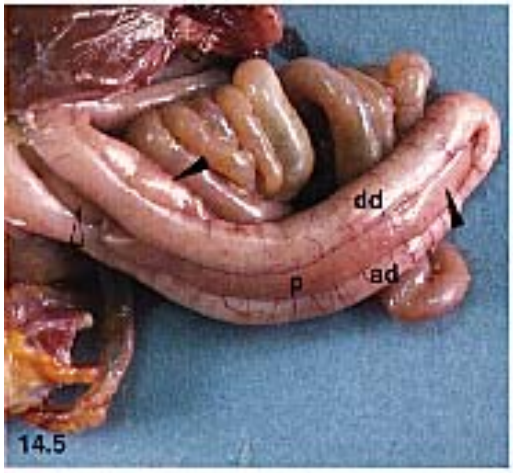

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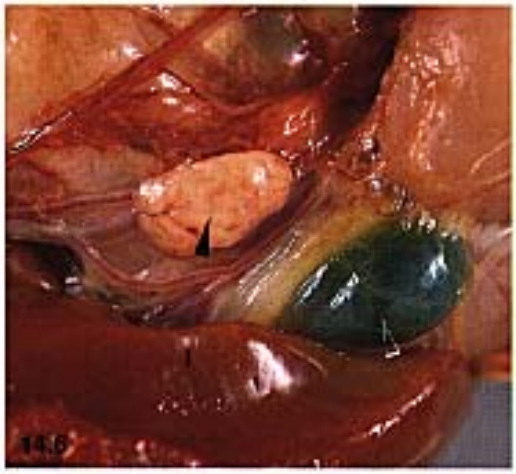
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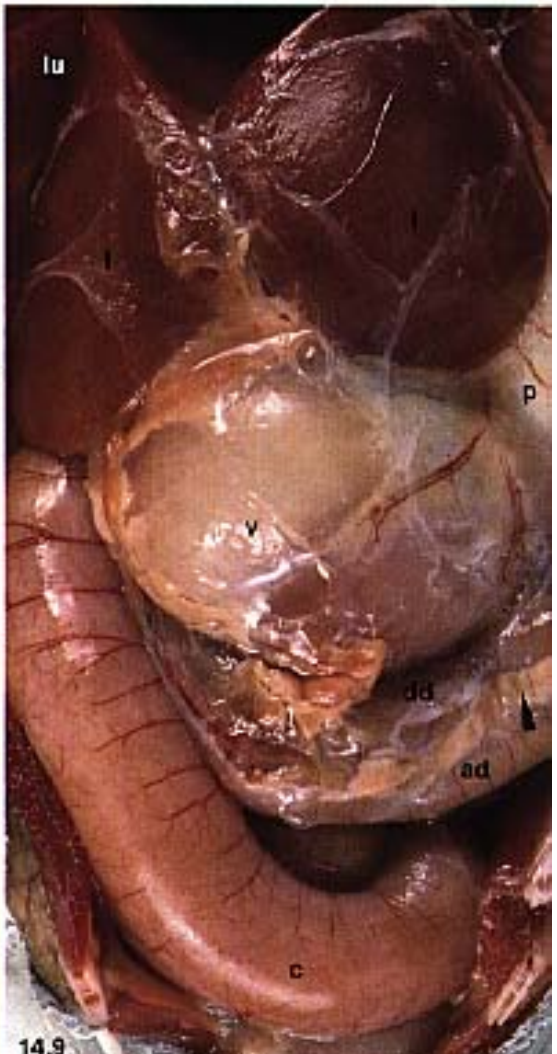

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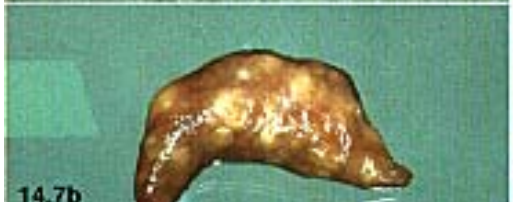
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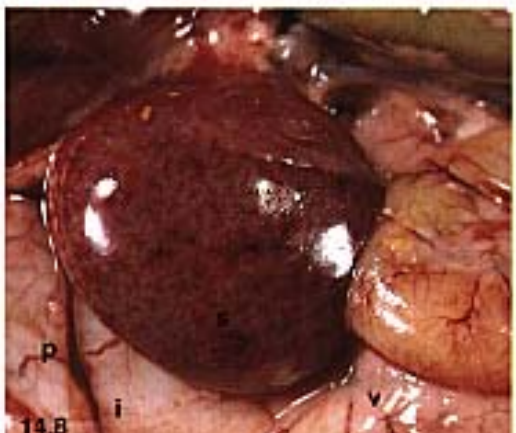

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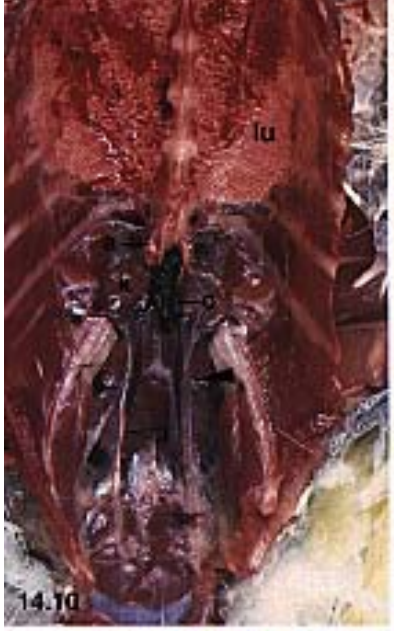
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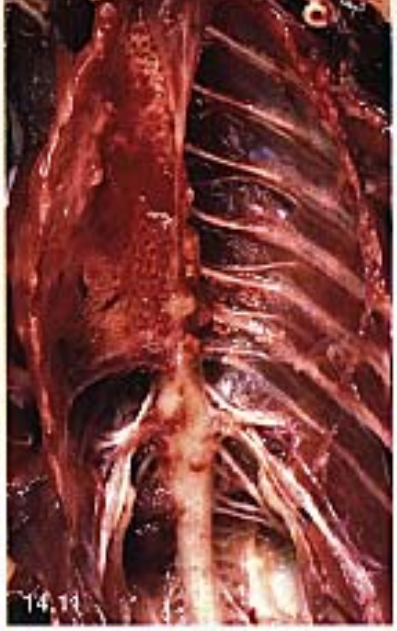
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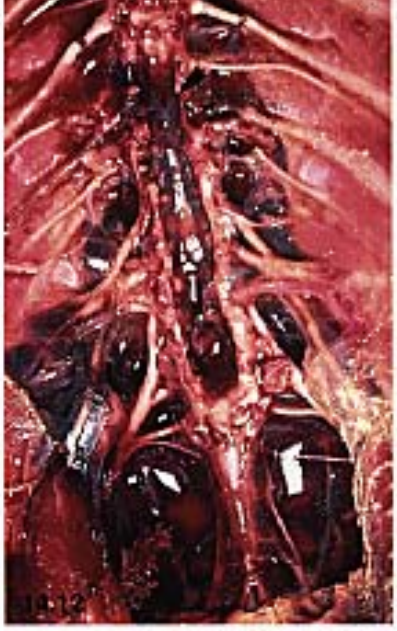
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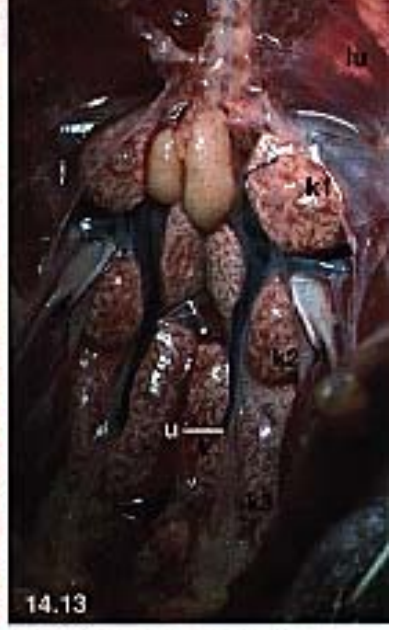
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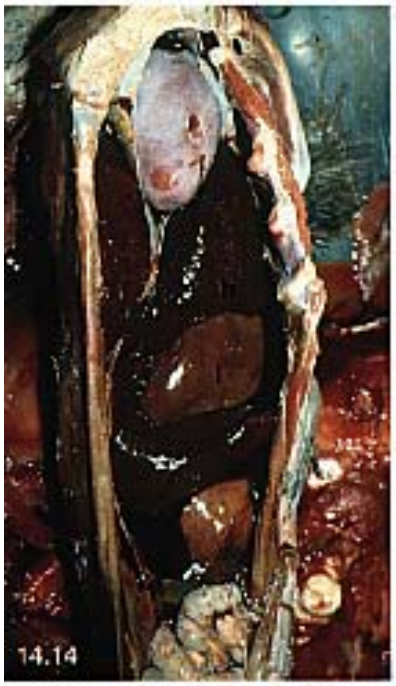
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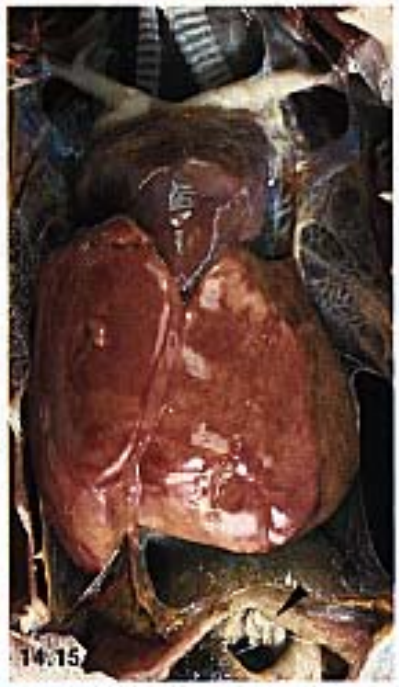
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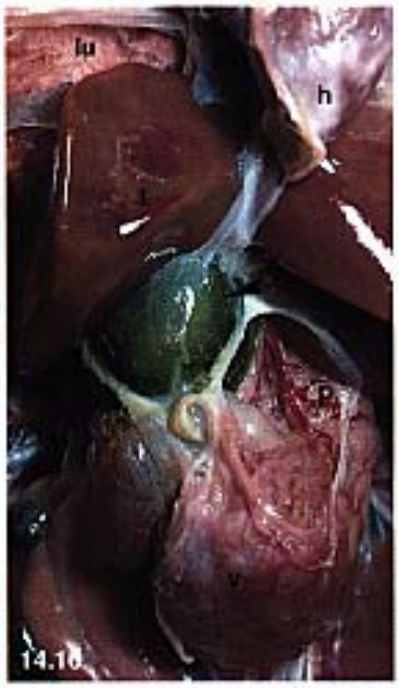
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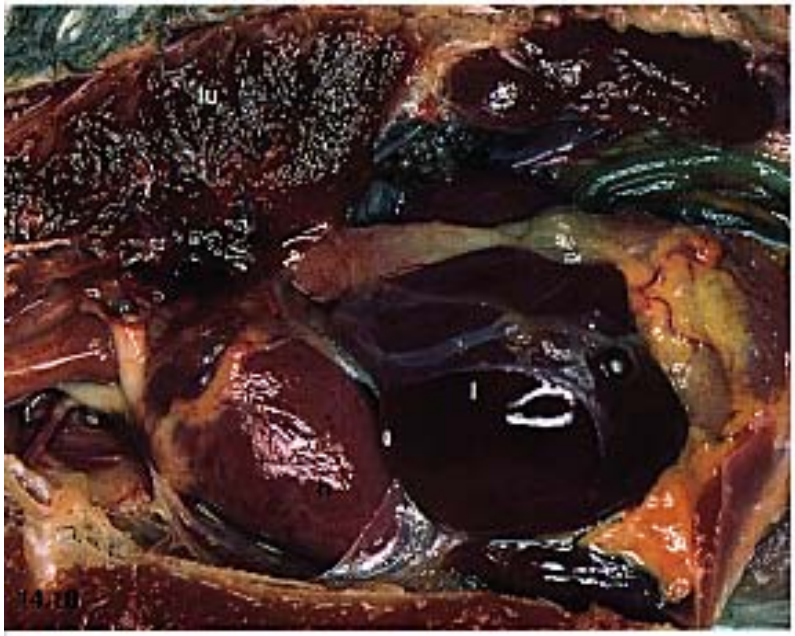
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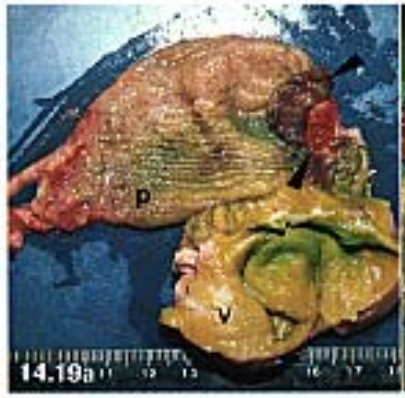
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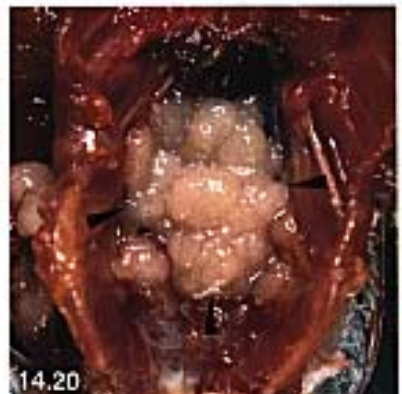
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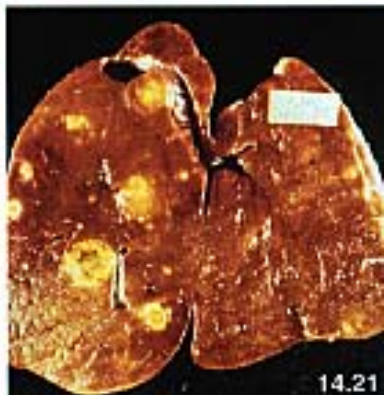
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Necropsy Examination

Color 14.10
The lungs (lu), kidneys (k), ovary (o) and adrenal glands (a) remain in the carcass following removal of the majority of the viscera. Normal lungs appear deep pink and kidneys appear red-brown. Note the inactive oviduct (arrow) and ureter (open arrow). In health, the kidneys appear dark red-brown and are embedded within the renal fossae. The adrenal glands are small, round, yellow structures at the cranial divisions of the kidneys. The quiescent ovary of this bird is granular and pigmented (melanin pigment) (courtesy of Ken Latimer).

Color 14.11
The left lung has been removed to demonstrate its normal anatomic position in the dorsal thoracic cavity. The lung is attached to the dorsal body wall and interdigitates with the spinal processes and ribs.

Color 14.12
Trauma-induced spinal cord hemorrhage (arrow) in a cockatoo. The ventral vertebral structures have been removed for visualization.

Color 14.13
Pale kidneys in an anemic male Amazon parrot. Cranial division of left kidney (k1), middle division of left kidney (k2), caudal division of left kidney (k3), lung (lu), common iliac vein (arrow), caudal renal vein (open arrow) and ureters (u).

Color 14.14
Hepatic rupture and hemorrhage (h) in a six-month-old emu with *Clostridium shovia*. Infected birds frequently die of exsanguination secondary to the tears in the liver (l) (courtesy of Brett Hopkins).

Color 14.15
A Barn Owl was presented with severe depression and weight loss. A palpable mass was present in the lower abdominal cavity. Abdominocentesis indicated the presence of a septic exudate containing numerous gram-negative bacteria. At necropsy, a perforating lesion was noted in the proventriculus (arrow), and the liver was enlarged, pale and mottled. Histopathology indicated a gram-negative septicemia with hepatitis and peritonitis.

Color 14.16
Cystic dilatation of the right bile duct (arrow) in an anorectic Amazon parrot. The accumulation of bile was detected radiographically as a fluid-filled mass slightly dorsal to the hepatic shadow. Lung (lu), heart with thickened, opaque pericardium (h), liver (l), proventriculus (p) and ventriculus (v).

Color 14.17
Congested, swollen kidneys in a male Scarlet Macaw with aspergillosis. Note the plaques (open arrows) on the right testicle. Note the plaques (arrows) and thickening of the dorsal wall of the intestinal peritoneal cavity.

Color 14.18
A mature Rose-breasted Cockatoo was presented with an acute onset of depression, dyspnea and syncope. The bird did not respond to supportive care. Necropsy findings included dark, congested lungs (lu), an enlarged, congested liver (l) (note the line of reflection of the lateral wall of the caudal thoracic air sac from the liver's surface, see Color 14.4), enlarged, congested kidneys (k) and an enlarged heart (h) with petechiation. Histopathology indicated *Sarcocystis* sp. The bird was housed indoors but the food was kept in an open container and was contaminated with roach feces.

Color 14.19
a) Proventriculus (p) and ventriculus (v) from a one-month-old ostrich. Note the hemorrhage and ulceration (arrows) at the isthmus, which is common in birds with *Clostridium perfringen* infections. This bacteria secretes an exotoxin that causes generalized vasculitis and is associated with atony of the proventriculus. **b)** Similar *C. perfringen*-induced lesions in the proventriculus of a 23-month-old ostrich (courtesy of Brett Hopkins).

Color 14.20
A mature cockatiel hen was presented with depression and severe abdominal distention. The bird did not respond to supportive care. At necropsy, multiple masses were identified in association with the pancreas and dorsal body wall. Histopathology indicated a pancreatic adenocarcinoma with carcinomatosis (arrows) of the abdominal cavity (courtesy of Cheryl Greenacre).

Color 14.21
Diffuse amyloidosis in the liver of an American Merganser. Focal granulomatous lesions characteristic of *Mycobacterium* sp. are also noted. Amyloidosis commonly occurs in waterfowl with chronic inflammatory diseases (courtesy of R. J. Montali).

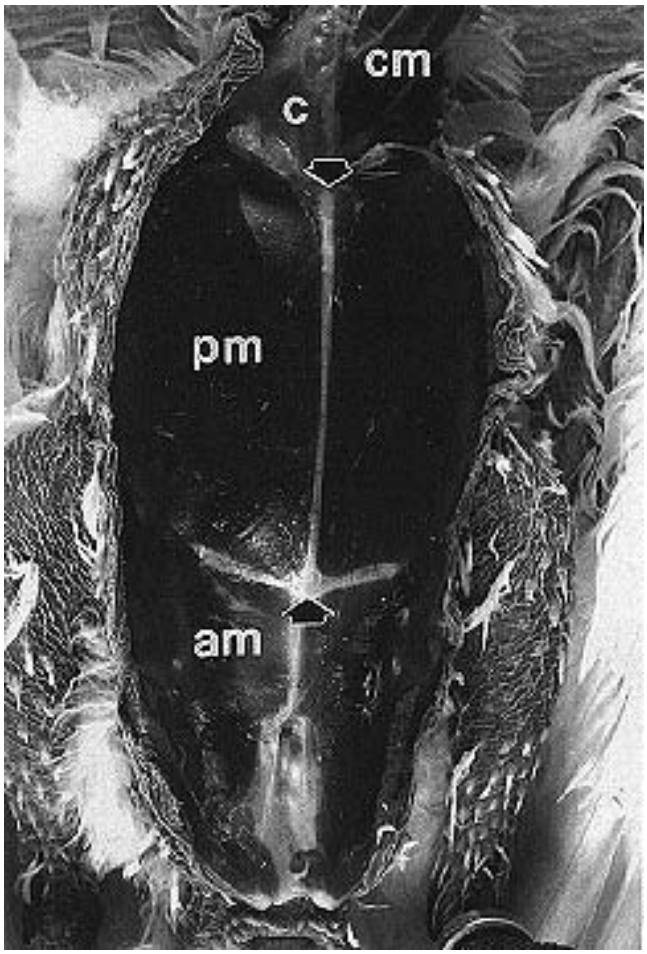


FIG 14.4 The skin has been incised and reflected. The proximal cervical musculature (cm), crop (c), pectoral musculature (pm) and abdominal musculature (am) have been exposed. The keel of the sternum identifies the ventral midline (arrows).

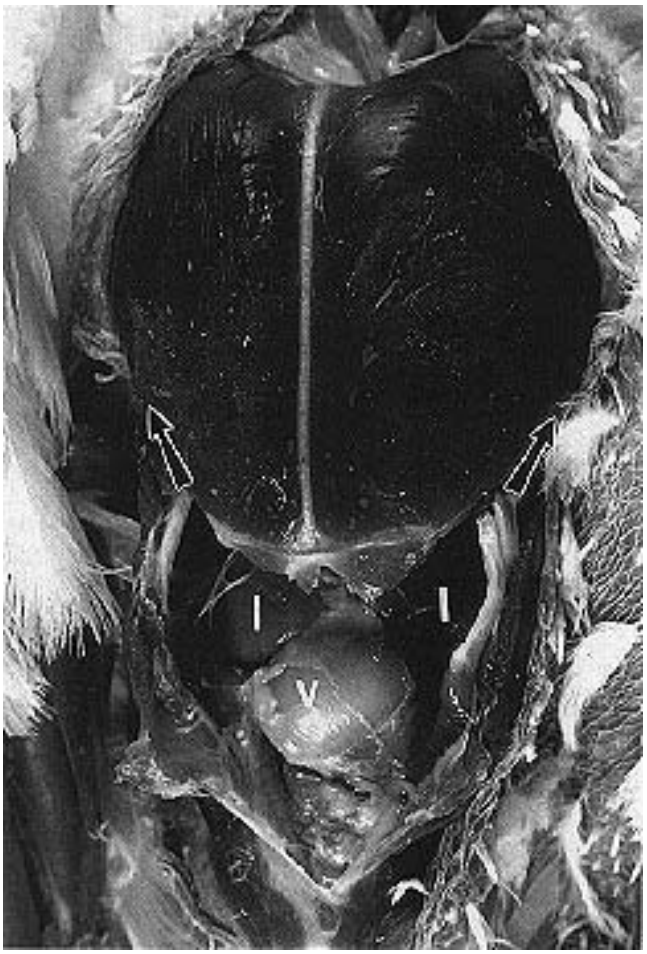


FIG 14.5 An incision has been made through the abdominal musculature and continued around the left and right margins of the sternal plate (arrows). The posterior portions of the left and right hepatic lobes (l) and ventriculus (v) are observed.

When external examination of the carcass is complete, survey radiographs may be taken if heavy metal toxicosis is suspected. These radiographs may assist the clinician in localizing metal densities that may be collected for analysis during the necropsy.

Initial Dissection

The bird is placed in dorsal recumbency for initial dissection (Figure 14.3). With very small birds, the wings and legs may be pinned to a dissecting tray or board to immobilize the carcass. With larger birds such as ducks or geese, the coxofemoral joints may be disarticulated by incising the skin, adductor muscles of the medial thigh and coxofemoral joint capsule. The knees are then forced craniolateral. Using a scalpel and scissors, a ventral midline incision is made from the intermandibular area to the pelvic area, encircling the vent. The skin is reflected by blunt dissection to reveal the underlying cervical musculature, trachea, crop, keel and pectoral and abdominal musculature (Figure 14.4).

Normal pectoral musculature of most companion birds is plump and appears red-brown. The musculature should be examined for hemorrhage, penetrating wounds, pallor, pale streaking or loss of total mass. Pallor or pale streaking may represent muscle necrosis, inflammation or neoplasia. Pale streaking of the pectoral musculature may be observed in feral birds with sarcocystosis. Muscle wasting is often a sign of inanition.

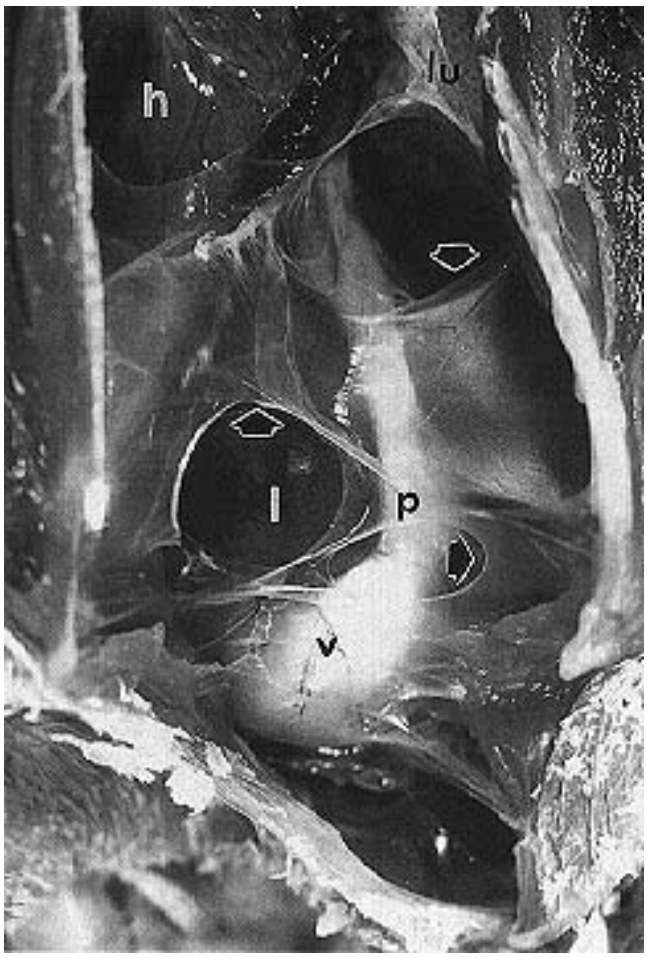


FIG 14.6 Normal air sacs (arrows) appear as glistening, transparent membranes that can be visualized as the sternal plate is lifted. Portions of the heart (h), lung (lu), liver (l), proventriculus (p) and ventriculus (v) also are visualized.

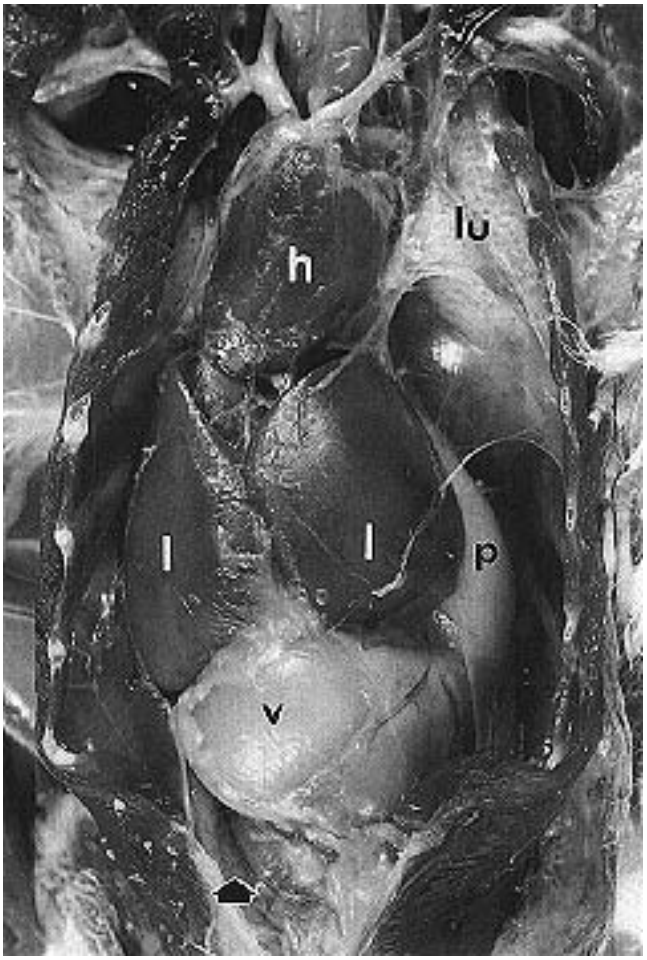


FIG 14.7 The sternal plate and a portion of the abdominal musculature have been removed to expose the thoracoabdominal viscera for gross examination. The heart (h), lung (lu), right and left hepatic lobes (l), proventriculus (p) and ventriculus (v) are identified. A small segment of the duodenum also is observed (arrow).

Exposure of the Thoracoabdominal Cavity

An incision is made through the abdominal musculature at the distal tip of the sternum. The incision is continued left and right through the pectoral musculature lateral to the sternum (Figure 14.5), which can be lifted craniodorsal to expose the thoracic and abdominal air sacs (Figure 14.6). Normal air sacs appear as glistening transparent membranes (Color 14.4). If air sacs appear opaque or contain accumulations of fluid or exudate, appropriate specimens should be obtained for microbiological culture or cytology before the field is contaminated (see Color 22). Air sac tissues collected for histopathology should be placed on a small piece of paper before fixation. This will facilitate identification of the tissue for processing and minimize the possibility that these transparent membranes will be discarded inadvertently.

The sternal plate is removed by continuing to incise the thoracic musculature and transecting the ribs, coracoid bones and clavicles using scissors, rongeurs or poultry shears (large pruning shears may be necessary for ratites). The midline incision is extended caudad through the abdominal musculature proximal to the vent; care should be taken to prevent incising the cloaca. The pectoral musculature of the sternal plate may be incised and examined; any abnormal tissue is collected for cytologic imprints and histopathologic evaluation. Abdominal wall musculature is then removed as necessary to expose the viscera within the body cavity (Figure 14.7).

Examination of Thoracoabdominal Viscera *In Situ*

Several gross observations should be made before the viscera are disturbed. The presence of fluid, exudates or fibrin tags within the thoracoabdominal cavity should be noted (minimal fluid is present in health). Air sac remnants can be examined further for opacity related to bacterial, chlamydial or fungal infection. Aspergillosis is observed commonly in the abdominal air sacs and appears as a velvet-like yellow to green mat (see Color 22). A small amount of fat may be observed normally in the abdomen, around the cloaca and within the coronary groove. Excessive fat may be present in obese companion birds, while serous (gelatinous) atrophy of fat may occur with inanition. The pericardial sac should be relatively transparent and contain little measurable fluid (Color 14.25). A white chalky discoloration may indicate visceral gout from urate deposition (see Color 21). White streaks occasionally are present on the pericardial sac and epicardium following euthanasia by intracardiac injection. Petechial epicardial hemorrhages may represent septicemia or be observed as an agonal event (Color 14.26).

The liver is mahogany brown and bilobed, extending around the left and right margins of the heart. In psittacine birds, the right lobe is larger, occasionally giving it an asymmetric appearance (Color 14.9). A swollen, pale-yellow liver may be observed in severe hepatic lipidosis or may represent a normal finding in neonates that are mobilizing egg yolk (see Color 30). Diffuse yellow-orange discoloration of the liver may be observed in severe hemosiderosis, which occurs with some frequency in mynah birds. Multifocal white-to-yellow discoloration of the hepatic parenchyma suggests necrosis secondary to chlamydial, bacterial or viral infection (see Color 20). Large umbilicated lesions in the liver, especially in peafowl, are strongly suggestive of histomoniasis. Pallor of the hepatic parenchyma may be observed in severely anemic birds. The gallbladder should be examined if present (some birds lack a gallbladder), and the patency of the common bile duct should be determined if possible (Color 14.16).

The heart and great vessels are examined next. The epicardium should be examined for petechiation. The heart is roughly triangular with the length slightly exceeding the width. Any alteration in the size or shape (eg, globose shape) of the heart should be noted.

As the great vessels are examined, any changes in the size of the thyroid and parathyroid glands also should be recorded. These glandular structures are located at the thoracic inlet lateral to the syrinx and adjacent to the jugular veins and carotid arteries. Normal thyroid glands are small, oval and reddish-brown (Color 14.22). The parathyroid glands are very small and best distinguished microscopically. In dietary-induced secondary hyperparathyroidism, the parathyroid glands will appear as enlarged circular off-white to yellow structures (Color 14.29).

A small portion of the ventriculus may be observed ventral to the liver. Much of the caudal portion of this organ is obscured by the duodenal loop and pancreas. The proventriculus is located beneath the left liver lobe and may not always be visible unless severely dilated (Color 14.9).[4]

FIG 14.8 The syringeal area is a common location for pathologic lesions of the respiratory tract. This area should be carefully examined and the trachea (t) and syrinx (s) should be opened under sterile conditions to collect samples for bacterial, fungal or viral isolation in birds with respiratory sounds. Note the reduction in size of the primary bronchi as they leave the syrinx (arrows). The thoracic esophagus (e) is dorsolateral to the trachea at the level of the syrinx and then courses from right to left to connect to the proventriculus. (vs) = ventral syringeal and (ds) = dorsal tracheobronchial muscles.

Disease-induced alterations in gastrointestinal morphology usually are quite subtle grossly and may be limited to congestion, hemorrhage or gas-filled intestinal loops (this latter change also is observed commonly following a long postmortem interval). Gas-filled intestinal loops and discoloration due to altered intestinal contents or hemorrhage should be noted. On rare occasions, gastrointestinal lesions may be quite striking. Examples include gastrointestinal tract obstruction and impaction in pheasants with proliferative typhlitis secondary to *Heterakis isolonche* infestation, severe nematode or trematode infestations, surgically-induced visceral adhesions, marked proventricular distention in birds with neuropathic gastric dilatation and severe egg-related peritonitis (Color 14.2, 14.3).

In hens, the viscera are reflected on the left side of the dorsal thoracoabdominal cavity to examine the communication of the colon and oviduct with the cloaca. The cloacal bursa may be partially visualized, especially in juvenile birds.

Removal and Examination of the Viscera

The heart is removed by transecting the great vessels. At this time the thyroid and parathyroid glands also may be collected while they are easily identified.

The epicardial surface should be examined for changes in size, shape and color. The heart of small birds may be transected near the apex and placed whole in formalin solution. In larger birds, the heart may be opened to inspect the valves and chambers; sections of tissue may be taken for formalin fixation.

The tongue and oral mucosa should be inspected for erosions, ulcers, plaques or masses. The tongue is freed by transecting the hyoid apparatus and pharyngeal tissues in the intermandibular region. Gentle traction is applied to remove the tongue, esophagus, crop, trachea and thymus with attached large vessels. The thymus may appear as pale tan to gray lobules of tissue extending along the cervical fascial planes adjacent to the trachea. This organ undergoes involution as sexual maturity is reached. The distal trachea is transected below the syrinx, leaving the lungs for later dissection (Figure 14.8). The esophagus is transected just below the syrinx and lifted upward. The ligamentous attachments, air sacs, blood vessels and ureters (including the oviduct if present) are transected and the vent area is excised with an intact margin of skin. The entire gastrointestinal tract, along with the liver and spleen, is removed from the carcass. The adrenal glands, gonads and kidneys remain in the carcass.

The spleen may be found dorsally in the angle between the ventriculus and proventriculus (Color 14.8). It appears as a variably-sized, round to elongate, red-brown structure. It should be removed and examined. Swelling and tan discoloration suggest inflammation or infection (viral, bacterial, chlamydial or protozoal such as atoxoplasmosis). Cytologic imprints may be made and a small portion removed for microbiological culture; the remainder is fixed in formalin solution.

The liver, gallbladder (if present) and patency of the bile duct connections to the duodenum should be examined. Excess accumulation of bile may cause gross distention of the bile ducts. The liver is removed, and its color, size and texture are examined in more detail. The parenchyma is examined by making several transverse slices through the organ with a sharp knife or scalpel. Lesions are imprinted and appropriate specimens are fixed for histopathologic examination, and fresh tissue is retained for other ancillary tests (microbiologic culture or toxicologic analysis) as necessary.

Lobules of thymic tissue, if present, are preserved for histopathologic examination. The esophagus, crop and trachea should be opened and the luminal surfaces and contents examined. Any abnormalities such as hemorrhage, erosion or ulceration and plaques or masses should be noted and appropriate portions of tissue imprinted, preserved in formalin solution and retained for other analyses (see Color 22). The crop contents should be examined carefully, especially in cases of unexplained death where poisonous plants may have been ingested. Crop contents may be collected for analysis if toxicosis is suspected.

The proventriculus and ventriculus are opened and examined for surface erosions or ulcers and foreign bodies. The morphology of the ventriculus varies

CLINICAL APPLICATIONS

- Maximum necropsy information can be obtained only by following a systematic approach and using ancillary support services as needed to establish a definitive diagnosis.
- The final diagnosis is directly proportional to the quality of the specimens submitted and the information provided with them.
- A telephone call or fax to the diagnostic laboratory prior to performing the necropsy is a prudent measure to ensure correct specimen collection, preparation and handling.

with the species of bird and its diet. The ventriculus of seed-eating and omnivorous birds has a thick muscular wall, and the mucosa has a koilin lining (thick horny material) that is often bile-stained. In carnivorous and piscivorous birds, the ventriculus may be fusiform, thinner-walled and blend with the proventriculus.[14]

The intestine may be opened in large birds and inspected for luminal hemorrhage, erosions, ulcerations or parasites. Direct visualization of parasites is noted and intact organisms may be preserved in appropriate fixatives for later identification (see Table 14.4). Wet mounts of intestinal contents and mucosal scrapings should be examined microscopically to identify protozoa (giardia, cryptosporidia), parasite ova or merozoites (coccidia). In large birds, various portions of the intestinal tract may be excised and preserved for histopathologic examination. In tiny birds, the intestine may be fixed *in toto* without gross examination, but it should be cut into multiple sections to allow adequate penetration of the formalin fixative. Portions of intestine also may be retained in a sealable plastic bag for microbiologic culture.

The terminal colon and cloaca should be examined externally and internally. Patency of the colon, ureters and oviduct, if present, should be determined. In some species of birds, such as pheasants and peafowl, the ceca should be examined for the presence of inspissated exudates, masses, parasites or other lesions.

The bursa of Fabricius generally may be found associated with the dorsal wall of the cloaca. Grossly, this organ may resemble a large lymph node in young birds (see Figure 5.6). In older individuals, the bursa may have involuted and will be difficult to identify. Histopathologic examination of formalin-fixed cloacal tissue may allow identification of bursal remnants following involution.

In juvenile hens, the reproductive tract will be minimally developed. The ovary will be small and have a slightly granular appearance (see Colors 13, 29). Adult hens that are sexually quiescent or severely stressed may experience atrophy of the reproductive tract, resembling a juvenile hen. In sexually active hens, the oviduct is a prominent, large, off-white, flaccid, vascular, hollow tubular organ with a rugose luminal surface.[17] Egg binding may induce inflammation wherein the distal wall of the oviduct will appear thickened. The oviduct also may be the origin of adenocarcinoma, especially in budgerigars. Hens that have undergone stress may have the uterus and ovaries reduced in size to that of juveniles due to alterations in hormonal secretions.

Removal of the majority of the viscera permits inspection of the lungs *in situ*. Normal lungs are deep pink. The lungs should be examined for areas of discoloration or other abnormalities. A dark red, wet appearance of the lungs suggests pulmonary edema and hemorrhage, which may accompany acute pulmonary sarcocystosis, polytetrafluoroethylene (Teflon®) toxicosis, inhalation of noxious gases, carbon monoxide asphyxiation or necrotizing bacterial pneumonia (see Color 22). Fungal pneumonia may present as cavitating nodules, the walls of which have a velvety green lining.

Because avian lungs are attached to the dorsal ribcage, removal requires gentle traction along with blunt and sharp dissection (see Chapter 22). The lung parenchyma should be transected at 0.5 cm intervals (as with the liver) to look for occult lesions such as bronchial exudates, particulate debris and areas of consolidation or cavitation. In small birds, use of a magnifying loupe may facilitate identification of particulate debris in aspiration pneumonia.

Next, the kidneys, gonads and adrenal glands are inspected *in situ* (Color 14.10). These organs are removed as a single unit by careful dissection, especially in regard to removing the kidneys from the renal fossae of the synsacrum. The sacral plexus is embedded within the kidney, which makes removal of the kidneys difficult. Normal kidney tissue is dark red-brown. The renal parenchyma is examined for discoloration, pallor, swelling or masses or linear white foci that may indicate renal gout. Removal of the kidneys may be impossible in some small birds; however, this portion of the synsacrum may be removed from the carcass and fixed *in toto*. The tissue subsequently may be decalcified, pro-cessed, embedded in paraffin and sectioned *en bloc*.

The testes of male birds are elongate to cylindrical organs near the anterior portion of the kidneys (Color 14.13).[15] Juvenile testes are yellow due to interstitial cell lipid.[17] These organs undergo cyclic atrophy and enlargement in sexually mature individuals and may be quite large in breeding birds.[14] The testes of adult male birds appear large and are commonly white with a vascular surface. Some species of male birds have melanistic testicles.

Only the left ovary normally persists in psittacine hens (Figure 14.9). In some species (eg, some raptors),

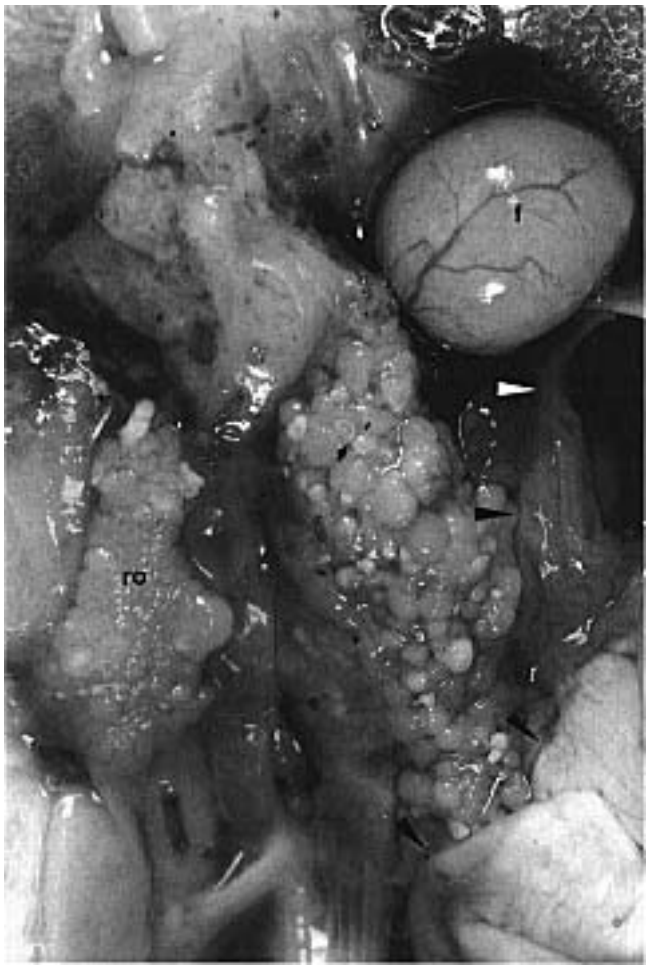


FIG 14.9 In a normal psittacine hen, only the left ovary and oviduct are present. Occasionally, the right ovary (ro) and oviduct will also be present. Note the large follicle (f) and enlarged left oviduct (arrows) indicative of a reproductively active hen.

the ovaries are frequently bilateral. The juvenile left ovary appears yellow and granular, resembling a piece of fat.[15] Variably-sized, vascular, yellow follicles are present in sexually active hens (see Color 13, 29). The yellow color is imparted by variable quantities of yolk.[17] In some species of female birds, the ovary may be pigmented.

The adrenal glands are identified as small, round, yellow structures to the left and right of the midline at the cranial pole of the kidneys. Adrenal gland enlargement may be observed in chronically stressed birds.

The remainder of the carcass consists of the musculoskeletal, integumentary and nervous systems. Specimens of skin, feather follicles and feathers may be taken for histopathology if they have not already been obtained. Abnormal, newly emerging feathers and associated follicles provide the best diagnostic specimens. Sections of the uropygial gland may be taken from appropriate species if masses are palpated or observed.

Examination of Special Organs and Tissues

Examination of the nervous system and associated tissues is governed by the presence or absence of neurologic or ocular disease. Although the brain and ischiatic (sciatic) nerves are routinely obtained for histologic evaluation, the spinal cord, brachial and sacral nerve plexuses and eyes are obtained only if pathology is present.

Brain

The brain is relatively accessible and is frequently obtained for routine histopathologic examination (Figure 14.10). The brain may be removed by plucking the feathers from the head, incising the scalp and reflecting it. A sagittal incision is made through the calvarium using a pair of blunt-sharp scissors. Using a forceps or rongeurs, the bony calvarium is removed as necessary to expose the brain.

Before removing the brain from the calvarium, it should be inspected for congestion or hemorrhage. Depending upon the rapidity of death or method of euthanasia, agonal hemorrhage may be observed in birds following severe terminal motor activity. Agonal hemorrhage must be distinguished from antemortem head trauma if possible.[6,14] Greenish bruising is more typical of old hemorrhage. The brain may be removed from the calvarium by severing the cranial nerves from rostral to caudal (Figure 14.11). The optic tectum (a bony plate that covers the large optic lobes) may present a problem in removing the brain from psittacine birds. In hatchlings, the calvarium is soft and may be transected through the midline with a scalpel. The halves of the calvarium may be fixed *in toto* or one-half of the calvarium may be retained for culture.

Vertebral Column

If neurologic disease involves spinal cord or nerve roots, appropriate sections of the vertebral column or synsacrum may be identified, removed *en bloc* and fixed in formalin solution.[6] The pathologist subsequently can decalcify these tissues and section them with a knife or scalpel to discern subtle gross lesions. These tissue sections can be processed and examined microscopically to evaluate nervous tissue, bone and attached soft tissues.

Necropsy Examination

Color 14.22
Normal thyroid glands (arrows) appear as small, oval, red-brown structures adjacent to the carotid arteries. The parathyroid glands are present at the caudal pole of the thyroid gland but are normally minuscule.

Color 14.23
A greater than 20-year-old Rosella was presented with a history of feather dystrophy and exercise intolerance. The bird was DNA probe-positive for PBFD virus. The bird died shortly after presentation, and at necropsy the great vessels were noted to be hard, irregularly shaped and yellow. The vessels were partially calcified and the histologic diagnosis was atherosclerosis.

Color 14.24
Normal heart (h), liver (l) and lungs (lu) demonstrating the relationship of these organs in the cranial portion of the thoracic cavity. Note the pericardial sac (arrow) and the left and right hepatic peritoneal membranes (open arrows). The ostium (o) of the caudal thoracic air sac is also clearly visible through the transparent, contiguous wall of the cranial thoracic and caudal thoracic air sacs.

Color 14.25
A mature Moluccan Cockatoo was presented for an acute onset of lethargy, dyspnea and weakness. The PCV was 12, and a large quantity of blood was noted in the right axillary and neck region. The bird was given a blood transfusion but did not survive. Necropsy indicated a pale heart and liver, and a ruptured brachial artery. The pale heart is shown resting in an increased quantity of clear pericardial fluid.

Color 14.26
A male duck from a zoological collection was found dead in its enclosure. Necropsy findings indicated multifocal, petechial hemorrhage in the epicardium. *Pseudomonas* sp. was isolated from the heart blood. Multifocal, myocardial, petechial hemorrhage can be an indication of septicemia or can represent agonal hemorrhage. Note the syringeal bulla (arrows) that is an extension of the trachea found in some male ducks.

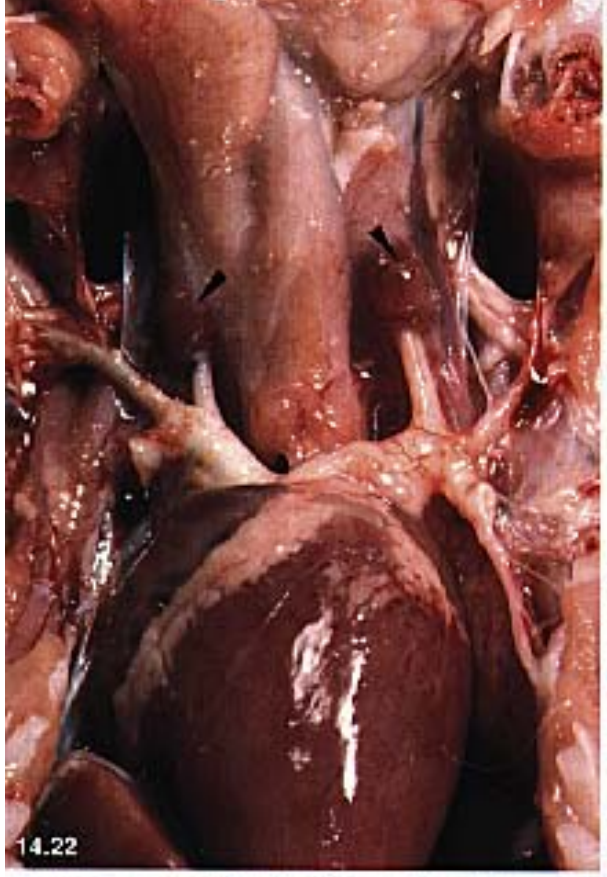
Color 14.27
Pericardial effusion (arrow) can occur from several bacterial or viral diseases. In this case, hydropericardium was associated with avian viral serositis in a Blue and Gold Macaw neonate.

Color 14.28
A wild-caught Ducorps' Cockatoo was presented with abnormal feather development. DNA probe testing of a blood sample confirmed the clinical diagnosis of PBFD. During routine necropsy, filariid worms (arrows) were removed from the right ventricle. The parasites were identified as a new species of filariid worms, *Chandlerella* sp. (courtesy of Ken Latimer).

Color 14.29
A mature, female Amazon parrot with a history of an all-seed diet was presented for evaluation. The hen had been a consistent egg producer for several years. The bird was provided cuttlebone that was seldom consumed. The bird flew into a wall and sustained multiple fractures. Radiographs indicated metabolic bone disease and egg-related peritonitis. Finding enlarged hyperplastic parathyroid glands (pt) suggested nutritional secondary hyperparathyroidism. The normal syringeal muscles (s), trachea (t), thyroid (th) and thoracic esophagus (e) can be visualized. Note how the thoracic esophagus passes dorsally to the syrinx at the level of the heart.

Color 14.30
Pericarditis can be caused by many bacterial, fungal or viral pathogens. In this Amazon parrot, the pericarditis with plaques was secondary to a gram-negative bacterial septicemia. Note the congestion of the liver.

Color 14.31
A 32-year-old Green-winged Macaw was presented for progressive weakness of several weeks' duration. The bird was recumbent, depressed and severely dyspneic. The bird died shortly after presentation. Necropsy indicated a pale, mottled heart. Histopathologic changes included atherosclerosis and myocardial fibrosis.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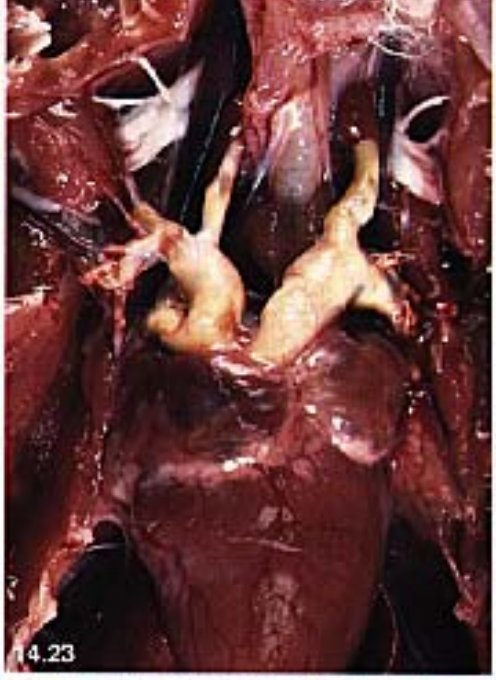
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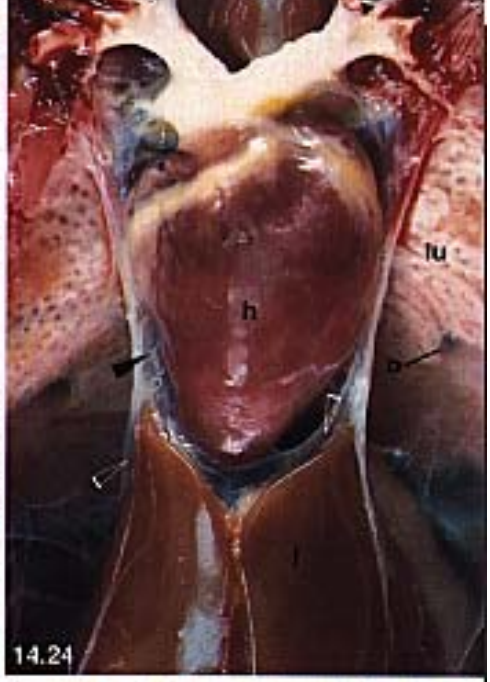

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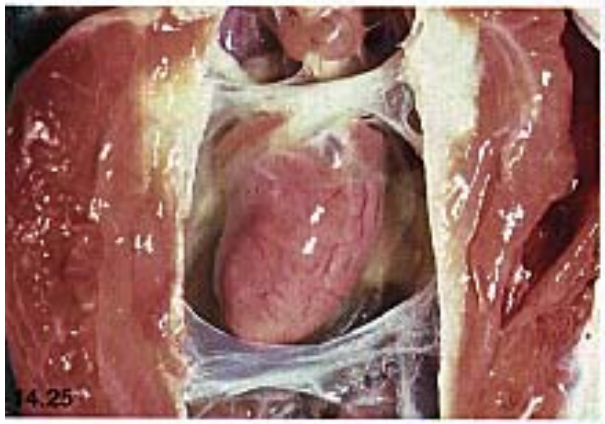
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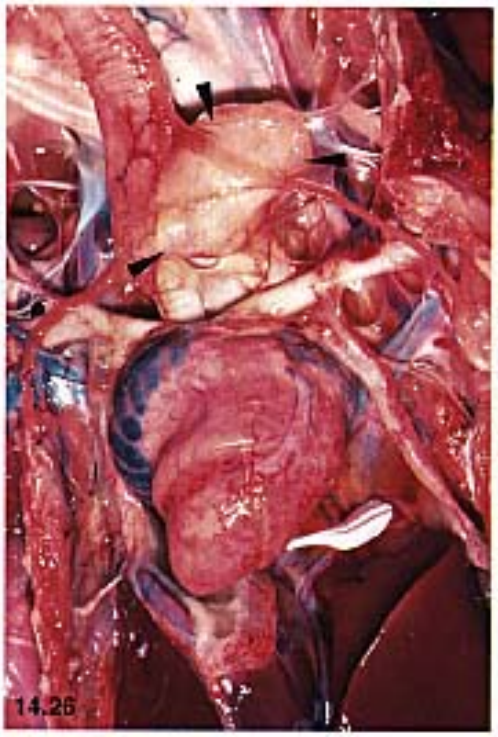
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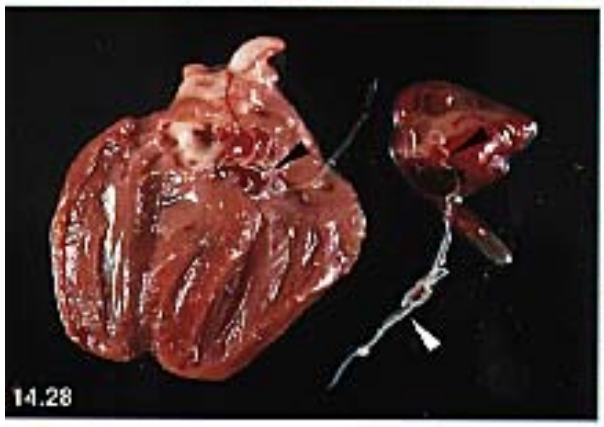
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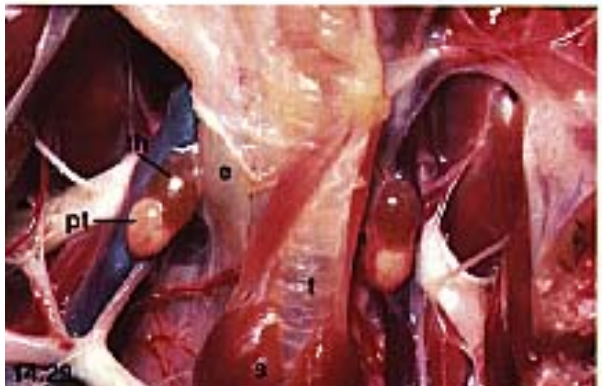

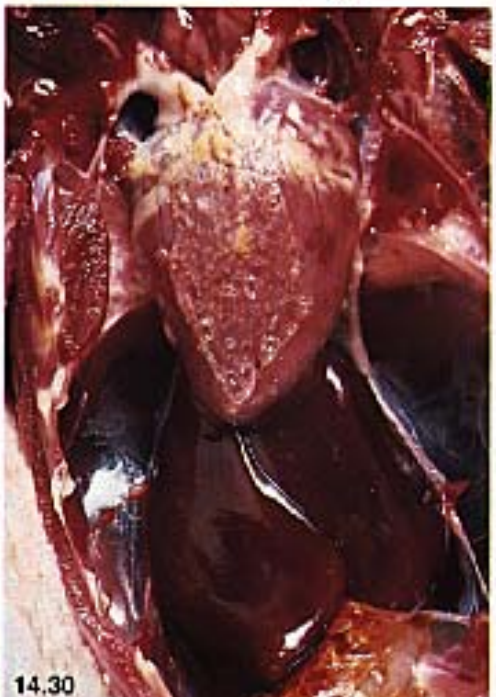
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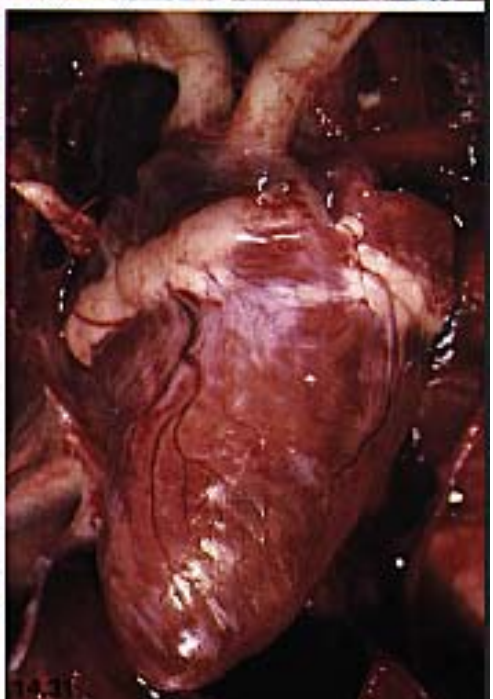

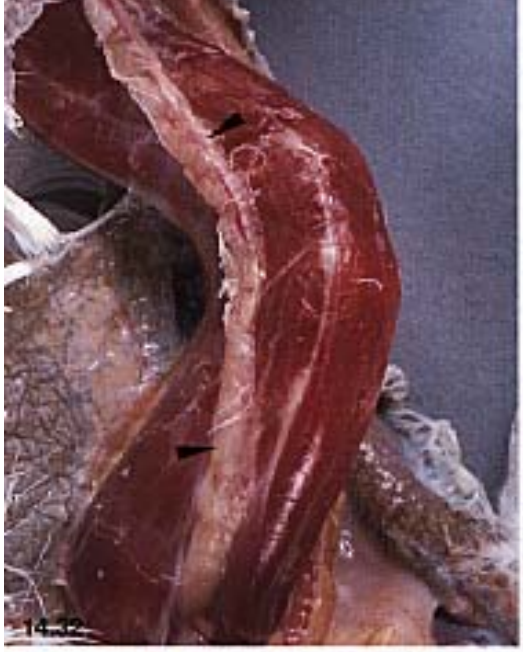


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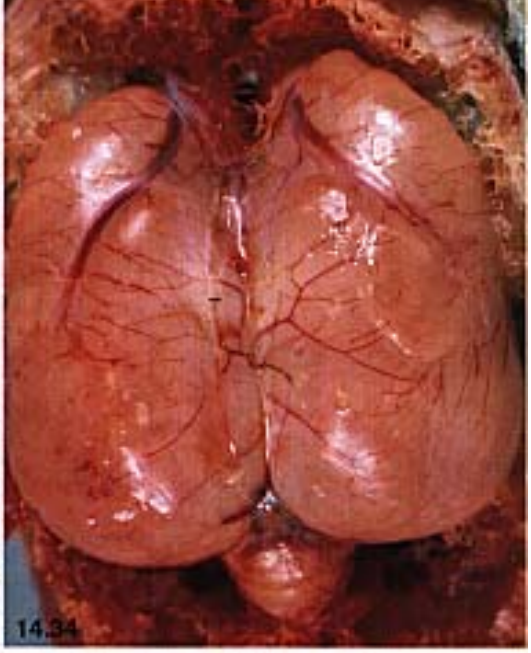
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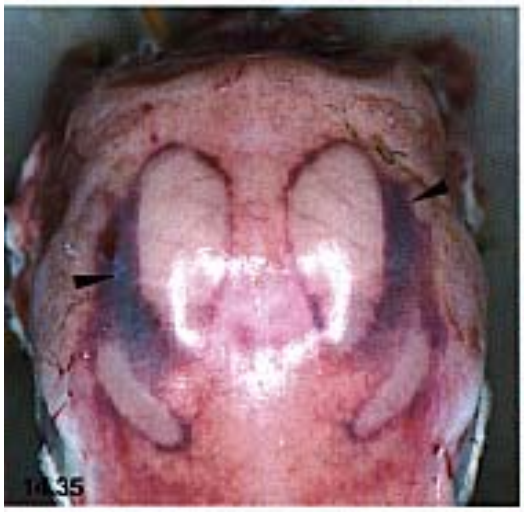
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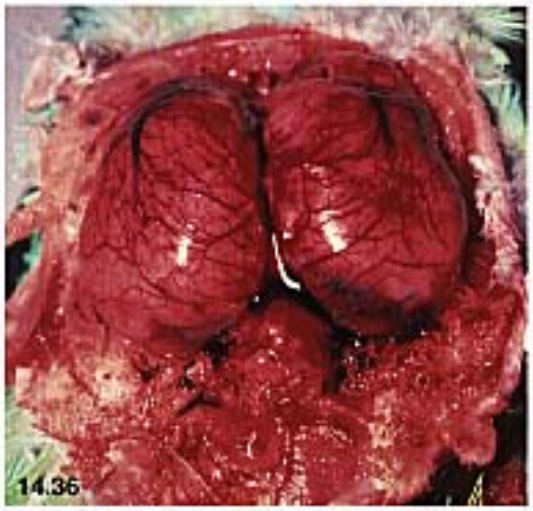
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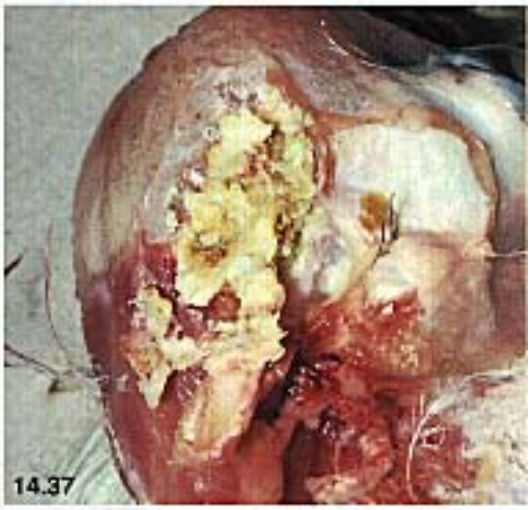
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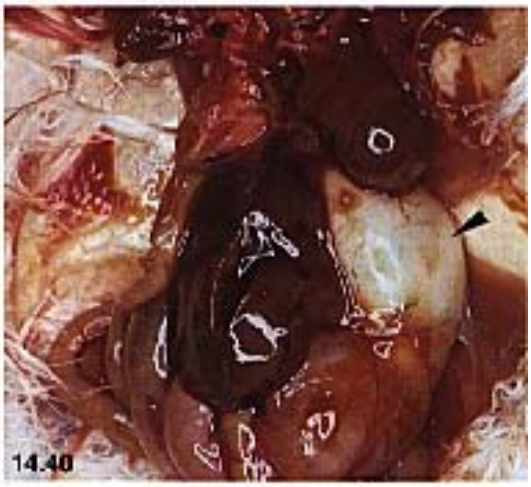
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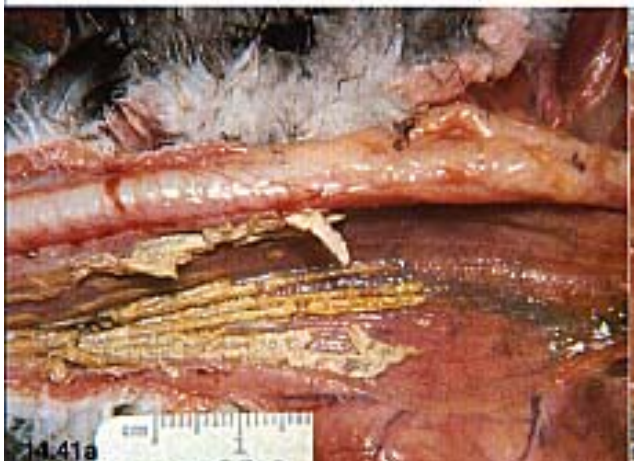
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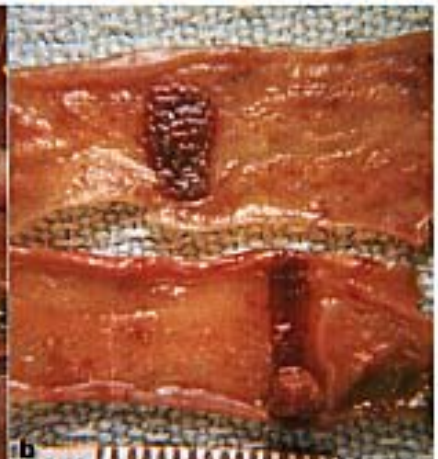
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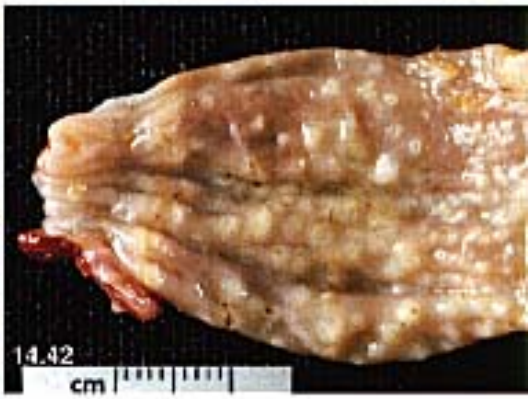
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Necropsy Examination

Color 14.32
Lobules of normal thymic tissue (arrows) within fascial planes adjacent to the cervical musculature in a young cockatoo (courtesy of Ken Latimer).

Color 14.33
Fibrotic areas of pectoral muscle (arrows) secondary to the injection of enrofloxacin. Note the yellowish discoloration of the subcutaneous tissue (open arrow) associated with the area where the bird had been vaccinated with an oil-emulsion vaccine.

Color 14.34
Normal brain. The cerebral hemispheres and cerebellum are exposed following removal of the posterior aspect of the cranial vault. Note that the tissues are moist but there is no accumulation of fluid (courtesy of Kenneth Latimer).

Color 14.35
Subdural hemorrhage (arrows) can be an indication of trauma or can occur as an agonal change.

Color 14.36
A mature Amazon parrot was presented with a progressive onset of ataxia and severe depression. The WBC was markedly elevated, and the bird did not respond to antibiotics and supportive care. Severe congestion and hemorrhage in the brain were caused by bacterial encephalitis.

Color 14.37
A ten-year-old Barn Owl was presented for a progressive head tilt and ataxia. Physical examination revealed numerous gram-negative rods in a necrotic discharge from the right ear canal. Auditory evoke potentials indicated a centralized inflammatory disease. Necropsy indicated an internal and external bacterial ear infection with progression to the brain.

Color 14.38
Acuaria skjabini is a common nematode parasite in finches maintained in aviaries in Australia. The nematode burrows into the koilin layer of the ventriculus, causing hypertrophy (arrows). A normal ventriculus is shown on the right to show the marked hypertrophy in the affected ventriculus (courtesy of Patricia Macwhirter).

Color 14.39
Proliferative, necrotic lesions (arrows) in the crop of a finch. These "turkish towel"-type lesions can be caused by candidiasis or aspergillosis.

Color 14.40
Proventricular dilatation (arrow) in a canary infected with megabacteria.

Color 14.41
a) Esophageal necrosis and diphtheritic membranes in a North American Black Duck caused by duck virus enteritis (duck plague) (courtesy of R. J. Montali). **b)** Necrotic, hemorrhagic bands of lymphatic tissues in the small intestines of a duck with duck virus enteritis (courtesy of John H. Olsen).

Color 14.42
Proventricular nodules in an Anseriforme caused by *Tetrameres* sp. (courtesy of R. J. Montali).

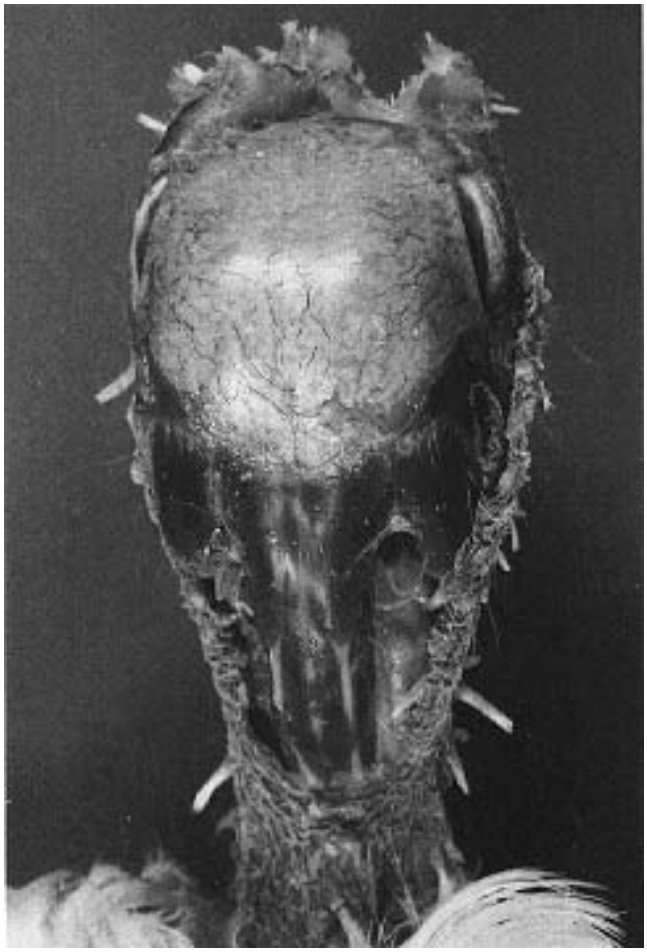


FIG 14.10 The scalp has been incised and reflected to expose the posterior portion of the skull.

FIG 14.11 A midline incision has been made through the cranial vault using blunt-sharp scissors. The cranium is then peeled away to expose the brain.

Brachial Plexus

The brachial plexus lies lateral to the thyroid gland in the vicinity of the subclavian artery (see Anatomy Overlay). Although the plexus commonly is inspected at necropsy, dissection and collection of tissues is limited except in cases of suspected neurologic damage from penetrating wounds, inflammation, neoplasia or trauma resulting in avulsion of the plexus.

Sacral Plexus

The sacral nerve plexus should be examined carefully in instances where pelvic limb paresis or paralysis has been noted (see Anatomy Overlay). This plexus is best inspected when removing the adrenal glands, gonads and kidneys because it is embedded in the midportion of the kidney just anterior to the ischiatic artery (Figure 14.12). The ischiatic nerve, which innervates the pelvic limb, may be damaged in severe nephritis or renal neoplasia where compression or infiltration of the nerve occurs.

Ischiatic (Sciatic) Nerve

In instances of pelvic limb paresis or paralysis, the ischiatic nerve should be examined grossly and histologically. The ischiatic nerve can be found beneath the medial thigh muscles caudal to the femur (see Anatomy Overlay).

Removal of the Eyes

If intraocular disease is present, the eye(s) should be removed from the orbit(s) for histologic evaluation. This process is slightly more difficult in birds because of the relatively large size of the eye. The eyeball is removed by sharp and blunt dissection of orbital soft tissues and transection of the optic nerve.

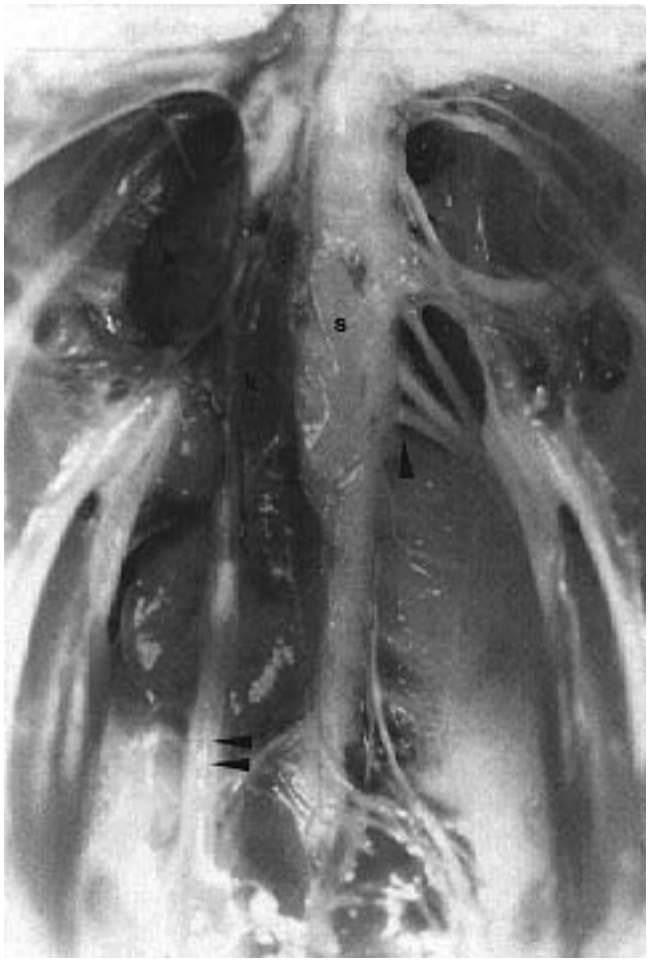


FIG 14.12 The sacral plexus (arrow) should be carefully examined in all cases involving weakness or ataxia (unilateral or bilateral) of the pelvic limbs. The sacral plexus and lumbosacral spinal column should be submitted for histopathology in these cases as well as those with clinical changes suggestive of neuropathic gastric dilatation. The left kidney has been removed to show the relationship of the kidneys and sacral plexus. The right kidney (k) and ureter (double arrow) are in their normal anatomic locations lateral to the spine (s).

Other Cranial and Skeletal Tissues

The nares, cere, beak, choanal slit, infraorbital sinus and ears should be examined. Abnormal tissues can be collected for formalin fixation or ancillary testing. Joints of the wings, legs and feet should be opened and examined. Articular surfaces should be off-white, smooth and glistening. If exudates are present, appropriate specimens should be taken for cytologic and microbiologic examination. White, chalky deposits may represent urate deposition. The presence of urate crystals can be confirmed by microscopic examination of cytologic preparations (under polarized light if available). Urate crystals will appear as refractile needles. Appropriate bony lesions may be collected with a dovetail saw, fixed in formalin, decalcified and processed for histopathologic examination.

Collection of Bone and Bone Marrow

Detailed examination of portions of the skeletal system may be necessary in instances of fractures, metabolic bone disease, osteomyelitis, arthritis or synovitis and anemia or blood cell dyscrasia. Collection of various skeletal tissues ultimately may be essential for a definitive diagnosis.

In the case of fractures, osteomyelitis and arthritis or synovitis, the tissues of interest may be localized with the assistance of survey radiographs. Blunt and sharp dissection will allow gross observations of these tissues. Callus formation, if present, should be noted and specimens for culture or cytology can be taken after the site is exposed by dissection. Cytology preparations will be useful to characterize inflammatory infiltrates, identify pathogens or identify urate crystals.

Joints can be disarticulated with a scalpel, knife or scissors. The joint capsule, ligaments and tendons can be inspected grossly. Articular surfaces should be examined for erosions of cartilage, eburnation of subchondral bone, tags of fibrin or the presence of exudates or hemorrhage. Rongeurs or a small dovetail saw can be used to excise portions of bone *en bloc* for histopathologic examination.

If the bird is anemic based upon laboratory studies or gross necropsy examination (pale liver and kidneys suggest anemia) or has a blood cell dyscrasia, bone marrow also should be examined. When bone marrow examination is necessary, it should be collected as soon as possible after death since bone marrow cells undergo rapid degeneration. Because many bones of the bird are pneumatized (including those of the thoracic girdle, humerus, sternum, sternal ribs and occasionally femur), the tibiotarsus or vertebral rib(s) should be used for collection. Because the tibiotarsus is larger, more marrow can usually be obtained for both cytology and histopathology. To obtain tibiotarsal marrow, the integument over the tibiotarsus is plucked and the skin is incised and reflected. The underlying musculature is dissected to reveal the shaft of the tibiotarsus. Using rongeurs, a portion of the shaft is excised. The cortex is cracked and small amounts of marrow are teased or gently squeezed from the marrow cavity. Smears or squash preparations of bone marrow are made for cytologic examination. Additional small segments of cortical

bone and marrow are taken for histologic examination. The cortex should be cracked to promote rapid penetration of fixative into the tissues.

Whole Carcass Submission

In instances where the entire carcass is extremely small, such as embryos, nestlings or very small adult birds, the entire carcass may be submitted for histologic examination. This is best accomplished by opening the thoracoabdominal cavity, gently separating the viscera and fixing the entire carcass in formalin solution.

Specimen Collection for Ancillary Testing

Ancillary testing often is essential to confirm or establish a definitive diagnosis. Tissue specimens should be collected routinely for histopathologic evaluation; however, additional specimens (eg, swabs for bacterial culture, fresh tissues for bacterial culture and virus isolation, crop contents for toxicologic analysis) are obtained as necessary based upon historical, clinical and necropsy findings. These latter specimens can be submitted along with the formalin-fixed tissues if the need for additional laboratory testing is obvious or they may be held under appropriate conditions for later submission if required. It is better to have taken specimens for ancillary testing and not need them, than to need the specimens and not have taken them. The following information is designed to expedite specimen procurement and handling to maximize the results obtained. A telephone call to the diagnostic laboratory prior to performing the necropsy is a prudent measure to ensure correct specimen collection, preparation and handling.

Histopathology

Tissue specimens for histopathology should be preserved in neutral-buffered ten percent formalin solution. Buffered formalin is necessary to prevent acid hematin formation, which can obscure microscopic examination. Furthermore, adequate preservation of tissues requires rapid and complete penetration of the fixative. This is best accomplished by procuring thin (four to five mm thick) slices of tissue. Excessively thick (one cm thickness) tissue slices or tissues that float (gas-filled intestine, fatty liver, lung) when immersed in formalin solution often do not fix and become autolytic. Representative tissue specimens from all organ systems should be collected (Table 14.1). When specific lesions are observed at necropsy, the tissue specimen collected should include a small margin of normal tissue adjacent to the lesion.

TABLE 14.1 Tissues Routinely Collected for Histopathology

Skin (including feathers, follicles)	Crop	Pancreas
Trachea	Proventriculus	Ovary and oviduct (female)
Lung	Ventriculus	Testis (male)
Air sac	Small intestine	Pectoral muscle
Heart	Large intestine	Bone marrow
Kidneys	Ceca (if present)	Cloacal bursa
Thyroid glands	Cloaca	Thymus
Parathyroid glands	Spleen	Brain
Adrenal glands	Liver	Ischiatic (sciatic) nerve
Esophagus	Gall bladder (if present)	

Selection of additional tissues will depend upon gross lesions observed at necropsy.

Specimens should be shipped to the laboratory in leak-proof containers that are well packaged. To decrease shipping weight, tissues that have been fixed in formalin solution for at least 24 hours can be wrapped in a formalin-soaked gauze square that is placed into a sealable plastic bag for shipment. In the authors' experience, a complete set of necropsy tissues provides the best diagnostic material. Because cost is often a consideration when submitting histopathologic specimens to the laboratory, the practitioner should consult a veterinary pathologist concerning the tissues to be submitted in a particular case. The remaining fixed tissues can be held for additional study if needed.

Hematologic and Cytologic Specimens

Preparation of blood and cytology specimens for microscopic examination is detailed in Chapters 9 and 10.[2] Smears of blood or exudates may be prepared in a routine manner by the wedge technique. Tissue scrapings may be smeared onto a clean glass slide, or squash preparations may be made if particles of tissue are present. Tissue imprints are prepared by blotting the tissue specimen on an absorbent surface (filter paper or paper towel) to remove excess blood and tissue fluid. The tissue specimen is then gently touched to a clean glass slide several times or vice versa. Imprints of liver and spleen can be prepared on a single slide and submitted for special stains (eg,

Macchiavello's or Gimenez staining for chlamydiosis, acid-fast staining for mycobacteriosis or fluorescent antibody staining for chlamydiosis or herpesvirus infection). Intestinal mycobacteriosis also may be diagnosed using cytologic imprints. Swab specimens are properly prepared by gently rolling the swab the length of the glass slide. Three such passes may be made on a single slide from top to bottom. All specimens are air-dried. If they are not stained before examination or submission to the diagnostic laboratory, they should be protected from excessive moisture or formalin fumes, which could cause cellular lysis or interfere with staining, respectively.

Microbiology

Microbiology includes culture and identification of bacteria, viruses and fungi as well as certain serologic assays to detect the presence of or exposure to these pathogens. Specimens procured for analysis may include culture swabs, fresh tissues, body fluids or exudates, cytologic smears and imprints (eg, fluorescent antibody staining for chlamydia and herpesvirus) and serum. These specimens are perishable and should be shipped to the laboratory without delay. Next-day courier service is recommended.

Fresh tissues submitted for bacterial culture should be at least two cubic centimeters to yield accurate results. At the laboratory the surface of the tissue is seared with a heated spatula to sterilize it, and a loop is inserted through the seared area into the center of the specimen to collect tissue for culture. If the tissue is too small, the entire specimen (including bacteria) is destroyed during the searing step, and a false-negative culture result is obtained. Tissues for routine bacterial culture can be placed in sterile, sealable plastic bags and submitted immediately or frozen if a delay of more than 12 to 24 hours before culturing is expected. If unusual pathogens are suspected, the diagnostic laboratory should be consulted regarding the best means of handling the tissue to optimize culture results.

Specimens for bacterial culture also may be obtained aseptically using swabs. Products such as Culturettes® are preferred because they are self-contained, minimize the possibility of specimen contamination and contain a transport medium that maintains organism viability while preventing saprophytic bacterial overgrowth.

Fresh tissues (especially liver, spleen, kidney, lung and brain) are collected for viral isolation. The selection of tissues for viral isolation depends in part upon the organ system affected. Tissue specimens may be placed in sealable plastic bags and frozen prior to shipment to the laboratory. If tissues are not sent to the laboratory immediately, they may be stored in the freezer until needed for diagnostic testing. After the definitive diagnosis has been made, remaining tissues can be discarded.

Tissue specimens for fungal culture and identification may be collected, placed in sealable plastic bags and refrigerated or frozen until analyzed. The choice of tissues is variable, depending upon the extent of infection.

Parasitology

Fecal flotation for detection of parasite ova is performed frequently as a portion of the minimum database to assess a patient's medical status. Additional fecal specimens may be taken for analysis at necropsy, especially in those patients with diarrhea, where protozoal infection is a consideration. Also, intact parasites such as cestodes, trematodes, nematodes or arthropods may be taken for specific identification when encountered in exotic birds or observed in unusual locations. Proper fixation of these parasites is essential for successful identification by a veterinary parasitologist.[20] Preferred fixatives for preservation of fecal material and parasites are detailed in Tables 14.3, 14.4.

Wet mounts of feces or a feces-saline slurry should be examined within minutes of death to detect organisms such as *Giardia* sp., which are identified by their characteristic rolling movement. Following initial examination, a small drop of Lugol's iodine can be added to kill and stain protozoa and their cysts for more detailed examination. These specimens are perishable and generally will not survive shipment to the diagnostic laboratory. Intestinal scrapings or imprints, which may be air-dried, stained and examined in-house or shipped unstained to the laboratory for examination, may be useful to diagnose coccidiosis, atoxoplasmosis and cryptosporidiosis.

Toxicology

Toxicologic analysis is generally labor-intensive, requires sophisticated analytical equipment and is often expensive. The clinician should have some suspicion of the substance involved before toxicologic analysis is requested, because tissue handling and the specimen(s) required vary with the type of toxi-

cologic analysis performed. A veterinary toxicologist or diagnostic laboratory should be contacted to ensure that the proper samples are collected and submitted for analysis. In addition, a particular laboratory may not perform a desired test or may not be equipped for analysis of small tissue specimens.

The most commonly ingested toxins in companion and aviary bird practice are heavy metals (eg, zinc, lead), aflatoxin-contaminated feeds and various ornamental houseplants. The most commonly inhaled toxins include the fumes of polytetrafluoroethylene produced from over-heated cooking pans or utensils and some varieties of red heat lamps.[1,9,11,13,18,24,28] The following discussion briefly covers sample submission for toxicologic analysis, especially for identification of certain heavy metals and aflatoxins (see Chapter 37).

Heavy Metals

Heavy metal toxicosis is most frequently associated with ingestion of zinc by companion or aviary birds and lead by foraging waterfowl. Sources of excess zinc include ingestion of particulate material from homemade galvanized wire mesh enclosures and ingestion of pennies thrown into captive bird displays.[9,13,23] United States pennies minted since 1982 are essentially copper-plated zinc wafers. Lead poisoning is usually due to ingestion of lead shot by waterfowl during normal feeding activities.[18] However, lead poisoning in companion birds may result from chewing leaded windows, lead-containing toys or costume jewelry, lead pellets and fishing sinkers.[32] Suspicion of heavy metal toxicosis may be based upon observing metallic foreign bodies in the crop and gizzard on routine survey radiographs or at necropsy.

Heavy metal toxicosis is best detected using graphite furnace atomic absorption spectrophotometry, which requires a small sample volume. Using this technique, quantitation of lead requires submission of 250 µl of blood in heparin or one-half gram each of liver and kidney. Quantitation of zinc requires 250 µl of serum (avoid hemolysis) or one-half gram each of liver and kidney. The above specimens may be submitted refrigerated or frozen. Blood and serum should be submitted in screw-cap plastic containers or stoppered test tubes. Control specimens are helpful in evaluating results because reference values have not been established for most birds. Liver and kidney specimens may be submitted in sealable plastic bags.

Aflatoxins

Aflatoxins B_1, B_2, G_1 and G_2 are metabolites of *Aspergillus flavus*. These substances may form in improperly stored feed and act as potent hepatotoxins. They may be identified in feed or tissue specimens using thin-layer chromatography or high performance liquid chromatography. An ELISA test is available for identification of aflatoxin B_1. Identification of aflatoxin in foodstuffs requires submission of 50 to 100 g of feed. The feed should be well mixed to prevent sampling errors, and should be derived from the same lot of material fed before the onset of disease. Detection of aflatoxin residues in tissues requires 100 g of fresh or frozen liver. Samples for analysis should be placed in sealable plastic bags. Although not ideal, tissues from several dead birds can be pooled for analysis if necessary.

Poisonous Plants and Chemicals

Suggestion of plant-induced toxicosis may be based upon the medical history and observation of crop contents. Although large lists of potentially toxic plants have been published, recent publications indicate that development of toxicosis is dependent on the species of bird, portion of plant ingested and season of plant growth.[1,8,28] Diagnosis of plant alkaloids or chemical-induced toxicosis should be pursued on an individual basis. A veterinary toxicologist should be consulted concerning appropriate specimens and handling prior to analysis.

Products Mentioned in the Text

a. Culturettes, Becton Dickinson, Cockeysville, MD
b. Whirl-Paks, Fort Atkinson, WI

TABLE 14.2 Fixative Solutions for Tissue Specimens[27]

- **Neutral-buffered 10% formalin solution:** This solution is used as a common fixative to preserve tissue specimens for histologic examination. Proper fixation requires a ratio of one part tissue to 10-20 parts fixative solution.

Concentrated formaldehyde (37%)	100 ml
Distilled water	900 ml
Sodium phosphate monobasic, monohydrate	4.0 g
Sodium phosphate, dibasic, anhydrous	6.5 g

- **Carson's modified Millong's phosphate-buffered formalin:** This solution may be used for routine preservation of tissue specimens for both histopathology and electron microscopy. Proper fixation requires a ratio of one part tissue to 10-20 parts fixative solution.

Concentrated formaldehyde (37%)	100 ml
Deionized water	900 ml
Sodium phosphate monobasic	18.6 g
Sodium hydroxide	4.2 g

TABLE 14.3 Fixative Solutions for Fecal Material[20]

The following fixatives are intended for preservation of fecal material for storage or mailing to the diagnostic laboratory. Comments on the usefulness of each fixative solution follow.

- **PVA fixative:** This fixative is recommended because stained preparations of fecal material subsequently can be made for identification of protozoa.

  PVA, Elvanol 71-24 . . . . . 10.0 g
  95% ethanol . . . . . 62.5 ml
  Mercuric chloride, saturated aqueous . . . . . 125.0 ml
  Glacial acetic acid, concentrated . . . . . 10.0 ml
  Glycerin . . . . . 3.0 ml

  Mix all liquid ingredients thoroughly. Add the PVA powder without stirring and allow to soak overnight in a sealed beaker. Heat solution slowly to 75°C, remove from heat and swirl for 30 seconds until a homogeneous, slightly milky solution is observed. Using applicator sticks, mix approximately 1 g feces with 7-9 ml fixative and store in a labeled brown bottle.

- **10% formalin solution:** This fixative is used primarily to preserve ova for identification. Stained smears cannot be made for identification of protozoa.

  Concentrated formaldehyde (37%) . . . . . 100 ml
  Deionized water or 0.85% saline . . . . . 900 ml

  Best preservation is achieved by mixing 1 part feces with 10-20 parts of hot (60°C) fixative.

- **MIF preservative:** Fecal specimens may be stored indefinitely in MIF solution and ova may be harvested by common concentration techniques. This fixative is useful for large surveys where fecal materials are collected from many animals over a long period of time.

Solution A (store in a brown bottle):

  Distilled water . . . . . 50 ml
  Concentrated formaldehyde (37%) . . . . . 5 ml
  Thimerosal (tincture of merthiolate, 1:1,000) . . . . . 40 ml
  Glycerin . . . . . 1 ml

Solution B (Lugol's solution; good for several weeks in a tightly capped bottle):

  Distilled water . . . . . 100 ml
  Potassium iodide crystals . . . . . 10 g
  Iodine crystals (after above crystals dissolve) . . . . . 5 g

Combine 9.4 ml of solution A with 0.6 ml of solution B just before use in a small vial. Add feces (up to 1 g) and mix thoroughly. If the suspension is allowed to sit undisturbed for 24 hours, 3 well-defined layers will be apparent. The microscopic specimen is collected from the interface and bottom layers using a disposable Pasteur pipette.

TABLE 14.4 Fixative Solutions for Specific Parasites

- **Trematodes and Cestodes:** Platyhelminths may be fixed in 10% neutral-buffered formalin solution or alcohol-formalin-acetic acid mixtures. The parasites should be flattened under a slide and coverslip during fixation. Fixatives are best used hot (60°C) for more rapid penetration.

Alcohol-formalin-acetic acid fixative (Galigher's fixative):

  Concentrated formaldehyde (37%) . . . . . 10 ml
  95% ethanol . . . . . 70 ml
  Distilled water . . . . . 15 ml
  Glacial acetic acid, concentrated . . . . . 5 ml

- **Nematodes:** Living nematodes should be placed in boiling (60-63°C) alcohol glycerin fixative to rapidly kill the parasites and prevent contraction of the specimen. Nematodes can remain in this fixative indefinitely.

Alcohol-glycerin fixative:

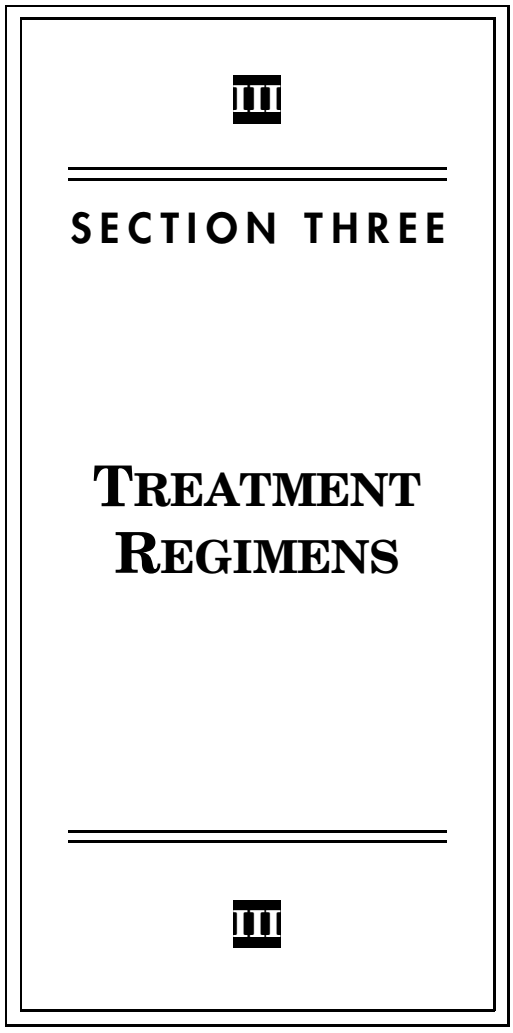
  95% ethanol . . . . . 70 ml
  Distilled water . . . . . 25 ml
  Glycerin . . . . . 5 ml

- **Arthropods:** Arthropods can be preserved in 70% ethanol or 70% isopropyl alcohol solutions (formalin is unsatisfactory for arthropod fixation).

References and Suggested Reading

1. **Arai M, et al:** Evaluation of selected plants for their toxic effects in canaries. J Am Vet Med Assoc 200:1329-1331, 1992.
2. **Campbell TW:** Avian Hematology and Cytology. Ames, Iowa State University Press, 1988.
3. **Fowler ME:** Comparative clinical anatomy of ratites. J Zoo Wildlife Med 22:204-207, 1991.
4. **Gaskin JM, et al:** Preliminary findings in avian viral serositis: A newly recognized syndrome of psittacine birds. J Assoc Avian Vet 5:27-34, 1991.
5. **Grimes JE:** Chlamydiosis in psittacine birds. J Am Vet Med Assoc 190:394-397, 1987.
6. **Graham DL:** Necropsy procedures in birds. Vet Clin N Am Small Anim Pract 14:173-177, 1984.
7. **Guthertz LS, et al:** *Myco-bacterium avium* and *Mycobacterium intracellulare* infections in patients with and without AIDS. J Inf Dis 160:1037-1041, 1989.
8. **Hargis AM, et al:** Avocado (*Persea americana*) intoxication in caged birds. J Am Vet Med Assoc 194:64-66, 1989.
9. **Howard BR:** Health risks of housing small psittacines in galvanized wire mesh cages. J Am Vet Med Assoc 200:1667-1674, 1992.
10. **Keymer IF:** Postmortem examinations of pet birds. Mod Vet Pract 42:35-38, 47-51, 1961.
11. **LaBonde J:** Avian toxicology. Vet Clin N Am Small Anim Pract 21:1329-1342, 1991.
12. **Latimer KS, et al:** Recognition and interpretation of selected gross necropsy lesions and anatomical variations. J Assoc Avian Vet 6:31-33, 1992.
13. **Lloyd M:** Heavy metal ingestion: Medical management and gastroscopic foreign body removal. J Assoc Avian Vet 6:25-29, 1992.
14. **Lowenstine LJ:** Necropsy procedures. *In* Harrison GJ, Harrison LR (eds): Clinical Avian Medicine and Surgery. Philadelphia, WB Saunders Co, 1986, pp 298-309.
15. **McDonald SE:** Anatomical and physiological characteristics of birds and how they differ from mammals. 1991 Manual of Basic Avian Medicine, Association of Avian Veterinarians, Atlanta, GA, 1991, pp 1-18.
16. **McKibben JD, Harrison GJ:** Clinical anatomy with emphasis on the Amazon parrot. *In* Harrison GJ, Harrison LR (eds): Clinical Avian Medicine and Surgery. Philadelphia, WB Saunders Co, 1986, pp 31-66.
17. **McLelland J:** A Color Atlas of Avian Anatomy. Philadelphia, WB Saunders Co, 1991.
18. **Murase T, et al:** Treatment of lead poisoning in wild geese. J Am Vet Med Assoc 200:1726-1729, 1992.
19. **Pass DA:** The pathology of the avian integument: A review. Avian Pathol 18:1-72, 1988.
20. **Prestwood AK:** Clinical parasitology techniques for veterinarians. University of Georgia, Athens, GA, 1992.
21. **Prince DS, et al:** Infection with *Mycobacterium avium* complex in patients without predisposing conditions. N Engl J Med 321:863-868, 1989.
22. **Randall CJ:** Color Atlas of Diseases of the Domestic Fowl & Turkey. Ames, Iowa State University Press, 1985.
23. **Reece RL, et al:** Zinc toxicity (new wire disease) in aviary birds. Aust Vet J 63:199, 1986.
24. **Richardson M:** Teflon toxicity from heat lamps. J Assoc Avian Vet 5:192, 1991.
25. **Ritchie BW, Dreesen DW:** Avian zoonoses: Proven and potential diseases. Part I. Bacterial and parasitic diseases. Comp Cont Educ 10:484-492, 1988.
26. **Ritchie BW, Dreesen DW:** Avian zoonoses: Proven and potential diseases. Part II. Viral, fungal and miscellaneous diseases. Comp Cont Educ 10:688-695, 1988.
27. **Sheehan DC, Hrapchak BB:** Theory and Practice of Histotechnology, 2nd ed. St Louis, CV Mosby Co, 1980, pp 45-46.
28. **Shropshire CM, et al:** Evaluation of selected plants for acute toxicosis in budgerigars. J Am Vet Med Assoc 200:936-939, 1992.
29. **Smith AW, et al:** 1986 report of the AVMA panel on euthanasia. J Am Vet Med Assoc 188:252-268, 1986.
30. **T-W-Fiennes RN:** Diseases of bacterial origin (Techniques of postmortem examination). *In* Petrak ML (ed): Diseases of Cage and Aviary Birds 2nd ed Philadelphia, Lea & Febiger, 1982, pp 498-499.
31. **van Ripper C, van Ripper SG:** A necropsy procedure for sampling disease in wild birds. Condor 82:85-98, 1980.
32. **Woerpel RW, Rosskopf WJ:** Heavy-metal intoxication in caged birds - part I. Comp Cont Educ 4:729-738, 1982.
33. **Zander DV, Mallinson ET:** Principles of disease prevention: Diagnosis and control. *In* Calnek BW, et al (eds): Diseases of Poultry, 9th ed Ames, Iowa State University Press, 1991, pp 27-36.

CHAPTERS



SECTION THREE

TREATMENT REGIMENS