NECROPSY

Procedures and Basic Diagnostic Methods for Practicing Veterinarians

ALBERT C. STRAFUSS, D.V.M., M.S., PH.D.

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This illustrated manual, designed to aid veterinary practitioners and students in establishing diagnoses, emphasizes necropsy gross pathologic diagnosis and supportive laboratory procedures. The orderly and systematic necropsy procedure outlined herein stresses a philosophy of "collect and do" instead of "collect and send," and eliminates the necessity of learning a different procedure for each species by providing appropriate modifications of the basic necropsy procedure for the dog. Specific chapters address postmortem changes versus antemortem lesions, specimen collection and submission, clinical bacteriology, diagnosis of parasitism in domestic animals, diagnostic evaluation of bovine ingesta, abortion problems, the disposal of dead animals and birds, and malpractice in veterinary medicine. The veterinarian will also find invaluable the special sections on avian necropsy and the common gross pathological findings in small farm flocks, commercial poultry, cage birds, and game birds.

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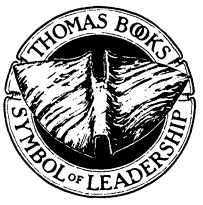

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To

The veterinary student and practitioner who will read and find this textbook to have aided their quest in becoming a better diagnostician by improving diagnostic skills through the necropsy and associated "hands on" laboratory supportive techniques.

PREFACE

THIS BOOK on necropsy diagnosis is written to aid veterinary practitioners and students in establishing diagnoses. Information scattered throughout the literature or learned by the author's experience has been collated and presented in a logical sequence. A necropsy procedure for the dog with appropriate modifications for other domestic animals is described, thereby eliminating the necessity of learning a different procedure for each species. Emphasis is placed on gross pathologic diagnoses with simple and quick supportive laboratory techniques, which can be performed during necropsy (impressions or smears, Gram stains, etc.). A common mistake in practice is to omit laboratory procedures or to do difficult tests that are needed infrequently without first perfecting tests that are supportive, simply done, inexpensive, and more rewarding.

In veterinary medicine, teaching and learning revolve around living patients, but in many instances necropsy can result in an intelligent and scientific understanding of disease processes. Before lesions can be studied, an orderly and systematic necropsy must be carried out and visualized by an orderly technique; such a procedure is described in this text.

It is important that necropsies be fully utilized as a means of understanding disease pathogenesis and for distinguishing one disease from another. Pathologic anatomy is one facet only (most likely an end result) of a process involving one or more mechanisms that elucidate etiology and provide a rational basis for prevention and treatment of disease. A skillfully performed necropsy allows laboratory procedures to be performed to yield maximal diagnostic information. A necropsy carelessly done or attempted to improperly trained people is more likely to provide misinformation or no information. The organizational concept of necropsy and associated laboratory procedures stresses a "collect and do" instead of a "collect and send" philosophy.

The text emphasizes quality control in establishing diagnoses. Although quality control in the practice of veterinary medicine is not decreed by law, it behooves the profession to police itself with quality control so that this will not happen. A practitioner can provide some degree of quality control by doing necropsies on animals that die. Secondly, he can send tissues from problem cases to a diagnostic laboratory not to get a diagnosis, but to confirm his diagnosis. Diagnostic laboratories around the country, however, are seldom used for this purpose. Thirdly, attendance at veterinary short-courses in bacteriology, cytology, etc., marks the beginning of individual quality control by adapting and using laboratory supportive procedures during necropsy.

In large animal practice, routine necropsies, particularly in swine and cattle confinement operations, permit monitoring the herd health status and managerial procedures. This, likewise, applies to dog kennels, aviaries, or commercial poultry operations. Veterinary practitioners frequently avoid doing necropsies because they take too much time or are too much trouble, or because the rendering companies will not pick up animals after necropsy. These personal factors arise from a lack of familiarity with efficient necropsy procedures, instruments, and availability of simple and quick laboratory tests to confirm a diagnosis.

Veterinarians are seldom taught the science and art of doing a necropsy examination. In veterinary school, we learn anatomy and surgery, but seldom relate anatomy to necropsies. We learn pathology by studying effects of disease, but usually are not taught how to best detect postmortem change or to distinguish abnormal from the normal in the cadaver, which is really the heart of a competent necropsy. All available laboratory tests are discussed in the classroom, but we really never demonstrate or require students to understand the appropriate samples to be collected from the cadaver at the time the necropsy is being conducted. We always say in our teaching, "Send in the appropriate sample, properly packaged so that it is adequately refrigerated and can be transported." The inconclusive diagnosis is derived from inconclusive evidence, either from the history or necropsy and the negative diagnosis may be directly associated with the fact that we were not thorough enough to make a diagnosis.

Chapters on necropsy of birds and common gross findings of diagnostic significance in poultry (small farm flock and commercial) and cage birds are included to provide basic information for diagnosing many avian diseases. Veterinary contact with small farm flocks, game birds (chukars, pheasants, and quail), pigeons, cage birds, and aviaries

is increasing and, therefore, there is need for veterinarians to grasp the information available in these chapters to develop and sharpen their diagnostic skills by doing necropsies and supportive laboratory tests. Most veterinary practitioners have the facilities and scientific background to diagnose avian diseases. Their reluctance to handle birds stems from a lack of knowledge about how their problems should be approached; such an approach is outlined in the avian chapters. Establishing a diagnosis is really no more difficult in birds than domestic animals once the *modus operandi* is established.

Necropsies are continual educational opportunities offered to veterinary practitioners in their own practices and can seldom be duplicated elsewhere. This type of continuing education is lifelong.

Albert C. Strafuss

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A SPECIAL THANKS to Dr. S. A. Jones for doing the illustrations on necropsy procedure which were superbly done with a diligent stroke of perception. Much appreciation to Sherri Kraus for the excellent typing of the manuscript and for organizing a format which projects the various headings throughout to have a better reading style. The interest, encouragement, and many suggestions by Dr. S. M. Dennis were most helpful. The timely editing by Eileen Schofield of the Kansas Agricultural Experiment Station was invaluable. The critique and suggestions of Dr. Loren D. Kintner were appreciated.

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NECROPSY
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CHAPTER 1

IMPORTANCE OF NECROPSY

VETERINARIANS who perform necropsies become better diagnosticians who can administer treatment with more precision and success. Necropsies document the incidence of disease in a community and are important in surveillance of diseases transmissible from animal to man. Information from necropsies support and aid programs to monitor herd, kennel, or flock disease status.

Scientific inquiry by necropsy should be regarded as an examination of a body to determine the pathologic processes in relation to "clinical examination," and to acquire information regarding the nature of disease. The more effectively these ends are accomplished, the greater the contribution of the necropsy in determining the cause of illness.

The expression "clinical examination" should not be misunderstood. It has three aspects; animal, history, and environment. Inadequate examination of any of these may lead to error. The future of the veterinary profession essentially rests on the ability to render service based on accurate diagnoses. Clinical examination is an important cog in the diagnostic process. In veterinary medicine, history taking is the most important of the three aspects of a clinical examination, so it must be accurate and complete. The more the veterinarian knows about veterinary medicine, the more proficient he becomes at taking a good history. He has the knowledge to ask the appropriate questions and to meaningfully interpret the owner's answers. A good history, properly taken, remains one of the most valuable diagnostic aids a veterinarian has at his disposal. Many questions pertinent to the history can be asked while performing a physical examination, and the answers the clinician receives should constantly be compared with what he is observing. If the physical findings and the history do not support each other, it is well to review the relevant portion of the history again, perhaps rephrasing the questions so that if

misunderstandings have occurred, they can be clarified. Statements, particularly those concerned with time should be tested for accuracy. Owners, especially herdsmen and hired help, often attempt to disguise their neglect by condensing time or varying the chronology of events. History-taking will vary considerably depending on whether one animal or a group of animals is involved in the disease problem under examination. As a general rule in food animals, any disease should be considered a herd problem until proven otherwise. It is often rewarding to examine the remainder of a herd or flock to find animals in the early stages of the disease.

It is frequently stated that a good history will give the diagnosis without any other data. The veterinarian who has been working with a particular client and a particular animal may not need to ask a litany of questions, since he already may have the history well in mind. Other cases may require a thorough history. It is important that the history be written down. First, the owner realizes the importance of the history and will do his best to answer the questions as accurately as possible. Secondly, it provides the owner with time to collect his thoughts and remember details that might be overlooked in ordinary conversation. By getting the history organized on paper, the veterinarian can pick up leads that need to be pursued.

When doing a necropsy without a history, evaluation of tissue changes in an open carcass may often reveal nothing; everything may look normal. However, when incorporating the history with the necropsy, certain differential diagnoses will suggest looking for the presence of specific lesions. Frequently, lesions otherwise hidden may now become obvious. The business of getting an accurate history along with differential diagnoses in one's mind, is essential for obtaining a diagnosis.

The performance of a necropsy is a science and requires a good knowledge of general and special pathology of organs and organ systems. It requires a standardized necropsy procedure to effectively illuminate all pathological conditions so they may be studied in a thorough and systematic manner. A standardized necropsy procedure allows for precision, neatness, and thoroughness so that definitive diagnoses are made. A familarity with normal color and size of organs and tissues is important for diagnosing disease. Grossly normal-looking organs may have early microscopic changes that can be overlooked. However, direct cytological impressions or smears may easily detect early tissue changes. Histopathologic, bacteriological, and chemical examinations may be indicated to further support cytological interpretations.

Necropsy looks easy when one watches an experienced prosector at work. Repeated practice of a procedure increases proficiency and prevents the useless pulling, cutting, and destruction of lesions. A veterinarian using a definite necropsy protocol will perform uniform, rapid, systematic, and complete necropsies that are the key to consistent interpretation of lesions. Using a standard protocol for a necropsy assures that each organ system will be grossly observed in turn. Shortcuts lead to a lack of thoroughness, resulting in a wrong diagnosis, or no diagnosis at all. Only occasionally will a case require a modification of a standardized protocol. For example, a bloated animal may necessitate relieving the gas pressure and removing organs from the abdomen before the thoracic viscera may be removed.

In cases where lesions are not present and the cause of death cannot be ascertained, the prosector should realize that although a "no diagnosis" is a measure of lack of total knowledge about a case, it is not due to lack of thoroughness. The percentage of correct diagnoses depends largely on one's skill and thoroughness in performing necropsies.

A necropsy is performed for one or more of the following reasons.

1. History, clinical signs, and necropsy aided by laboratory tests often determine the nature of an animal's disease.
2. Apart from diagnosis, necropsies play a vital role in understanding diseases and their pathogenesis. Such an understanding is essential for developing and applying rational therapeutic and control measures.
3. A skillfully conducted and intelligently interpreted necropsy will give the client (especially livestock owners) a surprising amount of satisfaction and confidence in his veterinarian.
4. In disease surveillance, collection of data for statistical analysis of pathological conditions may provide answers to management problems in large livestock operations or uncover a subclinical problem before it becomes economically costly.

Most errors in diagnosis are not the result of ignorance, but of haste, carelessness, or apathy. Pasteur's statement, "In the fields of observation, chance favors only the prepared mind," has withstood the test of time. Powers of observation are markedly enhanced when a *systematic* necropsy becomes routine.

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CHAPTER 2

NECROPSY PROCEDURES

INTRODUCTION

THE NECROPSY procedure described here is neat and quick. It exposes tissues so that lesions can be seen and not destroyed. It should be the goal of every prosector to follow the technique religiously at every necropsy. However, the procedures in this chapter can be modified for special circumstances, such as working alone in a diagnostic laboratory or in the field, where three or four clients have animals in trucks sitting out in the sun, waiting their turn. Inadequate time is no excuse for a practitioner to omit a necropsy. A large animal such as a cow or a horse can be opened and questions can be answered within a few minutes with little mess on the ground or to equipment.

The estimated cost for a necropsy done on an animal varies. A good necropsy is always cost effective.

Summarizing, there is no right or wrong way to do a necropsy. If time and costs permit, the procedure detailed here is an efficient approach. When other conditions prevail, one may wish to emphasize some areas of the dissection and hasten over the less important systems. Remember, you only have one chance to do a necropsy, so do it right.

Before starting a necropsy, an external examination should include:

1. Position of animal at time of death.
2. General appearance of carcass, namely bloat, rigor, postmortem decomposition, and dehydration.

3. Thriftiness or apparent nutritional status.
4. Condition of hair coat and presence of external parasites.
5. Color and appearance of all visible mucous membranes.
6. Presence or absence of discharges from all body openings and mammary glands (discharges, color, size, etc.).
7. Evidence of swellings, wounds, hernias, and fractures.

To gain confidence in doing a necropsy, it is suggested that one first become skilled in the necropsy of the dog and cat for three reasons. (1) The cadavers are readily manageable; (2) the digestive tracts are short and manageable; and (3) the following procedural sequence learned on the dog and cat can be applied to other species: midline incision and opening of the cadaver; removal of jaw, neck organs and thoracic viscera; removal of pelvis and urogenital tract; and removal of the brain and spinal cord. The anatomic differences of the gastrointestinal tract of various animals necessitates a different sequence in removal of the abdominal viscera.

DOG AND CAT

Dogs and cats are placed on their left sides. The prosector stands at the ventral abdomen to perform the necropsy.

Opening of Cadaver

1. Start a midline incision at the sternum and extend it to the symphysis of the mandible. Extend the incision from the sternum caudally to the groin above the umbilicus, mammary gland, or external male genitalia (Fig. 2-1).

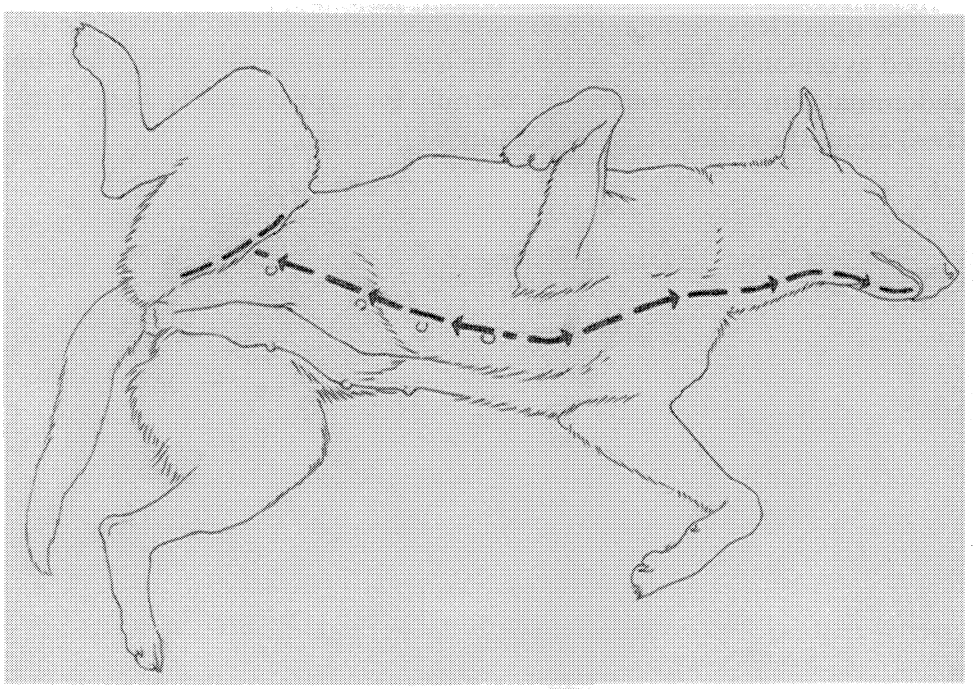

Figure 2-1.

2. Lift the front leg and cut the muscle attachments close to the thorax and lay the leg dorsally and flat. Reflect the skin dorsally from the midline incision of the neck to the base of the ear. Grasp the upper rear leg; incise the adductor, gracilis, and quadratus muscles; disarticulate the coxofemoral joint; and lay the leg dorsally and flat on the surface. Reflect the abdominal skin dorsally from the midline incision to the transverse processes of the spinal column (Fig. 2-2).

The upper legs (front and rear) and integument of the cadaver are reflected as one unit. Do not remove the legs from the cadaver or from the abdominal skin. Reflect the skin ventrally for approximately 5 cm (2 inches) along the entire length of the initial incision.

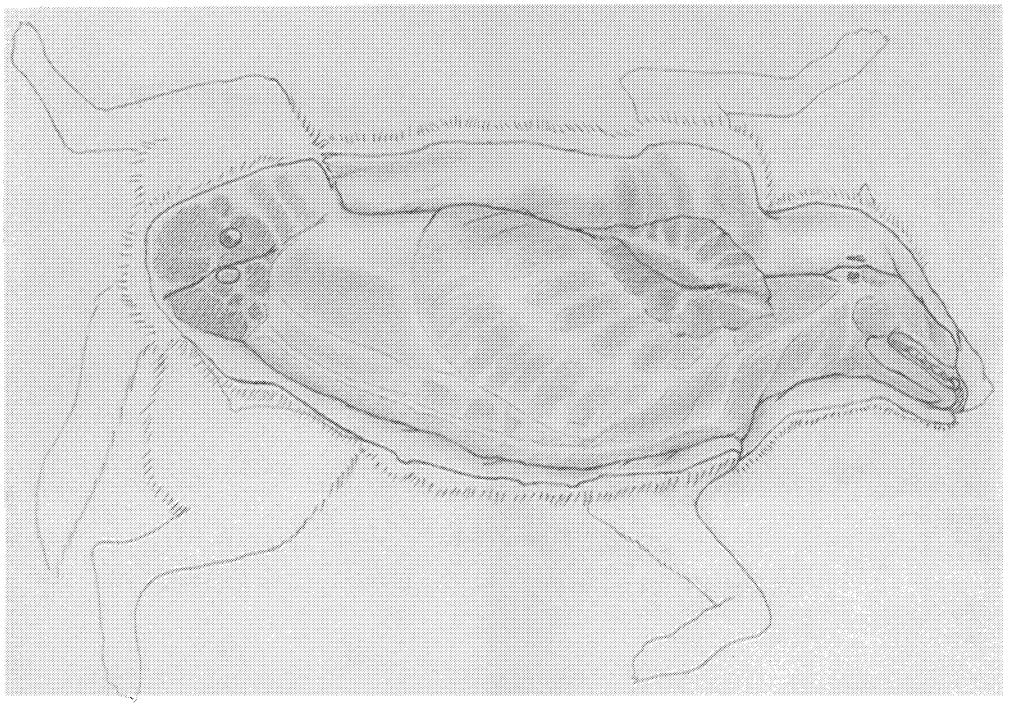

Figure 2-2.

3. Remove the upper jaw. To do this, three steps are necessary: (a) Cut the masseter muscle by inserting the knife at the commissure just lateral to the ramus, extend it to the angle of the rami, and sever the muscles (masseter) from the lateral surface of the angle of the ramus. The symphysis of the rami is cut with pruning shears or a saw. (b) Grasp and pull the upper ramus back. Cut the muscles behind the molar teeth and continue cutting the muscle attachments medial to the ramus and around the caudal border of the angle of the rami (Fig. 2-3). (c) Pull the upper ramus caudally by rotating it laterally in one movement to disarticulate it. Twist the disarticulated ramus and cut the remaining muscle attachments to remove it.

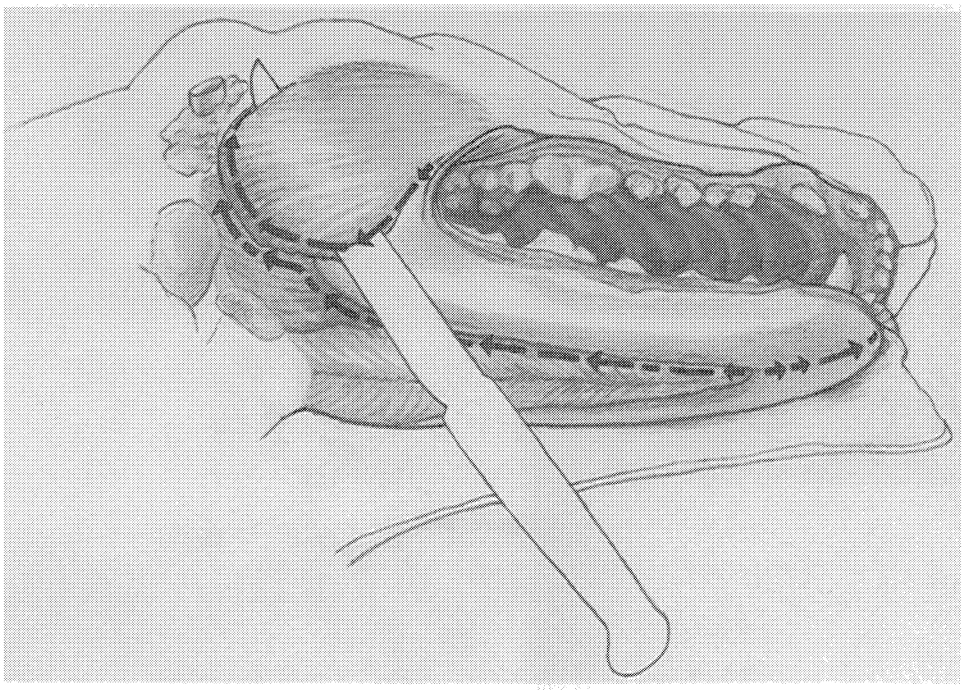

Figure 2-3.

4. Grasp the tongue and cut it loose from the medial surface of the left ramus. Cut behind the hard palate and around the pharynx to the level of the hyoid bones (Fig. 2-4). Disarticulate the hyoid bones and continue reflecting the tongue, pharynx, esophagus, and trachea to the thoracic inlet (Fig. 2-5).

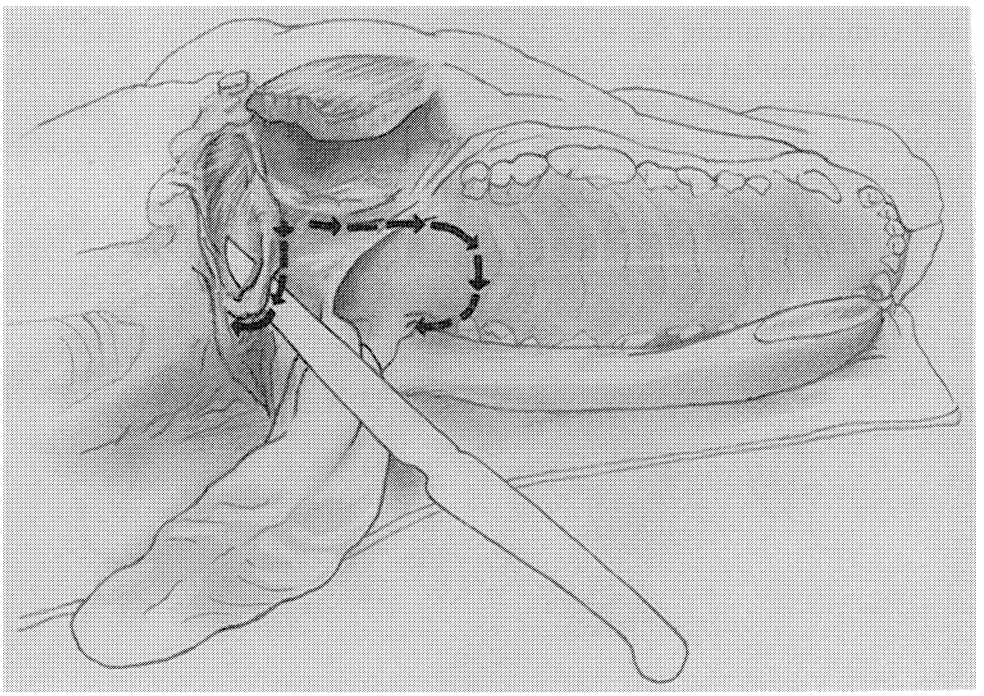

Figure 2-4.

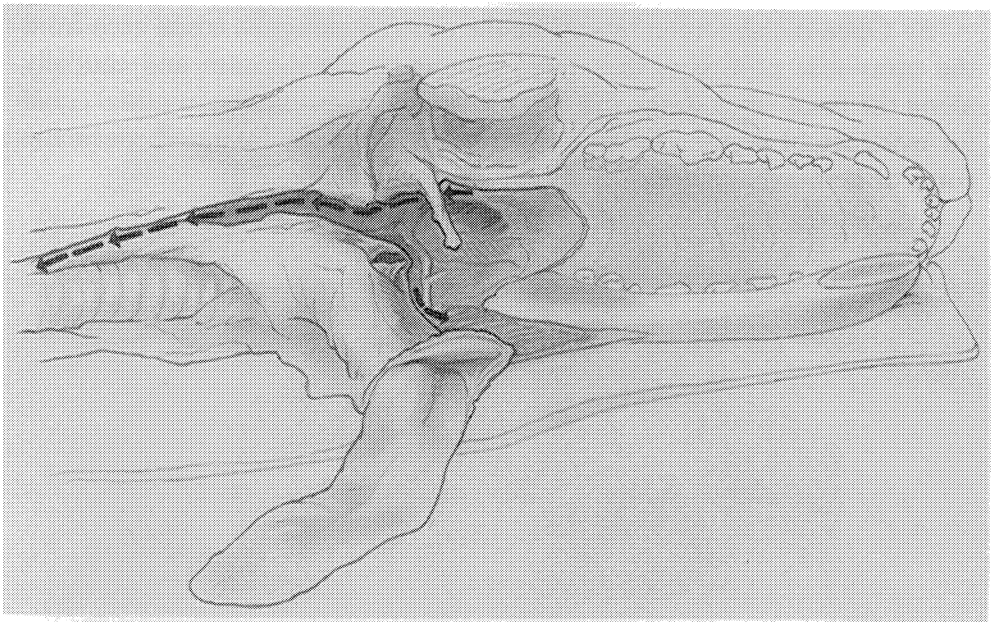

Figure 2-5.

5. Open the abdomen by cutting the muscles along the costal arch from the transverse process of the spinal column to the sternum and caudally along the linea albea to the brim of the pelvis. Examine the inguinal canal for evidence of cryptorchidism, incarcerated hernias, infarcted spermatic cord, or hemorrhage because of faulty castration (Fig. 2-6).

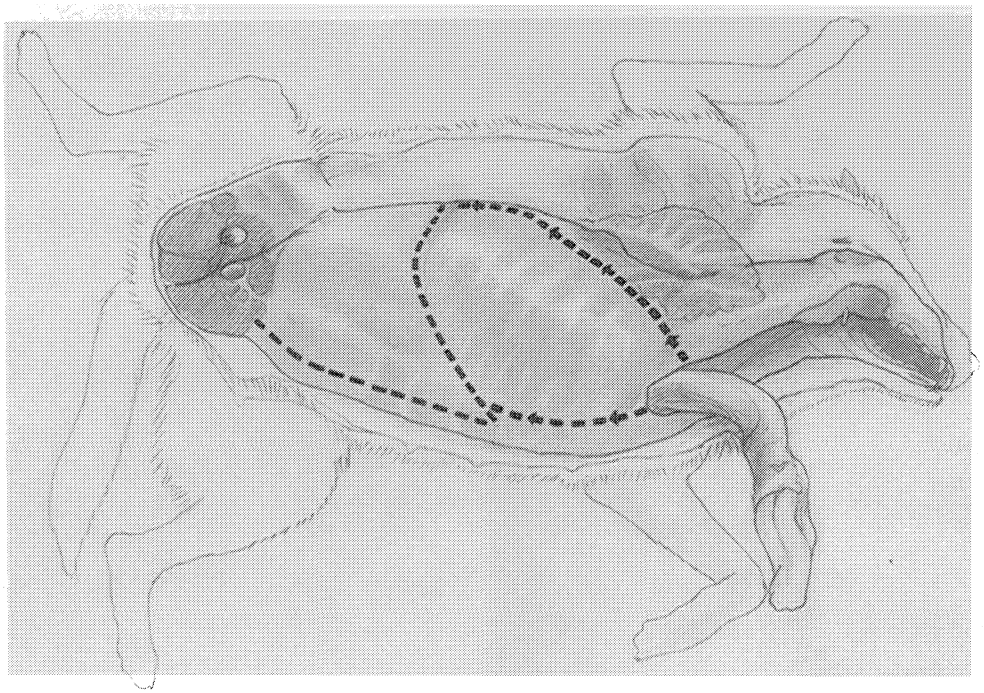

Figure 2-6.

6. Remove the rib cage by cutting the ribs with rib shears (pruning shears, cleaver, etc.) along the line of proposed cuts. The cuts are from the xiphoid cartilage cranial to thoracic inlet (just ventral to the esophagus, trachea, and tongue, that were reflected to the thoracic inlet). The next cut begins from the most dorsal aspect of the first rib (dorsal to the reflected neck organs), and extends caudally to the transverse process of the first lumbar vertebra. In heavy muscled animals, reflect muscles from rib cage along the proposed cuts. After the ribs are detached along these lines, the severed rib cage is removed by cutting it from the diaphragm (Fig. 2-6).

7. After removing the upper rib cage and abdominal wall, it is important to examine the thoracic and abdominal organs *in situ.* Note the

color of the exposed tissues and organs for hemorrhage, congestion, postmortem imbibition, and autolysis. Observe the anatomic relationships of organs prior to removal for signs of volvulus, torsion, intussusception, hernia, or other anomalies. Haste at this point could interfere with interpretation of a lesion later. Note the presence of hydropericardium, hydrothorax, hydroperitoneum, etc., and the characteristics of the fluid.

Collection of specimens for bacteriologic examination or direct culturing should be done at this time before contaminating any organ by manipulation or handling of viscera. Obtaining fluids, exudates, etc. from pericardial sac, pleural or abdominal effusions, cysts, gall bladder, and urinary bladder is best done before they are handled.

8. Grasp the tongue, esophagus, trachea, and reflect the thoracic viscera (heart, lungs, and thymus) from the thoracic cavity. Open the esophagus and check for ulcers or parasites. Check the thyroid and parathyroid glands that lie adjacent to the trachea and caudal to the larynx. Open the trachea and extend the incision into the bronchi and bronchioles. Palpate and incise each lobe of lungs for suspected lesions. *Do not detach* the heart from the lungs.

Examination of Heart

9. Heart Cuts:

Lay out the thoracic viscera so that the tongue, esophagus, and trachea are to the prosector's right and open the pericardial sac.

a. Hold the heart in the left hand with the apex towards you and with scissors make a cut in the right ventricle adjacent to the septum and extend the cut dorsally and caudally into the caudal vena cava (Fig. 2-7).

b. Extend the cut anteriorly from the posterior vena cava opening the cranial vena cava (Fig. 2-7).

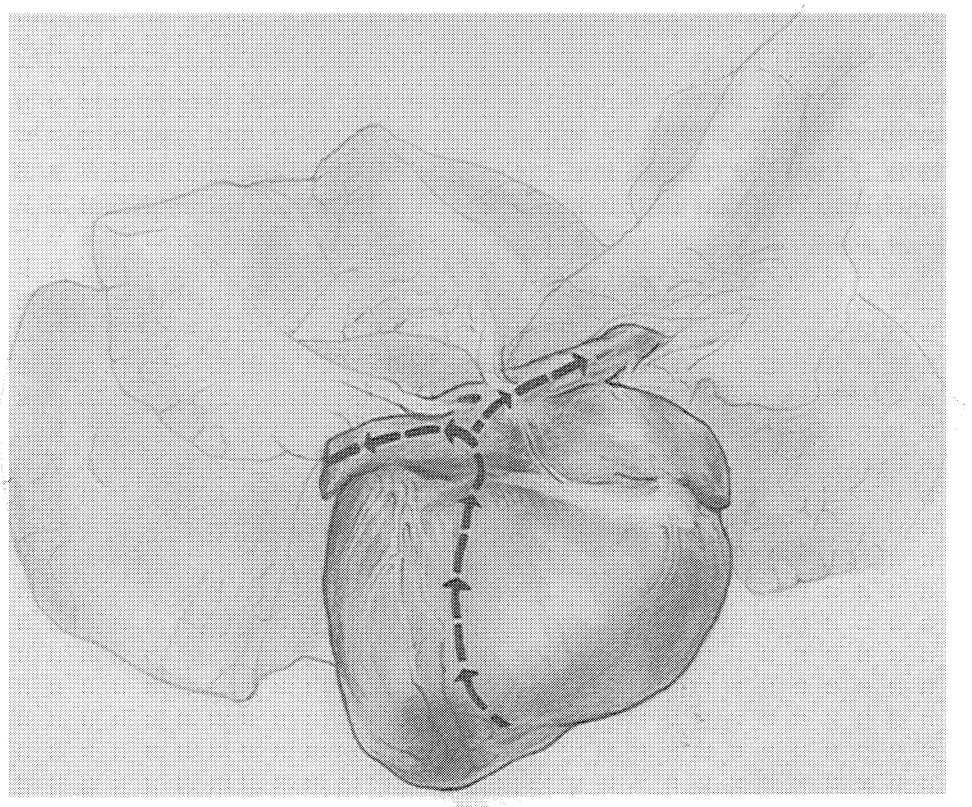

Figure 2-7.

c. Extend the initial cut (a) in the right ventricle dorsocranially adjacent to the septum continuing out to the conus anteriosus and pulmonary artery (Fig. 2-8).

d. Examine all valves and openings of the right auricle and ventricle.

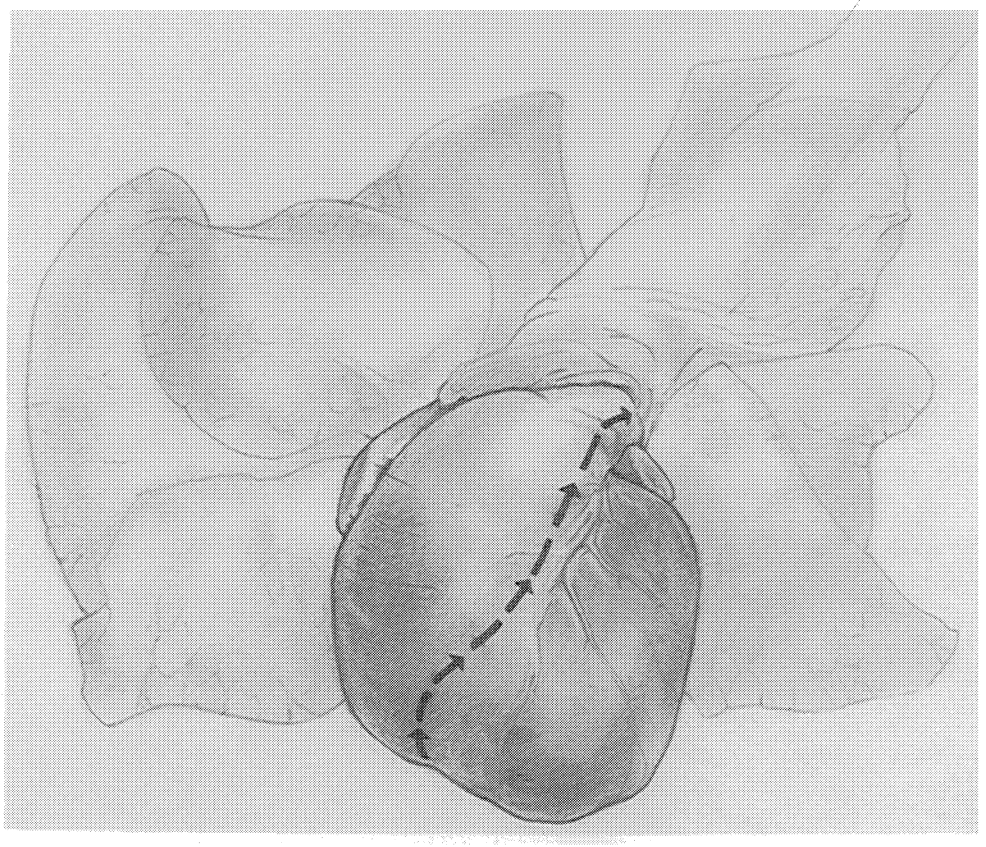

Figure 2-8.

e. Rotate the heart counter clockwise until the left auricle is uppermost. Hold the heart in the left hand with the thumb over the left auricle. Bisect the left ventricle near the apex and extend the cut dorsally into the pulmonary veins (Fig. 2-9). Examine the left AV valve.

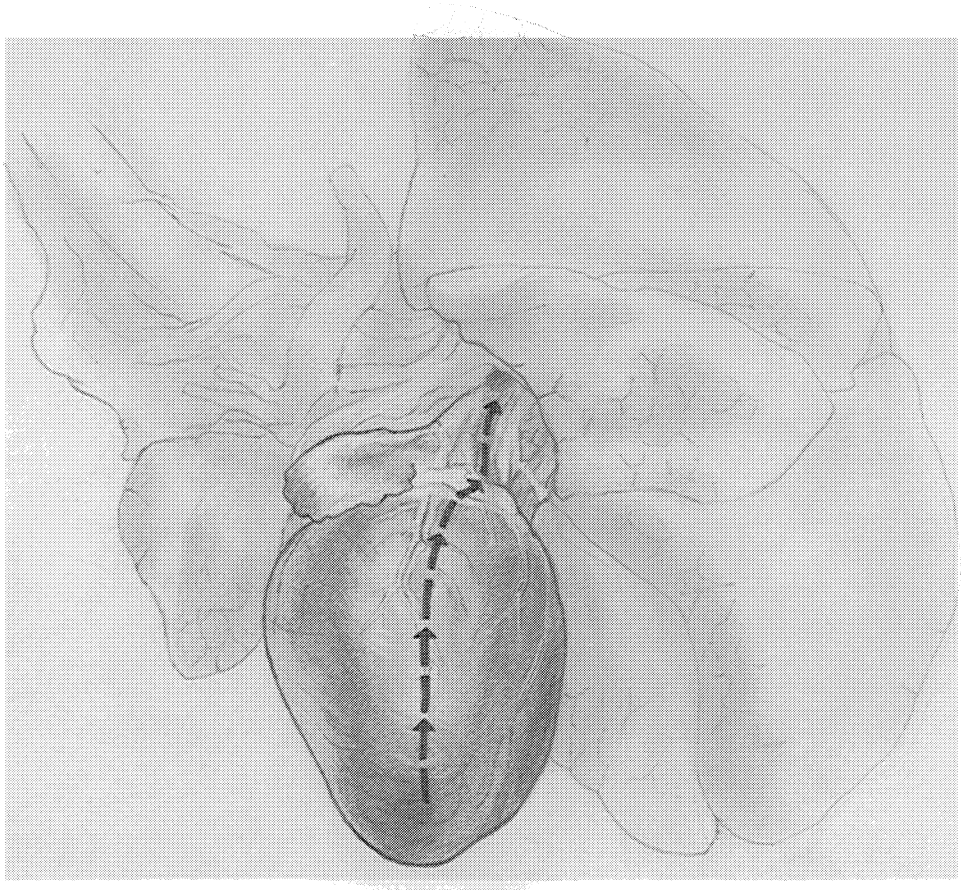

Figure 2-9.

f. With the left index finger and thumb grasp the left half of the ventricle, and with scissors in the right hand cut through the left ventricle going under the left AV valve (Fig. 2-10).

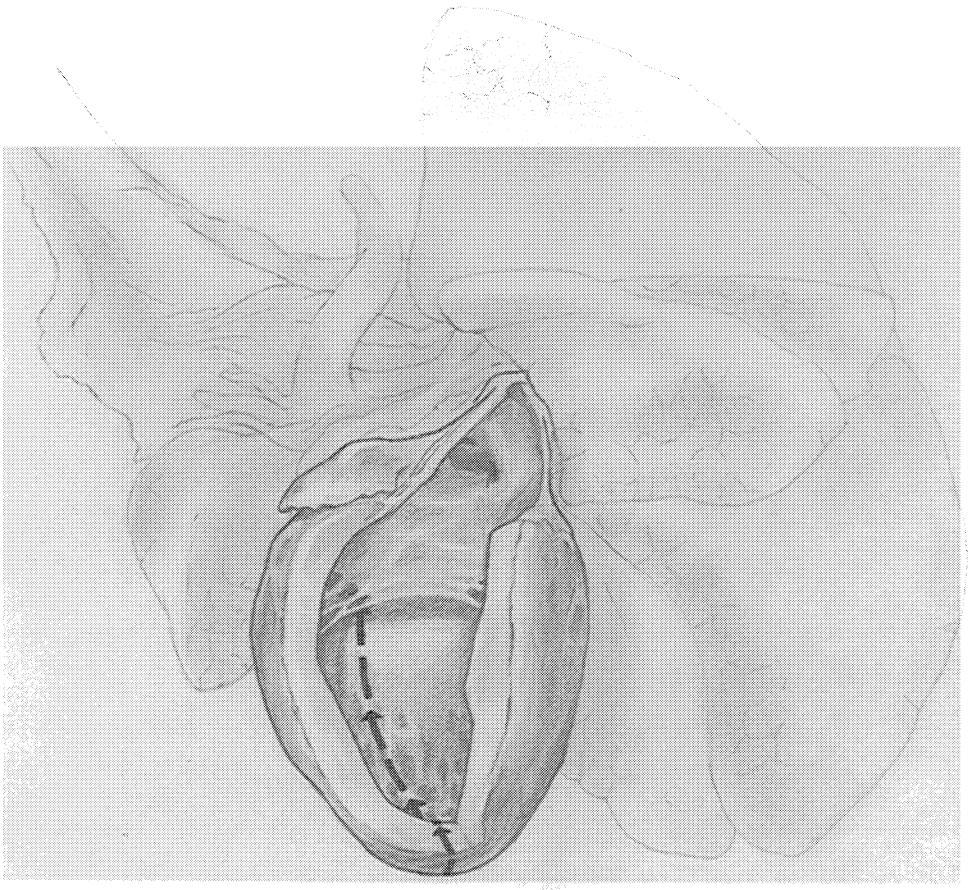

Figure 2-10.

g. Extend the incision out to the aorta. Examine the coronary, subclavian, and brachocephalic arteries (Fig. 2-11).

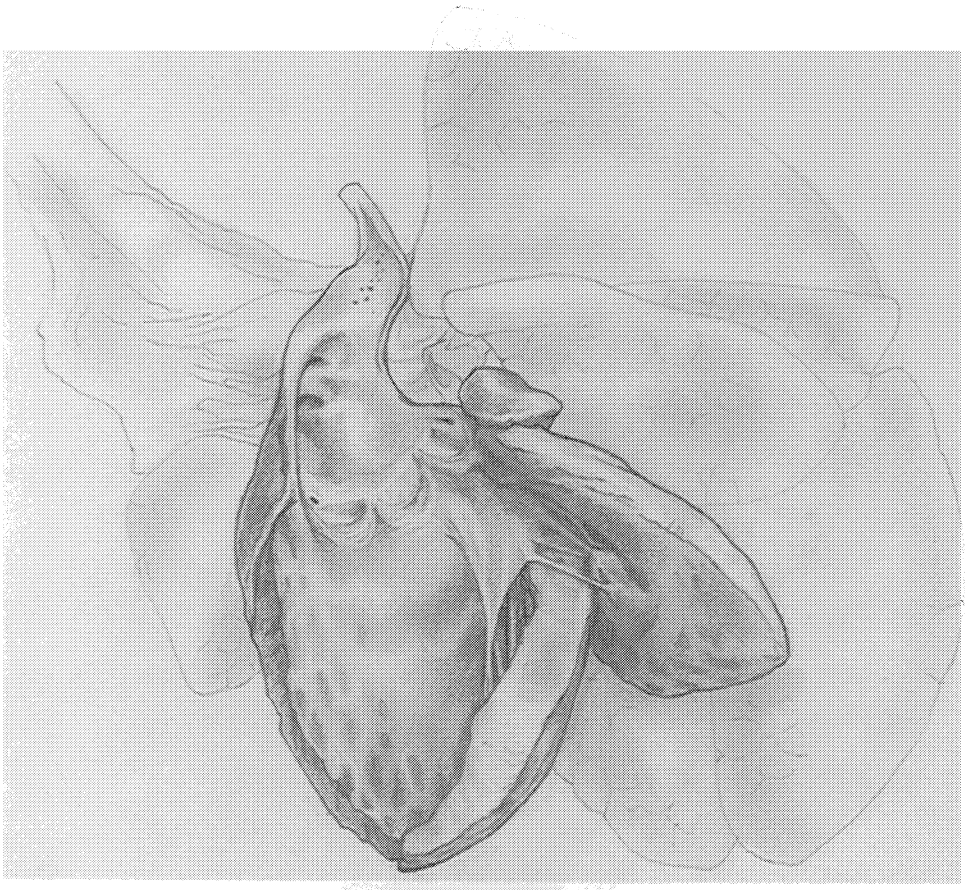

Figure 2-11.

Removal of Gastrointestinal Tract

10. Grasp the duodenum and pancreas and lay them dorsally over the vertebral column. Grasp the omentum and pull it ventrally. The cecum is usually visible as an inflated comma-shaped structure surrounded by intestine and ventral to the right kidney (Fig. 2-12). Find the ileum at the apex of the cecum, cut the small intestine from its mesenteric attachment to the posterior duodenum, and stop as the duodenum loops under the large intestine (Fig. 2-13).

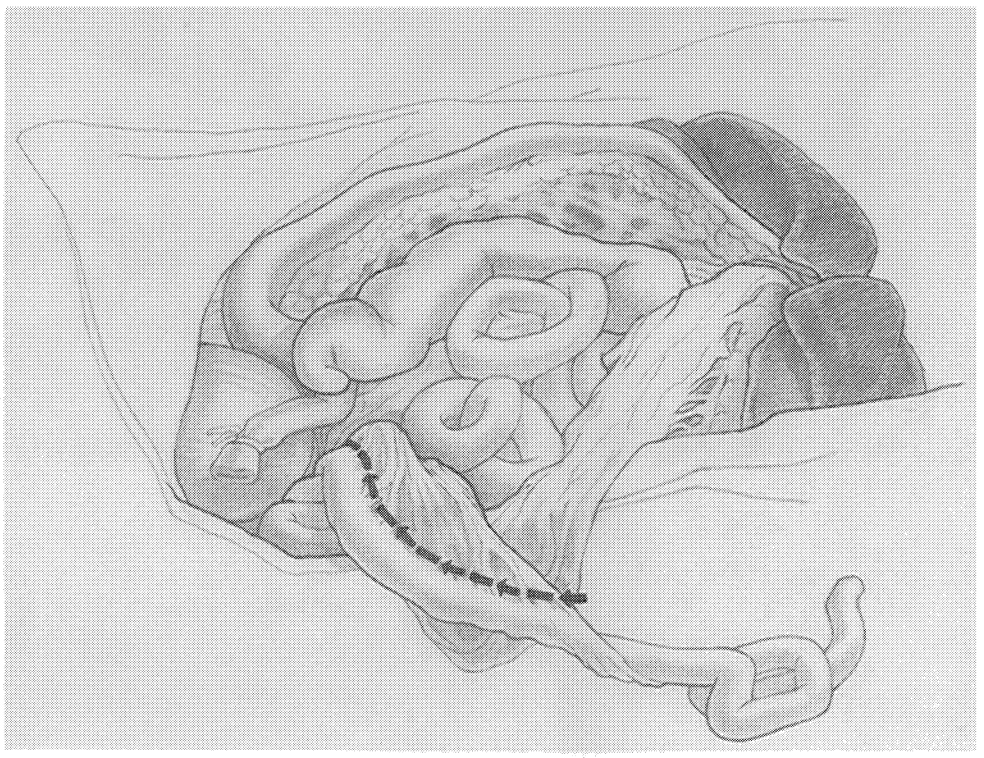

Figure 2-12.

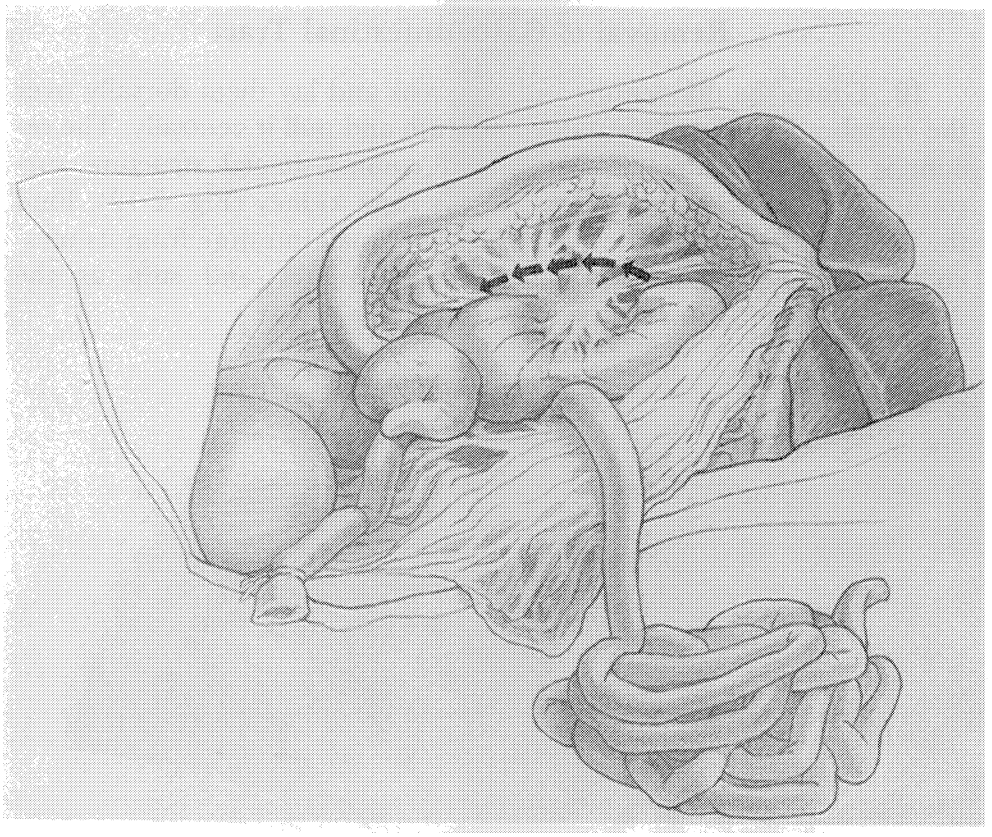

Figure 2-13.

10a. Grasp the cecum in the left hand, cut dorsal and cranial to the cecum and the mesenteric lymph node. Pull the cecum, terminal ileum, colon, and mesenteric lymph nodes towards you and cut the mesenteric attachment at the same time (Fig. 2-14).

11. Grasp the posterior duodenum, cut it loose from any remaining attachments, and lay it ventrally outside the abdominal cavity. The pancreas can be examined *in situ* (Fig. 2-14). Cut the duodenum at the tail of the pancreas and remove the small intestine for examination. Then place the cecum, terminal ileum, colon, and mesenteric lymph nodes over the paralumbar region.

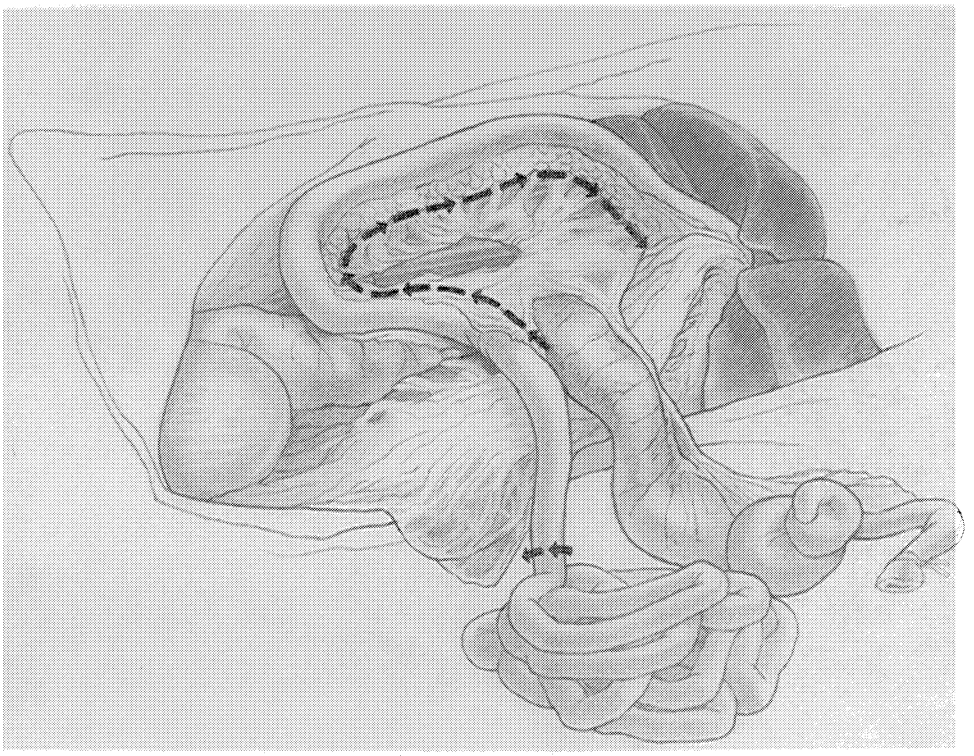

Figure 2-14.

12. Grasp the right kidney and cut laterally and anteriorly to the kidney and adrenal. Reflect the kidney and adrenal posteriorly with the ureter to the pelvic inlet and lay them dorsally over the paralumbar region. Repeat for the left kidney, adrenal, and ureter (Fig. 2-15).

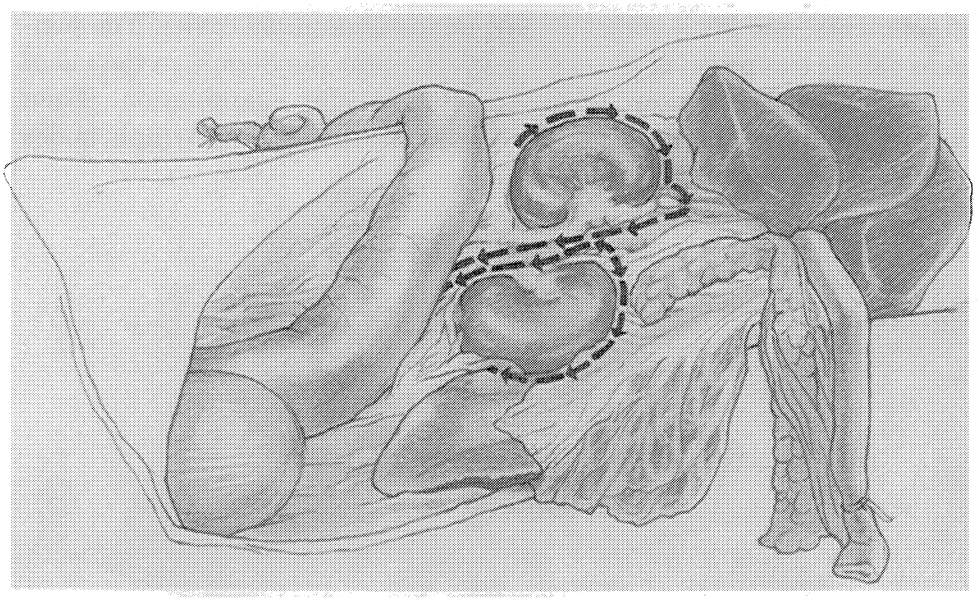

Figure 2-15.

13. Check patency of the gall bladder by squeezing it and note whether any bile flows into the duodenum. Remove the stomach by cutting it from the hilus of the liver. The spleen, duodenum, and pancreas are removed with the stomach (Fig. 2-16).

14. Remove the liver and diaphragm together by cutting the diaphragm from its costal attachment.

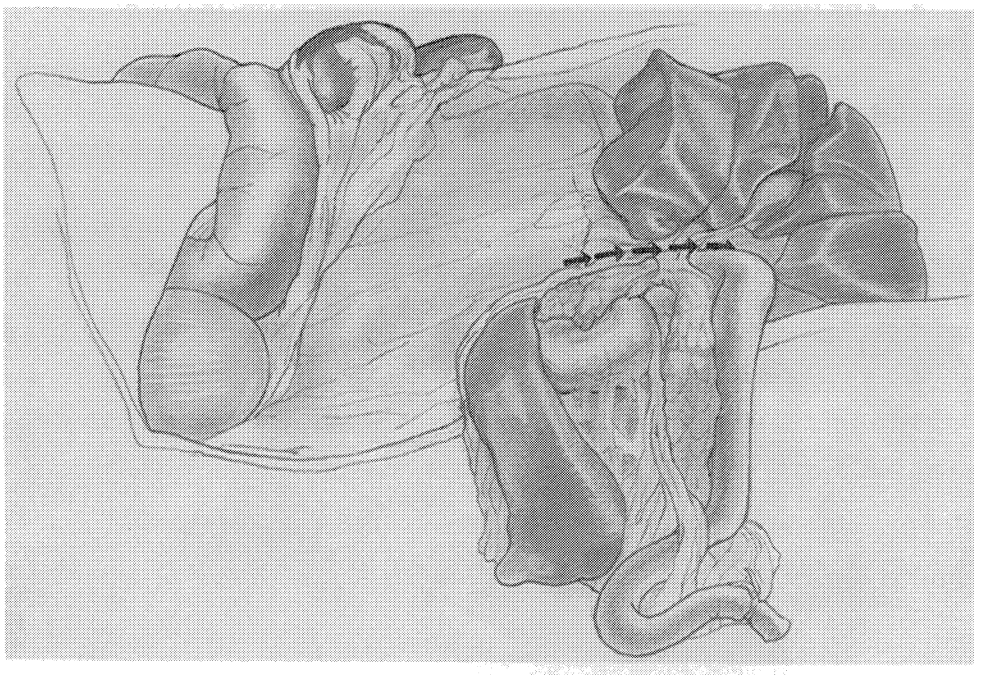

Figure 2-16.

Pelvic Cuts and Urogenital Tract Removal

15. Before the genital tract, urinary system, and posterior portion of the alimentary tract are removed, it is necessary to remove the lateral aspects of the pelvis. *Three cuts are required* and all are made close to the exposed coxofemoral joint (Fig. 2-17):

a. The first cut is made from the cranial brim of the pelvis to the obturator foramen.

b. The second is made from the obturator foramen to the ischial arch parallel to the long axis of the vertebral column.

c. The third is made across the shaft of the ileum anterior to the disarticulated coxofemoral joint.

The ligaments and muscles surrounding the detached lateral bony pelvis are cut. Remove the detached bony pelvis from the cadaver.

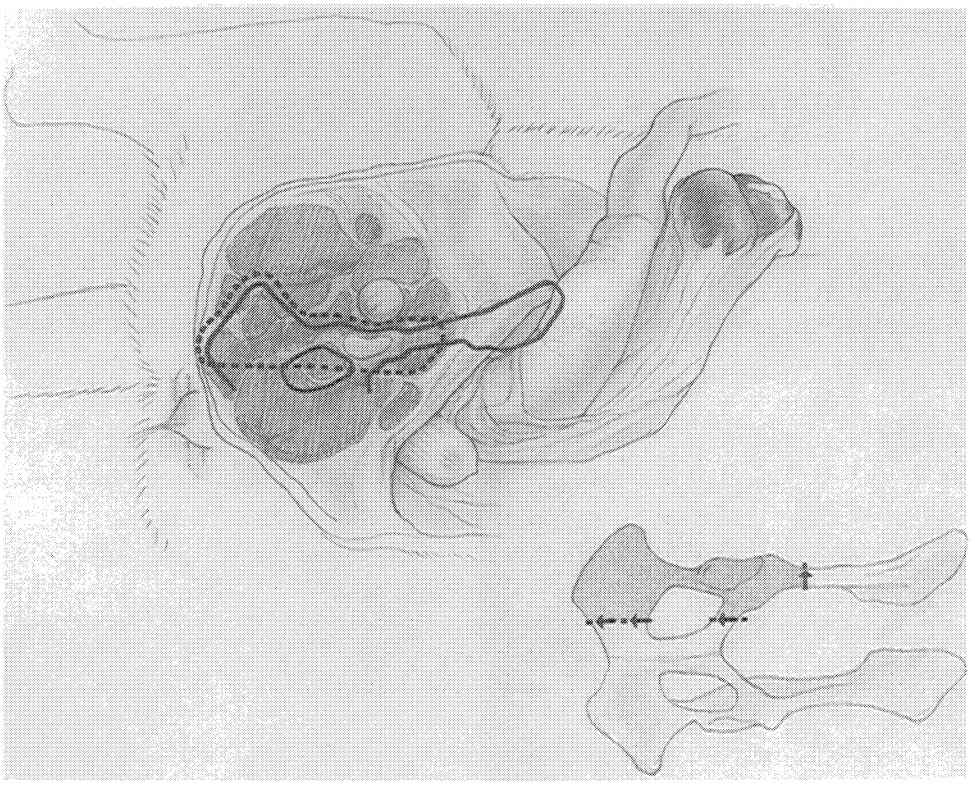

Figure 2-17.

16. In females, grasp the kidneys, attached adrenal, ureter, uterus, and large intestine and reflect them posteriorly to the level of the ischial arch, cutting all attachments close to the abdominal wall and bony pelvis. Cut the fascial attachments from the ventral floor of the pelvis, extending the cut adjacent to the ischial arch to remove the vulva with the internal reproductive tract intact (Fig. 2-18). A gravid uterus with full-term fetuses may be examined *in situ,* cut at the cervix, and removed separately.

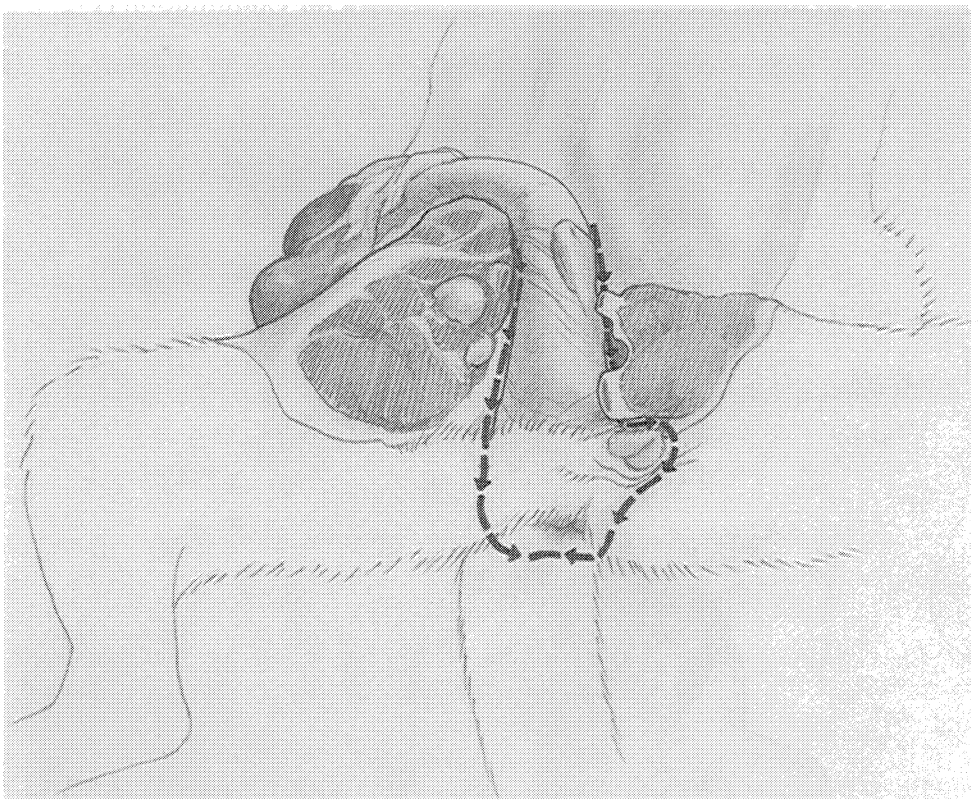

Figure 2-18.

17. In males, grasp the kidneys, attached adrenals, ureters, and large intestine and cut their pelvic attachments at a point just cranial to the ischial arch. Reflect the external genitalia caudally (scrotum included) to near the caudal-ventral border of the ischial arch. Grasp the external genitalia and reflected pelvic organs and cut the ligaments and crura close to the caudal border of the symphysis of the pubis and ischial arch, and continue to reflect ventrally around the anus. The urogenital organs and large intestine are removed together. This step permits removal of an intact internal and external urethra for examination (Fig. 2-19).

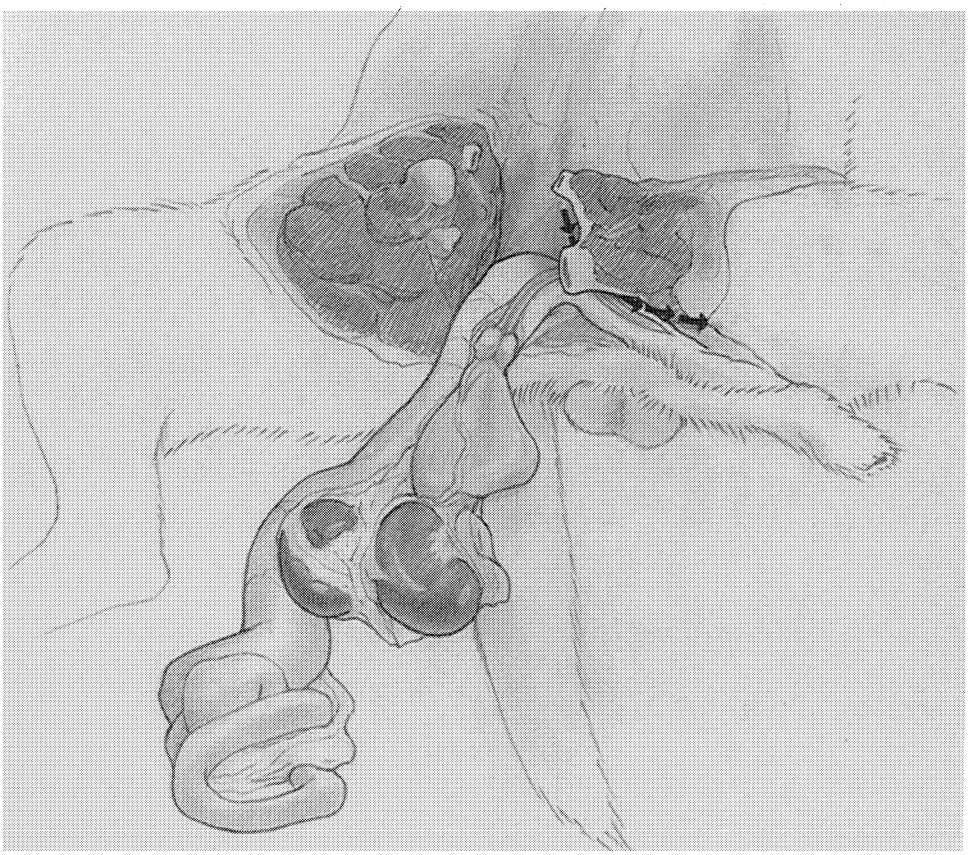

Figure 2-19.

Collection of Spinal Fluid

18. Cut behind the angle of the mandible deep to the posterior border of the occipital condyle, exposing the foramen magnum (Fig. 2-20). Sterile cerebral spinal fluid (CSF) may be removed at this time for microbiological and clinical pathological examinations (Fig. 2-20 — Inset).

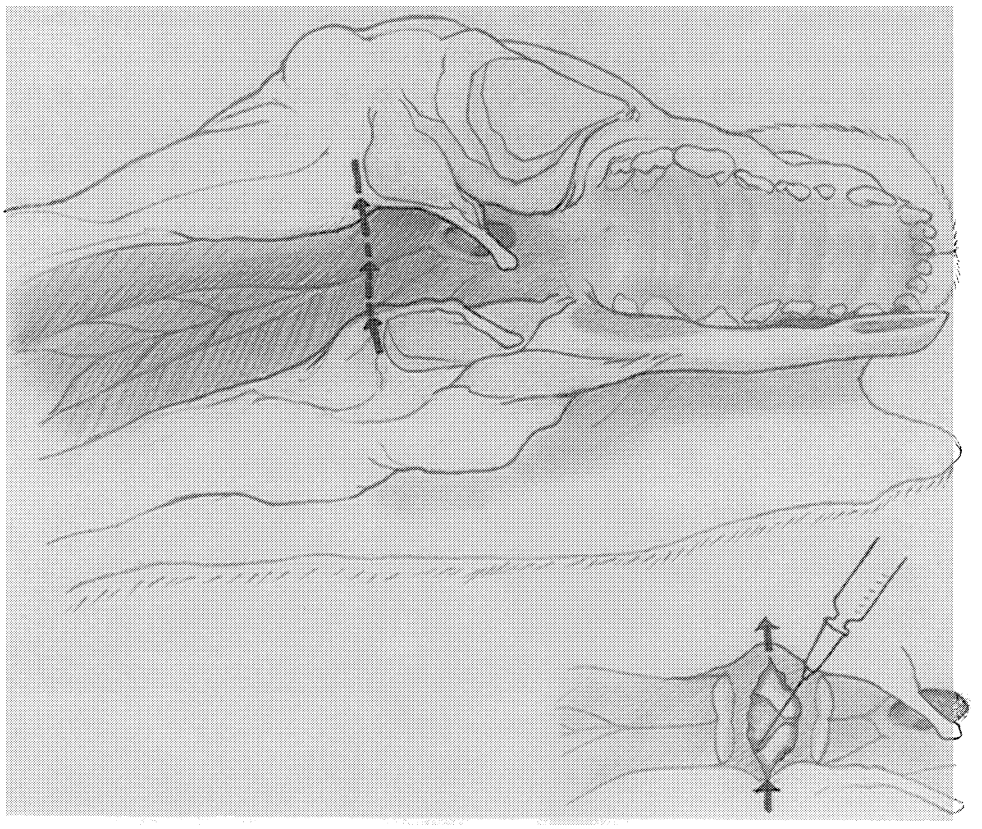

Figure 2-20.

Removal of Brain

The brain should be examined in all cases in which the clinical history suggests neurological disturbances.

18a. Leave the head attached to the spinal column and reflect the skin from the head. This may be a possible compromise, but an easy way to remove the brain is a near mid-saggital incision (¼ to ½″ to either side of the midline). Saw the head from the ventral side and extend the incision posteriorly to the level of the occipital condyle (Fig. 2-21).
After the calvarium and brain are exposed, each half is removed by gentle traction and passive gravitational force. The brain will peel out of the calvarium after cutting the cranial nerves and dural attachments with a pair of curved pointed scissors. *Do not* squeeze or handle the brain roughly, because it is easily damaged.

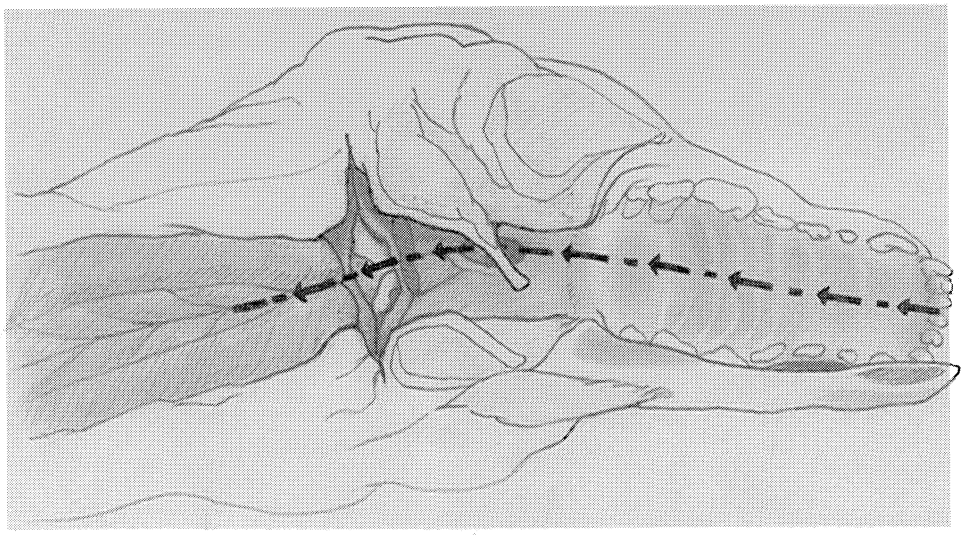

Figure 2-21.

18b. The head may be removed from the cadaver and the brain removed *in toto*. Make a midline cutaneous incision from the frontal prominence to a point behind the occiput. Reflect the skin back on either side to expose the temporal muscles to the zygomatic arch. Cut the temporal muscles free from their proximal attachments and from the bone over the dorsal and lateral surfaces of the skull in the temporal fossa. Remove the head by making a deep transverse cut through the extensor muscles immediately caudad to the occipital protuberance to the atlanto-occipital articulation and disarticulate.

After the head is removed, three cuts are made through the calvarium to open the cranial cavity:

(1) Saw on a line from the dorsal and lateral periphery of the foramen magnum, cutting the dorsal ⅓ of the occipital condyle and medial to the orbit, extend the incision rostrally and anteriorally on a line to the medial canthus of the opposite eye.
(2) Repeat this cut for the other side.
(3) Cut the frontal bone transversely at the anterior limit of the cranial cavity (line varies with species, age, and breed) on a line posterior to the supraorbital process.

Lift or pry the severed calvarium off of the cerebral hemispheres and cerebellum. With a scissors, cut the meninges, falx cerebri (dura between cerebral hemispheres), and tentorium cerebelli (dura between cerebral hemispheres and cerebellum), and reflect the meninges from the dorsal aspect of the brain. Invert the head to gain gravitational assistance for easing the brain out of the cranium. By cutting the cranial nerve roots and ventral dural attachments, the brain is easily removed from the cranial cavity. Handle the brain carefully as it easily becomes an amorphous mass.

The brain may be sectioned immediately or placed *in toto* in 10% buffered neutral formalin (BNF) and sectioned after preliminary fixation of 12-24 hours. The brain may be placed in a container to which concentrated formalin (37% w/v) is added until brain floats. This will rapidly firm the brain for sectioning. To section a fresh brain, place on its ventral surface and press the olfactory lobes against a block or lip of the table. Moisten the knife in physiologic saline or water before each cut so that tissue will not adhere to it. Carefully make a series of transverse cuts (1 cm apart) through both lobes. When diffuse changes or large focal lesions are present, quicker information and better fixation is acquired by sectioning when brain is fresh. Small focal lesions are best detected grossly in fixed brains. Lesions in fixed brain tissue are grossly observed as darkened foci and frequently are easily distinguishable.

Removal of Spinal Cord

19. Cut the muscles and tendons from the dorsal, ventral, and transverse processes of the spinal column. A cleaver or ax may be used to chip away the exposed transverse processes along the vertebral column in large animals. Continue chipping parallel to the transverse process until

the right side of the vertebral column is removed and the spinal cord exposed. In small animals a rongeur may be used to accomplish the same end. A Stryker saw may be used if one is not adept to handling a cleaver or ax. Starting at the atlanto-occipital joint, the spinal cord is removed by grasping the meninges with a forceps to hold the spinal cord at an obtuse angle and cutting the dorsal and ventral roots to free the cord from the vertebral column.

Do not squeeze, mash, or pull the spinal cord at acute angles from the spinal column. After the cord is removed from the spinal column, cut the meninges the entire length of the spinal cord and examine grossly. Place the cord in 10% BNF to fix for 24 hours before sectioning. Transverse cuts may be made of the fresh cord with gentle handling and a sharp knife to examine for lesions.

Opening Joints

20. Make a medial skin incision, reflect the skin, and open the joint to examine the type and amount of fluids and to look for erosions of the articular surfaces.

Method of Culturing Joints

21. Three approaches are recommended:

(a) Saw across the long bone midshaft above and below the joint and send the joint intact to a diagnostic laboratory for culture.

(b) Reflect the skin from the surface of the joint and with a sterile syringe and needle aspirate joint fluid or exudate and send it to the laboratory.

(c) Reflect skin from the surface of the joint, sear the surface, open the joint with a sterile blade and with a sterile loop, streak the joint contents directly on the blood agar plate, or take a sample with a sterile swab and send it to the diagnostic laboratory.

SWINE

The same procedure described for the dog and cat is used for opening swine cadavers (Steps 1-7) and for removing and examining the neck and thoracic organs (Steps 8-10). Removal of the entire pharynx is necessary, because the tonsil is a flattened structure embedded in it and easily overlooked.

Removal of the Gastrointestinal Tract

10. Grasp the ileum, near its entry into the cecum. Cut the small intestine away from its mesenteric attachment until the small intestine goes under the large intestine. At this point, sever the ileum and caudal duodenum and remove the small intestine. By cutting the small intestine close to its mesenteric attachment, the attached border is freed so that the intestine is easily opened for examination (Fig. 2-22).

11. Bluntly dissect the rectum and colon cranially from the pelvic inlet to the right kidney. Sever the colon and place over the paralumbar fossa (Fig. 2-22).

11a. Check the pancreas and sever the duodenum caudal to the gall bladder and bluntly dissect it from the stomach.

12. Grasp the large intestine (spiral colon) in the left hand and pull it tautly from its mesenteric attachments, sever and remove it from the abdominal cavity.

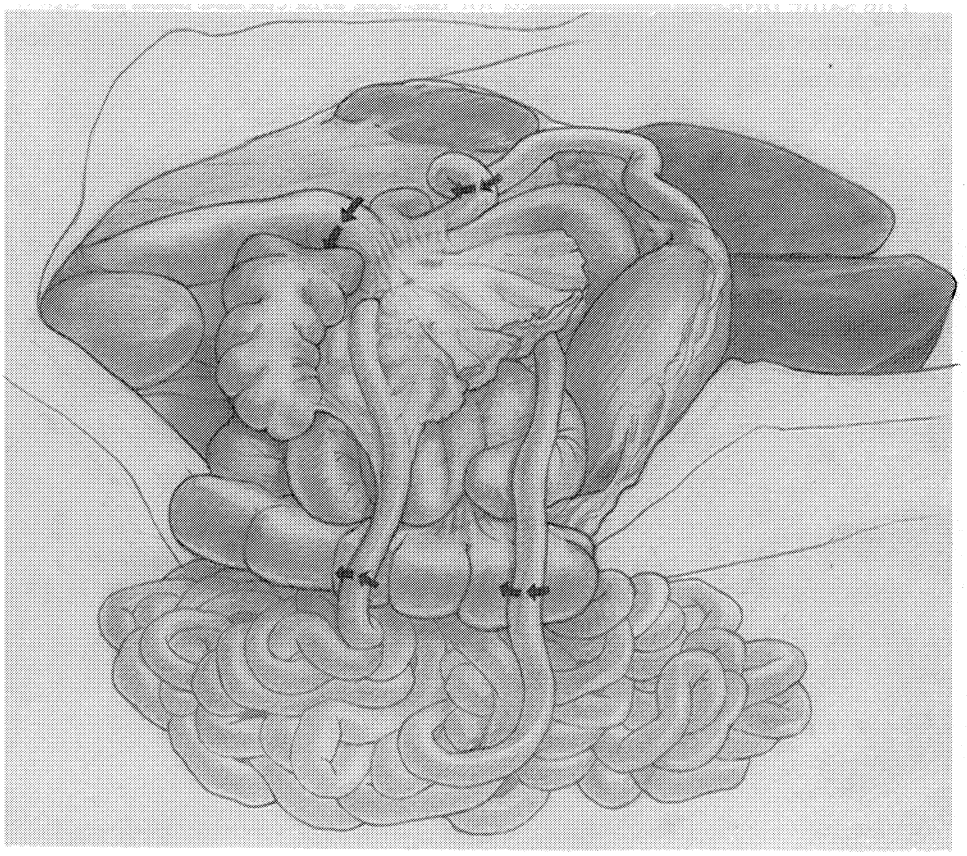

Figure 2-22.

13. Reflect the kidneys, adrenals, ureters, urinary bladder, and nongravid uterus caudally to the pelvic inlet and place over the paralumbar region. A gravid uterus with fetuses may be examined *in situ* and removed at the cervix.

13a. Check gall bladder patency and remove the stomach, spleen, and duodenum together.

14. Sever the costal attachments of the diaphragm and remove the liver and diaphragm.

Pelvic cuts and removal of urogenital, posterior gastrointestinal tract, brain, and spinal cord are the same as for the dog and cat (Steps 15-21).

CATTLE, SHEEP, AND GOATS

All ruminants are placed on their left side and prosector is at the ventral abdomen.

The same procedures described for the dog and cat are used for opening cadavers of ruminants (Steps 1-7) and for removing and examining the neck and thoracic organs (Steps 8-10).

Removal of the Gastrointestinal Tract

10. Grasp the ileum with the left thumb and index finger near its entry into the cecum and cut the small intestine away from its mesentery attachment until the small intestine disappears under the large intestine (duodenojejunal flexure). Cut the small intestine close to its mesenteric attachment, freeing the attached border so that the intestine is easily straightened and opened for examination. At this point, sever and remove the small intestine (Fig. 2-23). Cut the duodenum caudal to the gall bladder, check gall bladder patency and bluntly dissect the duodenum to the abomasum (Fig. 2-23).

11. Bluntly dissect the rectum cranially from the pelvic inlet to the right kidney, and sever the rectum and place over the paralumbar region (Fig. 2-23).

11a. Grasp the large intestine in the left hand, pulling it taut from its mesenteric attachments, sever and remove it from the abdominal cavity.

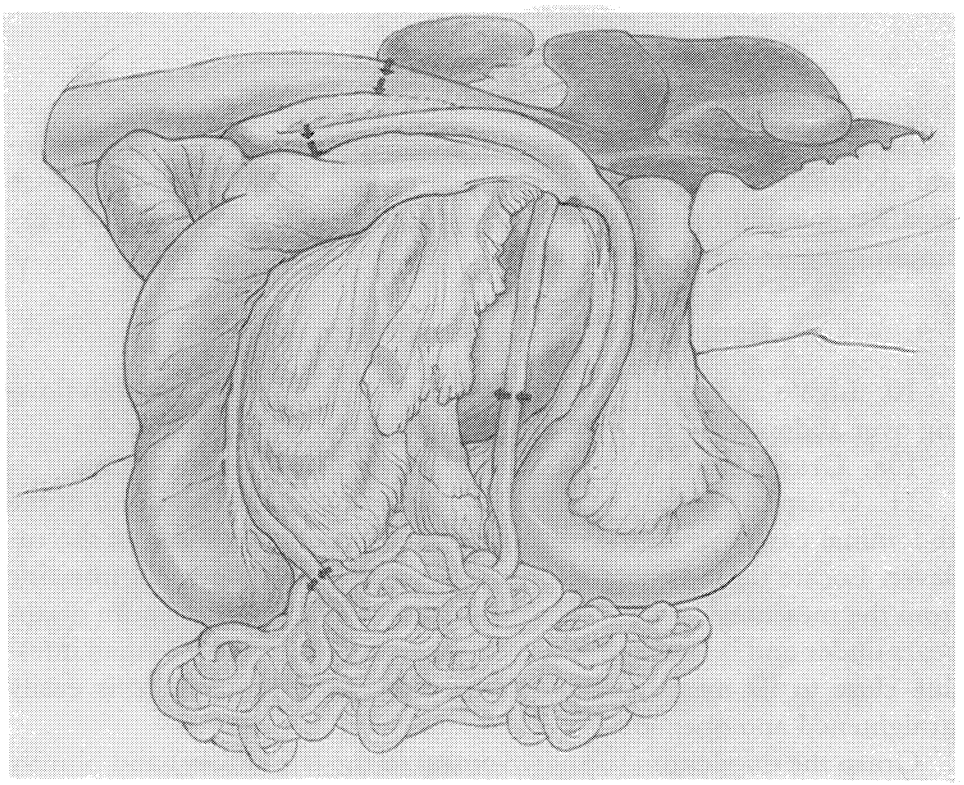

Figure 2-23.

12. Reflect the kidneys, adrenals, ureters, urinary bladder, and nongravid uterus caudally to the pelvic inlet and place them over the paralumbar fossa. Near term fetuses in the uterus are removed and the uterus examined *in situ.* The uterus may be severed at the cervix and removed.

13. Palpate for hardware lesions between the reticulum and diaphragm before removing the rumen. Sever the dorsal attachments of the rumen from the abdominal wall and pull the rumen counterclockwise. Sever the attachments between the liver and omasum and ventrally and clockwise pull the omasum and abomasum from the abdominal cavity. Grasp the caudal dorsal area of the rumen, sever the remaining dorsal attachments, and pull the rumen counterclockwise until it is out of the abdominal cavity. The spleen lies on top of the rumen and can be examined.

14. Sever the costal attachments of the diaphragm and remove the liver and diaphragm.

Pelvic cuts and removal of urogenital, posterior gastrointestinal tract, brain, and spinal cord are the same as for the dog and cat (Steps 15-21).

HORSE

All horses are placed on the right side with the prosector at the ventral abdomen. The same procedures described for the dog and cat are used for opening horse cadavers (Steps 1-7) and for removing and examining the neck and thoracic organs (Steps 8-10).

Removal of the Gastrointestinal Tract

10. Ligate and remove the spleen (ligation prevents blood from flowing over abdominal viscera).

10a. Grasp the small colon and lay it over the paralumbar area.

11. Grasp the pelvic flexure of the great colon and pull it out of the abdominal cavity so that it is at right angles to the long axis of the cadaver. Locate the ileum adjacent to the cecum. Cut the small intestine from the mesenteric attachment forward to where the duodenum disappears under and is attached to the transverse colon. (Cut the small intestine close to its mesenteric attachment so that the intestine is easily straightened and opened for examination) (Fig. 2-24).

Grasp the duodenum (usually ventral to the left kidney) and bluntly tear it loose from its mesenteric attachments and from the large intestine cranial to the stomach.

Pull the stomach cranially and cut the esophageal portion loose from the diaphragm. Grasp the duodenum at the pylorus and bluntly dissect it from its mesenteric attachments caudally until it is freed. Sever the duodenum below the right kidney, and tie the proximal end in a knot. Grasp the stomach and pull it and the first portion of the duodenum cranially from the abdominal wall.

Sever and ligate the small colon 4 cm caudal to the transverse colon and lay it over the paralumbar region (Fig. 2-24).

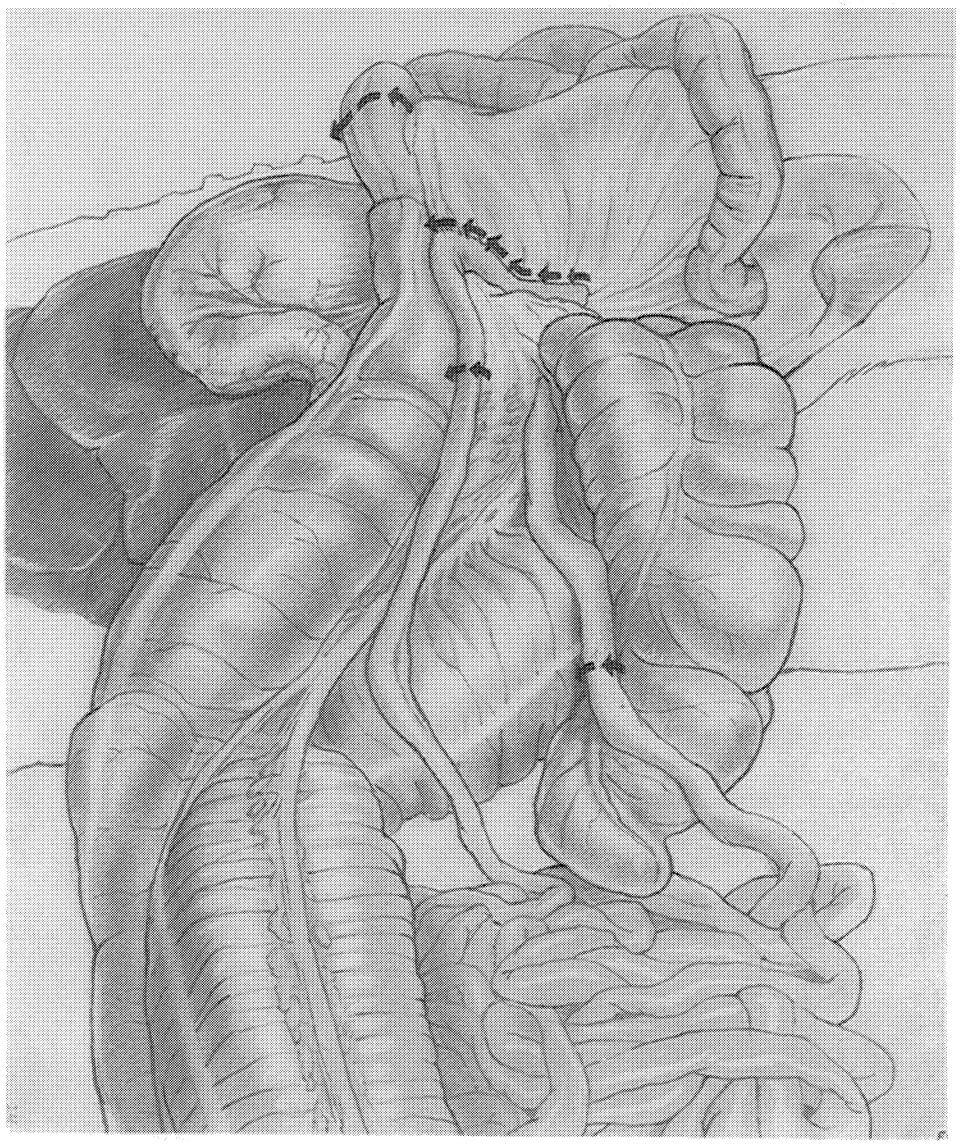

Figure 2-24.

12. Cut lateral to the left kidney and reflect the kidney medially. Extend the incision cranially and medially to remove the left adrenal gland, left kidney, and its ureter as a unit posteriorly and lay it over the anterior brim of the pelvis or paralumbar region (Fig. 2-25).

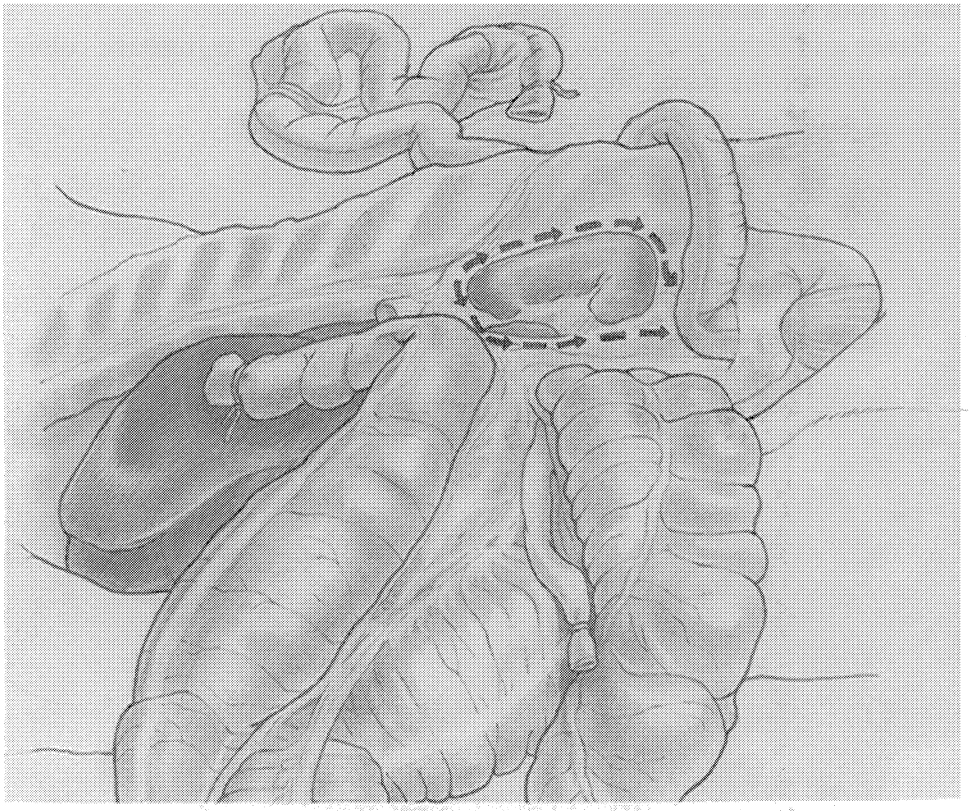

Figure 2-25.

13. Check major abdominal blood vessels for thrombi. Start at the beginning of the abdominal aorta and open it to its termination (internal and external iliacs). Open the coeliac, cranial mesenteric, and renal arteries and check the major (3) branches of the cranial mesenteric artery (Fig. 2-26).

13b. Sever the abdominal aorta caudal to the cranial mesenteric artery and remove this section along with the large colon from the abdominal cavity (Fig. 2-26). When reflecting the large colon, *do not* remove the right kidney and adrenal with it. The right kidney and adrenal are partially under the liver and may be torn loose when removing the large intestine and the abdominal aorta.

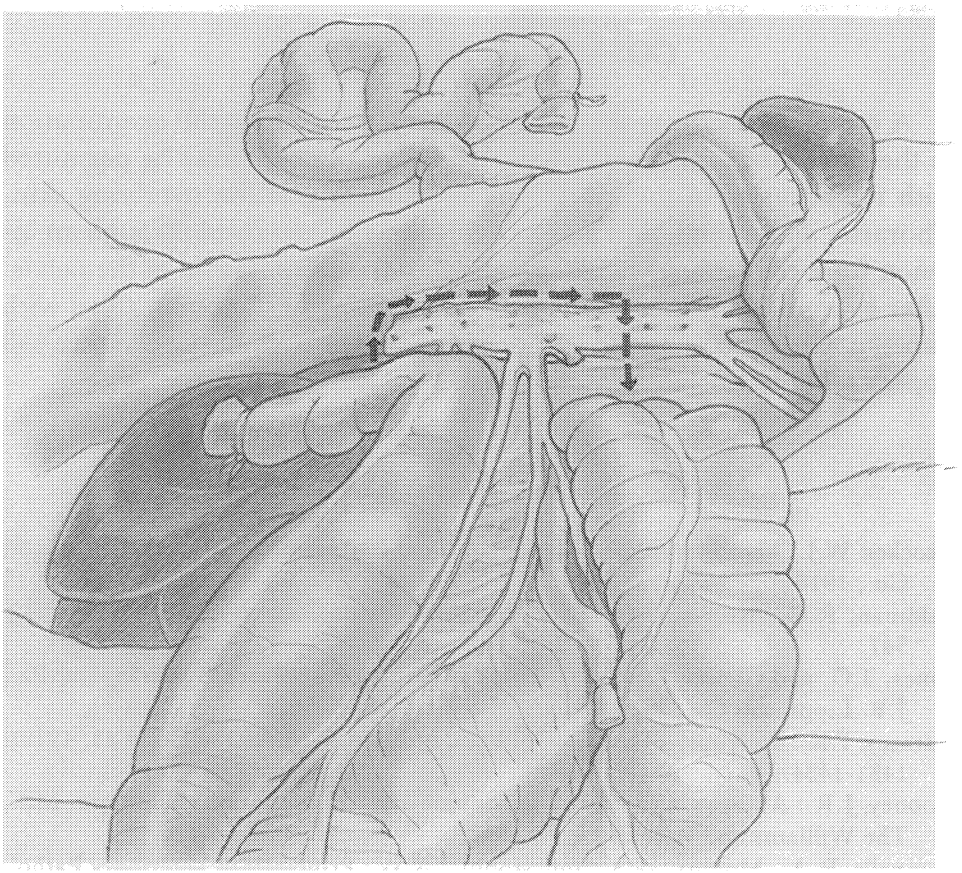

Figure 2-26.

13c. Cut lateral and cranial to the right kidney and adjacent adrenal gland (under the liver) and reflect it caudally to the cranial brim of the pelvis. Reflect the kidney, adrenals, and ureters, and nongravid uterus caudally to the pelvic inlet and place them over the paralumbar region. A gravid uterus with a full-term fetus is examined *in situ.* The gravid uterus may be removed at the cervix.

14. Sever the costal attachments of the diaphragm and remove the liver and diaphragm.

Pelvic cuts and removal of urogenital organs, caudal gastrointestinal tract, brain, and spinal cord are the same as for the dog and cat (Steps 15-21).

COSMETIC NECROPSY

A cosmetic necropsy is restrictive, because it limits the examination to the extent that the *client will tolerate.* Brain removal may be permitted with replacement of the calvarium and suturing of the overlying skin. Thoracic, abdominal, and pelvic organs may be removed through an incision from the xiphoid cartilage caudal to the cranial brim of the pelvis. After examination, the organs may be replaced or abdomen filled with paper, gauze, or cotton and the incision sutured, so that the cadaver will appear without unsightly mutilations.

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CHAPTER 3

DESCRIPTIVE WRITING OF PATHOLOGIC CHANGES

IN DESCRIPTIVE writing of pathologic changes, every effort should be made to produce a clear and complete mental picture of all the findings. The description should be objective and establish a report so that the pathologic diagnosis will have meaning for anyone who may later review it. Interpretation should not appear in descriptions of lesions. Objectivity in descriptive writing of gross lesions can be achieved by looking the material over and then describing it. Read the description back as the material is scanned again to see if what is described can be demonstrated. A description of the lesion recognizes the necessity of immediate recording of lesions.

DESCRIPTIVE WRITING

Descriptive scientific writing is an art rather than a science, and it can be acquired and improved by study and practice. In anatomic pathology, certain patterns of lesions appear again and again, and phrases used to describe them will recur in reports. Descriptive terminology and phrases used in recording lesions are listed in this chapter. The appropriate word should be used as suggested by the characteristics of a lesion. Encountering that word should recall a precise mental picture of an organ or lesion.

Before a description of the case can be written, all organs and lesions must be evaluated. A normal anatomic profile of each organ must be known. For example, if one were to describe gross liver lesions, the evaluation would be based on size, color, smoothness, or granularity of the surface; color and texture of the cut surface; and degree of friability as

judged by resistance compression between the forefinger and thumb. Many of these features are used in evaluating other organs. If the organ was grossly normal, the phrase "no gross lesions were seen" might well be used. Include the size, weight, shape, color, consistency, odor, and general description of capsule and cut surfaces of each organ. All departures from normal position and relationship should be listed. All lesions recognized should be fully and carefully described. An example is as follows: "The right kidney appeared small, weighing 40 grams. The capsule was white, roughened, and tightly adherent. The parenchyma cuts with increased resistance, and gray radial streaks about 3 mm wide were present in the medulla, etc."

When the artist sees a building, he very quickly notices the number of windows and their relative size and symmetry in relation to the rest of the building, the color of the building, and the texture of the surface. Most of this goes unnoticed to the casual observer. It is not until the prosector describes lesions that he starts seeing changes from normal in more detail. For example, it is not enough to recognize that an animal is anemic or has hemoconcentration, one needs to have a hematocrit (either a laboratory result or an estimate) reading in mind even though there may be some error in the estimate. It is not until one has the hematocrit in mind that the significance of a gastric ulcer in a pig can be evaluated. One needs an estimate of the hematocrit to diagnose anaplasmosis, colitis-X in the horse, neonatal isoerythrolysis, and so on. A simple observation of anemia or hemaconcentration does not suggest any special disease, whereas a hematocrit of five may suggest several specific diseases. The observation of pneumonia means practically nothing to the prosector until he determines the approximate or exact percentage of the lung involved, the distribution of the lesion, the estimated duration, and type of exudate. Once he has this information, he will have some criteria for determining whether the pneumonia was severe enough to cause death or whether it was a secondary terminal event. It may not be clear to the prosector whether a column of clotted blood in a vessel is antemortem or a thrombus until he describes the lesion. It is frequently helpful to know whether the stomach contents are fresh or stale. It gives some clue about the duration of the illness. It is inadequate to state that the stomach contains food; the contents need to be described. In doing so, the quantity and quality of food in the stomach is determined. When one uses the sense of smell to evaluate gastric contents and then describes the results, he learns whether the stomach contents have been recently consumed and contain acid or whether there is achlorhydria, or whether the gastric contents are overgrown with bacteria, giving it a putrid odor.

An organ system may appear normal to the prosector until he starts making a description at which time he may discover all sorts of interesting things. This is particularly true for lymph nodes. All lymph nodes appear normal until one starts reporting their diameter, color, and homogeneity of the cortex and medulla. Swollen lymph nodes may suggest sites of inflammation previously overlooked.

The ability to observe carefully and accurately, to describe accurately what is observed, and to record such descriptions precisely and succinctly are essential in necropsy examinations. In examination of gross pathologic changes, *observation, description,* and *recording* are essential steps that can be developed with mental practice and discipline.

Observation

Observations must be methodical, careful, and done in a proper sequence so that description and recording also may be done in a logical manner.

Description

The use of specific technical terms adds greatly to conciseness and clarity. In describing pathologic changes, accurate measurements of dimensions, weights, etc., should take the place of vague terms such as "large" or "small", the "size of a hen's egg", etc. Always record measurements by the metric system.

Recording

It is necessary to properly record lesions at the time of necropsy, otherwise essential facts may be forgotten or omitted from the record. This point is of special importance when the number of pathologic changes observed during an examination may be numerous. In developing a technique for descriptive writing to record lesions, a list of adjectives may help to express the lesions.

DESCRIPTIVE TERMINOLOGY

In succinct descriptive writing, the appropriate words should be suggested by the characteristics of a lesion and, conversely, encountering the word should recall a precise mental picture of an organ or lesion.

Size

Size is conveyed to the reader by weight, linear measurements, and occasionally volume. Linear expression in the metric system is usually in millimeters (mm) or centimeters (cm). One inch equals 2.54 cm. If a metric ruler is not available, use other reference objects. Example, width of (my) thumb, 1″ or 2.5 cm; extension of (my) thumb and middle finger, 20.5 cm; width of one penny, 1.5 mm; width of 4 pennies, 6 mm. One does well to avoid calling things "small," "large," "shrunken," or "enlarged." Organs having a variable or a nondescript outline may be quantitated for the reader by weight. The lung is an organ for which measurements of length, breath, and thickness vary widely within normal limits, depending on the size and age of the cadaver and the functional capacity at the time-of-death. Expressing the size of the lung in relation to its occupying dimensions of the thoracic cavity or by weight would probably be more suitable. While it is not widely done in veterinary medicine, weighing the heart and measuring the thickness of its chambers may be judged more acceptable in understanding any variation of the heart musculature from normal.

Color

Use precise words in designating color, indicate degree and distribution, and qualify as dark, brilliant, light, pale, mottled, streaked, or stippled. Many tissue changes are multicolored. Avoid using the vocabulary of decorators and paint manufacturers by referring to certain yellow-white paints as "egg-shelled."

Position

When possible, lesions should be described as having a definite relationship or location to other organs or structures. For example, a 2 cm spherical mass, 10 cm cranial to the ileo-cecal valve.

Surface

Usually referring to the organ surface as viewed prior to incision or manipulation, the following terms may be used to describe such a surface: smooth, rough, nodular, hairy, shiny, dull, pitted, ulcerated, covered with (specific) exudate, eroded, elevated, depressed, glistening, rugose (wrinkled, ridged, corrugated), undulant, scaly, and horny.

Cut Surface

Note the appearance of the exposed surface of organs such as liver, spleen, and kidney immediately after sectioning. Terms such as buldging, nodular, elevated, dry, oozing, rough, depressed, glistening, dull, hairy, ulcerated, undulant, scaly, and membranous are descriptive.

Shape

The shape of organs and other specimens may frequently be assumed to be normal when it appears so to the observer. In recording this "of the usual shape" may be entered into the record. Terms descriptive of shape include ovoid, oval, spherical, conical, elliptical, triangular, flattened, nodular, lobulated, tortuous, discoid (a flat circular plate), punctate (covered with points, dots, spots, etc.), crateriform (like the mouth of a volcano, a funnel-shaped cavity), bulbous (a spherical dilatation of any cylindrical organ or structure), wedge-shaped, spindle-shaped (tapering from the middle towards both ends; equivalent to fusiform), filiform (having the form of a thread; long, slender, round and equal in diameter throughout), lace-like, whorled, interwoven, fungoid (having a spongy nature), mushroom-shaped, dome-shaped, irregular, shapeless. Amorphous can mean shapeless or irregularly shaped or in biology it can mean not conforming to a normal standard.

Consistency and Texture

Consistency requires only a small vocabulary. Soft, hard, firm, and resilient are useful basic terms, to be modified by slightly, moderately, or markedly. Within tubular glands, terms such as fluid, compressible, or friable are applicable.

Special features of organs may be the products of disease in their anatomic location.

Tubular Structures

Patent, dilated, obstructed, obliterated, narrowed, diverticulate, branches, communicating, and tortuous are used for describing tubular structures.

Weight

If weighing is impossible, estimated weights should be recorded. One pound equals 454 grams (g) and 2.2 pounds equals 1 kilogram (kg).

Contents

Quantity should be measured in metric system, e.g., 1 gallon is approximately 4 liters (L). The nature of contents is described as cloudy, turbid, bloody, clear, or viscous. These terms are important for structures such as pleural sacs, intestinal tracts, gall bladder, abdominal and pericardial cavities, etc.

Odor

Smell is an important sense that we possess, but often neglect at necropsy. The description of an odor is difficult to describe, but odors may be identified by comparison to a more identifiable smell or associated with disease conditions per se. Some odors you may encounter are:

Nembutal—an alcoholic, sweet odor absorbed in lungs or heart of euthanatized animals.

Turpentine, creosote, turcapsol—pine-related odors most distinct and related to treatment of digestive disorders mostly in ruminants.

Motor oil—related to the toxic factor of lead contained in it.

Rancid butter—diseases such as *Clostridium chauvoei* in cattle and *Clostridium septicum* in pigs and horses.

Apple cider—distinct fermentative odor associated with large clots of blood in the stomach as the result of a gastric ulcer.

Ammonia—in urea poisoning, ammonia may be given off when the rumen is opened.

Septic tank odor—in all species, the affected, nontreated intestine will give this odor in salmonellosis.

Hydrogen sulfide—usually associated with postmortem bacterial decomposition of the cadaver.

Lactic acid—fermentative odor in rumen contents observed in grain overload.

Other diseased conditions listed below give off distinct odors, but are hard to describe.

Mange—mange mites give off a peculiar odor, especially in dogs with advanced lesions.

Myiasis—in wool or matted hair, a sickening odor of their own.

Footrot—a distinct characteristic fetid odor.

Infected wounds (anaerobic environment)—a septic foul odor.

Urine—in uremia.

Parvo infection—observed in many dogs with this infection, especially with enteritis.

NECROPSY REPORT

The following outline is suggested as a guide for recording necropsy findings. The importance of an outline is that it can be used as a checklist to insure examination of all organs, collection and processing of specimens and a simple method for recording all gross observations. This report would produce a complete and clear word picture of all findings along with their processing.

Date ..... Owner ..... Address .....
Breed ..... Sex ..... Age ..... Species ..... Wt .....

NGL = √ : Tissue for section = S : Bacteriological exam. = B : Photography = P

nose	skin	esophagus	bronch. LN	omentum	s. intest.	bladder	penis	spinal cord
mouth ✓	subcutis	thyroid	mediast. LN	liver	cecum	adrenals	oral cav.	muscles
ears	blood	parathyroid	pericard.	gall bladder	colon	abdom. LN	teeth	bones
eyes	head LN	thymus	heart	spleen	rectum	testes	nasal cav.	bone marrow
anus	neck LN	thorac. cav.	diaphragm	pancreas	mesent. LN	ovaries	sinuses	joints
vulva	tongue	pleura	abdom. cav.	forestomachs	kidneys S,B,P	uterus	meninges	limb LN
prepuce	pharynx	lungs	peritoneum	stomach	ureters	vagina	brain	

ORGAN FINDINGS

EXAMPLE:

A check (✓) indicates the organ was examined and "no gross lesions" were observed (example, mouth)

S - indicates that tissue was taken for microscopic studies
Kidney B - indicates that the kidney was being cultured
P - indicates that the kidney was photographed

An example of a morphological description of the kidney could be as follows:

The right kidney appeared small, weighing 40 g. The capsule appeared white, roughened, and tightly adherent. The parenchyma cuts with increased resistance, and gray radial streaks about 3 mm wide were present linearly from the cortex to the medulla. The left kidney had, in addition, a dilated pelvis in which a calculi, 0.5 cm in diameter, with a smooth surface was present.

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CHAPTER 4

POSTMORTEM CHANGES VERSUS ANTEMORTEM LESIONS

THE CHANGES that a body undergoes after death are important to veterinarians performing necropsies. Because of their effects on normal and diseased tissues, postmortem changes are constant problems in interpretating lesions. Without recognizing or being familiar with postmortem changes, it is possible to mistake them for antemortem lesions.

Postmortem changes are dependent on a number of variables:

1. Number of hours between death and necropsy.
2. Presence of widespread bacterial infection.
3. Nutritional status (amount of body fat).
4. Body weight (size of the animal).
5. Age and species (gas and heat production in ruminants vs. monogastric animals).
6. Antibiotic levels in the animal at the time of death (many treatments inhibit bacterial fermentation and putrefaction).
7. Environmental temperature prior to and following death.
8. Body temperature at the time of death.
9. Type of hair coat (external insulation of wool, feathers, or hair).
10. Health and metabolic state at death.

Animals dying quickly and without previous illness have a large viable bacterial population in their gastrointestinal tract and a quantity of fermentable ingesta that give off heat and digest tissues. The enzyme systems in tissues will undergo self digestion within minutes. Bacterial fermentation and autolysis cause rapid deterioration of tissues on relatively cool days. Animals that have been ill for several days have reduced cellular enzymatic activity in tissues, which diminishes autolysis.

In addition, there is relatively little fermentable food in the digestive tract and the intestinal bacterial flora is markedly reduced. Consequently, these animals deteriorate slowly in contrast to animals dying suddenly on full feed. However, a cow killed by lightning and necropsied 12-24 hours later might appear to have been dead for three or four days. This error is commonly made because of comparison with the rate of decomposition in cattle dying from blackleg or shipping fever with which the veterinarian has had more experience.

Postmortem changes begin to develop as soon as a animal dies. If the ambient temperature is high or if the animal has heavy external insulation, such as a fleece in sheep or a thick layer of fat in pigs, the changes may develop so rapidly that they mask antemortem lesions even when the necropsy is performed within a few hours after death.

To avoid confusion and to differentiate antemortem from postmortem changes, it is necessary to know what normally happens in a carcass after death. The main changes are: (1) rigor mortis; (2) postmortem clotting of blood; (3) autolysis; (4) putrefaction; (5) rupture and displacement of the gastrointestinal tract; (6) hypostatic congestion; and (7) color changes.

1. ***Rigor mortis*** is helpful in determining the time of death, but is not reliable. It results from partial contraction of skeletal musculature (stiffening) after death and is related to generalized anoxia, metabolic activity and body temperature. It is a unique example of function being not only maintained, but exaggerated for a period after death. After a short agonal period of relaxation, muscles harden and fix joints firmly so that attempts to move the limbs encounter resistance. Rigor usually begins in the jaw muscles and spreads caudally along the trunk and extremities; rigor disappears in the same order. Rigor usually appears two to four hours after death, reaches its height in less than forty-eight hours, and disappears in another forty-eight hours. It does not recur.

The eventual disappearance of rigor is due to exhaustion of energy necessary to sustain chemical activity within the muscle fibers. As the chemical systems disintegrate, muscle proteins degenerate and become soluble, and rigor is not sustained.

When the environment temperature is warm, rigor occurs early and disappears early. In cold atmospheres, the rate of appearance and disappearance is retarded. If death occurs during a high temperature, disease such as porcine stress syndrome (PSS), heat stroke or anthrax, rigor can occur almost simultaneously with death. It begins earlier and is most pronounced if there has been considerable muscular activity (excitement

or severely stressed) prior to death as in tetanus, strychnine poisoning, poisoning with prussic acid, and sun stroke. This is not true for animals that are moribund or cachectic prior to death. Rigor may not occur for hours in these instances.

Rigor mortis involves smooth muscles in the media of middle-sized and smaller arteries, pylorus of the stomach, urinary bladder, intestines, and eye (smooth muscle of the iris) producing pupillary constriction.

2. Postmortem clotting of blood occurs early after death, and in cases of slow death may actually begin during the agonal period. Postmortem clots are red, elastic, or jelly-like and are not adherent to the lining of blood vessels or the heart. In agonal blood clotting, blood elements stratify before coagulation, resulting in a layered clot. The heavier red cells form a thick layer at the bottom, above is a grayish layer of leukocytes, and on top of this, a yellowish layer of plasma containing platelets and leukocytes. These layered clots are most likely to be observed in the heart and large vessels. The clot is not adherent to the endocardium and usually separates into a lower friable *"current jelly" clot,* whereas the upper part is a tougher elastic, yellowish translucent mass resembling chicken fat, hence, a *"chicken fat" clot.* Red clots form rapidly, yellow ones slowly. Chicken-fat clots are considered normal for the horse because of the increased sedimentation rate. If found in other species, some disease process (anemia, infections, etc.) producing an increased ESR (erythrocyte sedimentation rate) should be suspected.

An antemortem clot or *thrombus* has a dull and irregularly roughened or somewhat stringy surface in contrast to the shiny, smooth glistening surface of a postmortem clot. Antemortem clots are a mixture of red and gray, usually in irregular layers or laminations, and part or all are attached to the endothelial wall of the blood vessel. In summary, a thrombus is inelastic, firm, friable, granular, adheres to endocardium or intima, and when removed, leaves a roughened area at the place of attachment.

Table 4-1.

COMPARISON BETWEEN A THROMBUS AND A POSTMORTEM CLOT

Thrombus	*Postmortem Clot*
1. Dry in consistency	1. Moist in consistency
2. Granular and rough surface	2. Smooth and glistening surface
3. White or buff	3. Intense red or yellow

Table 4-1 *(continued)*

Thrombus	*Postmortem Clot*
4. Stratified in structure	4. Uniform in structure
5. Attached to the vessel wall	5. Not attached to the vessel wall
6. Vascular endothelium below the thrombus is damaged and rough	6. Vascular endothelium below the clot is undamaged, smooth, and glistening
7. Composed primarily of platelets and fibrin	7. Composed primarily of fibrin
8. Forms in flowing blood	8. Forms in stagnant blood
9. Formed in a living animal	9. Formed in a dead animal
10. May be partially organized	10. No indication of organization
11. Caused by endothelial injury	11. Initiated by thromboplastin after death

3. Autolysis (self-lysis) is defined as digestion of tissues by their own enzymes and is recognized by tissue softening and friability. At the same time, the tissue usually becomes pale. Autolysis proceeds rapidly in tissues with high enzymatic activity such as intestinal mucosa, pancreas, and adrenal medulla, but slowly in bone or skin. Autolysis proceeds at an accelerated rate, especially in liver where *Clostridium* spp. multiply rapidly in an anoxic environment. Necrosis occurs antemortem when tissue is deprived of its normal supply of nutrients or its metabolism is interrupted by toxic, physical, and chemical agents.

Tissues may liquefy postmortem (autolysis) without undergoing putrefaction. A good example is softening of kidneys (pulpy kidneys) or desquamation of intestinal villi resembling a catarrh-like consistency.

4. Putrefaction results from bacterial activity postmortem. Following death, bacteria from the gastrointestinal tract invade surrounding tissues, grow, and participate in digesting the tissues. Putrefactive decomposition by bacterial enzymes is often accompanied by gas formation (hydrogen sulfide, ammonia, etc.) within the tissues where it causes *postmortem emphysema.* Putrefactive emphysema produces gas blisters raised above the surface of the mucous membranes and serosa of the intestinal tract, resulting in an irregular, dirty, grayish-red appearance.

Postmortem softening may be due partly to autolytic tissue enzymes and partly to proteolytic enzymes from saprophytic bacteria causing putrefaction. As a result, the tissues are softened and then liquefied. Saprophytic bacteria enter the blood stream from the intestine shortly after death and cause putrefactive decomposition of parenchymatous organs such as liver and lungs and skeletal muscles similar to that of antemor-

tem, moist gangrene from *Clostridium* spp. The sum of the changes occurring after death including both autolysis and putrefaction is often simply referred to as postmortem decomposition (PMD).

5. Hypostatic congestion results from gravitation of blood into the ventral or dependent side of the cadaver during agonal deaths. This is most noticeable in paired organs, particularly kidneys and lungs, where the ventral organ is darker. Segments or loops of intestine may be markedly congested, and staining of luminal contents by imbibition of blood may occur. This may be confused with blood in the gastrointestinal tract from antemortem hemorrhage. The segmental pattern usually is not observed in hemorrhagic enteritis. A thickened gut wall and fibrin will be present in hemorrhagic enteritis and not with segmental hypostatic congestion postmortem. Imbibition of blood with staining of luminal contents from hypostatic congestion may mimic focal hemorrhage in the gastrointestinal tract. In both instances, the luminal contents and intestinal walls are a dark muddy red; however, in hemorrhagic enteritis, the gut contents and wall of the intestine are a darker red, fibrin is present, blood clots may be present, and the gut wall is thicker.

6. Color changes

1. *Imbibition of hemoglobin* involves discoloration of tissues with blood pigments and their derivatives after death. Red blood cells hemolyze and the released hemoglobin stains the vessel walls and surrounding tissues. This is most marked in areas of congestion, shock, and septicemia because of hemolytic bacteria. In septicemia, some pink staining of the lining of large vessels may occur antemortem. Generalized hemoglobin staining of tissues and fluids is common in fetuses dead *in utero;* it begins 12 hours after death and is marked by 30 to 36 hours. Generalized hemoglobin staining only occurs *in utero,* but not in neonatal or adult deaths.

2. *Pseudomelanosis* is discoloring of tissues along and adjacent to the alimentary tract, resulting from hydrogen sulfide produced by putrefying ingesta combined with iron released from hemolyzed red blood cells. Iron sulfide is formed and stains tissues grayish-blue, green, or black. Immediately after death, the blood with its breakdown products and iron sulfide may gravitate into veins to give a greenish discoloration with a blackish background to the skin of the abdominal wall.

3. *Imbibition of bile* through the wall of the gallbladder results in yellowish discoloration of tissues focally in the liver around the gallbladder and adjacent gastrointestinal tract. The resulting yellowish discoloration

of the gastrointestinal tract should not be confused with a diffuse yellow staining pattern in the intestine following certain drugs given orally (Terramycin, etc.).

4. *Liver mortis* (lividity) is a bluish-red staining or discoloration of the body because of postmortem gravitation and seepage of blood and engorgement of dependent tissues. Lividity or hypostatic congestion is most evident in skin, lungs, kidneys, and most ventral loops of intestine. Lividity may appear within one to two hours after death and is fixed after 12 hours in one position. It indicates the position of the body at time of death, which can be an important aid in forensic pathology.

7. Rupture and displacement of the gastrointestinal tract. After death, peristalsis may continue for a time and terminate in intestinal displacements (torsion, intussusception, and volvulus). When gases collect in the intestinal tract following death, the gas-filled portions have a tendency to rise, causing unusual twists while fluid-filled portions are displaced to the ventral side of the abdominal cavity. However, the displacements are not accompanied by the vascular and inflammatory changes (engorgement of tissues with blood, infarction, increased friability) characteristic of similar antemortem lesions.

Continued fermentation of ingesta with gas accumulating within the gastrointestinal tract after death may become so extensive as to cause rupture of the rumen, stomach, or other portions of the gastrointestinal tract so that contents are localized in the abdominal cavity. In ruminants, extreme distention frequently causes rupture of the diaphragm and muscles in the inguinal region. In antemortem rupture of the gastrointestinal tract, there is always evidence of inflammation around the site of rupture, and ingesta is likely to be scattered throughout the peritoneal cavity and associated with an accompanying peritonitis. In postmortem rupture, accumulations of ingesta remain localized. Both ante- and postmortem rupture of the stomach occur at the greater curvature of the stomach.

Postmortem decomposition of the gastrointestinal tract usually begins at the tips of the folds of the mucosa. The swollen, soft, transparent, and glassy mucosa strips off easily, leaving the collapsed lamina propria. Within one-half hour of death, the epithelium of the rumen loosens and can be easily stripped from the submucosa. The stripping of epithelium is dramatic in the omasum. The rapidity of autolytic and putrefactive digestion of mucous membranes is dependent upon bacterial fermentation and the amount of heat and gas generated.

Segmental hypostatic congestion of the intestine must be distinguished from intraintestinal hemorrhage. Frequently in ruminants, one or more segments of atonic congested intestine will be filled with blood-stained fluid. The mechanism by which a segment of intestine becomes atonic, congested, and filled with blood-stained fluid probably cannot be documented in specific animals on the necropsy table. The ruminant, unlike some monogastric animals such as dog and cat, cannot empty the digestive tract with the onset of disease and this may contribute to the overgrowth of potential pathogens. Hemorrhage by diapedesis in bovine cases of shock produces a segmental pattern. Failing or reverse peristalsis or impairment of circulation during terminal illness may result in contents and blood poolling in segments of the intestine. Biologically active materials from bacteria and digestive enzymes may digest the mucosa shortly after death, resulting in blood seeping into the lumen from exposed capillaries. Likewise, increased permeability in the walls of the small vessels may occur to permit escape of blood.

Postmortem decomposition in the liver. The liver may become a soft pulpy mass because of autolysis and fermentation of saprophytic bacteria via the portal circulation from the intestinal tract. Frequently, a reduction in size of the liver is noted from advanced decomposition or autolysis, and foci, 1-3 mm in diameter, and tan-brown color, are commonly observed in the periphery of the liver lobes. The foci appear to coalesce near the hilus, producing a uniform tan-brown appearance. The pattern of the foci suggests a portal entry of saprophytic organisms from the intestine at the hilus and subsequent agonal dissemination of organisms throughout the vascular network of the liver, with few foci and less coalescing at the periphery. Frequently, the foci have a spongy appearance and texture because of gas production by the saprophytic bacteria. Pseudomelanosis (black or bluish-black) will be observed on the visceral and not the parietal surface of the liver because of bacterial activity in the intestine (H_2S) reacting with released hemogloblin (Fe) from the vascular system. The pigment (FeS) usually penetrates the surfaces of organs for a distance of 1-3 mm. A pale yellow or greenish discoloration on the liver and intestine adjacent to the gall bladder is the result of imbibition of bile.

Postmortem decomposition of the kidney. Pseudomelanosis and hypostatic congestion may be evident at times. The kidney cortex appears to undergo rapid autolytic changes, becoming soft (pulpy kidney) shortly after death. There is greater release of enzymes in cortical than medullary tissue. This process is enhanced in fat animals, sheep, and fetuses. It can be confused with the soft, so-called pulpy kidney that has been called a

diagnostic feature of *Clostridium perfringens* infection in sheep. In other animals such as dogs and cats, the kidney maintains its structure and consistency better. Kidneys with fibrosis or acute tubular necrosis maintain their consistency in the cadaver longer and do not undergo autolysis as rapidly as a normal kidney. This is probably the result of increased connective tissue and enzyme inactivation by coagulation necrosis.

Since the kidney is a paired organ, information can be obtained by comparing the color of the two kidneys. Normally, the kidney on the down side will be darker than the upper kidney because of hypostatic congestion. In a cadaver with fatty kidneys, the upper kidney will appear pale and whitish (fat) and the kidney on the down side, a normal rusty red (fat plus hypostatic congestion). However, gross observations of normal color do not necessarily mean that the kidney is normal.

Postmortem observations of the heart begin by observing rigor mortis occurring within the first hour. The left ventricle becomes almost empty and the right ventricle about half-full. Forcing blood out of the ventricles is directly correlated to the amount of musculature. Under normal conditions, rigor mortis disappears in about 18 hours. A flabby heart with an empty ventricle is usually observed. However, if at this time the heart is flabby and the left ventricle is filled with coagulated blood, this indicates antemortem myocardial weakness and no or very little rigor mortis. If the heart is flabby and filled with uncoagulated tarry, dark blood, rigor mortis may have occurred and disappeared but hemolyzed blood seeped into the ventricle as the result of gravity. The following general guides may be helpful in examining the heart:

Table 4-2.

Left ventricle	*Right ventricle*	*Significance*
Empty	Clotted Blood	Rigor mortis present
Unclotted, nonhemolyzed blood	Same	Rigor mortis has not set in, death was recent.
Unclotted, hemolyzed blood	Clotted and hemolyzed blood	Rigor mortis has passed, death was not recent.
Clotted blood	Same	Myocardial weakness (many causes such as heart disease, debilitation, cachexia, etc.)

Postmortem coagulation of blood occurs slowly with separation of clots into two, an upper "chicken-fat" clot and a lower "currant jelly" clot. Predominance of chicken-fat clots indicates an increased ESR that is normal in the horse. The endocardium is frequently blood stained because of imbibition of hemoglobin. Occasionally, gas bubbles appear in the myocardium as a result of postmortem decomposition by gas-producing saprophytic bacteria.

Postmortem decomposition of the lung. Imbibition of blood and postmortem emphysema frequently are directly associated with postmortem bloat. Saphrophytic bacteria are forced cranially via the vascular system from the abdominal viscera to infiltrate the lung, producing emphysema and bacterial foam (in presence of fluid) in the bronchi and trachea. The foam (fermentation of hypostatic fluids) is usually present unilaterally in bronchi on the down side of the cadaver. In pulmonary edema, foam is present bilaterally.

The following guidelines help in differentiating hypostatic congestion from pneumonia.

Table 4-3.

DIFFERENTIATION BETWEEN HYPOSTATIC CONGESTION AND PNEUMONIA

	Hypostatic Congestion	*Pneumonia*
Location	Unilateral	Usually bilateral
Distribution of congestion	Widespread (entire lung involved)	Foci - coalescing, may be dorsal diaphragmatic, or vental dependent portions of lung. May be unilateral in aspiration pneumonia, *Mycoplasma* and *Haemophilus* infections in pigs.
Texture	Spongy	Firm, liver-like (hepatization)
Bronchi	May have fluid and foam (hypostatic congestion plus bacteria if animal dead for several hours.	Mucopurulent exudate, foam (edema and hyperventilation)
Septa	Close apposition	Separated—fluid (exudate), gas (emphysema)
Color	Entire lung dark reddish brown, black	Lobules darkened, uneven, red, brown, black ventral dependent portions involved

Table 4-3 *(continued)*

	Hypostatic Congestion	*Pneumonia*
Size of lung	Both lungs may *not* be of equal size. Tidal air entrapped in congested lung and will not collapse.	Both lungs usually same size since lesions are bilateral. Emphysema may be present. Lungs may occupy all thoracic cavity.
Specific gravity	Floats in water	May sink in water*
Stain smear	Blood cells, bacteria	Neutrophils, macrophages, bacteria

* Will not sink if piece of lung is too large and includes lung with adjacent compensatory emphysema.

In evaluating pneumonic lungs, palpation and incision are necessary. Palpation will reveal more accurately the degree of pneumonic involvement. With pneumonia, the affected areas are firmer than adjacent tissues. Do not confuse small bronchi with focal nodular pneumonic lesions in the lung.

Discoloration of skin. Dark areas of unpigmented skin (livor) are the result of postmortem gravitational draining of blood (hypostasis) to these areas. Pale white areas result from any compression restricting the entry of blood. The position of dearth can be determined this way and is important in forensic pathology. A well delineated and widespread reddening of skin with the same distribution internally is the result of decreased venous return.

Hemorrhages or hematomas resulting from diathesis (bleeding disease) can be located in subcutaneous tissue. Be aware that severed congested vessels may mimic hemorrhages. One should consider bleeding diseases such as warfarin poisoning, vitamin K deficiency, liver disease with prothrombin deficiency, and toxic agents such as snake venom. Iatrogenic lesions from accidental needle injury to a major artery should also be considered.

Postmortem epidermal sloughing results from heat from the sun on the ear after death. Under the sloughed tissue, congestion is evident.

Lesions of Bloat

A difficult and common interpretative problem encountered in ruminants at necropsy is differentiating tissue change occurring in either

ante- or postmortem bloat. Most ruminants when necropsied have varying stages of postmortem bloat. Lesions attributed to antemortem bloat may have occurred postmortem. With time, bloat occurs in all animals with the proper body and environmental conditions. The rapid postmortem putrefaction occurring in ruminants from rumen bacterial fermentation and distension of rumen with gas makes differentiation difficult between postmortem bloat and antemortem changes associated with sudden death.

Primary ruminal tympany (frothy bloat) is usually associated with cattle on full feed in a feedlot or grazing a legume pasture. Secondary ruminal tympany (free gas bloat) usually results from physical interference with eructation and rumen atony. Possible causes of ruminal atony and failure of eructation include esophagitis, acidosis, ruminitis associated with an all-grain diet, continued feeding on coarse indigestible roughages, indigestion from accumulation of readily fermentable milk foods, and traumatic reticuloperitonitis that may be associated with vagal indigestion.

Physical obstruction to eructation occurs in esophageal obstruction caused by a foreign body, by stenosis or by external pressure on the esophagus (e.g., enlarged thymus in young calves or abscess in the caudal mediastinal lymph node), or by obstruction of the cardia from the interior. Interference with esophageal groove function in vagus indigestion, diaphragmatic hernia, and tetanus from a spasm of esophageal musculature are possible causes of secondary bloat. Carcinoma and papillomata of the esophageal groove and reticulum are unlikely causes, but lymphoma along the esophageal path (around heart) is a likely cause. Actinobacillosis of the reticulum may cause secondary bloat.

In primary ruminal tympany, foaming ingesta will be expelled through an incision in the rumen. Similar ruminal contents are present in the reticulum; however, ingesta of the omasum and abomasum are normal. The frothiness disappears within 10 to 15 minutes after the rumen contents have been expelled. The foaming ingesta in the rumen is not observed if a necropsy is delayed for 10 to 12 hours. Diagnosing primary bloat (frothy bloat) at necropsy is much easier if the animal is necropsied soon after death.

Necropsy findings recorded for antemortem bloat include the following:

1. Tongue may be swollen and protruding from the mouth.
2. Epistaxis occasionally present.

3. Congestion of the sclera and conjunctiva.
4. Numerous subcutaneous petechial and ecchymotic hemorrhages on the trunk and neck.
5. Congested cervical muscles with dark, partially clotted or unclotted blood in tissues, imparting a thickened appearance to the neck.
6. Musculature of the anterior cervical region, pectoral limbs, and muscle mass along the vertebral column beginning in the thoracic region being markedly congested and hemorrhagic.
7. Muscles of the hindquarters normal to pale and may be edematous due to venous stasis.
8. Prescapular, axillary, submandibular, and retropharyngeal lymph nodes and soft palate swollen, congested, and frequently hemorrhagic.
9. Prefemoral and popliteal lymph nodes are pale and normal size.
10. Gas distended intestine pale with loops containing sanguineous ingesta because of blood flow constriction.
11. Liver pale, ischemic, and usually autolytic with a congested caudate lobe.
12. Spleen compressed and pale.
13. Kidneys pale and autolytic with subcapsular congestion.
14. Rupture of diaphragm may or may not be observed.
15. Lungs atelectatic occupying cranial part of thoracic cavity.
16. Epicardial and endocardial petechial hemorrhages present and in some cases up to 100 cc of dark, blood-tinged fluid within the pericardial sacs.
17. Mucosal petechial and ecchymotic hemorrhages present the entire length of the trachea, with submucosal hemorrhages present in cranial half of the trachea ending abruptly at the thoracic inlet; the hemorrhages become diffuse after death. Mucosal congestion and ecchymoses extend into smaller bronchi.
18. Unclotted blood in trachea extending into bronchi and bronchioles.
19. Cervical portion of the esophagus consistently congested with submucosal petechiae and ecchymoses. Some edema in connective tissue surrounding the esophagus and trachea in lower neck.
20. Thoracic part of esophagus pale and blanched; the boundary distinction is abrupt.
21. Congestion and hemorrhage always present in cranial sinuses and nasal mucosa; clots of blood in paranasal and frontal sinuses.
22. Meninges congested.

23. After a few hours (depends on environment and temperature), marked distension of the subcutis with gas from lower neck to the perineum.
24. Marked erythema is evident beneath the ruminal mucosa especially in the ventral sacs, in which subsequently there is an exfoliation of the cornified epithelium with marked congestion of submucosal tissues.
25. Diffusion of fluid under pressure through vessel walls into connective tissue giving appearance of antemortem edema in the anterior aspects of the cadaver and along the ventral midline. It usually is purplish-pink and found in fascial area between muscle fibers and not associated with muscle lesions.

Necropsy diagnosis of bloat, blackleg, death from lightning, electrocution, anthrax, snakebite, etc., is difficult, especially in animals that have been dead for several hours on pastures in warm weather. Bloat occurs quickly because of rapid rumen bacterial fermentation and tends to obliterate antemortem lesions.

Cattle dying suddenly with fresh fermentable ingesta in the forestomachs decompose rapidly as the ruminal flora activity continues to produce heat, biochemically active products, and gas. The diaphragm is forced forward sometimes to the point of rupture as the rumen expands with gas. Blood with anaerobic bacteria is forced from liver, heart, vessels, and lungs through the thoracic inlet into the cervical tissues where fascia, areolar tissue, and muscles in the ventral aspects soon become swollen and congested with black, bloody fluid from postmortem congestion, hemorrhage, and edema. Jugular blood or sanguinous fluid from cervical tissues collected several hours after death from such cattle will contain numerous Gram-positive bacilli, a variety of anaerobes including *Clostridium* spp. This can be difficult to distinguish from an antemortem clostridial myositis.

Ruminants with full rumens dying suddenly from lightning, grass tetany, prussic acid poisoning, adverse drug reaction, acute respiratory failure, bloat, suffocation (edema of larynx), heat exhaustion, milk fever, and insecticide poisoning will probably have bloody fluid in the ventral cervical tissues by the time they are presented for necropsy. It may be impossible to differentiate these clostridial-invaded artifacts of blood imbibition from actual cases of clostridial disease or bloat, either primary or secondary.

A diagnosis of primary or secondary bloat depends largely on the absence of local or general lesions characteristic of disease, the presence of marked ruminal tympany, and the absence of clinical signs (struggling) and postmortem changes.

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CHAPTER 5

GROSS PATHOLOGIC EVALUATION OF TISSUES

GROSS LESIONS *in situ* are easier to interpret when a necropsy procedure allows removal of organ systems in a properly defined sequence. A selective choice of tissues can be collected for later morphologic, bacteriologic, and chemical studies. A careful and thoughtful scrutiny of gross lesions in human medicine is reported to provide an accurate diagnosis in 90% of cases; the remaining 10% are generally close to a correct diagnosis.

It is important to develop the art and science of evaluating gross lesions for pathologic diagnosis. First, one must understand the color and architecture of normal tissues. Before lesions can be accurately interpreted, the effects of postmortem changes (Chapter 4) must be evaluated. Exfoliative cytology (impressions or smears) of lesions aids in classifying lesions into the following categories:

1. Inflammatory vs. neoplastic lesions
2. Benign vs. malignant neoplasms
3. Primary vs. metastatic malignant neoplastic lesions

GROSS APPEARANCE OF INFLAMMATORY AND NEOPLASTIC LESIONS

The most frequently occurring pathological alterations to be distinguished by veterinarians doing a necropsy are inflammation and neoplasia.

A. Gross appearance of neoplasms are not as informative as one would desire. Some rules-of-thumb are:
   1. A benign neoplasm is circumscribed and resembles a sphere embedded in tissues. A capsule is usually present separating it

from the tissue in which it is growing, allowing it to be shelled out from surrounding tissue. On section it may be firm (fibroma) or soft (sebaceous gland adenoma, lymphoma), usually homogenous, and white-gray in color.

2. A malignant neoplasm infiltrates irregularly and usually lacks a definitive border or capsule. It is usually multiple, and may be large or small. On section, its appearance varies from area to area because hemorrhage, degeneration, and necrosis are common. Superimposed inflammatory and ischemic changes produce a variety of colors such as red, black, and yellow. Certain neoplasms produce their own pigment, e.g., melanoma (black); interstitial cell tumor (yellow). Metastases of a similar lesion (color and consistency) to distant tissues or organs, e.g., lung or lymph nodes indicates neoplasia rather than inflammation (abscesses).

B. Gross appearance of inflammation varies according to cause, exudate, location, duration, and quality and quantity of the inflammatory reaction. Necrotic tissue occurring in inflammation is usually sharply demarcated by altered color and loss of structure. A hyperemic (red) zone, variable in size (1 mm to 1 cm or more), surrounding the necrotic tissue is seen in acute infarcts. The necrotic tissue is generally pale grayish-white if caused by ischemia and yellowish if it contains leukocytes and histiocytes with lipid breakdown products. Consistency may be soft (necrosis of parenchymal cells and abscess formation), firm (fibrous scarring), or gritty (deposition of calcium salts and bone formation).

Table 5-1.

GENERAL DIAGNOSTIC CRITERIA
FOR DIFFERENTIATING INFLAMMATORY AND NEOPLASTIC LESIONS

	Inflammatory	*Neoplastic*
Weight and size	Normal in mild insults Increase due to accumulation of cells and fluid	Increase due to presence of new growth
Shape	Usually normal Uneven if focal lesions present Example—abscesses, scarring	Usually altered due to diffuse cellular infiltrations in focal tumor swellings
Color	*Acute:* Red due to vascular congestion Pinkish-tan due to fibrinous exudate Yellow due to purulent exudate *Subacute:* Red due to granulation tissue and vascularization	Grayish-white is predominant color Black associated with melanoma, red with hemangiosarcoma, yellow with interstitial cell tumor Combination of colors due to secondary inflammatory and ischemia changes

Table 5-1.

	Inflammatory	*Neoplastic*
Color	*Chronic:* Yellow due to lipid breakdown Grayish-white due to fibrosis	
Consistency	Soft due to necrosis and abscess formation Firm due to fibrous scarring and metaplasia of glandular cells Hard due to deposition of calcium salts and/or bone formation	Soft due to necrosis associated with ischemia Firm due to fibrous stroma Hard due to calcium salt deposits or bone formation
Architecture	Usually preserved unless excess scarring or abscess formation	Usually preserved in diffusely infiltrating neoplasia Capsule formation or widespread focal lesions obliterate parenchyma
Location	Usually a diffuse involvement of parenchyma or surface and lining membranes of an organ or tissue Focal lesions may occur	Usually focal involvement of parenchyma or surface implants and lining membranes of organ Diffuse lesions may occur

Table 5-2.

DIAGNOSTIC VALUE OF CONSISTENCY IN GROSS PATHOLOGY

Consistency	*Reason for consistency*	*Diagnostic examples**
Soft	Cell death and destruction of supporting stroma	Necrotic tissue White muscle disease Porcine stress syndrome
	Autolysis	Postmortem change
	Cellular neoplasms with absence of fibrous stroma (fish-flesh consistency)	Embryonal carcinoma Malignant lymphoma Seminoma
Firm	Collagen formation	Fibrosis
	Neoplasms with fibrous stroma	Cirrhous carcinoma Adenocarcinoma Fibrosarcoma
Hard	Cartilage	Cartilaginous tumor
	Bone	Bone tumor
	Calcification	Ossification within an inflammatory lesion
Rubbery	Chronic passive congestion	Congestive heart failure
Waxy	Amyloid deposits	Amyloidosis of kidney, spleen and liver
Cream cheese type consistency	Caseous necrosis	Tuberculosis
	Sebaceous material	Dermoid cyst Sebaceous cyst Abscesses

*These examples are not all-inclusive

Table 5-3.

DIAGNOSTIC VALUE OF COLOR IN GROSS PATHOLOGY

Color of tissue or organ	*Associated cell type, pigment or mineral deposits*	*Diagnostic examples**
Gray-white	Lymphocyte	Benign lymphoid hyperplasia, e.g. Malignant lymphoma and lymphatic leukemia
	Segmented leukocytes and precursors	Granulocytic leukemia
	Carcinoma cells	Cellular carcinoma with little or no fibrous stroma, and occasionally with lymphoid stroma, e.g. seminoma, malignant lymphoma
	Fibroblasts	Fibrosis, e.g. old healed infarct or fibrosarcoma
White (chalk-like)	Calcium deposit in or about cells	Dystrophic calcification, e.g. fat necrosis, lipomas, liposarcomas
Yellow	Lipid (lipochromes)	Fat deposits in horse, and dairy cattle breeds.
	Interstitial cell (testicle)	Interstitial cell neoplasma
	Segmented leukocytes	Purulent material, e.g. abscess
	Bile pigment	Jaundice, e.g. hepatitis, bile duct obstruction, hemolytic disease
	Hematoidin	Old hemorrhage (infarct)
Yellow-brown	Lipochrome pigments	Brown atrophy—age
Yellow-green	Eosinophils, neutrophils	Eosinophilic myositis, abscesses from visceral larval migrans
Brown	Hemosiderin pigment	Old hemorrhage
	Lipochrome pigment	Brown atrophy of organs, serosal discoloration of ducts, pancreatic atrophy in dogs
Black	Lipofuscin accumulating in hepatocytes	Black liver in old horses
	Hemosiderin	Old hemorrhage
	Melanin pigment	Melanosis, melanoma
	Anthracotic pigment	Anthracosis
Black-green	Pseudomelanin (H_2S + porphyrin)	Pseudomelanosis
Pallor	Decrease in erythrocytes	Anemia, ulcers, animal bleeding for several hours
Red (bright)	Erythrocytes	Acute hemorrhage, active hyperemia of inflammation
Red (dark to black)	Erythrocytes	Hypostatic or passive congestion, shock, hematomas
Green	Eosinophils	Hemangiomas or hemangiosarcomas, eosinophilic myosititis in cattle, abscesses, e.g. visceral larval migrans or other parasites, bacteria, etc.

*These diagnostic examples are not all-inclusive.

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CHAPTER 6

SPECIMEN COLLECTION AND SUBMISSION

CARE IN collecting specimens is the most important step in the entire procedure of utilizing a diagnostic laboratory. Veterinarians using a laboratory, at times, may consider the laboratory results to be unreliable and misleading. To avoid this misunderstanding, first, the right questions must be asked by both parties (veterinarian and laboratory personnel) so that they communicate directly, and secondly, the right specimens must arrive at the laboratory in good condition. In the final analysis, it is the veterinarian submitting the specimen who has the responsibility for proper specimen handling of the case and for correlating all results, both clinical and laboratory, for the final diagnosis.

The axiom "garbage in, garbage out" applies not only to the use of computers and data processing, but also to collecting and sending specimens to the laboratory for bacterial or virus isolation, toxicological analysis, histopathological examination, etc.

CHOICE OF SPECIMENS

The live, sick, untreated animal exhibiting typical signs of the problem along with dead animals (if available) are the specimens of choice. The submission of one or more animals usually enhances the chance for a rapid and accurate diagnosis. Owners should be encouraged by veterinarians to submit the proper animal (if a herd problem) so that proper specimens may be collected for laboratory examination.

If typical recumbent animals cannot be brought to the laboratory, specimens collected during necropsy are to be properly collected and handled from such animals. Frequently, time and distance make

submission of live animals impractical. Therefore, specimens must be representative and reflect the problem in question. If a central nervous system (CNS) disturbance is suspected, submit brain and cerebrospinal fluid (CSF) in addition to parenchymatous organs.

Avoid the all too common practice of sending intestine (even if tied off, microorganisms from the gut can escape into their surroundings) and parenchymatous organs together in the arm of an obstetrical sleeve with a knot in the end.

SELECTED SITES FOR SPECIMEN COLLECTION

Cerebral Spinal Fluid Cultures and Cerebral Tissue

Fluid available from cerebral spinal fluid (CSF) for culturing is usually limited to 1 to 2 ml, but this allows a few organisms to be isolated. A Gram's stain should be performed on all CSF specimens collected for culture. It is a reliable, convenient, and rapid diagnostic tool. The few microorganisms present in infected CSF, should be concentrated by centrifugation. Remove the supernatant fluid for chemical or serological studies and use the sediment for culture and smears.

Cultures on agar media are kept for three days and are inspected daily. Broth cultures are retained for two weeks and inspected daily. Development of any turbidity should lead to subculture on agar media and preparation of microscopic smears.

Centrifugation is adequate for fluids in which there is an increase in inflammatory cells; however, in early cases of bacterial and fungal meningitis, the cell count may be normal despite a positive culture. Leukocytic response in the CSF is polymorphonuclear in acute bacterial meningitis, and lymphocytic and less intense in protozoan, leptospiral, and fungal diseases.

Cell and chemical changes in CSF may occur with a brain abscess; however, smears and cultures are usually negative unless the abscess has ruptured.

In fungal meningitis, a drop of CSF sediment is mixed with a drop of India ink or nigrosin solution on a clean glass slide, covered with a cover-slip, and examined with a decreased intensity of light. Nigrosin is preferable to India ink because it is free of discernible particulate matter. The presence of encapsulated, budding, yeast-like

cells in the wet preparation is virtually diagnostic for cryptococcal meningitis; however, special care must be exercised in differentiating nonencapsulated yeast from red or white cells, air bubbles, or even talc.

Fluids Other than Cerebral Spinal Fluid

Percutaneous aspiration of pleural, pericardial, peritoneal, and synovial fluids must be obtained aseptically to avoid contamination. This is best done by a sterile syringe and needle. A small amount of sterile heparin may be added to prevent coagulation, since clots may trap microorganisms. The fluids should be smeared directly on a slide, stained, and examined for bacteria.

Fluid removed from body cavities can be used for direct observation of cellular content, enumeration of cells, preparation of wet mounts, and for smears. Samples of fluid may be obtained by sterile swabs to prepare smears. If there is much fluid, as from a serous cavity or a spinal tap, centrifuge the material and examine the sediment. If cryptococcosis is suspected, examine the sediment under a microscope after staining with nigrosin or India ink. If nocardiosis is suspected, make a smear and stain by Gram's method.

Urinary System

Urinary tract infections include diseases of the kidney, urethra, ureter, and bladder. Insert a needle into the bladder or renal pelvis and aspirate fluid. A sterile screw-cap container or tube should be used for urine. Urine is an excellent culture medium and a small or insignificant number of bacteria can multiply rapidly to give a false finding. If urine cannot be cultured within an hour after its collection, it can be refrigerated with satisfactory results for at least 24 hours, and up to 5 to 10 days. Microscopic examination of a wet preparation of urinary sediment easily and inexpensively demonstrates yeast and pseudohyphal elements.

Splitting a urinary calculus will allow culture of the interior. It is not uncommon to isolate bacteria from within the stone that are not present on its surface.

A number of screening kits to detect clinically significant bacteriuria and a culture system including sensitivity testing are available commercially. The results should always be correlated with the clinical findings.

Respiratory Tract

Cultures of the respiratory tract must be interpreted cautiously because of a wide spectrum of microflora. Isolation of organisms from cultures of respiratory secretions does not represent *a priori* evidence of their etiologic roles in respiratory infections. Examination of Gram-stained smears of material obtained by nasal or pharyngeal swab may be helpful in providing preliminary indication of the etiology of atrophic rhinitis or other pulmonary infections, if there is a predominance of one type of organism.

At necropsy, exudates from a cadaver are easily obtained from the bronchi and used as a diagnostic aid in dogs, cats, horses, and cattle. Causes of pneumonia are recovered more often by using bronchial exudates especially in swine, e.g., *Mycoplasma hyopneumoniae.*

Eye

In the examination and culturing of the eye, especially with conjunctivitis and keratitis, there are special problems in specimen collection and processing. Swabs are generally inadequate for establishing presence of microorganisms because of the small sample size. The frequent application of topical drugs is usually inhibitory to isolating the organism.

Examination of Gram- and Giemsa-stained smears of corneal scrapings may provide preliminary clues as to the nature of the disease. If necessary, stains for acid-fast bacteria can be done. Swab cultures may be inoculated onto one half of each agar plate and scrapings on the other half.

Bovine Mastitis

If milk samples are taken aseptically, blood agar alone may be used. The teat orifice must be properly cleansed with alcohol before the milk sample is collected in a sterile container. Selective media are available and are of particular value for veterinarians interested in milk bacteriology. Generally, plating milk on blood agar and MacConkey agar is adequate for most culturing.

Wounds

Material from a previously undrained wound abscess should contain the etiological agent or agents in most instances. Open wounds, ulcers, or sinus tracts are frequently contaminated with skin and mucosal or

air-born microorganisms. In general, the use of swabs to collect material from these sites is of limited value because it is likely to represent an inadequate sample. A sterile needle and syringe should be used to collect a generous quantity of liquid material from a closed abscess. Since anaerobes are commonly recovered from certain wounds, this material should be immediately injected into an anaerobic tube or vial for transport. Sinus tracts usually originate in bone or in lymph nodes and material should be collected from as close to the base as possible. Ulcerative lesions of skin and mucosa should be scraped and/or swabbed for stained smears and culture. Actinomyces, mycobacteria, and fungi must be considered in the differential diagnosis. Biopsy and histopathologic examination are valuable adjuncts in the diagnosis of the latter type of infections. The importance of microscopic study of tissues is emphasized by the possible resemblance between neoplasms and infectious processes. Gram-stained smears and histopathological studies may be helpful in providing an early clue to the nature of the disease.

Intestinal Contents

To obtain a specimen, tie a segment of intestine in two places and remove the segment from the intestinal tract. Aspirating intestinal contents through the serosal surface with a sterile syringe is recommended. This can only be done if luminal contents are liquid.

The collection of feces in tetrathionate broth for isolating *Salmonella* spp is the primary reason for most culturing of fecal contents. This should be done with a freshly acquired sample. Sterile swabs are used in obtaining fecal specimens and are inserted into the lumen via a cut surface from the serosa of the intestinal wall, carefully rotated, and withdrawn. The swabs may be added to a screw-capped tube containing an enrichment or transport medium. The vast majority of bacteria in feces are usually anaerobic, Gram-negative rods. They are usually ignored in diagnosing infectious intestinal disorders. Enrichment media such as tetrathionate broth or Selenite F are used if diarrhea from *Salmonella* spp is suspected. The use of Gram-stained smears for demonstrating bacteria in fecal contents is useful in diagnosing enterotoxemia, pseudomembraneous colitis, or acidosis, in which an overwhelming number of Gram-positive cocci are present. Yeast-like cells would easily be demonstrated in a Gram stain of the intestinal contents or rectal swab.

The filter paper method is acceptable for fecal specimen collection for bacteriological culture. Spread fresh fecal material over a strip of filter

or blotting paper. Allow to dry and fold strip inward to cover fecal material. Insert in plastic envelope and send to the laboratory. Pathogens usually are unaffected by this technique and many normal intestinal flora organisms die.

Enteric specimens are frequently examined only for so-called enteric pathogens, namely, *Salmonella* and *Shigella* spp. This limited approach no longer reflects our knowledge of the pathogenesis of enteric infectious diseases. Selenite F enrichment broth is superior in the selection of *Salmonella* and *Shigella* spp in the first 24 hours, whereas tetrathionate enrichment broth enhances detection of organisms even when only a few are originally present. It can be incubated for as long as one week and is useful for detecting carriers. For routine clinical specimens, Selenite F broth, subcultured after 24 hours incubation on Eosin Methylene Blue (EMB) and Salmonella-Shigella (SS) agars* is adequate. Some of the original specimen should be kept refrigerated until isolation procedures are completed.

Blood Cultures

Sear the surface of the heart to provide a sterile area 5 cm in diameter. Insert a sterile needle through the seared area and aspirate the desired amount of blood into a sterile syringe. Transfer the blood to a blood culture bottle or place a drop on blood agar and streak.

Joint Cultures

Joints may be submitted in a refrigerated container and left intact by sawing the long bone above and below the joint. To culture, the skin over the joint is reflected, the joint opened with a sterile blade, and a swab or sterile syringe used to collect the joint fluid contents. The joint fluid may be cultured directly onto blood agar plates. Cultured plates may be sent to the diagnostic laboratory for colony identification.

COLLECTION AND PRESERVATION OF SAMPLES

Histopathology

1. Place tissues in 10% buffered neutral formalin (BNF). This is probably the best overall fixative and is recommended for routine use. (See appendix for formula).

*Remel, Regional Media Laboratories, 12076 Santa Fe Drive, Lenexa, Ks. 66215

2. Tissue samples should be about 1 cm thick and include lesions and adjacent normal tissues; one volume of tissue to at least 10 volumes of 10% BNF.
3. After the tissues have fixed for 24-48 hours, the specimens may be removed from the BNF, and put in a whirl-pak, and sent to the laboratory. This reduces shipment costs and avoids extra packaging to prevent leaking because of breakage during shipment.
4. In submitting intestinal specimens for histopathologic examination, take a syringe and needle and inject the lumen of intestine with 10% BNF while the intestine is in the animal. Tie and cut the formalized loop and place in 10% BNF. This minimizes autolysis of the tips of the villi. Many enteric problems (bacterial, viral, and protozoal diseases) require histopathologic interpretation of villi in which autolysis has been minimized. If not properly fixed, the villi and lamina propria undergo autolysis, quickly destroying criteria important for interpretation.

Bacteriology

Selecting proper specimens and collecting adequate samples are the most important steps for successful bacteriological examination of tissues. When there is an obvious site of infection, the choice of specimen is straightforward. However, clinical judgment, history, and agonal events influence the choice of specimens and their handling.

The best specimens for bacteriologic examination are obtained from cadavers in the following situations: (1) acute illness or febrile reaction; (2) sudden death; (3) abortion; (4) diarrhea; and (5) inflammatory and exudative skin lesions.

The most frequently obtained specimens for bacteriologic culture are lung, liver, spleen, kidney, heart blood, and exudates. Swabs from the ear, throat, nasal cavity, eye, vagina, and skin may improve diagnosis.

Specimens should be obtained, if possible, prior to administration of antibiotics and contamination must be kept to a minimum. During necropsy, surface contamination from intestinal content or other extraneous material from gloves and knives is common. This type of contamination may be prevented or minimized by veterinarians being more careful in how they obtain specimens to place in containers for bacteriological examination. Be sure not to spill intestinal contents over the abdominal or thoracic viscera prior to swabbing or collecting tissues for culture.

After death, the bacterial flora of the digestive tract enters the portal system and the liver quickly becomes seeded with cadaver organisms. In a decomposed cadaver, postmortem tympany forces bacteria via vascular channels cranially and caudally, thus, seeding thoracic and abdominal organs with enteric contaminants and making these organs undesirable for culture within a few hours of postmortem autolysis. Cadaver bacilli will frequently overgrow causative pathogens because of their greater numbers and rapidity of growth.

Clostridial species are ubiquitous in healthy cattle and may be demonstrated in numbers after death. Elter and Scharner (1969) found *Clostridium perfringens* in the tissues of 27% of 182 cattle killed for slaughter; mesenteric lymph nodes and livers were the most commonly infected tissues. Niilo, Dorwood, and Avery (1969) electrocuted 3 healthy Hereford steers and left them unopened at 71 to 82°F for 24, 20, and 4 hours, respectively. *Cl. septicum* and *Cl. novyi* were demonstrated by immunofluorescence in the liver of steer #1; *Cl. novyi* in the liver of steer #2; and *Cl. novyi* in the liver of steer #3 after the liver was left at room temperature for another 24 hours. They also reported that 62.3% of 308 bovine submissions to the diagnostic laboratory over a 4-year period yielded *Cl. novyi;* 20% of 64 cattle dying of pneumonia, peritonitis, nephritis, rhinotracheitis, and other causes, had *Cl. novyi* in their tissues.

Ruminants that die suddenly with full rumens from lightning, winter and grass tetany, prussic acid poisoning, drug reactions, founder, bloat, suffocation (laryngeal edema), heat exhaustion, adenomatosis, milk fever, and insecticide poisoning will probably have bloody fluid in the ventral cervical tissues by the time they are presented for necropsy. These tissues yield potentially pathogenic clostridia, particularly *Cl. perfringens, Cl. novyi,* and *Cl. septicum.* Areas of blood imbibition are not limited to the cervical area; they may occur elsewhere such as flank or muscle of the thighs.

It may be impossible to differentiate clostridial postmortem artifacts of blood imbibition from antemortem cases of clostridial disease. Close gross observations frequently reveal intact muscle fibers in cases of postmortem blood imbibition, whereas muscles are usually black and necrotic with antemortem *Cl. septicum* and *Cl. novyi* infections similar to blackleg. Microscopically, clostridial lesions contain hemolyzed erythrocytes, necrotic muscle fibers, thrombi in vessels, and some peripheral leukocytic infiltration. The bacteriologic findings are of secondary importance.

Cl. perfringens can usually be isolated from the contents of a hemorrhagic segment of intestine, but neither the lesion nor bacterial findings are specific for enterotoxemia. Niilo and Avery (1963) obtained 379 *Cl. perfringens* isolates from various ruminant tissues; 40% were isolates from acute deaths. Of the isolates, 97% were *Cl. perfringens* type A. Clostridial toxin is quickly inactivated in the bovine intestine and probably causes little harm.

One tissue frequently used for culture from decomposed animals is bone marrow from femur, radius, or sternum, because it is sequestered from putrefactive processes from the abdominal cavity. A section of midshaft of femur, radius, or sternum may be submitted to a laboratory. Bone marrow may be cultured after sawing the bone in half, searing the exposed surface, and swabbing the bone marrow. Frequently, an inoculating loop is required if the marrow cavity is small. A swab of bone marrow may be taken and sent in a Culturette* to the diagnostic laboratory.

A good bacteriological specimen is an inoculum from a representative organ not contaminated with surface organisms. A noncontaminated or carefully cleansed area (surface) is necessary before inserting the swab into an organ. Swabs collected at necropsy do not require a seared surface, if they are inserted into tissues immediately after the cadaver or body cavities are opened and *before* the organs are handled. Swabs frequently can be thrust into tissues without making an incision, if there is a minimum amount of connective tissue present. When the surfaces are contaminated, specimens may be prepared for culturing by either searing the surface with a hot spatula or by making an incision into the parenchyma with a sterile knife, so that either a swab or inoculating loop may be inserted to obtain an inoculum.

A number of bacteria are inhibited by substances present in processed cotton, therefore, specimens on dry, cotton-tipped swabs should not be used. Lesions swabbed with a Culturette are ideal. After tissue is swabbed, the swab is inserted into a transport media chamber that will maintain bacterial populations.

The use of a transport medium is essential, if causative microorganisms are present in low numbers. It is also important for specimens in which normal flora may be mixed with infectious bacteria and/or other microorganisms foreign to the location. Microbiological

*Culturette-Fisher Scientific, 711 Forbes Ave., Pittsburg, PA 15219; Marion Scientific Corp., Rockford, IL 61101

specimens may be transported to a diagnostic laboratory in a variety of nonproliferating, buffer-type media such as Stuart's. Nutrient or thioglycolate broth may be used when swabs or aspirates are involved. Fecal specimens for *Salmonella* culture should be submitted in tetrathionate.

The chamber can be easily sealed with tape, put in a heavy envelope, and sent by mail to the laboratory without refrigeration. This avoids a specimen container and packing in ice, and it is also cheaper.

Swabs collected at necropsy do not require a seared surface, if they are inserted into tissues immediately after the cadaver or body cavities are opened and *before* the organs are handled. Swabs frequently can be thrust into tissues without making an incision if there is a minimum amount of connective tissue present. When the surfaces are contaminated, specimens may be prepared for culturing by either searing the surface with a hot spatula or by making an incision into the parenchyma with a sterile knife so that either a swab or inoculating loop may be inserted to obtain an inoculum.

Use of Sterile Syringes

Sterile syringes with the proper needle gauge are convenient for aspirating fluid or exudate from joints, abscesses, intestinal lumens, and CSF from the foramen magnum. Aspirating lumen contents from intestine can frequently be done by locating a section of gut where the contents are fluid, usually no problem in animals with enteritis. This is an excellent way to send intestinal contents for culture. Aspirated specimens in syringes are not subject to the drying and subsequent loss of viability of bacteria that frequently occurs in swabs. Syringes with exudates may be sent to diagnostic laboratories with little additional preparation to prevent leakage, etc.

Whirl-pak containers* are widely used, but specimens collected in these containers usually have surface contamination that readily seeds the entire specimen.

Transferring Swabbed Material to Culture Plates

This is done when primary culturing is desired. The bacterial colonies on petri dishes may be used for bacterial identification or sensitivity testing. The petri dish may then be sealed and sent to a diagnostic laboratory for futher identification.

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*American Scientific Products, Chicago, Ill.; Fisher Scientific Company, St. Louis, Mo.

The lid of the plate is opened just wide enough to admit the swab minimizing contamination from the air. Swabs are rubbed across the surface of a suitable agar medium next to the edge of the plate. Then a flame-sterilized inoculating loop is used to streak the swab inoculum onto the remaining areas of the agar plate. The primary objective is to spread the inoculum to obtain separate colonies.

Staining Specimen Smears

Smears from specimens for staining should be prepared at necropsy. Prepare *thin* smears, because thick smears are of little value when stained. Thin smears can be prepared from material that seems thick or tenacious by firmly pressing two slides together over the area containing the material. Pull the slides apart leaving a thin film to be stained. This is recommended for urethral discharges, wound exudates, aspirated material from abscesses, etc.

Virology

Swabs or tissues collected from animals during the acute stage of illness prior to antibody formation are ideal. The choice of specimen for virus isolation is the organ that exhibits the severest lesions of the suspected disease, or organ secretions.

Cold but unfrozen specimens are preferred. Specimens that cannot be delivered to the laboratory within 24 hours of collection should be frozen and delivered frozen, preferably maintained with dry ice.

Viruses are denatured by heat, light, dehydration, changes in salt concentration, and pH (caused by dry ice fumes). Be sure specimens are placed in sealed containers if submitted with dry ice as the refrigerant.

Serology

Paired serums are required for meaningful serological results except for pseudorabies virus (PRV), Venezuelan equine encephalomyelitis (VEE), western equine encephalomyelitis (WEE), and eastern equine encephalomyelitis (EEE). Four to six animals or 10% of the group should be bled. Three (3) ml of serum is optimal for each serotest, but submit at least 6 ml if infectious bovine rhinotracheitis (IBR) and bovine viral disease (BVD) are requested. To safeguard from hemolysis (which will give clouded results), centrifuge and submit the supernatant serum only.

Mycology

Do *not* place material for mycologic examination in transport media or in a moist tube. Skin scrapings or hair should be placed in a dry, sterile, screw-capped tube.

Parasitology

Fecal samples are best kept cool until they are received at the laboratory. The specimen may be shipped in 10% BNF formalin if refrigeration is unavailable. If mucus is excessive, 10% sodium bicarbonate can be added to the water used for suspending the parasites. External and internal parasites should be submitted for identification in 70% ethyl alcohol. Skin scrapings of various manges can be submitted in a stoppered glass vial or bottle.

Toxicology

Never send specimens accompanied by a request "check for poisons" when an animal dies of unknown causes. Analyses for all the hundreds of chemical toxicants would be impossible and the cost of attempting to do it would be prohibitive. Many toxicants are not readily detected by any analytical procedure. By obtaining a thorough clinical history and doing a good necropsy, one can narrow the choices for analytical toxicology, and give the toxicologist a logical idea of the tests to perform. Extreme attention should be given to details at the scene surrounding loss of animals to make a judgment as to possible cause.

Samples submitted by veterinarians must be properly tagged and sealed in the presence of a second person, if there is a possibility of legal implications. Before collecting and sending specimens for analysis, check with the toxicologist as to the proper methods of handling, since he will be required to testify in court.

Electron Microscopy

Intestinal contents from animals with diarrhea are frequently submitted for visualization of viral particles. Submit the intestinal specimen from animals that have had diarrhea for less than 24 hours. Fix the specimen in a preservative such as formalin, with a final concentration of 2-3%. If intestinal contents are chilled and submitted, the ELISA test can be performed on the same sample.

Rabies Specimens

In the event of human exposure, the animal must immediately be impounded and placed in quarantine according to the regulations of accepted procedure in handling animals suspected of rabies. The victim's physician should be contacted immediately as well as the local public health department. This places the responsibility for the victim's health under supervision of the physician and for animal deposition under the veterinarian. As the veterinarian on the case, be sure that you have adequately communicated with all the proper officials so that there is total understanding of your responsibility.

Don't shoot the animal in the head and then submit the head to a reference laboratory for diagnosis. Before killing any suspected rabid animal, be sure proper quarantine procedures have been followed, especially for domesticated animals (dogs, cats, etc.). Communication with the diagnostic laboratory, local public health authorities, and the family physician is helpful in arriving at a decision on whether to destroy or quarantine the suspected rabid animal. If the animal is to be destroyed and not quarantined, the reference laboratory should be phoned regarding specimen submission. The specimen (head and/or animal) should always be brought to the laboratory by interested party(ies) so that a prompt diagnosis can be rendered. Sending such specimens by bus or parcel post should be discouraged. The specimens may leak from their containers and, therefore, open the door to a liability law suit. If the specimen is not presented to the laboratory by courier, then proper preparation of the specimen is essential. Put the head in a plastic bag, close and secure by placing a rubber band or string around the open end. Be sure the plastic bag is leak-proof. If the animal is small (guinea pig, rat, mouse, or hamster), the entire animal may be submitted. Remove the head of suspect rabid skunks; do *not* send the entire animal.

Freezing the specimen is urged if it will be in transit for more than 24 hours. Use frozen, pack type refrigerants, *not* wet ice. Approved shipping containers for submitting rabies specimens are available from most County or Regional Health Department Offices.

Blood Smears

Prepare a thin smear on a clean slide followed by immediate air drying (use hair dryer). A slide prepared in this manner will maintain cell morphology for a few days, providing insects do not damage the cells.

Blood smears and impressions may be fixed in 95% or absolute ethanol or methanol for 3 minutes, dried, and then submitted to the laboratory for staining.

Blood Samples

Unless blood is delivered within a few hours, results on blood counts, hemoglobin, packed cell volumes, etc., will be unsatisfactory. When blood samples are submitted, EDTA is the anticoagulant of choice; one drop of 10% EDTA in 5 ml of blood is effective.

TRANSPORT OF SPECIMENS TO THE LABORATORY

Criminal Statue (18 USC 1716) pertains to shippers of diagnostic materials. This statue is of interest to all shippers of diagnostic materials, whether the materials are potentially pathogenic or not. If spillage occurs from nonpathologic materials and injures or damages mail, equipment, or personnel, the shipper is liable and may face prosecution, even though there is no human hazard from a noninfectious agent. Therefore, meticulous packaging with sufficient absorbent material around the specimen is essential for preventing accidental leakage or spillage of both noninfectious and infectious material.

The safest and most expeditious method for transporting specimens to a diagnostic laboratory may be for the client to bring the specimen directly by automobile rather than submitting it by mail or express, thus obviating the need for elaborate packaging. Sending specimens to a laboratory by mail requires procedures to ensure *viability* of bacterial, viral, and chemical agents and maximum protection to anyone handling the specimens in transit.

Packaging of Specimens. Proper packaging protects not only specimens in transit but also personnel handling them. Protection is especially important to prevent breakage. Never mail viable specimens in glass petri dishes and never enclose dry ice in hermetically sealed containers.

Screw-capped tubes are not recommended for blood, serum, or other fluid specimens because leakage frequently occurs, particularly when outside pressure decreases during air transportation. Screw-capped tubes, however, are acceptable for agar or similar culture media. Screw caps should have a resilient, sealing gasket or insert and should also be firmly secured by tape.

Shipment of Specimens. For long distances, ship all specimens by air mail or air express or motorized express service.

Specimens submitted to diagnostic laboratory should be accompanied by an appropriate history giving name and age of patient, source of specimen, disease suspected, brief statement of clinical signs, and, in the case cultures, tentative identification.

Shipment of specimens should be timed so that they will not arrive at the diagnostic laboratory on or just before a weekend or holiday; this will avoid deterioration of specimens. Always check the schedule so that specimens arrive at the destination within scheduled working hours.

In some localities surface mail, bus, or United Parcel Service, etc., may be faster than air transport, but in any case, the most rapid method should be used.

Call the diagnostic laboratory and let them know when the specimens are being shipped; better service will result.

Identification of Specimens. Identify individual specimen tubes or containers by encircling them with typed or penciled legends on adhesive tape. Give the name of client, type of specimen, and date of collection. This is particularly important with clear fluid and paired serums. Ink, ball point pen, wax, or indelible pencil should not be used because writing may become illegible. It is advisable to include with the shipment a legible copy of a list of specimens, with identifying name or number, date obtained, and tests desired.

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CHAPTER 7

NECROPSY AND CLINICAL BACTERIOLOGY

BACTERIOLOGY is a major adjunct to necropsy in obtaining definitive diagnoses. Culturing and sensitivity testing require minimum equipment and effort but add efficiency to your practice. Bacteriology is also helpful in advising on herd health programs, evaluating geographic boundaries of disease, diagnosing diseases transmitted from animals to man, aiding prognosis or furnishing information for a definite plan of disease control, and in minimizing diagnostic errors.

The identification of causative bacterial agents and accompanying antibiotic sensitivity tests provide a basis for rational treatment. Many current chemotherapeutic agents are specific for certain conditions; therefore, the diagnosis must be specific. Two examples are penicillin for treating bovine streptococcal mastitis and blackleg and streptomycin for leptospirosis.

In establishing herd health programs, the use of reference laboratories is important to epidemiologically know the genus, species, and phage or serotype of a particular organism so that specific recommendations can be made.

Routinely doing bacteriology by a set protocol as suggested here will make it easier for veterinarians to recognize common contaminants and common normal flora, so as not to confuse these organisms with pathogens. Identification of bacteria is aided by knowledge of the source of specimen and clinical history.

Negative bacteriological cultures of tissues may occur with faulty technique, e.g., inoculating loop too hot, inappropriate media, previous antibiotic treatment, or a noninfectious process. Negative results may suggest the absence of pathogens but should not be considered absolute.

In reality, the causative bacteria may not have been isolated, because of failure to culture either aerobically or anaerobically or supply the required nutrients for growth.

Information necessary for the precise and detailed identification of organisms is available in several excellent textbooks, and is beyond the scope of this chapter. Systems of diagnostic bacteriology have been reported previously.

COLLECTION OF SPECIMENS FOR BACTERIAL EXAMINATION

Representative specimens of the problem under investigation should be collected with minimal or no contamination by extraneous organisms, and plated onto appropriate agar plates as soon as possible.

Ear, eye, fecal, throat, tonsil, wound, and intestinal (obtained at necropsy) cultures are best collected with sterile cotton swabs. The best procedure is to have agar culture plates (blood and MacConkey's) on hand when the animal is examined. When the swab is taken, it should be immediately plated onto the agar. With a little experience, it is rare to apply too heavy an inoculum. With urine, cerebrospinal, and serous fluids; heart blood; and humor from eye chambers, collect with a sterile syringe, add a drop of fluid directly onto the culture plates at the edge, and spread the inoculum for individual colonies by using a sterile loop or swab.

Before culturing specimens, it is advisable to make an impression or smear of the tissue and stain by Gram's method to ascertain the presence of bacteria and then procede with a culturing system for identification.

GROWTH OF ORGANISMS

Some organisms require a microaerophilic atmosphere for growth. A simple, inexpensive method for creating such an atmosphere is to place the inoculated plates in a large jar having an airtight lid, place a burning candle in a small container on top of the plates, and screw the lid on tightly. When the candle exhausts, a CO_2 rich, low oxygen atmosphere results. *Campylobacter coli, Campylobacter fetus, Brucella abortus,* and *Haemophilus somnus* require this atmospheric condition. All

plates inoculated from organs of aborted fetuses also should be incubated in a CO_2 atmosphere. In practice, it is advisable to do all primary isolations in a CO_2 environment.

The colony morphology of bacteria and result of growth on selective media are guides to identification. The somewhat colorless gray colonies of Gram negative rods on MacConkey's agar will be helpful in *Salmonella* recognition, as will the odor produced by *Pseudomonas* sp. To develop confidence, obtain stock cultures of known organisms and frequently use them along with your routine scheme of identification to establish a working base-line knowledge about common bacteria. This assures proper quality control for identifying unknown agents. A stereoscopic microscope or hand lens are valuable aids in recognizing bacteria by their colony appearance, type of hemolysis on blood agar, pigmentation, and size.

GRAM-REACTION OF MICROORGANISMS

Colony growth on agar plates, slants, etc., should always be examined by Gram stain to determine bacterial morphology and whether the organism is Gram-positive or negative before additional selective tests are done. When doing Gram stain, use a drop of clean water to emulsify the bacterial colony on a clean slide. The suspension of bacteria should be permitted to dry in the air. Passing the wet preparations through a flame to hasten drying is commonly done but may lead to distortion in cell morphology. The Gram stain permits recognition of colony purity and alerts the laboratory worker to a contaminated culture.

An alternative to Gram staining is to place 1 drop of 3% potassium hydroxide (KOH) on a clean microscope slide and mix with a loop containing a generous number of organisms. Gram-positive organisms form a uniform suspension in contrast to Gram-negative organisms which form a viscous solution that strings out when teased.

Cultures of 24 hours or less should be used for Gram staining. Club shapes or rounded ends of rods; arrangement of cocci in packets, pairs, or chains; relative length and width of the bacterium; and presence of an obvious capsule, spore, or inclusion are significant characteristics for identification.

There are a number of special stains, such as the acid-fast stain, that can be performed to aid in the recognition of bacteria. Wright's or Giemsa stains are readily available to demonstrate the position and size of spores relative to the bacterial cell.

PRIMARY ISOLATION OF MICROORGANISMS

For most routine work, a universal medium such as 5% ovine or bovine blood agar and an enteric bacteria selective medium such as MacConkey's agar are commonly used. The agar plates are streaked with a swab in a 4-flame streak (Fig. 7-1). The swab may then be used to prepare smears for cytology and Gram staining.

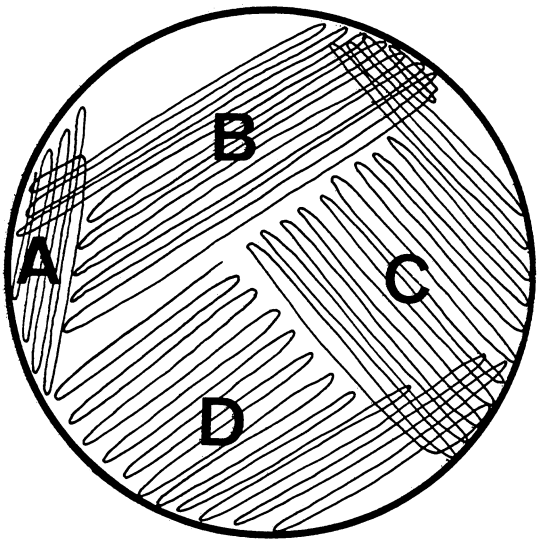

Figure 7-1. Method of Inoculating Plate of Medium.

Interpretation and identification of colonies on blood agar is determined by colony size, shape, color, and consistency and by hemolysis (*beta, alpha* or double zoned). All organisms growing *only* on blood agar are Gram stained and subdivided into Gram-positive and Gram-negative.

MacConkey's agar is an excellent selective and differential medium. It contains bile salts and crystal violet to prevent growth of Gram-positive and some Gram-negative microorganisms. Most Gram-negative microorganisms grow rapidly. The organisms are further subdivided into colorless colonies (nonlactose fermenters) and red to pink colonies indicating fermentation of lactose. A blue-green pigmentation indicates a *Pseudomonas* sp. If an organism grows on MacConkey's agar, it also grows on blood agar, but not always vice versa.

Triple sugar iron (TSI) agar slants are made with a butt and slant so that two areas can be evaluated (Fig. 7-2). A single colony from a

24-hour growth on blood agar plate is inoculated into a tube of TSI agar by stabbing into the butt and streaking the slant. The agar slant is a pink color when sterile. After 24 hours of incubation, the tube is examined to see if the slant and/or butt has turned yellow (indicating fermentation) or black if hydrogen sulfide has been produced. Some organisms do not produce any change in TSI and these are shown as a pink color in branch #1-8. Organisms fermenting glucose only turn the butt of the tube yellow.

After being cultured on TSI agar, Gram-positive organisms may be tested for catalase production. This is done by adding a few drops 3% hydrogen peroxide solution to the 24 hour TSI growth; a bubbling reaction is positive.

Triple Sugar Iron Agar: 24-Hour Reading

Inoculation—streak slant and stab butt.
Must be incubated with a loose cap or cotton plug.

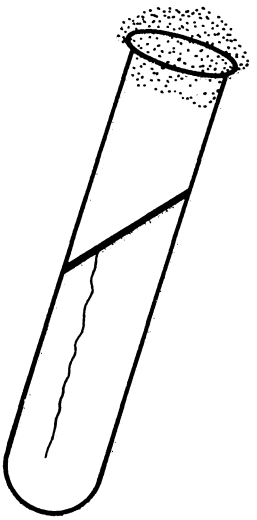

Figure 7-2. Triple Sugar Iron Agar Slant. Yellow - fermentation (acid). Red - no fermentation (alkaline). Black butt - H_2S positive. Bubble - gas formation.

Interpretation of reaction (slant/butt)
Alk = red Acid = yellow
(1) No change/no change (NC/NC)
No growth or no action on substrates

(2) Alkaline/no change (Alk/NC)
Amino acid degradation on slant surface
(3) Alkaline/acid (Alk/A)
Glucose fermentation with amino acid degradation on slant surface
(4) Alkaline/acid with hydrogen sulfide (Alk/A + H_2S)
Same as (3) but H_2S produced from NA Thiosulfate blackens the medium
(5) Acid/acid (A/A)
Lactose and/or sucrose and glucose fermentation
(6) Acid/acid + gas (A/A + Gas)
Same as (5) but CO_2 or other gas produced from carbohydrate
(7) Acid/acid + hydrogen sulfide (A/A + H_2S)
Same as (5) but H_2S produced from NA Thiosulfate blackens the medium

Other Selective Tests

Indole production is determined by inoculating bacteria into a 1% tryptone media and incubating overnight for fast growing organisms. It may require 3-4 days for slower growing organisms. Add a solvent to form a layer (xylene or chloroform), shake, and add Ehrlich's reagent to form a layer between medium and solvent. A positive reaction is indicated by Ehrlich's reagent turning red.

A ***citrate slant*** is streaked lightly with culture. If sodium citrate is utilized, an alkaline reaction and a color change from green to blue is noted.

A ***urea slant*** is inoculated and a positive reaction is evident when urea is degraded to ammonium by an alkaline reaction color change to brilliant red.

Cytochrome oxidase in bacterial cells is determined by smearing organisms from a blood agar plate onto filter paper by a sterile swab. One drop of oxidase reagent is added and a positive reaction is noted within one minute, blue or pink depending on the reagent used.

Catalase production is characterized by the enzyme of the bacteria (if present) attacking peroxides. The use of 3% hydrogen peroxide on the surface of a TSI slant results in a positive reaction of O_2 bubbling off.

Motility is determined by examining a wet mount preparation. Additionally, one may use a motility medium that is a semisolid (0.3% agar). A stab is made in the center of the tube and, if positive, a growth radiating out from the stab will be present.

SIM Medium is used for determination of sulfide production, indole formation, and motility of enteric bacteria. Inoculate by stabbing the center of the medium to about one-half its depth by means of a straight needle. Tubes are incubated for 18-24 hours or longer. Sulfide reaction is indicated by blackening of the medium along the line of inoculation. Oxalic acid papers (filter paper strips soaked in saturated oxalic acid and dried) suspended over the medium turn pink during growth of organisms that form indole. The conventional tests (see indole production) may be employed at the end of the incubation period. Mobility is evidenced by spreading of growth away from the line of inoculation.

One clinically important problem is recognition of *Salmonella* infections. Whenever an isolate grows rapidly as a colorless colony on MacConkey's agar, shows no "swarming" or hemolysis on blood agar, lacks the characteristic *Proteus* odor, and gives a yellow butt and pink (alkaline) slant in TSI, *Salmonella* is likely even though H_2S production is not demonstrated. A few salmonellae are negative or weakly positive for H_2S production. Confirmation of suspected *Salmonella* colonies can be made by positive agglutination with commercially available polyvalent antiserum.* Suspected colonies are mixed with typing serum on a clean glass slide and a gross flocculant precipitate indicates a positive reaction. Whenever a *Salmonella* sp. is suspected or identified, the culture should be sent to a reference laboratory for specific serological typing and identification.

In fecal cultures from animals with diarrhea, *Salmonella* spp. as etiological agents must be differentiated from *E. coli.* This is based on the ability of *E. coli* to rapidly produce red colonies on MacConkey's agar, often within 4-6 hours after inoculation. *Salmonella, Proteus,* and *Pseudomonas* spp. produce clear or colorless colonies on MacConkey's agar plates. *Proteus* is recognized by its "swarming" characteristic on blood agar. It swarms but less so on MacConkey's agar. *Campylobacter* sp. grow poorly on MacConkey's agar and require a microaerophilic atmosphere.

ANTIBIOTIC SENSITIVITY TESTING (Kirby-Bauer)

This one procedure, more than any other, has stimulated practitioners' interest in office clinical bacteriology. Difficulty encountered in

*Salmonella O-antiserum polyvalent A-I BBL, Division of BioQuest, Cockeysville, Md.

conducting and interpreting sensitivity tests is caused by mixed or contaminated cultures. Sensitivity testing should be performed routinely on all cultures and should be done concurrently with steps for bacterial identification. To evaluate antibiotic susceptibility without identifying the organism involved can be misleading, because the organism could be a nonpathogen.

The procedure commonly employed in antibiotic sensitivity is to obtain a pure culture by suspending 4-5 identical colonies from a blood agar plate in tryptic-soy broth, which is incubated for 2-8 hours at 37°C. Then the culture is aseptically, heavily, and uniformly swabbed on a Mueller Hinton agar* plate and the surface is allowed to dry for at least five minutes. Discs with appropriate concentrations of antibiotics are placed firmly and evenly over the inoculated plate. Sensitivity readings for most organisms can be read from six to 24 hours after inoculation, using the procedures and standardized chart available from the suppliers of antibiotic discs. Comparison of the widths of zones of inhibition of different drugs as a measure of *in vivo* efficacy is of little value, but the width of the zone of inhibition does provide an evaluation of the relative sensitivity of an organism to a particular drug.

The disc method of *in vitro* antibiotic susceptibility testing corresponds with clinical effectiveness when adequate quantities of the particular antibiotic are given by the correct route of administration to achieve therapeutically effective blood, tissue, or urine concentrations. Veterinarians should use drugs showing clear-cut zones of inhibition. The size of the zone of inhibition is often closely related to the susceptibility of the organism being tested. To ensure confidence and competence in understanding this procedure, consult with a diagnostic laboratory so you can be sure of quality control.

IDENTIFICATION OF IMPORTANT AEROBIC BACTERIA

This section is concerned with bacteria likely to be encountered frequently in veterinary practice and does not include a complete scheme for identifying all bacteria. There are reference text books for this. However, it does present a simple and rapid approach for utilizing reactions for identification, which are usually diagnostic. Presumptive

*Remel, Regional Media Laboratories, 12076 Santa Fe Drive, Lenexa, Ks. 66215

recognition of bacteria is made from knowledge of (a) history and animal, (b) colony development on blood and MacConkey's agars, (c) TSI agar reaction, (d) Gram stain, (e) catalase reaction, and (f) other selective tests.

To suggest a protocol is not without certain risks. A flow chart is outlined in the appendix which is simple and adequate for most common bacteria encountered in practice.

The advantages of this protocol are as follows:

1. It requires MacConkey's and blood agar culture plates, TSI agar in tubes, Gram stain reagents, hydrogen peroxide, and potassium hydroxide. Other special media can be incorporated into the identification scheme, if necessary.
2. Simple techniques such as observation of growth on solid media, color changes in TSI and MacConkey's agar, O_2 release from addition of hydrogen peroxide, and Gram stain are used.
3. Most organisms can be identified within 48 hours after collecting the specimens.
4. The system has wide application, since an estimated 95% of all isolates likely to be encountered in practice can be identified.

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CHAPTER 8

LABORATORY PROCEDURES AT NECROPSY

DIAGNOSTIC CYTOLOGY AT NECROPSY

EXFOLIATIVE cytology has proven to be reliable for obtaining definitive information in many areas of human medicine, e.g., gynecology and urology. The techniques for evaluating cytology in medicine have been applied to veterinary medicine, particularly for diagnosing many canine diseases, but less so in large animals.

Cytology as a diagnostic procedure during necropsy has unlimited potential for evaluating gross lesions. It is concerned with the microscopic evaluation of cell populations from organs and tissues. Cytological interpretation of fluids, secretions, scrapings, and tissue impressions (tumors) during the necropsy examination enhances one's diagnostic capability and sharpens gross interpretation skills. Biopsy for histopathologic interpretation of the lesion is frequently required and is complementary in providing a definitive diagnosis.

Practicing veterinarians are frequently confronted with situations in which an immediate rational treatment can only be determined by knowing whether a lesion is neoplastic or inflammatory. Cytologic evaluation can often provide prompt diagnosis and, hence, appropriate treatment. Most comprehensive cytological efforts in veterinary medicine are directed toward the usefulness of blood smear-type cytology as an adjunct in aiding clinical diagnosis.

Direct Examination of Tissues

Impression or fluid smears may give valuable clues regarding the cause of disease, particularly bacterial, mycotic, and viral (especially

those characterized by inclusion bodies). Direct impression smears should be used as *Routinely* as microbiological culture. The intent of direct impression is twofold: to ascertain the cytological features of gross lesions (neoplasia or inflammatory), and to determine the causative agent (parasite, bacterium, or virus).

Direct impressions of lesions or swabbed specimens for cytologic evaluation are made and stained after the organ or tissue has been cultured and examined grossly. Wright's, Gram's, acid-fast, Giemsa, etc., are common stains that can be performed easily on impressions from the same piece of tissue that was cultured or swabbed. Determining the morphological characteristics of organism(s) from a lesion is invaluable in correlating with what is cultured. This was aptly demonstrated in a case of ovine hemorrhagic and necrotizing mastitis. A smear from the lesion stained by Gram's method and examined microscopically had abundant numbers of Gram+ rods and a few gram+ cocci. On routine aerobic culturing, only B-hemolytic *Staphylococcus* sp was isolated. The abundance of Gram+ rods on the slide suggested additional anaerobic culturing, which resulted in isolation of *Clostridium perfringens,* the cause of the necrotizing mastitis. This finding correlated with the Gram's stained slide of mammary tissue, and clostridial mastitis was diagnosed. Examination of the mammary tissue impression slide focused attention on organisms present and directed an effort toward anaerobic culturing. A slide from any lesion may direct efforts towards making a specific diagnosis.

Preparation of Cytology Specimens

Impressions can be prepared by streaking a cotton applicator impregnated with material from the lesion across precleaned slides to leave a barely visible thin film. If a thick exudate or transudate is collected directly on a slide, thin smears can be prepared by carefully teasing the material to another slide and spreading with an inoculating loop. If the slides are to be stained for bacteria, they should be fixed by passing them *Quickly* through a flame several times. Even then, distortion may occur.

On direct impressions, bacteria will be seen against a background of tissue cells or precipitated proteinaceous fluids. Although intact leukocytes, macrophages, and epithelial cells are clearly recognizable, lyzed and crushed host cells contribute to a variety of subcellular fragments especially in purulent exudates, spinal fluids, and urine, which may be falsely identified as bacteria. The morphology of coccoid or rod-like

forms encountered in smears may be resolved if a second smear is available and fixed in methanol and stained by Wright's. This is especially useful for urine smears. Decoloration with acetone of direct smears of material rich in inflammatory cells is helpful in unmasking the Gram characteristics of any bacteria present. A specimen for culture may be bisected and utilized for impression cytologic and histopathologic evaluation.

At necropsy, touched tissue imprints are easily made and provide a satisfactory population of cells for examination. The specimen is cut into a rectangle shape with a sharp blade (razor or scapel blade) to give a maximum width of 1.5 cm and placed on a wood tongue depressor. The surface of the specimen to be imprinted must be blotted gently with absorbent paper to remove excess fluid and blood so that cells may be easily imprinted onto a slide. A series of impressions are made by gently touching the specimen on a clean, dry, glass slide. Moving or dragging the specimen or using too much pressure while making the preparation will result in damaged and distorted cells. Usually a drop of New Methylene Blue (NMB) or Wright's is placed on the prepared slide and a cover slip is applied. There are a variety of commercial staining kits available such as Diff Quik* that are satisfactory and easy to use. Rapid hematoxylin and eosin (H&E) or other stains may also be used.

The staining procedure with new methylene blue is quick and consistent. After the impression slide is made, place 2-3 drops of NMB on the slide and coverslip. The slides retain staining and cytological qualities for approximately 3-4 hours until cells become dry and crenated and produce artefacts. The disadvantage of NMB is that the slides are not permanent.

Tissues composed of collagen and connective tissue will usually result in too few cells for adequate imprint impressions. Light scraping of hard tissue surfaces with a sharp knife or a tongue depressor will remove sufficient cells to lightly smearing on a glass slide. Exudate smears can be made by standard hematologic procedures, being careful to ensure that the feathered end is not carried to the end of the slide. If large particles are present in an exudate, they may be squashed between two slides that are then drawn apart to make the smear.

In impression cytology, the first goal in differentiation is to identify the cell types as inflammatory, noninflammatory, or neoplastic.

*Harleco's Diff Quik, Harleco, 480 Democrat Road, Gibbstown, New Jersey.

Knowledge and identification of cell types increase with each lesion studied. As more experience in cytology is acquired, a better understanding and correlation of gross, cytologic, and histopathologic interpretation will follow.

A mixture of neutrophils, macrophages (large cells with foamy cytoplasm), lymphocytes, and plasma cells usually denotes an inflammatory cell population. The predominant cell type characterizes the inflammation as to type (e.g., purulent, eosinophilic, granulomatous) and may suggest the cause. The ratio of neutrophils or macrophages to plasma cells suggests whether the inflammatory process is acute or chronic. Degenerative neutrophils characterize septic processes and their presence suggests searching for the etiologic agent, especially bacteria. Degenerative neutrophils are characterized by large, swollen, pink-staining nuclei. Vacuolated cytoplasm and karyolysis occurs to the point that the cell cannot be recognized as a neutrophil. Karyorrhexis may be present. Remember, many surface neoplasms may be accompanied by inflammation, especially if ulcerated.

Cell populations of one type (excluding inflammatory cells) suggest neoplasia. Neoplasms are usually composed of clusters or sheets of cells. Cells with large nuclei with one to three nucleoli and mitoses are characteristic of rapidly dividing neoplasms. Malignant cells usually possess some of the following characteristics: large size, pleomorphic size and shape, large nuclei and nucleoli, high nuclear to cytoplasmic ratio, and heterogeneous, disorderly chromatin. Some neoplastic cell populations lack nucleoli. In some neoplasms, there may be a few neutrophils or macrophages; however, the predominant cell population is of one type and not inflammatory.

Diagnosis of Neoplasms

The following types of tumors are readily diagnosed by cytologic techniques: mast cell tumors, melanomas, lymphosarcomas, lipoma, anal gland adenomas, and histocytomas.

Mast cell tumor is easily diagnosed with an impression stained by NMB, which shows the numerous, fine, blue-black granules in the cytoplasm of mast cells. The cells usually occur singly or in sheets depending on the cellular density in the specimen. Eosinophils and some other inflammatory cells are often present. The intercellular background will contain many free granules from cells damaged in the preparation of slides.

Melanomas are readily recognized in stained preparations. The cells of malignant melanomas are quite pleomorphic. The nucleus is usually round to oval and the cytoplasmic membrane is indistinct. Melanin granules appear as dust-like greenish-black, oblong cytoplasmic granules. Amelanotic melanomas usually will have many fine melanin granules in cytologic preparations. The tinctoral nature of the granules will cause a minimum of confusion with mast cell sarcomas.

Hemangiomas, hemangiosarcomas, and hematomas frequently resemble melanomas grossly in that they all appear black. Hemangiosarcomas can have a metastatic pattern similar to that of melanomas. The cytological aspects of malignancy and the presence or absence of melanin granules will aid in differential diagnosis. A simple procedure is to blot the cut surface of the tumor on white, absorbant paper. Melanomas will leave a black impression and blood vascular tumors a rusty-brown impression, without fail.

Lipomas are common tumors that do not present a problem in diagnosis. Smears are usually characterized by small oil droplets of varying size on the slide. The droplets tend to coalesce with time to form larger droplets in the presence of the water-based stains. The cellularity of lipomas is low.

Anal gland adenomas in the typical location seldom present a diagnostic problem. They usually have characteristic large, well defined, "liver-like" cells. Secretion droplets may be found in the cytoplasm.

Carcinomas in general are readily distinguished as to tissue of origin. Carcinoma cells are generally large, pleomorphic, and hyperchromatic and tend to exfoliate in sheets because of their cohesiveness. Abnormal mitoses are common in highly malignant carcinomas.

Lymphosarcomas are characterized by a homogeneous population of lymphocytes with a high mitotic index; the normal and abnormal mitotic cells may be numerous, three to four per high power field or greater. The presence of primitive hyperchromatic cells and a high mitotic index are usually sufficient evidence for a definitive diagnosis of lymphoma. Malignant lymphocytes in a lymph node vary considerably in morphology from case to case and, yet, individual cases usually have homogeneous cell populations. Differential diagnosis of lymphadenopathy includes lymphosarcomas, metastatic neoplasia, and inflammatory diseases. Metastatic neoplasms are easily recognized when the node is well infiltrated. Inflammatory lymph node lesions are associated with

infiltration of large polymorphonuclear or mononuclear leukocytes. In many cases, the etiologic agent may be found, particularly in bacterial and mycotic lymphadenitides.

DIAGNOSIS OF INFLAMMATORY DISEASES

Inflammatory lesions are classified generally on the basis of their cellular constituents. Acute inflammation is composed almost entirely of neutrophils and macrophages. Degenerative changes in neutrophils such as pyknosis, karolysis, and karyorrhexis may indicate a bacterial etiology. Some bacteria elaborate potent cytotoxins that cause marked degenerative changes. Chronic inflammatory lesions are characterized by lymphocytes, plasma cells, and mast cells in association with macrophages and polymorphonuclear leukocytes. Stromal elements are less frequent; however, fibroblasts and capillary endothelial cells are observed. In granulomatous inflammation, epithelioid cells (modified macrophages and giant cells) may be associated with the previously mentioned cells.

Cytological studies of pleural and ascitic fluids. The principal objective is to distinguish between transudates and inflammatory exudates. Transudates usually contain few cells. A few polymorphonuclear cells, macrophages, and lymphocytes may be present. Exudates are cellular, except fibrinous where a paucity of cells may exist. Macrophages, neutrophils, lymphocytes, and plasma cells are abundant in most exudates. Degenerative cellular changes are frequent and when present, a careful examination of the feathered edge of smears for bacteria should be done; they may be intracellular or clustered among the cells. Fibrin among cells is identified as fine granular strands in stained preparations. Synovial fluids are examined in the same way as pericardial, ascitic, and thoracic fluids.

Cytology of other organs. Eosinophilic, crystal-like, nuclear inclusions are sometimes observed in histologic preparations of the liver and kidney of dogs. These intranuclear inclusions are nonspecific and are readily demonstrated in stained imprints of liver. Viral inclusion bodies, both intracytoplasmic and intranuclear, are found in specific diseases and can be seen in stained impressions. Examples are intracytoplasmic inclusions in the epithelium of urinary bladder, lung, and stomach in canine distemper and intranuclear inclusions in hepatocytes in canine hepatitis, the liver of aborted fetuses with infectious bovine tracheitis (IBR), and equine rhinopneumonitis (EHV-I).

In salt toxicity in pigs, an eosinophilic cuffing may be present in stained smears of the cerebral cortex. In cattle and sheep, along with small bacteria rods, foci of neutrophils in the brain stem can be seen with listeriosis.

Cytological findings in specific infectious processes. In some diseases, the etiologic agent may be seen in smears. Examples include nocardiosis, actinomycosis, blastomycosis, cryptococcosis, histoplasmosis, coccidiomycosis, and aspergillosis. Bacteria may be seen intracellularly or clustered among cells and with Gram's staining may be more precisely identified.

Diagnosis of bacterial diarrhea. Place a small fleck of mucus (or stool, if no mucus is present) on a clean glass slide, mix thoroughly with two drops of Loffler's methylene blue stain, coverslip, wait 2 to 3 minutes for good nuclear staining, and examine. The test works best with fresh specimens, but cellular detail is still well preserved in stools that remained at room temperature for up to 24 hours. The presence of leukocytes signals a break in the integrity of the intestinal mucosa, suggesting that a bacterial agent has penetrated the mucosa. Possible causes include *Salmonella,* invasive *E. coli,* etc. Absence of leukocytes in the smear suggests a noninvasive diarrhea caused by an enterotoxin, a virus, or a noninfectious cause.

CLINICAL LABORATORY SUPPORT AT NECROPSY

Using a laboratory, private or commercial, to support necropsies is becoming a necessity, if a veterinarian desires to practice quality medicine. Obtaining laboratory data from animals (in the terminal stages of illness) prior to necropsy may reveal invaluable data for diagnosis. Each practitioner must decide what is needed for supportive laboratory medicine. He alone knows how much time he can devote to diagnostic techniques. Laboratory work in practice depends on simple and efficient methods, availability of equipment and reagents, ability to interpret results, proximity of a reference laboratory for quality control, and reasonable monetary compensation. The key to productive laboratory work is careful planning. Laboratory tests will not be used in practice unless they are readily available and simply and easily interpreted. Frequently, animal technicians in a veterinary practice assist in laboratory services because the practitioner feels he has neither time nor self-confidence to perform the various procedures. It must be stated, however, that we cannot expect good laboratory work by a technician unless the practitioner has sufficient interest and skill to promote the technical methods necessary to meet everyday problems. Therefore,

anyone planning to establish a laboratory must educate himself sufficiently in these matters to adequately fulfill his supervisory responsibility. A wider use of laboratory methods may be made by utilizing reference laboratories for quality control and additional sophisticated testing.

However, one should *never* forget that the history and clinical examination are *important* factors in making a diagnosis. Laboratory results are of little or no value if the veterinarian is unable to interpret the results and correlate them with the history and clinical picture. Laboratory tests should be used for diagnostic confirmation and not solely for diagnosis. Clinical laboratory findings should be utilized to support a necropsy as well as a clinical workup.

The veterinary literature contains numerous reports dealing with laboratory diagnostic methods. The laboratory tests discussed here are quick and reliable. For example, the time required for estimating the hemopoietic status of an animal within your own laboratory is approximately 2 to 3 minutes. Variations in laboratory values found in different textbooks may be associated with different techniques or instrumentation. It is recommended that each practice establish its own laboratory values of normal ranges, which may depend on the type of practice. For example, different hematological values may occur in race horses in training compared to childrens' ponies in a 4-H club.

Quality Control

The quality of laboratory results are related to the quality of the samples collected and submitted. A uniformity of sampling technique must be emphasized; only then will consistent values be obtained. Directions must be followed carefully. In doing chemistries, use quality control serum to check equipment function, reagent quality, and technique. Commercial quality control serum with known constituent concentrations is available. When used in precisely the same way with a patient's sample, the results should agree within 2-3 standard deviations of the average value. If not, the method must be checked to determine why it is not compatible with the quality control.

Most practice laboratories are concerned with the following basic areas: hematology, urinalysis, fecal examination, blood chemistry, and microbiology. Not all of the above tests will be required for every necropsy. Each of these areas and certain related problems will be discussed individually.

Hematology

Frequently, hematological examinations of sick animals submitted in the terminal stages of illness will reveal important information. Several examinations are performed quickly and easily and give information regarding hydration, hemoglobin concentration, plasma color (icterus or lipemia), etc.

Microhematocrit

The microhematocrit offers practicing veterinarians more potential information than any other laboratory diagnostic aid, including: (1) packed cell volume (PCV); hydration and anemic status; (2) sedimentation rate; (3) buffy coat; (4) plasma color and icterus index; (5) presence of lipemia; and (6) filaria content. In addition, sufficient plasma may be obtained for several other microchemical procedures.

To ensure quality control in hematocrit readings, the brushes on the rotor of the hematocrit should be checked and replaced, if necessary, every 3-4 months. Other inaccuracies in PCV determinations are excess EDTA anticoagulant and a poorly mixed sample.

The PCV undergoes daily fluctuations depending on temporary changes in the hydration status. For valid interpretation of daily changes, the relationship of the PCV to total protein level must be considered. A refractometer quickly measures plasma protein, and the drop of plasma needed for the test can be obtained from the microhematocrit tube. If the plasma color is suspicious, a direct and indirect bilirubin assay may be required. Lipemic serum from a fasting animal would indicate the need for performing additional chemistries when clinical signs concur. In evaluating PCV, consider the physical evaluation of the animal for dehydration, urine specific gravity, and plasma protein.

In obtaining plasma from the hematocrit for heartworm examination, consider the differential microscopic characteristics of *Dipetalonema* and *Dirofilaria.* A disc micrometer reticle is utilized for determining length of the microfilaria, one identifying characteristic.

Total and Differential Leukocyte Count from Stained Blood Film

Blood smears may be made from the microhematocrit tube. The differential count and cell morphology are easily done and are probably the most overlooked and least expensive of all basic laboratory procedures. With experience, a laboratory technician can get a relatively accurate

impression about the cell distribution just by scanning the slide. This impression may be more accurate than an actual count performed on an inadequate number of cells. To be significant, the differential count should include a minimum of 200 cells. The area recommended for evaluating a blood smear correctly is the feather edge of the slide.

If the blood film shows a shift toward immature neutrophils, it indicates an infectious or necrotic process. Increased numbers of hypersegmented neutrophils and decreased numbers of lymphocytes and eosinophils suggest a corticosteroid effect. Abnormal, immature neutrophils or lymphocytes may suggest neoplasia. Plasmacytoid or "reactive" lymphocytes suggest active antibody response, whereas increased eosinophils indicate an allergic type of antigen-antibody response. Increased eosinophils may also occur within 48-96 hours after corticosteroid withdrawal, with depression of lymphocyte numbers. Increased basophils strongly suggest *Dirofilaria* infection in the dog. Circulating mast cells of mast cell leukemia appear different from blood basophils and, therefore, a diagnosis of mastocytoma could be made.

Erythrocyte Morphology

Erythrocyte morphology can determine whether anemia is regenerative or nonregenerative. The presence of reticulocytes or polychromatic red blood cells indicates a regenerative response. Most nonregenerative anemias are caused by bone marrow depression resulting from infections, toxemias, neoplasia, etc.

Platelet Numbers from Stained Blood Film

Platelet (thrombocyte) evaluations are determined easily with a complete blood count (CBC) and are normally found in peripherial blood smears. Anticoagulative samples give a more even distribution with less clumping of platelets than films prepared directly from venous blood. Check the edges of blood films for platelet clumps before diagnosing thrombocytopenia. Normally, there are 5 to 15 platelets in the average oil immersion field (OIF) (above 150,000/cu mm) examined. Hemorrhage resulting from thrombocytopenia rarely occurs until the thrombocyte count is less than 50,000/cu mm (less than 2/O.I.F.). Platelet counts may be moderately low (50,000-150,000/cu mm) immediately following acute hemorrhage from any cause.

Diagnosis of Hematozoan Parasites in Stained Blood Films

Examination for hematozoan parasites, which are demonstrable in circulating blood, is relatively simple. The essential basic requirements

are a good microscope with oil immersion objective, thin blood films stained as for differential leukocyte counting, and familiarity with the morphologic characteristics of the parasites. Thin blood films stained with Romanowsky stains, such as Wright's or Giemsa for staining smears for differential leukocyte counts, are satisfactory for demonstrating specific causative organisms in blood. The more common intracellular parasitic conditions in dogs include babesiosis, haemobartonellosis, ehrlichiosis, and histoplasmosis. Anaplasmosis and eperythrozoonosis are the more common intracellular blood parasitic diseases in large animals.

Platelets superimposed on a red blood cell may have morphologic features resembling trophozoites of *Babesia.* Howell-Jolly (H-J) bodies are usually a prominent feature in cases with marked anemia. Howell-Jolly bodies conceivably may be mistaken for singularly occurring coccoid forms of *Haemobartonella* organisms; however, H-J bodies are more deeply basophilic and larger. Finely dispersed precipitated particles of staining material adhering to the smear surface may also resemble intracellular parasites. Smears with diffuse stain precipitate are unsuitable for demonstrating haemobartonella, eperythrozoon, and anaplasma organisms and should be discarded.

Urinalysis

This can be done on fresh specimens from animals that recently died, because urine is easily collected from the bladder upon opening the animal. Insert a needle into the bladder and aspirate the urine into a syringe. If the animal has been dead for any length of time, considerable desquamation of cells from the lining of the urinary bladder will have occurred and may cause confusion in interpreting urine sediment smears. Animals may void urine during extremis or early in death, resulting in an inadequate volume in the bladder for examination. In a urinalysis profile, volume, color, and specific gravity should be determined, and a smear of the sedimentation after centrifugation for cell morphology should be made. Chemical analysis of urine should include protein, glucose, ketones, bilirubin, blood, and pH. These tests can be done on urine during necropsy with commercially available rapid screening kits. The urine specimen should be fresh, because if urine is allowed to stand, the pH rises as result of bacterial activity. The amount of sediment may increase because of precipitation of crystals from the altered pH and accumulation of desquamated cells from the lining of the urinary bladder. The use of a refractometer allows the specific gravity and the protein content to be

determined with one drop of urine. A reliable conclusion can be drawn from inspecting the size of the sediment button on the bottom of a centrifuge tube, providing that the same amount of urine and the same amount of centrifugation are employed. After smearing urine sediment onto a slide, check for oxalate crystals by using polaroid film. Place a piece of polaroid film above the slide and rotate another piece of polaroid film beneath the slide until all of the light is filtered out; if oxalate crystals are present, they will polarize as white to yellow.

Cerebral Spinal Fluid

Pandy test is performed by dropping a drop of cerebral spinal fluid in a tube of concentrated phenol solution. If excess protein is present, as in brain infections, the solution denatures it to an opaque precipitate.

To make Pandy reagent, fill bottle with phenol crystals. Pour in boiling water, wait several hours until crystals settle, and use the clear supernatant.

POSTMORTEM EVALUATION OF BODY FLUIDS

Necropsy chemistry is in its infancy. Studies on normal postmortem profiles of blood, cerebrospinal fluid, and vitreous humor in relation to time have been done in the dog. There are few data available on other domestic animals.

Although a thorough gross and microscopic examination is best for necropsy diagnosis, it may not always be possible for a number of reasons, such as lack of client permission or a cadaver too severely traumatized or decomposed. In such cases, necropsy diagnosis may be inadequate and require support from analysis of body fluids such as blood, cerebrospinal fluid (CSF), and vitreous humor. Determination of chemical values also may be beneficial in establishing the cause of death when necropsy has revealed no significant findings. Chemical parameters in adjunct with necropsy may contribute to the evaluation of physiologic effects of recognizable lesions.

Some biochemical materials remain remarkably stable in the blood after death, whereas others undergo various degrees of change. Both the nature and concentration of many chemical substances in the body may change rapidly because of the unpredictable interplay of processes such

as dehydration, diffusion, hemolysis, enzymatic activity, and putrefaction. The postmortem interval is an important factor influencing these processes.

The chemical and physical changes in blood may vary from site to site because of the close association with autolyzing tissues. Movements of blood after death are also likely to increase the interchange of chemical substances between blood and surrounding tissues. Blood viscosity and hematocrit values begin to increase sometime after death, whereas osmotic resistance of erythrocytes decreases rapidly. Effusions and exudates in body cavities may be expected to remain unaltered longer after death.

Postmortem biochemical values can also be used to determine the time-of-death. This forensic biochemical technique has been used more in human medicine, but has potential in veterinary medicine especially with wildlife killed illegally by poachers, insurance cases, and in situations where a necropsy cannot be done for various reasons.

Choosing and Obtaining Body Fluids

Blood is probably the best fluid to use, because more established values are available than for CSF or vitreous humor; however, it is usually the hardest to collect. Vitreous humor is obtainable longer than blood and is easy to collect without altering body structure; however, most laboratories have little experience dealing with it and often have little or no enthusiasm to try. CSF analysis adds nothing to what can be determined from blood; furthermore, it is not as readily obtainable as blood. It does, however, add support to findings from other body fluids. All fluids should be centrifuged, and the supernatants should be frozen until examined.

Vitreous humor is removed with a 15-gauge needle attached to a 12 cc syringe. *CSF* is removed ventrally from the cysterna magna by penetrating the ventral atlanto-occipital membrane after the skin, muscles, esophagus, and trachea have been removed. Postmortem *blood* is removed by opening the thoracic cavity, incising the right ventricle, and inserting a 12 cc syringe without a needle. All body fluids should be centrifuged, as soon as possible, frozen, and kept that way until they can be analyzed. When possible, the postmortem interval and environmental temperature should be recorded.

Vitreous Humor

To interpret postmortem values of vitreous humor, they have to be compared to normal values. Antemortem chemistries for the dog are listed in Table 8-1.

Table 8-1.
ANTEMORTEM CHEMISTRIES OF CANINE VITREOUS HUMOR

Constituent	*Sample size**	*Mean*	*Range*	*Standard deviation*
Chloride (mEq/L)	57	127.14	114-138	4.56
Sodium (mEq/L)	60	154.18	146-175	5.88
Potassium (mEq/L)	60	6.58	5-9.7	1.12
Urea nitrogen (mg%)	60	14.55	4-31.7	5.54
Glucose (mg%)	59	73.46	54-102	10.04
Creatinine (mg%)	40	.7	.36-1.01	.17

*Initial attempts to use an SMA/12-60 resulted in "plugging" the instrument with too little vitreous left for reanalysis; therefore, some sample sizes are less than 60.

Values are more stable if the animal is refrigerated after death. Summarized postmortem biochemical changes in the dog are as follows:

1. *Potassium* levels rise with increases in temperature and time.
2. *Sodium, chloride,* and *urea nitrogen* values are stable at 4°C for 48 hours; they are less stable at higher temperatures.
3. *Glucose* drops to less than half within 3 hours at all temperatures.
4. *Creatinine* values are inconsistent.

Cerebrospinal Fluid

Postmortem values must be compared to antemortem normals (Schoning and Strafuss, 1980b). The changes in the dog are summarized as follows:

1. *Sodium* and *urea nitrogen* values are stable.
2. *Chloride, glucose,* and *carbon dioxide* values drop.
3. *Potassium* and *phosphorus* values increase greatly; *calcium* and *creatinine* values increase slightly but consistently.

Blood

Postmortem values must be compared to antemortem normals (Schoning and Strafuss, 1980c). Summarized postmortem changes for the dog are as follows:

1. *Urea nitrogen, calcium, protein, amylase,* and *lipase* remain stable.
2. *Potassium, creatinine, phosphorus,* and *alkaline phosphatase* levels increase with time.
3. *Sodium, chloride* and *total carbon dioxide* decrease with time.

4. *Glucose, alanine aminotransferase,* and *γ-glutamyltransferase* levels fluctuate and are unreliable.

Determining Time of Death

Values of constituents of blood, CSF, and vitreous humor that increase or decrease consistently can be used to determine the time-of-death. It is helpful to have more than one body fluid and to know the environmental temperature of the dead animal. Tables with expected values at 4°, 20°, and 37°C have been published (Schoning and Strafuss, 1980c). Constituents that help to determine time-of-death are listed below with their expected changes.

Vitreous Humor

Potassium values increase

CSF

Chloride values decrease
Potassium and phosphorus values increase markedly
Calcium and creatinine values increase slightly

Blood

Potassium, phosphorus, and creatinine values increase
Sodium and chloride values decrease

Diagnosis Using Postmortem Body Fluids

Postmortem body fluid chemistry values that remain stable or change consistently can aid diagnosis. Tables with expected values at 4°, 20°, and 37°C have been published (Schoning and Strafuss, 1980d). Listed below are diseases and accompanying body fluids that can support or refute the diagnoses.

Renal Disease

Urea nitrogen values remain stable in blood, CSF, and vitreous humor. Creatinine values increase in blood, CSF, and vitreous humor but remain below values associated with renal disease, so high levels would support the diagnosis. Aqueous humor urea concentration of more than 9 mmol/liter is reported to be indicative of antemortem urea retention. Serum urea levels are closely correlated to aqueous humor levels in uremic dogs. Therefore, postmortem aqueous humor values are useful in substantiating the significance of morphological kidney changes.

Pancreatitis

Amylase and lipase values of blood remain stable.

Hypocalcemia

Blood and CSF calcium levels remain stable.

Hypoproteinemia

Blood values remain stable.

Hypernatremia

High sodium levels in vitreous humor suggest this diagnosis.

Hyperglycemia

High levels of glucose in vitreous humor and perhaps CSF are suggestive of hyperglycemia, but this constituent fluctuates more than most.

Hypoglycemia

High glucose levels early after death eliminate a diagnosis of hypoglycemia.

Hypomagnesemia in Cattle

Hypomagnesemia is a major cause of disease and death in dairy and beef cattle. Death often occurs before clinical signs are observed. At necropsy, specific lesions are minimal or absent. Postmortem blood samples are of little value for diagnostic purposes, because serum (Mg^{++}) levels increase rapidly after death because of leakage of Mg^{++} from cells. However, the postmortem vitreous humor Mg^{++} level in cattle parallels closely antemortem Mg^{++} levels and remains relatively stable. It can be a useful diagnostic aid for detecting Mg^{++} imbalances in cattle for at least 48 hours after death, provided the postmortem environmental temperature did not exceed 23°C after 24 hours. The range of postmortem vitreous humor Mg^{++} levels in healthy cattle is 1.8 mg/dl to 2.72 mg/dl at 23°C for 48 hours postmortem. A low normal or deficient PM vitreous Mg^{++} concentration correlated with an appropriate history and clinical signs is very helpful in establishing a diagnosis of hypomagnesemia.

Water Deprivation in Swine

Water deprivation for periods of 18 to 72 hours can result in salt poisoning even in the absence of excess salt in the diet. The pig is known to tolerate as much as 13% NaCl in the diet, if fresh water is *freely* available. In swine with classic water deprivation, sodium levels in serum and cerebral spinal fluid (CSF) usually exceed 150 m Eq/L. In normal

swine, sodium content in CSF is consistently less than in serum. In swine deprived of water, increased sodium levels occur, with CSF levels being greater than those in serum. Data are based on samples collected within 10 minutes after death. Samples are easily obtained and may be submitted to many diagnostic facilities.

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CHAPTER 9

SELECTION AND HANDLING OF TOXICOLOGIC SPECIMENS

LABORATORY ANALYSIS of specimens selected from living or dead animals and/or the environment is necessary for confirming a diagnosis of suspected poisoning. The veterinarian must be thorough and have an awareness in selecting and handling toxicological specimens. The most experienced toxicologist is of little help when presented with either the wrong specimens or specimens in poor condition.

In many instances, a careful history is sufficient to establish the nature of the poison for immediate treatment. Circumstantial evidence may be sufficient to indicate that death was caused by a particular chemical, especially if the clinical signs and necropsy findings are compatible with the suspected agent. Accurate diagnosis of toxicoses is made by obtaining the following information: (1) circumstantial or historic; (2) symptomatic; (3) pathologic; (4) experimental; and (5) chemical.

Avoid selecting specimens for chemical analysis only because histologic examination of such tissues frequently reveals information useful for diagnosis. Used properly and in the right perspective, chemical analyses may provide the single most important source of evidence. One should never request a laboratory to simply "analyze or check specimens for poisons" because an animal died of unknown causes. There are hundreds of toxic chemicals and plants, and analyses for all would be impossible and the cost prohibitive. Besides, there are no analytical procedures for many toxic plants and some chemical agents.

Photographic evidence carries much weight in court and since many toxicological cases are court-oriented, it is well to have movies or photographs of affected animals demonstrating clinical behavior.

Unless a laboratory is adequately staffed and equipped, many qualitative screening tests for toxicants are not worth the time it takes to perform them. False-positive or false-negative results can be disastrous, especially when one considers that a majority of toxicoses involve potential litigation.

The following information is necessary if a toxicology laboratory is to avoid lengthy and wasteful efforts in chemical analysis:

1. Species of animal, age, sex, and time of death.
2. Owner's name and address.
3. Complete description of signs observed and length of time (hours or days) that they were observed.
4. If no signs were observed, give length of time prior to being found dead that the animal was last seen and its general condition at that time.
5. When specimens are submitted, give as complete a postmortem report as possible.
6. Mention type of area where animal was kept (housed, feedlot, pasture, etc.).
7. List all pesticides known to have been used in the general area where the animal might have had some contact.
8. List all medications administered within a week prior to death and time prior to death that they were administered.
9. Suggest any poisons you think might be involved.
10. Add any other information you feel might be pertinent.

CHOICE OF SPECIMENS

Specimens should be collected free of chemical contamination and should not be washed because of the possibility of removing residues of the toxic agent or of contaminating the specimen with water. Trace amounts of a particular chemical or even the slightest contamination may produce erroneous results.

Specimens sent to laboratories should be packaged in clean, nonabsorbent, leak-proof containers such as heavy plastic bags. Tissue specimens should be frozen and packaged to arrive while still frozen. Serum and blood should not be frozen, but kept refrigerated. Blood samples may be citrated or heparinized. The type of packaging may vary with different laboratories; therefore, one should always contact the laboratory prior to submission.

With litigation possible, specimens *must* be obtained and transported to the laboratory by an unbiased party and no contamination or adulteration of specimens should occur. The laboratory *should be notified* that a legal court case may ensue. The need for supplying the history, clinical signs, and necropsy findings with specimens submitted for chemical analyses *cannot* be overemphasized. Complete information enables the toxicologist to intelligently select the appropriate tests, provided the appropriate specimens are submitted for chemical analysis.

Specimens from a Live Animal:

- blood urine, vomitus, or food
- feces are of little value in most cases
- liver biopsy may be performed, if animal's health not jeopardized

Specimens from a Dead Animal:

- liver, kidney, stomach and its content tied off, or stomach contents, urine
- serum
- brain

Approximate Amounts of Specimens to be Submitted:

serum	5 ml
whole blood	10 ml
urine	50 ml
liver	50-100 g
kidneys	50-100 g
spleen	50 g
brain (entire; ½ fixed in formalin; ½ frozen) amount varies.	50-100 g
stomach or rumen contents	150-500 g
feed or incriminated material	200-500 g
body fat	50-100 g

Specimens Required for Some of Common Toxicosis:

Suspected sources of toxic substance may be included: food, feed, water, sacks, etc.

Nitrate-nitrite test—Several small samples of roughage should be pooled for analysis. Submit water samples if suspected.

Cyanide test—Samples of roughage from several areas pooled for analysis, stomach contents, liver, oxalated blood.
Urea toxicity—(Ammonia toxicity) A citrated sample of blood in a air-tight tube (full tube) from animal with clinical signs.
Arsenic—Liver, kidneys, urine, hair, and stomach contents.
Aflatoxins—Suspected sample of feed. Keep dry and refrigerated if possible.
Calcium—Serum, avoid hemolysis.
Sodium chloride—Serum, and brain for histopathological examination.
Phosphorus—Serum.
Mercury—Kidneys, urine, liver, and stomach contents.
Lead—Kidneys, liver, urine, blood (heparinized), and stomach contents.
Strychnine—Liver, urine, and stomach contents.
Warfarin—Liver and kidneys.
Antifreeze poisoning (ethylene glycol)—Submit kidney in formalin for histopathological examination.

PRACTICAL TESTS FOR FIELD DIAGNOSIS OF TOXICOSES

These tests could *not* be considered definitive, since they are only utilized to assist in rapid diagnosis and treatment of toxicoses. They are to be used *only* as an aid in diagnosis and are not usually adequate in litigation.

1. Reinsch Test (Detects arsenic, mercury, bismuth, antimony, or silver)

Place approximately 25 g of chopped hay, forage, rumen contents, urine, liver, stomach contents, or other tissue to be tested in a chemically clean, Pyrex® container. Add 10 ml of concentrated hydrochloric acid and 90 ml of distilled water and mix. Introduce a strip of bright, pure copper foil or wire that has been cleaned with 15% nitric acid or burnished with steel wool (a copper penny cleaned in the same manner may be used). Place the container in a boiling water bath for 30 to 60 minutes, remove copper foil, rinse in water, and allow to dry. A discoloration or coating on the copper is presumptive evidence for a heavy metal. The deposits can be divided into four main groups based on color: (1) gray, blue-gray, black—selenium and sulfur; (2) silver—mercury, platinum, and silver; (3) gold—gold and platinum; and (4) violet—antimony and tungsten (fades after drying).

If arsenic is present, the discoloration will be steel-gray to grayish-black because of the formation of copper arsenite. To prove that the coating is arsenic, the coated copper is placed in a small test tube and the lower end heated gently. Arsenic forms a characteristic sublimate of glistening octahedral or tetrahedral crystals on the cooler portion of the tube, which can be seen under magnification.

2. Ph of Rumen Content for Urea

Since ammonia is formed in urea poisoning, ammonium hydroxide results and the rumen contents become basic. A rumen pH of 8 or more is strongly indicative of urea poisoning. Ordinary pH paper may be utilized. Normal rumen contents have a pH of approximately 7 with some variation with diet (roughage has a pH of 7 - 7.5 and concentrates have a slightly acid pH, 6.5 - 7). Acidosis usually results in a rumen pH of less than 6.

3. Response to Atropine to Diagnose Organophosphate or Carbamate Poisoning

Pharmacologically, atropine is a specific blocker of the physiological effects of organophosphates or carbamate poisoning in all species of animals. Salivation and drooling in animals suspected of organophosphate or carbamate insecticide poisoning is stopped dramatically by intravenous atropine. Increased digestive and respiratory secretions, extensive peristalsis, muscular tremors, and increased neuromuscular activity associated with organophosphate should be considerably decreased, if not eliminated, by a large dose of atropine. If the clinical signs are not diminished considerably (i.e., no response observed from sufficient atropine to cause dry mucous membranes), then the patient probably was not suffering from organophosphate or carbamate insecticide poisoning.

4. Nitrate Field Test

Reagents: Mix 0.5 gm diphenylamine in 20 ml of distilled water. Add sulfuric acid to bring the total volume to 100 ml, cool, and store in a brown bottle. This full-strength solution may be diluted with equal parts of 80% sulfuric acid to obtain a working solution and is stored in a brown bottle.

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*Sigma Chemical Company, St. Louis, MO.

Plants: Put one drop of the test reagent on the cut surface of a plant. Several plants from different locations should always be tested. A green to blue color with half-strength solution indicates a 2+ nitrate, which is the danger point for feed.

Body Fluids: Place a drop of serum, urine, or other body fluid on a white plate and add 3 drops of reagent. An immediate intense blue is positive. Can be used on animals that have been dead for up to 24 hours.

Water: Place a drop of water on a white plate and add 1 drop of reagent; a gray-blue color is positive. The limit of detection is 30 ppm nitrate.

5. Nitrite Field Test

Reagents: Sulfanilic acid solution is 0.5 g sulfanilic acid in 150 ml 20% glacial acetic acid. Alpha-naphthylamine hydrochloride solution is 0.2 g of the salt dissolved in 150 ml of 20% glacial acetic acid by gently heating.

Cornstalks: Apply 3 to 4 drops of sulfanilic acid solution to freshly cut surface, allow it to soak in, and then add 3 or 4 drops of alpha-naphthylamine solution. A resultant pink to red color is positive for nitrites.

Fluids: Place 2 ml of unknown solution in a clean tube and add 2 ml sulfanilic acid and 2 ml alphanaphthylamine solutions. A pink to red color is positive for nitrites.

6. Cyanide in Rumen Contents

A positive diagnosis of prussic acid poisoning is often necessary. The following technique gives excellent results.

Reagents: Sodium bicarbonate 0.5 g and picric acid 0.5 g dissolved in 100 ml of distilled water. The solution may be kept for up to four months, if well stoppered and kept cool.

Rumen contents or finely diced pieces of the right half of the liver are placed in a flask and enough water added to make the contents slushy. Strips of filter paper are saturated with sodium bicarbonatepicric acid solution and allowed to become almost dry. One end of the picric acid strip is carefully placed in a split cork stopper and the stopper carefully inserted in the flask without letting the strip touch the sides of the flask. A blank is usually run on another sample at the same time. Incubate the tube in a pocket or other warm place for at least 24 hours before reporting it negative. A positive test is indicated by red to violet on the filter paper strip.

Picric acid strips may be prepared, dried, and placed in stoppered vials for immediate use. Strips prepared in this manner lose their sensitivity in about a week's time. Such strips should be moistened with distilled water prior to use.

7. Cyanide in Plant Material

To a test tube add a couple of grams of moist shredded plant material and add four drops of chloroform. Insert picrate test paper and proceed as with the HCN test for rumen contents.

8. Insecticide Test

Useful in animals with signs suggestive of insecticide poisoning. Smear stomach contents on the side of beaker and dry. Trap flies in a beaker. A positive test is evident within two hours by abnormal flight and death of flies.

9. Fish Test

Several herbicides and pesticides are toxic to fish. A portion of stomach, rumen, or intestine may be made into a slurry with distilled water. Add about one teaspoonful of slurry to an average size aquarium containing several goldfish or other small fish. Observe fish for behavioral and toxic reactions. If no reaction is observed in 30 minutes, add about one-half fluid ounce more of slurry and continue observation for one hour.

10. Strychnine Type Drugs (Strychnine, brucide)

Strychnine continues to be used as a poison for dogs and cats, or at least it is frequently suspected as being the toxic material. If an animal has died from strychnine poisoning, a large quantity of the drug frequently remains in the stomach and/or small intestine. Stomach or intestinal contents should be placed in a clean glass container. The bait is often green or red and dye will be seen in 20 to 30% of the cases, upon postmortem examination of stomach contents. Add to stomach contents or wash off a suspected specimen with an equivalent amount of approximately 1/10 normal HCl (8.5 ml concentrated HCl—37%—in 991.5 ml water—pH adjusted to about 3.0). Mix well and centrifuge or allow to settle. Filter through glass wool or paper. Inject 1.0 ml of filtrate into dorsal lymph sac of a frog using a 26 hypodermic needle and place frog under a large beaker for observation. Strychnine will cause the frog to go into convulsions upon receiving stimuli, such as a tap on the table or container covering the frog.

11. Phenol

To 10 ml of urine, add 1 ml of 20% ferric chloride. A purple color is positive for phenol.

10 ml of urine is boiled with Millon's reagent (prepared by dissolving 10 g mercury in 20 ml nitric acid). Dilute with an equal amount of distilled water, allow to stand for two hours, and decant. A red supernatant indicates high levels of phenol.

12. Phenothiazine

To 2 ml of urine, add 6 drops of concentrated sulfuric acid and 2 drops of 10% ferric chloride. An overdose of phenothiazine produces a light pink to purple color. Some phenothiazine derivatives, such as chlorpromazine and promazine, give positive tests.

13. Sulfanilamide Derivatives

Place a drop of urine on a piece of paper (unprinted portion of newspaper or cheap wood pulp paper), add a drop of concentrated hydrochloric acid to the urine-impregnated area. An orange color indicates presence of a sulfanilamide derivative.

As a control, place a drop of hydrochloric acid on an unused portion of the paper. A faint straw-yellow color only should appear.

This test also works directly on tablets of sulfanilamide derivatives and is positive with acetozoleamide and other sulfone compounds.

14. Presumptive Test for Salicylate Derivatives

Urine: To 2 ml of urine, add 1 ml (20 drops) of ferric chloride (10%). If salicylates are present, a purple color results.

This test is very sensitive and is positive within 60 minutes after ingestion of 5 g aspirin (1 tablet). It is also positive for sodium, phenyl, or methyl salicylates or phenol derivatives.

Blood: Acidify 5 ml of blood with 0.2 ml of hydrochloric acid and extract with 10 ml of ethylene dichloride. Allow layers to separate, then discard upper blood layer. Add 4 drops of ferric chloride (10%) and 2 ml of water to the ethylene dichloride layer and shake. A purple change is positive for salicylates. The color change can be compared with standards and reported as faint, moderate, or large amounts present, or negative.

Serum: To 2 drops of serum in a white dish, add 1 drop of ferric chloride (10%); a purple color is positive.

The finding of salicylates in urine or blood is of no great emergency, unless the levels are high. This test should be followed by a determination of the CO_2-combining power of blood. It is of prime importance to determine the degree of acidosis (if any). Even one aspirin will give a positive urine test within a hour.

Aspirin poisoning may be deceptive in that the patient's condition may appear better than it actually is.

Salicylate blood levels above 20 mg% may be toxic.

Toxicological Terminology Frequently Utilizes the Following Expressions

1 mg/kg	=	1 ppm (part per million)
1 mg/kg	=	1 ppb (part per billion)
5000 ppm	=	0.5%
1%	=	10,000 ppm

ppm or ppb in feed or water
mg or g per kg of body weight
% of diet (feed) on a dry weight basis

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CHAPTER 10

DIAGNOSIS OF PARASITISM IN DOMESTIC ANIMALS

ACCURATE DIAGNOSIS of parasitic infections is imperative for effective chemotherapy and control. In making a diagnosis of parasitism in domestic animals, body products of the host are examined. These include skin scrapings, blood, urine, and feces. Feces is the most important, since eggs or larvae of a vast number of parasites leave the body in feces. Although comparatively rare, an examination of body products can be negative, even though an animal may be heavily parasitized. Inhibition of ova production may be caused by crowding of parasites or the effects of drug treatment. A positive diagnosis based on a fecal examination is possible only when mature female parasites are present in the host, since eggs produced by them are the basis for diagnosis. This is why newer serological tests, available for some parasitic infections, are valuable under some circumstances.

Most parasitological examinations are made on a single fecal flotation and/or smear. There are instances when a single sample should not be depended on and it may be advisable to take samples on three successive days. Variations in the egg cycle of the parasite or conditions of physiology (diet, enteritis, etc.) of the host may result in a negative sample. Positive findings indicate the presence of worms, but the absence of eggs does not necessarily mean that parasites, mature or immature, are not present. Again, serological tests can be useful under these circumstances.

COLLECTION AND PRESERVATION OF SAMPLES

Techniques in collecting, preserving, and examining feces vary from simple methods requiring little equipment (direct smear) to extremely

elaborate ones (quantitative egg counts) in which extensive and expensive equipment is used. Freshly passed fecal material should be used in diagnosis because under favorable conditions some parasite eggs rapidly embryonate and hatch; the larvae do not float or are rendered unrecognizable by hypertonic flotation solutions. Fecal samples must be kept free of dirt, straw, or other extraneous material, since these may be sources of contamination with free living eggs and larvae. If specimens must be taken from the floor or ground, avoid collecting the fecal material actually in contact with the substratum. If possible, rectal samples should be obtained, especially with ruminants.

Fecal material may be taken from the rectum by a gloved hand in large animals, or by a swab, blunt curette (tongue depressor) or fecal loop in small animals. Excitement of an animal associated with restraint will frequently stimulate defecation and material may be caught in a suitable container. In cattle, the pressure of two fingers on the floor of the rectum at finger's length will stimulate defecation.

Samples *should not* be taken after administering a laxative or during diarrhea associated with enteritis, since the concentration of eggs in the feces is much reduced. Collect samples for fecal examination before using oil lubricants, to avoid interference with methods of fecal examination. Glycerin suppositories should not be used to secure fecal samples, because their effects are similar to those of oil lubricants.

Feces can be fixed in 10% formalin for shipment, i.e., one part feces to 10 parts formalin. Refrigeration of feces is necessary if examination is not performed right away. Adult worms may be fixed in glycerol and 70% alcohol (1:10) after relaxation in cold saline for identification. Identification is difficult or impossible if worms are fixed when contracted and coiled up. Many external parasites (lice, ticks, flies, etc.) are detected on the body by direct observation, either with or without a hand lens. Addition of 5-10% NaOH or KOH can be added to the slide to digest skin remnants, hair, etc., and to help clear the parasite for examination.

METHODS OF PARASITOLOGICAL EXAMINATION

In *Taenia* and *Monieza* species, eggs remain within proglottides and are not passed as eggs, because there is no uterine pore. Proglottides are usually found in a distorted or partially dried condition in or on feces. When suspected, dehydrated proglottides are put in water for about an

hour prior to identification. Skin and hair of the perianal region should be examined for proglottides. Suspected cestode segments can be crushed between two slides and examined for characteristic ova. *Diphyllobothrium* and *Spinometra* species have a uterine pore and pass eggs into the intestinal tract of the host.

Smear Method

Fecal smears can be used to detect protozoa, trematodes, cestodes, and nematodes. A small amount of fecal material is mixed with a toothpick in a drop of water or physiological saline on a slide. The material is covered with a cover glass for a uniform thickness of fecal material and to prevent clouding of the lens objective. The fecal suspension should be thin enough so that newspaper print can be read through it. The method will usually allow detection of infections of a clinical grade and should be done in duplicate. The method is also good for motile protozoa (e.g., Trichomonads, *Giardia* sp., *Entamoeba* sp. trophozoites, etc.).

Sedimentation Method

Sedimentation is most valuable to recover trematode eggs, for which the other methods are of little use. The purpose of sedimentation is to concentrate eggs from large volumes of material. The method should be used only when liver flukes are suspected in sheep, cattle, or related hosts.

Dilute fecal material with water and shake the mixture in a closed container with or without glass beads or stir in an open container. Then pass the material through a sieve and repeat this process as many times as desired. The sieve may be bolting cloth, gauze, or a metal food strainer. When samples are coarse or the material is large, it is desirable to begin with coarse screens and use graded ones to the mesh desired. Pour plenty of water through sieves after the sample has passed to wash out additional eggs caught in the sieve. Cold water is better than warm; do not use water supersaturated with air (full of bubbles). Tiny bubbles imprison the eggs or larvae and carry them to the surface of the liquid where they are lost during decanting. The filtrate is allowed to stand a few minutes before decanting, so the eggs may settle. If the filtrate is fecal colored it may be cleared by repeated decantations with clean water. The final residue is put in a test tube or a sedimentation cone for the last decantation, after which sediment is placed on slides for microscopic examination.

A centrifuge may be used to hasten the process of concentrating eggs in the residue. If larvae are to be collected, the centrifuge should not be run at a speed greater than 2,000 r.p.m. (600 g).

Flotation or Levitation Method

This is the most valuable and accurate method for veterinarians, and with some modification may be used for nematode and cestode eggs, as well as some larvae and coccida oocysts. In general, however, flotation techniques fail to float trematode (fluke) eggs and will distort protozoan trophozoites beyond recognition. The procedure uses a flotation medium of sufficiently high specific gravity so that when the fecal sample is suspended in it, eggs or larvae with a lower specific gravity will float, while the fecal material gradually sinks.

Among solutions used are sugar, salt, sodium nitrate, magnesium sulfate, zinc sulfate, and sodium dichromate. Check each levitation solution with a hydrometer, since specific gravity varies from batch to batch. If specific gravity is too low, eggs won't float and if it is too high, distortion occurs. Use a flotation solution that produces the desired results: saturated salt will not float lungworm eggs; sugar is best for oocysts; and operculated egg flukes are not recovered with $ZnSO_4$. Specific gravity should be 1.18-1.20 for best results.

Techniques of examination are the same irrespective of the solutions used during flotation. A small amount of fecal material (1-2 g) is transferred to a small, straight-sided, glass receptacle (10 cc shell vial) and diluted with one of the above flotation solutions. Diluent is added to make a moderately thick paste when the sample is stirred, the container is filled to about three-fourths capacity, and mixed until the suspension is uniform. Stir with a wooden applicator, a piece of wire, or a small rod. Additional diluent is added until a slight convex meniscus is formed. Complete and adequate stirring to ensure uniformity in the mixture is essential. A cover slip is used to cover the vial touching the surface of the meniscus and is left for 15 minutes. Be sure the vial is *not* too full, or excess material with eggs will run down the sides and be lost. Instead of using a cover slip, one may use a wire loop or glass rod to transfer the top of the solution in the vial to a cover slip or slide. Examinations should always begin under low power of the microscope. The slide is best examined with a cover slip because it decreases evaporation, puts the medium in one plane for examination, and protects the lens objective.

A noncentrifugal flotation method (Ovaassay)* utilizing a disposable plastic collection container and spatula, mixing tube, and strainer has been introduced to the veterinary profession. The supplier of the equipment also supplies sodium nitrate solution (S.G. 1.200) as the flotation medium. The noncentrifugal sodium nitrate method of flotation examination of feces for helminth eggs and coccidial oocysts compares favorably with the sodium dichromate centrifugal flotation method. The apparent difference in the two methods was found to be due to centrifugation. From a clinical diagnostic viewpoint, the differences in egg recovery were of little significance. Fecal examination for nematode eggs and coccidial oocysts is satisfactory for the qualitative needs of most clinical practices.

Fecal material from horses and cattle, particularly older animals, contains coarse material that may interfere with flotations. Coarse material or abundance of fecal material at the surface of the container may be dealt with in several ways. With the fecalyzer, a plastic sieve strainer is utilized for removing coarse material and the egg count is not affected. Similar counts were observed with and without the use of the sieve. One can centrifuge in water first with the shell vial technique to get rid of "junk."

NEGATIVE FECAL EXAMINATION

All fecal examination techniques sometimes fail to reveal parasitic ova. The conclusion to be drawn from a negative fecal examination is not always obvious. With negative fecal examinations, the following conditions may be present:

1. Ova are too heavy to float. Many ova are fragile and readily rupture in solution. This is true of tapeworms contained in segments and is true for most fluke eggs (Operculum pops off with fluke eggs, so they fill up and sink!).
2. No ova are produced. Many parasites produce living larvae of small size that may closely resemble microscopic plant roots.
3. Parasites are present but are sexually immature and not producing ova. Subsequent examinations may demonstrate ova.
4. The parasite population is made up of males. Clinical parasitism by an all male population would not be diagnosible by fecal examination.

*Pitman-Moore, Inc., Washington Crossing, NJ 08560.

5. The population is all females, which produce eggs that are infertile.

6. Parasites are present but not in the digestive tract. There are numerous examples of parasites causing pathology that have no connection with the digestive tract through which their ova may leave the body (examples: *Setaria* sp. and kidney worm).

7. Parasites are too old and infertile. This occurs in conditions where longevity of the worm is greater than fertility.

8. Parasites produce so-called "showering" effect of eggs at which time they may be very numerous. In between the showering effect, there may be an absolute absence of helminth ova, oocysts (coccidia), or cysts *(Giardia).* Coccidia are classical examples of this phenomenon, and it has been shown that animals negative one day may be strongly positive the next.

9. Parasites produce forms that do not leave the body by the fecal route. This would occur where parasites are in the intestine but immature forms enter the blood, body fluids, or encyst elsewhere in the body. For example, *Trichnella* causes a severe parasitism, but larvae encyst in muscles, so no form is recoverable in feces. Others, e.g., *Stephanurus dentatus* and *Capillaria plica* are voided in urine.

10. Parasites are not present. This is most common when a fecal examination is negative. This conclusion can be erroneous and practitioners should always keep the other possibilities in mind before concluding the animal is nonparasitized.

11. Faulty technique may result in failure to detect eggs. Many things influence appearance of parasitic ova and a representative sample that is fresh is a basic necessity to good examination. Improper smearing or concentrating may also fail to demonstrate ova in infected animals.

In the final analysis, the use of all available techniques is necessary to establish a definitive diagnosis. Laboratory examinations for parasitism are equally important as clinical signs and history.

DETECTION OF OTHER TYPES OF PARASITISM IN DOMESTIC ANIMALS

Protozoa: Protozoan infections can be identified by smear; the flotation methods described above will allow coccidian oocysts to rise.

Lung worms: Methods described for determining presence of gastrointestinal nematodes occasionally indicate lung worms. Additional

examination of secretions of the posterior nares and mouth for larvae and eggs by flotation or smears can be done. Eggs of some lung worms hatch while passing through the digestive tract and may be detected in feces by direct examination. Place a small amount of feces in the center of a small container of warm water. Warm water should be used, since it has a tendency to activate larvae. The water should be no deeper than the highest point in the fecal mass. Larvae will leave the feces and may be found either active or inactive in and around the water in 15 minutes to 2 hours. A microscope is necessary for observation.

Renal Nematodes: Parasites of the renal organs may be identified by examining for eggs and sediment in urine samples. These parasites occur only in pigs, dogs, and cats.

Strongyloides sp: Strongyloides in dogs are passed as larvae and can be diagnosed in a fresh fecal sample by using a Baermann apparatus to recover the larvae.

Hookworms are passed as eggs, which are easily recognizable. They are most frequently diagnosed by fecal flotation techniques. Because of the potential health significance of strongyloides that can be transmitted from dog to man, it may be necessary to differentiate larvae of hookworms from strongyloides in older fecal samples in which hookworm eggs have hatched. This is done by examining the buccal capsules. In *Strongyloides* sp, the larvae are easily recognized by the long filariform esophagus that is approximately one-third the length of the body.

Filarial Worms: In skin infections with *Onchocerca sp.,* skin scrapings placed in physiological saline solution should reveal both adult and larval worms.

EXAMINATION FOR MICROFILARIAE IN BLOOD

Modified Knott's Technique

This is probably the most useful and reliable method for recovering and identifying microfilariae of *Dirofilaria immitis* and detecting microfilariae of other nematodes such as *Dipetalonema* sp. and *Setaria* sp. etc., in the peripherial blood. This is an antemortem procedure but may at times be utilized in a dog euthanatized for necropsy. The procedure is adequately described in clinical pathology texts. Commercially available filter disk techniques are available. Use at least 1 ml of blood to avoid false negatives, which may bring about legal action.

In examining for heartworm in dogs, there is a phenomenon of periodicity to consider. This periodicity of microfilariae in blood does not occur in all parts of the United States but is obvious in some areas. Blood should be drawn between 4 p.m. and 2 a.m. the following morning. An injection of epinephrine will result in abundant microfilariae in peripheral blood in a matter of minutes.

Other filarid worms, a species of *Dipetalonema,* have been found in dogs. These nematodes are small and the adults live in subcutaneous and surrounding tissues. The larvae are similar to microfilariae and circulate in blood. Differentiation is best based on width and presence (*Dipetalonema* sp.) or absence (*Dirofilaria* sp.) of a cephalic hook. Remember, finding microfilariae in a canine peripheral blood smear is not definitive for heartworm infection!

GROSS EXAMINATION FOR PARASITES

Demonstration of *Trichostrongylus, Osteragia, Cooperia,* and *Nematodirus* spp. can be done by rolling a loop of duodenum inside out on a test tube or a piece of glass rod, submerging this in an aqueous iodine solution (iodine 30g and potassium iodine 4g in water 100ml) for several minutes, and then in a 5% solution of sodium thiosulphate for a few seconds. The mucosa is decolorized but the brown-stained worms retain their color and are easily seen. This method has been satisfactorily adapted as an efficient rapid field technique for qualitative identification.

Another method is to collect a small amount of feces (5-10 g), or stomach or intestinal contents, including mucus scraped from the mucosa and place it in a Pyrex baking dish (5″ × 8″ × 2″). The fecal material is then diffusely scattered out and examined under direct sunlight or lamp light against a dark background. The light colored parasites stand out well. Care must be taken not to add too much material to the dish, because this will make examination difficult. For small parasites (⅛-¼″ long), a hand lens or dissecting microscope is helpful in locating them in the material. A few parasites should be picked out with a needle, examined, and identified under a dissecting or compound microscope. This is relatively easy using a key from a parasitology textbook.

It is not usual in routine and diagnostic work to differentiate between *Trichostrongylus, Osteragia,* and *Cooperia* spp., because treatment and control measures are similar. However, it is important to identify *Nematodirus* sp. in sheep infections because treatment is different.

External Parasites

No special techniques are required for detecting most external parasites of domestic animals because, except for mange mites, they may be observed directly. A good hand lens is almost indispensible for identifying external parasites on affected animals.

Mange mites may be found by examining skin scrapings from suspected areas. Scrapings may be taken with a scalpel or similar instrument. The scrapings should be deep enough to draw a slight amount of blood and should be taken from several areas, particularly the periphery of suspected lesions. The direction of scrapings for material may be either with or against the direction in which the hair lies. Transfer the scrapings to a drop of water on a slide, where mites may be detected by using the low power of the microscope. They may be differentiated under high power. It is best to use a drop mineral oil or Three-in-One oil on a slide. The scraping instrument may be dipped in the oil so that a film is present. This allows debris to adhere to the blade for transfer to the drop of oil on the slide. Mites stay alive and are in good condition for a long period of time when mounted in oil. Glycerin may be used as a substitute for oil.

In sarcoptic mange, vigorous scraping and combing is necessary to obtain enough material for examination. The scrapings are collected in a beaker. The skin scrapings may be transferred to a 10% KOH or sodium hydroxide solution and left for a few hours or until all organic matter has gone into solution. The material can be centrifuged, and the sediment either transfered to a microscope slide and examined, or the mites can be floated using a levitation solution as with helminth ova. The sediment is then transferred to a slide and examined under a microscope. Reduced light should be used, since only the nearly transparent exoskeleton of mites remain. If an animal is sensitized to mange mites, only a few organisms may be present, which makes positive diagnosis difficult. In such cases, daily examination for several days may be necessary. Ear mites may be detected by carefully swabbing ears with cotton rolled on the end of a toothpick or with an applicator stick. The cotton tip of the applicator may be rolled onto a slide and examined. Examination with a hand lens or a microscope is desirable. A black background is helpful under any light conditions. In bright light, the mites appear as tiny white moving specks.

In cattle suspected of having demodectic mange, the skin should be folded, squeezed, and the whitish material resulting should be mounted and examined for mites. In dogs with demodectic mange, scraping is adequate to obtain a good sample for examination.

ESTIMATION OF TIME-OF-DEATH BY MYIASIS

Fly maggots (larvae of Blow Flies) that invade dead bodies have a short incubation period and can be used in determining how long an animal has been dead.

Blowflies are shiny and have a very characteristic color that is used for identification. The genera *Lucilia* and *Phaenicia* are identified as greenbottle flies, the genus *Calliphora* is called bluebottle flies, and the genus *Phormia* is called blue-black or black blowflies. Others in the genus *Sarcophaga* are called gray flesh flies and have three black longitudinal stripes on the thorax, with the tip of the abdomen being orange in many.

The life cycle of blowflies is similar to that of the screwworm fly, *Cohcliomyia americana,* except that in all blowflies, the cycle is completed in approximately half the time. In the three main groups of blowflies (bluebottle, greenbottle, and flesh flies), the period required for development from egg or larval stage to the adult fly is only a few days. There is some variation in development among species but the greatest variation is caused by prevailing atmospheric conditions. Cold weather slows down their metabolic rate and they remain in the larval stage longer. Bluebottle and greenbottle flies may hatch in six hours and the larval stage may be completed in three days. The pupa stage requires more than three days in most, if not all, of these flies. The greenbottle flies may complete the whole cycle in 12 days, the flesh flies require at least 14 days, and the bluebottle flies about 22 days. Adult blowflies live for approximately one month. This time-table may aid in determining when the animal died, which is most helpful in determining insurance claims.

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CHAPTER 11

DIAGNOSTIC EVALUATION OF BOVINE INTESTINAL INGESTA DURING NECROPSY

DURING NECROPSY, gross observations of feces may detect digestive tract disorders and may provide valuable clues for the differential diagnosis of diseases elsewhere in the body. Fecal quantity in the intestinal tract is dependent on food and water intake and rate of passage.

The ability for prehension, mastication, and swallowing of food should be noted. The forestomachs should be examined for signs of functional or anatomical stenosis or paresis. Intestinal obstruction is indicated by traces or total absence of feces in antemortem observation. The intestine anterior to the obstruction is always dilated.

A paresis of the tail and urinary bladder, along with stagnating masses of excreta in the rectum, indicates paralysis of this bowel segment. This may result from contusion or stretching of the nerves of the cauda equina in the spinal cord through difficult labor or during estrus, infiltrating neoplasia (malignant lymphoma), and hypocalcemic parturient paresis.

Following surgical incision of the abdomen, little or no feces will pass for some time because of the pain. This results in dehydration of rectal contents and leads to formation of fecal balls. Diarrheic animals with an adequate appetite defecate frequently and have feces with a higher water content than animals with inadequate appetites.

COLOR OF FECES

Fecal color is influenced by factors such as age, type of feed (chlorophyll content), amount of bile (stercobilin), and passage rate through the digestive tract. The addition of drugs and feed additives may alter the color of feces.

Feces in calves on cow's milk are normally gold-yellow, but become a pale brown when hay or straw is ingested. Calves fed milk substitutes have a grayish feces varying in intensity depending on the amount of milk substitute ingested. In neonatal calf diarrhea (white scours), the feces are more liquid and appear gray-white to gray-yellow with occasional greenish or reddish-brown portions because of bile or blood.

Feces of adult cattle maintained on green forage are dark olive-green and on hay, brown-olive. The slower passage rate of feces with a thickening of ingesta (insufficiency of fluid) causes the color to darken. The resultant feces are ball-shaped and dark-brown on the surface with a shiny appearance because of the coating of mucus. Diarrheic evacuations are slightly paler than feces of healthy cattle on identifical rations, because of the more rapid passage through the digestive tract and higher water content. In severe rumen acidiosis, bovine feces may be a light gray-green.

Large amounts of bile produce dark olive-green to blackish-green feces, a common finding in animals with hemolytic anemia. With addition of water, these feces are quickly discolored to a yellowish-green. Feces of cattle with obstruction of the common bile duct are pale olive because few or no bile pigments are present. Mud-colored feces will not be present since chlorophyll coloration remains. Acorn poisoning is characterized by mustard to gold-brown feces (urobilin). Chocolate-brown to a blackish tarry appearance indicates hemorrhages (melena) into the upper part of the digestive tract. The color varies with the amount of blood and the length of time in the intestine. Hemorrhage in the rectum causes a diffuse red-brown feces or red streaks from adherent clots. Of various drugs causing discoloration of feces, charcoal powder should be mentioned (black).

ODOR OF FECES

Fresh feces are relatively odorless. Objectionable odors indicate presence of inflammatory products (epithelial cells, serum, fibrin, and blood) that decomposed in the digestive tract as a result of putrefaction. The presence of foul-smelling intestinal ingesta indicates primary diarrhea because of a severe catarrhal hemorrhagic or pseudo-membraneous enteritis before gross lesions are noticeable. A similar odor accompanies salmonellosis, diffuse putrefactive peritonitis, or inflammatory products discharged from obstructed and infected common bile ducts.

Chronic diarrhea in cattle with a normal fecal aroma indicates renal insufficiency (for example, pyelo- or glomerulonephritis) or insufficiency in the systemic circulatory system of the right heart because of valvular endocarditis, traumatic pericarditis, pericardial or cardiac lymphoma, or obliterative pyogenic thrombosis of the posterior vena cava. In pyelonephritis, a liquid intestinal ingesta and enlarged kidneys with urine of low specific gravity, proteinuria, hematuria, and bacteria are present. In diarrhea associated with congestion of the systemic circulatory system, check for any gross anomality impinging on blood flow. The absence of fecal odor in diarrhea will differentiate renal or circulatory disturbances from the fetid, primary cellular alterations in the alimentary tract. In rumen lactic acidosis with overload of early digestible carbohydrates, the feces have a sour, pungent smell.

Poisons can be identified on the basis of fecal odor, because mineral oils (crude oil, fuel oil, diesel oil, or lubricating oil) give the feces a petroleum-like odor and coal tar oil imparts a strong odor of phenol.

CONSISTENCY OF FECES

In ruminants, fecal consistency depends on water content of the ration and the length of time the ingesta spends in the large intestine where reabsorption of water takes place. Severe dehydration causes formation of firm balls of feces with a dark surface coated with shiny mucus, and arranged in facets inside the rectum. The feces of cows with left abdomasal displacement appear greasy or pasty, while those of animals with marked melena are more pulpy to tarry in consistency. Sticky and tenacious feces consisting mainly of mucus and fibrin are voided in chronic, regional enteritis or coccidiosis.

PARTICLE SIZE OF INGESTA

Plant particle size in the ingesta of cattle is influenced primarily by duration and thoroughness of rumination and the passage rate through the forestomachs. Traumatic reticuloperitonitis will allow undigested feed to pass the reticulo-omasal orifice. Unground grains often transverse the digestive tract of large ruminants whole and with no pathological significance. If large amounts of grain are present, the animal might be suffering from lactic acidosis, and evaluation of

ruminal juice is required. In acorn poisoning, feces may contain clearly recognizable acorn fragments; in acorn impaction, acorn fragments are present.

Mucus, when evenly distributed within feces, gives a muddy to pulpy consistency, but on hardened fecal material, a shiny coating of the surface is noted. The presence of a hardened plug of mucus in the rectum is always indicative of ileitis. In salmonellosis, glassy, particle-free mucus of watery consistency may be voided early and later clotted to form gelatinous masses. Intestinal casts of fibrin may be excreted in long strands molded in a negative print of the intestinal lumen. In coccidiosis, regional enteritis, and infectious or toxic enteritis, flakes or shreds of mucus may be formed. This is grossly observed when fecal material is mixed with water and allowed to stand.

BLOOD SMEARING OF RUMP AND TAIL

Skin of the rump, tail, and perineum being distinctly smeared with dark, partially digested blood from the gastrointestinal tract is a pattern seen from tail swishing.

The presence of hemolyzed blood in feces may be due to:

1. Swallowing of large quantities of blood. This is rare and usually occurs with pulmonary hemorrhage. The respiratory system should be checked.
2. Hemorrhage in the forestomachs because of a necrotizing lesion by a foreign body with erosion of a vessel. Postoperative hemorrhage following a rumenotomy may cause hemorrhage in forestomachs.
3. Hemorrhage in the abomasum may result from a mucosal ulcer, lymphoma-induced ulceration with recurrent bleeding, or right abomasal distension and displacement.
4. Hemorrhagic enteritis may be bacteriological, toxicological, or parasitological.
5. Escape of blood into cecum, because of dilation or torsion.
6. Neoplasia in the intestinal tract, or polyps.

The appearance of reddish-black blood dotted over the surface of feces is called micromelena. Usually the feces contain few plant particles and consist of mucus and/or fibrin. In young cattle, this indicates coccidiosis, and in adult cattle, chronic regional enteritis in ileum, cecum, and first part of the colon.

Blood appearing in the feces as red streaks or clots usually originates in the rectum, particularly after rectal examination or other injury to the rectum or anus. In young animals, coccidiosis may be the cause and in cattle of all ages, anthrax must be considered.

Gas bubbles may be present in a soup-like liquid, discharged in paratuberculosis. Calves suffering from enteritis and adult cattle with ruminal lactic acidosis may exhibit foamy characteristics because of tiny gas bubbles.

PH LEVELS AND PROTOZOAN ACTIVITY OF RUMINAL CONTENTS

The pH level and protozoan activity of ruminal contents are useful for evaluating rumen overload or lactic acidosis. The pH of ruminal contents will remain above 6.0 up to 24 hours after death. Usually a pH level of 5.0 or lower indicates fatal acidosis in untreated cases. Evaluation by pH paper is sufficiently accurate to make a diagnosis in most instances. Values of pH may change quickly when ruminal contents are exposed to air. Protozoan activity falls to zero in 24 hours, is less accurate than pH, but still satisfactory as a diagnostic aid. In most naturally fatal cases of acidosis, the protozoan activity of rumen content is nil at the time of death. Foamy feces with a whipped cream consistency is a sign of severe ruminal lactic acidosis.

Evaluation of Rumen Contents

The pH of rumen contents at necropsy or from a live animal by stomach tube may aid in making a diagnosis.

The pH expected in the following dietary situations is:

Acute urea poisoning	7 to 9.2
Protein engorgement	7 to 8.6
Fasting animals	6.8 to 7.3
Low grain, high forage rotation	6.0 to 6.8
High grain, minimal forage (fattening beef-type ration)	4.5 to 6.2
Moderate grain overload	4.0 to 5.0
Acute grain engorgement	3.0 to 4.0

Most rumen pH will remain stable for several hours after death and indefinitely if frozen. In urea toxicity, the pH will continue to rise after death.

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CHAPTER 12

GROSS OBSERVATIONS OF DIAGNOSTIC SIGNIFICANCE

DISEASES in which no distinctive lesions are present, but the history is of diagnostic significance:

1. Sodium fluoroacetate poisoning
2. Mushrooms poisoning
3. Lead poisoning
4. Strychnine poisoning
5. Botulism
6. Tetanus
7. Grass tetany
8. Lightning
9. Electrocution
10. Sudden death syndrome
11. Polioencephalomalacia
12. Endotoxic shock
13. Lesions of conduction system of heart
14. Rabies
15. Listeriosis
16. Encephalitis

Diseases in which no lesions are present, but odor may be of diagnostic significance:

1. Urea poisoning
2. Petroleum poisoning
3. Turpentine (acute) poisoning

DISEASES ASSOCIATED WITH ANEMIA

Mucous membranes that are pale during life will be generally the same at necropsy. Extravasated blood may be noticeably pale and *watery.* Be cautious in judging this observation, because tissues become pale during the postmortem autolytic process. A hyperplastic bone marrow (particularly long bones) in adults is more indicative of anemia than these other factors. Anemia can result from loss or destruction of

excessive numbers of circulating erythrocytes or from functional failure of the hematopoietic tissue of bone marrow.

Anemia resulting from hemorrhage (acute blood loss) may be caused by trauma, idiopathic thrombocytopenia purpura, sweet clover, warfarin poisoning, and bracken fern poisoning. Anemia from chronic blood loss may be due to unhealed ulcers (pigs, cattle); heavy infestations with blood-sucking parasites that include hookworms, stomach worms in ruminants *(Hemonchus contortus),* and intestinal strongyles in horses; and hemorrhagic bowel syndrome and dysentery in swine.

Excessive destruction of circulating erythrocytes within the blood stream, usually accompanied by hemolytic icterus, is a common sequela to many infectious toxic or immunologic disorders. Infections involving the erythrocyte directly include: (1) piroplasmosis; (2) anaplasmosis; (3) eperythrozoonosis of swine and cattle; (4) hemobartonellosis of dogs and cats; (5) malaria of birds; (6) viral equine infectious anemia; and (7) acute bacterial infections (*Clostridium* spp., *Streptococcus* spp., and *Leptospira* spp.. Hemolytic disease of the newborn (erythroblastosis fetalis, neonatal isoerythrolysis, etc.) are incriminated in this group. Chemical poisons (include potassium and sodium chloride), chronic lead and copper poisoning, and snake venom are potent toxic causes for destruction of blood cells.

Anemias caused by deficiency of ingredients necessary for hemoglobin production include iron, copper, and cobalt. The most striking of these is iron deficiency anemia in baby pigs. While copper deficiency is a cause, excess molybdenum will alter the balance between itself and copper and produce a deficiency of available copper for utilization of iron in the production of hemoglobin.

Aplastic anemias brought about by toxic suppression of bone marrow result from bracken fern poisoning and irradiation. Nephritis and associated uremia apparently will bring about a direct toxic action of retained waste products on hematopoiesis. Chronic infections and neoplastic diseases may also result in selective depression of erythrogenesis.

Space-occupying lesions of bone marrow result in anemia from physical destruction of erythropoietic tissue. Metastases (e.g., malignant lymphoma) and osteopetrosis both destroy marrow space.

DISEASES ASSOCIATED WITH EDEMA

Edema is a disorder characterized by an excessive accumulation of fluid (water) in intercellular spaces including body cavities. Grossly,

external locations of edematous swelling occur in the ventral, dependent areas of the body because of gravity. The preputial region in males, sternal or brisket area and feet (vitamin A in deficiency in cattle), and submandibular area or "bottle-jaw" in sheep associated with parasitism are common examples. Eyelid edema, edema of stomach wall and mesocolon in edema disease of swine, or wet pale groups of muscles in rear legs, and psoas or transverse dorsi muscles along with pulmonary edema are examples of fluid accumulations in tissues. Edematous tissue is swollen and relatively firm and has a "doughy" consistency. Upon incision, edematous area contain pale yellowish fluid which will seep (if extensive), separating tissue components. Accumulations of clear watery fluid (transudate with less than 3% protein that is easily determined by a refractometer) occurs in serous cavities such as peritoneal (ascites), pleural (hydrothorax), and pericardial sac (hydropericardium).

Two basic causes of non-inflammatory edema are *hypoproteinemia* and *venous stasis*.

Hypoproteinemia results from a loss of a large part of albumin and globulin from the blood in nephritis (nephrosis) and is characterized by severe albuminuria. With the loss of protein and consequent loss of osmotic pressure, renal edema will occur. Serious nutritional deficiencies of blood protein occur in starvation, protein deficiency in diet, and cachexia and result in nutritional edema. The continued daily removal of blood protein as a result of heavy infestation with parasites such as trichostrongyles or hookworms without adequate replacement frequently causes parasitic edema.

Venosis stasis indicates passive congestion so severe that serious impairment of blood flow (sometimes blockage) has occurred, resulting in edema. Examples are tumor masses (neoplasms or abscesses) restricting blood flow (portal or vena cava blockage), widespread cirrhosis, neoplasia of the liver, or necrosis of the liver. These may lead to cardiac edema because of impaired cardiac function. Cardiac impairment leading to edema is caused by valvular endocarditis, myocarditis, septal defects, tetralogy of Fallot, traumatic reticulitis; fibrosis of lungs, and selenium deficiency (myocardial necrosis).

For practical purposes, edema formation usually falls into categories of renal, nutritional, parasitic, or cardiac. The first three are the result of hypoproteinemia and the last of cardiac dysfunction and generalized venosis stasis by obstruction.

Localized edema involving an organ or part of the body is usually caused by venous stasis because of obstruction by a thrombus or external

compression (abscess or neoplasm), or obstruction to lymph outflow. Tracheal and aveolar edema because of bloat and pulmonary edema caused by exhaustion are other examples. Edema of the gall bladder is frequently associated with canine hepatitis. Gastric, colonic, and eyelid edema associated with gut edema in pigs are other diagnostic examples.

The kidney is frequently targeted as a site of perirenal edema caused by several toxic effects. Poisonings such as oak, pigweed, and ethylene glycol (antifreeze) are frequently associated with perirenal edema.

Accumulation of fluid in serous cavities is usually an inflammatory edema or serous inflammation (infectious feline peritonitis). The accumulation of fluid in serous cavities frequently results from stasis of the portal circulation caused in most instances by obstruction of intrahepatic flow by proliferated connective tissue (cirrhosis).

Pulmonary Congestion and Edema

Almost never present without an observable lesion to account for its presence (i.e., heart lesion). In (ANTU) poisoning, massive edema is present with excessive accumulations in pulmonary alveoli, bronchi, and trachea.

Bronchial and Tracheal Froth

Present to some degree in most animals as they die. Hardly ever of diagnostic importance except in hypoproteinemia and heart failure. Frequently observed in dead animals because of bacterial fermentation. May be unilateral in bronchi in hypostatic congestion and bilateral in pulmonary edema.

Starvation

With chronic starvation, the fat depots, especially around the heart and kidneys, are watery and semitranslucent (serous atrophy of fat). The kidneys and liver are dark. In neonatal starvation, the fat depots are reduced and reddish because of brown fat. In more acute cases, the liver will be fatty (yellow).

DISEASES ASSOCIATED WITH FIBRIN FORMATION

Fibrin, a protein found in blood or lymph clots (antemortem or postmortem), is frequently associated with certain diseases characterized by

fibrinous inflammatory exudates. On gross examination, when blood cells or exudative components have been washed away, a dull white, stringy material is evident as strands adhering to serous surfaces. The presence of fibrin will act as a stimulus and a scaffold for fibroblasts, resulting in fibrous adhesions.

Fibrinous inflammation usually arises in response to certain severe infections caused by microorganisms. Prominent among these are fibrinous enteritis (*Salmonella* spp), infectious feline enteritis (panleukopenia), polyserositis in pigs *(Haemophilus),* feline infectious peritonitis (FIP), fibrinous pleuritis (*Pasteurella* and *Haemophilus*), fibrinous tracheitis associated with avian tracheitis and infectious bovine tracheitis (IBR), sporadic bovine encephalitis (large amounts of fibrin in peritoneal and pleural cavities), and enterotoxemia (fibrin in pericardial sac).

Fibrin is frequently associated with exudates that give it a different gross appearance even though the predominant feature is fibrin. Such inflammations are frequently observed when mucous membranes are invaded by *Corynebacterium diphtheriae, Salmonella* spp or *Fusobacterium necrophorum, Haemophilius parasuis* (Glasser's Disease), and *Mycoplasma hyorhinis.*

DISEASES ASSOCIATED WITH HEMORRHAGE

Hemorrhage is escape of blood from a vessel. If a cut or break occurs in a vessel, it is hemorrhage by rhexis, and if blood escapes through minute or imperfections in blood vessel walls, it is hemorrhage by diapedesis.

Common causes of hemorrhage include:

1. Any mechanical trauma or break in a blood vessel.
2. Necrosis or destruction of a vessel wall associated with an ulcer or eroding neoplasm.
3. Toxic injury to capillary endothelium, chiefly responsible for petechial and ecchymotic hemorrhages.
   a. septicemias (pasteurellosis, anthrax, blackleg, clostridial infection or enterotoxemia, leptospirosis, Herpes infections, swine dysentery, and hemorrhagic bowel syndrome)
   b. poisoning by plants such as bracken fern or sweet clover
   c. poisoning by chemicals such as arsenic and insecticides
   d. anoxia associated with various anemias or electrocution, which, in fact, may be sequela to above conditions.

4. Disorders of clotting mechanisms resulting from dicoumarin poisoning, sequela to septicemic diseases, destructive lesions of liver, vitamin K deficiency (mycotoxins in swine and poultry), purpura hemorrhagica (following streptococcal infections).

In evaluating hemorrhage, fluid volume is replaced in an hour or two by fluid withdrawal from intercellular spaces of tissues (animal may appear dehydrated clinically). Erythrocyte replacement may take four to six weeks. Anemia will be noticeable quickly if blood loss is severe or slowly if prolonged (gastric ulcer).

Hemorrhages in pleura, lungs, myocardium or epicardium are common lesions in most animals that die with or without struggling; they are diagnostic of almost nothing.

Red or hemorrhagic appearing gastric and intestinal mucosa is mostly physiological *hyperemia,* not hemorrhage and is of little significance. It is important only when *free* blood is present in the lumen with flecks of necrotic debris and fibrin attached to the wall.

DISEASES ASSOCIATED WITH ICTERUS

Icterus, a generalized yellowish discoloration of tissues, is obvious normally in whitish tissues such as brain, joints, aorta, and sclera. Icterus must be differentiated from normal yellowish pigmentation (carotenoids) seen in breeds such as Jersey and Guernsey and to a lesser degree in horses. Depending on the causative mechanism, icterus or jaundice, is divided into three types: hemolytic, toxic, and obstructive.

Hemolytic jaundice results from massive hemolysis of circulating erythrocytes. Important causes of hemolytic icterus include piroplasmosis, anaplasmosis, leptospirosis, and equine infectious anemia. These diseases may progress so rapidly that the patient does not survive the two or three days usually necessary for icterus to develop.

Toxic jaundice is caused by toxic substances acting on hepatocytes and producing hydropic degeneration, fatty change, and necrosis. The hepatic cells may be damaged to such an extent that they cannot perform their biliary excretory function, allowing unconjugated bilirubin to accumulate in the blood. Secondly, swelling of the hepatic cells may be sufficient to block bile canaliculi. The bile is excreted from the cells but cannot pursue its course to the gallbladder and intestine and conjugated bilirubin is regurgitated into the blood. Both

of the above processes usually go on simultaneously. Causes of acute toxic hepatitis include chemicals such as copper, arsenic, tetrachlorethylene, carbon tetrachloride, coal tar pitch (clay pigeon), and gossypol. Plants causing toxic jaundice include species of *Senecio, Amsinckia* (tar weed), lupines, and vetches. Aflatoxin is a toxin produced by a fungus in grain and has a marked necrotizing effect on hepatic cells. A metabolic condition producing an acute hepatitis is "pregnancy disease" of ewes that results from the combined effects of pregnancy and ketosis. Vitamin E and selenium deficiencies may result in acute hepatic necrosis.

Obstructive jaundice results from any obstruction to the normal outflow of bile. Bile (conjugated bilirubin) may be retained anywhere in the biliary circulation and, therefore, may be reabsorbed into the blood. The obstruction may be caused by blocking of bile canaliculi by swollen hepatic cells, obstruction of the ducts either internally or externally by flukes, fimbriated tapeworms or ascarids, or pressure on ducts by fibrous tissue (biliary cirrhosis), neoplasms, granulomas, or abscesses.

DISEASES ASSOCIATED WITH ULCERS

An ulcer is a break in the continuity of epithelium with exposure of underlying dermis or submucosa. It is sometimes difficult to differentiate erosions from ulcers, but most frequently the deeper eroding of tissue results in an ulcer. Ulcers may be a sequela to petechial hemorrhages in mucosal surfaces. Aplastic anemia because of either chronic sulfa toxicity or bone marrow suppression from a toxic or infectious process or bracken fern poisoning may result in petechiae with subsequent ulceration.

Common diseases with tongue ulceration include bovine viral diarrhea (BVD) and vesicular stomatitis (VS) in cattle and calicivirus virus infection in cats. Oral and esophageal ulceration is associated with diseases such as BVD, uremia in dogs, generalized debilitation, and sequela to petechiae. Other sites include abomasal ulcers in cattle, fundic ulcers in pigs with *E. coli,* cardiac ulcers with stress, and gastric ulcers associated with uremia. Cattle in feedlots being fattened on concentrations with corn and alfalfa are prone to abomasal ulcers.

Table 12-1.

DIAGNOSTIC FEATURES OF THE ORIGIN OF VARIOUS TYPES OF FLUID FOUND IN BODY CAVITIES

A. *Pleural Cavity* *Type of Exudate*	*Possible Origin*
Serofibrinous	Early bronchopneumonia. Pulmonary infarct.
Fibrinopurulent	Bronchopneumonia. Abscess(es) in lungs. Extension of acute inflammation from adjacent structures. Perforating traumatic reticulitis.
Hemorrhagic	Neoplasia. Hemorrhagic diatheses.
Putrid	Gangrene of lungs. Rupture of esophagous. Foreign body aspirations.

B. *Pericardial Cavity* *Type of Exudate*	*Possible Origin*
Serous	Baby pig anemia.
Serofibrinous	Uremia in dogs and cattle, viral pneumonia.
Fibrinopurulent	Extension of inflammation from neighboring structures (mediastineum, pleura, vertebrae, myocardium). Trauma.
Hemorrhagic	Neoplasia. Rupture of heart, aortic aneurysm, or pulmonary artery aneurysm.

C. *Peritoneal Cavity* *Type of Exudate*	*Possible Origin*
Serous	Cardiac failure. Cirrhosis of liver. Parasitism. Malnutrition (hypoproteinemia).
Serofibrinous	Enteritis, inflammation of organs.
Fibrinopurulent	Perforation, postoperative, extension of inflammation from neighboring organs.

Table 12-1 *(continued)*

C. Peritoneal Cavity *Type of Exudate*	*Possible Origin*
Hemorrhagic	Neoplasia in peritoneal cavity. Rupture of spleen, liver. Hemorrhagic infarct, strangulation of intestines (volvulus, torsion). Blood clotting disorders (hemorrhagic diatheses). Hemorrhagic pancreatitis.
Putrid	Perforation of intestinal tract. Infection following abdominal surgery.

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CHAPTER 13

DIAGNOSING ABORTION PROBLEMS

THE DIAGNOSTIC challenge of the aborted fetus is best described by one author who stated, "The aborted fetus is like the tip of an iceberg floating in a sea of diagnostic confusion." The confusion results from the fact that approximately 70% of examination of aborted bovine fetuses fail to yield an etiological diagnosis. This, combined with the fact that bovine abortion can be an emotional problem, often results in frustration on the part of both client and veterinarian. To ease these frustrations and achieve a higher degree of diagnostic efficiency, a routine procedure should be set and followed.

Before any examination is made, it is very important to obtain an adequate herd history from the client including specific questions such as diet, new additions to the herd, age of dam and previous history, vaccinations, environmental stresses, herd pregnancy rate, and any other previous abortions. If the cause of the abortion is from intrauterine infection, the herd history often reveals other sequelae that help comprise the rest of the "iceberg" of the abortion complex such as premature births, congenital defects, parturient deaths, stillbirths, birth of weak calves, and neonatal deaths.

Attempt to accurately assess the incidence of abortion in the herd. An incidence of 1-2% is considered normal and uneconomical to prevent, 2-3% is a borderline problem, and 3% plus indicates a definite problem that should be pursued. Remember that the causes of abortion are multiple and can result from infectious or noninfectious factors or their combinations. One important point to remember is that the abortion often occurs at some time after the original insult. The etiologic agent may have done its damage and left in its wake an autolyzed fetus. Noninfectious etiologies such as stress, iatrogenic factors and nutritional factors are the most difficult and remain a diagnostic challenge in abortion testing.

The importance of examining aborted fetuses, stillbirths, and early neonatal deaths should be emphasized to clients. By classifying them as to time-of-death and keeping accurate records on as many perinatal mortalities in an area as possible, practitioners can have a better understanding of the disease trends and patterns in their area.

Next, after the history and incidence have been assessed, the examination should be performed. The examination should consist of two parts: aborted fetus(es) and placenta(s). Both are equally important.

FETAL EXAMINATION

All too often the client brings you a dead calf that has been found and assumed to be aborted. Unless the calf is undoubtedly preterm gestational age, it is important to determine when the calf died: before birth (antepartum death), during birth (parturient death), or after birth (postpartum death). To be able to accomplish this the prosector must understand two concepts: (1) postmortem changes of body tissues; and (2) the normal physiological sequence of events before, during, and after birth. Many times the findings or lack of findings used to determine the time-of-death will lead you to the cause of death, especially if it is not a true abortion (antepartum) death. The time-of-death can be determined even from a relatively autolytic carcass.

Postmortem Changes Characteristic of Antepartum Death

The two major influences on postmortem changes is the death-to-necropsy time interval and temperature of and around the calf.

The postmortem changes characteristic of antepartum death (tissue autolysis) will depend on how long the fetus has been dead *in utero* before expulsion. All antepartum deaths lack signs of viability, i.e., umbilical artery clot, aeration of lungs, and localized edema. Therefore, the only thing that you have to assess is the degree of tissue autolysis.

In antepartum deaths, autolysis of the renal cortex starts within 30 minutes after death, and is marked from 24 hours. At this time the liver undergoes softening. At 12 hours after death, the red blood cells begin to hemolyze and hemoglobin begins to leak out of the vessels and stains body tissues. Generalized hemoglobin staining is complete by 30 to 36 hours after death *in utero*.

Fetuses dying more than 48 hours before expulsion will be in an advanced state of autolysis and will show varying degrees of generalized blood-tinged subcutaneous edema.

Fetal emphysema occurs when the cervix is open and putrefactive bacteria invade from the anterior vagina. Fetal maceration occurs following fetal death and autolysis in amniotic fluid and the uterus is invaded by putrefactive bacteria. The dam reabsorbs the fetal fluids resulting in dehydration of the fetus and its membranes. The fetus may dry out and shrink down around its bones, resulting ultimately in an unrecognizable mass of leathery tissue and bones called mummification.

If fetal death occurs before 90 days gestation and the fetus is not aborted, complete fetal resorption occurs as fetal bones have not yet ossified.

Postmortem Changes Characteristic of Parturient Death

Fetuses dying during parturition may be mistaken for abortion deaths. Parturient and antepartum deaths occur *in utero*. Autolysis is the most common finding and edema the second. Fetuses dying during parturition have gross signs of viability with absence of signs indicating survival after parturition. Antepartum death will lack both these signs.

Localized edema is the most important finding indicating viability. Meconium staining of the carcass indicates viability during parturition. A fetus that is stressed *in utero* from prolonged hypoxia during parturition will respond with vasoconstriction, hyperperistalisis of the intestines, and relaxation of the anal sphincter, thereby voiding meconium *in utero*. As hypoxia continues, the fetus attempts respiration, resulting in inhalation of uterine fluids. A decrease in oxygen tension, hypoxia to anoxia, produces petechial hemorrhages on the pleura, epicardium, endocardium, and thymus. Frequently, the lungs of a calf dying during parturition will be partially expanded and are often confused with those of calves dying immediately after birth. Calves dying during parturition lack the most important and first sign of survivability, a clot within the umbilical arteries.

Fetuses dying during parturition can be classified by balancing out the signs of viability with those of death and autolysis. The extent of localized edema is directly related to the length of time the calf lived during the birth process with a functional heart. Common sites for localized fetal edema are the head, tongue, submandibular space, fore or

hindlegs, and rear quarters. Edema in the fetus accumulates in tissues or organs protruding from the vagina during difficult birth because of venous constriction.

The degree of renal autolysis indicates the length of time the fetus was dead during birth. By balancing out the degree of renal autolysis with the degree of localized edema, one can accurately classify whether the fetus died early, in the middle, or towards the end of a moderate to long birth.

Gross Signs Characteristic of Postpartum Death

All postpartum deaths will have a clot in the umbilical arteries indicating that the calf survived birth with a functional heart. Fetal hypoxia (meconium staining), localized edema, and petechial hemorrhages may be observed.

Estimate the degree of lung aeration to assess if the calf effectively converted from hematogenous to aerogenous respiration. Open the heart and check for developmental defects and estimate the opening of the ductus arteriosus. If it is open (greater than 6 mm), in a calf 5-7 days old or older, it is considered significant. The reason is that a decrease in oxygen tension in blood (hypoxia) will stimulate the ductus to remain open, returning the calf to a form of fetal circulation and often causing death.

If the eponychium (horny tissue covering the bottom of the fetal claw) has been worn down or removed, the calf has stood and walked. Open the stomach and see if milk is present in the abomasum, indicating that the calf had nursed. If milk is not present, observe the abomasal contents. Examine the lacteals of the small intestinal mesentery for evidence of milk absorption. Meconium is normally voided within 24 hours after the calf has suckled. Observe the amount of fat present around the kidneys, pericardial sac, and on the heart and assess the degree of metabolism as none, moderate, or completely metabolized. Calves are born with enough brown fat energy stores to keep them going without appreciable maternal nutrition for 3 days in mild weather.

Fetal Lesions

Figure 13-1 summarizes the various causes of infectious bovine abortion and lists the diseases that produce specific or suggestive gross fetal lesions. The majority of fetal lesions are nonspecific and laboratory support is required for definitive diagnosis.

(Mycotic) – Specific				Specific – (EBA, Mycotic)
(Leptospirosis, – Suggestive Mycotic, EBA, IBR)	Placentitis	Abortion	Fetal lesions	Suggestive – (IBR, EBA, BVD, BT, Listeriosis,
(Majority) Non-Specific				Defects – (BVD, BT)
				Non-specific – (Majority)

Herd Problem

Epizootic	*Sporadic*	*Low Incidence*	
Brucellosis	Listeriosis	Vibriosis	
Leptospirosis	Salmonellosis	Trichomoniasis	
IBR	IBR	Mycotic	
BVD	BVD	Miscellaneous –	*Corynebacterium*
	FMD		Streptococci
	PI_3		Staphylococci
			E. coli
			Haemophilus
			Pasteurella

*Modified from Dennis, 1979

Figure 13-1. Summary of Infectious Abortion in Cattle*

There are only two abortions that produce specific gross fetal lesions in cattle; EBA and mycotic infections. Approximately one-third of fetuses aborted from mycotic infections will have focal patchy areas of mycotic dermatitis in which fungal agents can be demonstrated. Enzootic bovine abortion (EBA) produces a strikingly nodular liver in less than half the affected fetuses that are usually fresh and not autolytic. More commonly seen alone or in combination with the nodular liver is severe vasculitis, with diffuse hemorrhages throughout the body.

Infectious conditions that produce suggestive fetal lesions include IBR, listeriosis, BVD, and EBA. Infectious bovine rhinotracheitis (IBR) and listeriosis both produce scattered small necrotic foci in the liver. The necrotic foci may be visible microscopically only in IBR. IBR is also reported to produce necrotic foci in the spleen and kidney, whereas, listeria is reported to produce small abomasal erosions. A congested and mottled liver is suggestive of EBA, IBR, aflatoxicosis, and has also been found with BVD abortions. BVD is also reported to produce hypoplasia of the thymus.

Congenital defects such as hydrocephalus and others can be produced from infection *in utero* by BVD and blue tongue viruses, as well as genetic or other environmental factors. BVD infection is known to

produce cerebellar hypoplasia in fetuses infected before 150 days of gestation. Other gross lesions suggestive of infectious abortion include excess fluid in serous cavities, fibrin strands or sheets in serous cavities, intestinal hyperemia, mucoid intestinal contents, hyperemic and/or edematous mesenteric lymph nodes, and reticuloendothelial hyperplasia.

PLACENTAL EXAMINATION

The proper handling of the fetus is important, but is considered by many to be of secondary importance when compared to proper handling and submission of all or part of the placenta and a portion of the material caruncle. The placenta is often the most useful diagnostic specimen.

The only lesion common to all cases of infectious abortion is placentitis. Therefore, examination of fetal membranes often will determine whether the cause of abortion is infectious or noninfectious. Placentitis may be acute but tends to be chronic.

When evaluating fetal membranes, they should be stretched out over a flat surface and gently washed with water if necessary. Normal placental membranes are thin and translucent with the cotyledons a dull red. Note the size (uniform or varying), shape, color, consistency, signs of mottling (white-to-yellow or dark), and degree of necrosis or autolysis of the cotyledons. Most lesions begin around the periphery of cotyledons.

The intercotyledonary areas (ICA) should be inspected for thickness, consistency (edematous or leathery), any opacity, color, hyperemia, hemorrhage, necrosis, and degree of autolysis. Most infected placentas are not uniformly involved.

Placental Lesions

Mycotic infections produce a characteristic severe necrotizing placentitis. Cotyledons and caruncles may be found still attached in expelled fetal membranes, dramatizing the severity of the lesion. Secondary adventitious placentation is common and the ICA is leathery, with extensive superficial necrosis. This is the only placental lesion that tends to be specific for a causative agent in cattle.

Leptospirosis, IBR, and EBA produce placental lesions that are suggestive. They consist of cotyledons that are white to yellow to tan, atonic, avascular, and uniformally affected. The ICA is thickened and opaque with yellowish to brown gelatinous fluid.

Other nonspecific placental lesions that indicate infectious placentitis include hemorrhages around cotyledons and pericotyledonary areas of the ICA, thickening and opacity of the ICA, and areas of necrosis.

SPECIMENS TO BE SUBMITTED

After examining the fetus and fetal membranes, one should collect specimens, which may be needed to confirm or support a diagnosis. Specimens should include part of the chorioallantois, fetus, fetal blood or fluid sample, maternal blood sample, and sample of uterine discharge.

Fetal blood sample for serology is easily obtained from axillary blood vessels upon reflection of a foreleg. Some pressure on the thorax may be needed to force enough blood out. Fetal serology is usually neglected but is often a rewarding procedure, because a positive titer is diagnostic. Fetal tissues should be taken for laboratory procedures and histopathology. Table 1 lists the tissues required for diagnosing infectious abortion in cattle.

Table 13-1.

SPECIMENS REQUIRED
FOR DIAGNOSING INFECTIOUS BOVINE ABORTION

Frozen or Refrigerated	*10% Buffered Neutral Formalin*
Lung	Liver
Liver For FA and virus	Lung
Kidney isolation	Kidney
Spleen	Intestine
Cotyledons 1 or 2	Eyelid
Abomasal contents - 5 ml	Cotyledon 2 or 3
Fetal serum or body fluid	Strip of ICA
Dam's serum	Any other organ with lesions
Vaginal discharge	

If the fetus is too decomposed, brain contents or contents from the medulla of the long bones should be taken aseptically and submitted for bacterial culture. In cases where you do not wish to examine the fetus or placenta, it is best to send both directly to the diagnostic laboratory.

Many practitioners overlook investigating the serologic aspects of abortion. Maternal blood should always be collected and titered for

common causes of abortion. Acute and convalescent titers should be performed. These titers are compared to titers of unaffected animals in the same herd.

LABORATORY PROCEDURES

Many laboratory procedures used to arrive at or confirm a diagnosis can be carried out in a practitioner's laboratory. By performing them in your clinic you can better utilize your own technical staff and more importantly, you dramatically decrease the time it takes for results. Prevention of future abortions is dependent on a quick and accurate diagnosis.

Cotyledonary smears and smears of abomasal contents and uterine discharges should be stained by Gram and modified acid fast methods. Brucellae, rickettsiae, and chlamydia elementary bodies stain red with modified acid fast stain; other organisms such as *Campylobacter sp.* (cause of vibriosis) are stained blue by the counterstain. Gram-positive bacteria such as *Corynebacterium, Listeria, Bacillus, Streptococcus,* and *Staphylococcus spp.* are identified by Gram stain as are Gram-negative bacteria like *Campylobacter, Haemophilus* and *E. coli.* Darkfield or phase contract examinations of wet preparations of abomasal contents, pericardial fluid, and cotyledon smears can quickly be made to identify leptospiras, campylobacters, trichomonads, and mycotic mycelia or hyphae.

To confirm a diagnosis of mycotic placentitis, simply make a smear from an affected cotyledon on a microscope slide, add a few drops of 10% KOH, and allow the mixture to digest 5-10 minutes, add a cover slip, and examine with a microscope using low power and subdued light. If present, branching mycelia are usually easily seen, providing a positive diagnosis.

The sodium sulfite precipitation test for immunoglobin can be rapidly performed to gain some indication if the fetus was infected *in utero.* This is a semiquantitative test for total immunoglobulin concentration. It consists of preparing three test tubes with 1.9 ml of three different concentrations of sodium sulfite (Na_2SO_3); 14%, 16%, and 18% (w/v). To each tube add 0.1 ml of fetal serum, mix, and let stand for one hour. The key used to interpret the results is given in Table 13-2. Fetuses not antigenically stimulated *in utero* should have weak to no precipitation in the tube containing 18% Na_2SO_3. Fetuses that have been stimulated to produce antibodies *in utero* will have a mild to strong precipitation in the same tube. This evidence will justify further specific serological testing.

Table 13-2.

ESTIMATION OF
SERUM IMMUNOGLOBULIN CONTENT FROM NA_2SO_3 DATA

Concentration range of Ig (mg/ml)	*Sodium Sulfite Concentrations*		
	14%	*16%*	*18%*
less than 5	-	-	+
5 to 15	-	+	+
greater than 15	+	+	+

Finally, bacteriology can be done in the laboratory, by culturing fetal lungs, cotyledons, uterine fluid, abomasal contents, or any other organ with lesions.

More sophisticated laboratory procedures have been developed for most organisms but you should contact your diagnostic laboratory to find out what tests they offer and what specimens they require. Fluorescent antibody tests have been developed for IBR, BVD, PI_3, blue tongue, leptospirosis, listeriosis, and campylobacterioses. Serology is available for almost all infectious diseases that cause abortion and any positive titer from fetal serum is significant. Virus isolation is available; however, the chances for a positive isolation are directly related to the degree of fetal autolysis and the procedure is time-consuming and costly. Finally, enzyme-linked immunosorbent assay (ELISA) tests are gaining popularity because of their relative ease, speed, and sensitivity. They promise to play a major role in faster and more sensitive diagnosis of diseases and may be simple enough that they can be performed in practitioners' laboratories.

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CHAPTER 14

NECROPSY OF BIRDS

VETERINARY practitioners often become students again when they are involved in avian diagnoses. Pet bird (caged bird) necropsies usually are an outgrowth from clients bringing pet birds to a small animal practice, whereas necropsy of other avian species (chicken, pheasant, quail, chukar, etc.) usually result from a persistent client in need. Generally, reluctance to handle avian problems arises from a lack of understanding the *modus operandi* for avian species. The differences between chickens and dogs are not any greater than the differences between cats and horses. The diversity of veterinary medical training qualifies veterinarians to cope with significant differences between groups of animals. Veterinarians have the facilities and scientific background to diagnose and handle avian disease problems. There is little reason for necropsy of birds to be vastly different than necropsy of domestic or wildlife mammals. Becoming an avian diagnostician requires only an interest and initiative to pursue a course for acquiring knowledge. There are differences between birds and mammals, but overall they are more similar than different. Understanding husbandry practices is a prerequisite, since many problems originate from lack of accepted management procedures. Experience gained in avian diagnosis orients thinking in terms of syndromes, age groups, groups of diseases, organ systems affected, and seasonal diseases.

To evaluate tissue changes one must differentiate lesions from normal variation in tissues, and to recognize either, one must understand sequential alterations produced by postmortem decomposition and agonal changes. Components of inflammatory reactions in birds are somewhat different from those occurring in mammals. In avian species, inflammation is frequently more fibrinous than suppurative,

tends to form granulomas rather than abscesses, and heterophils, not neutrophils are involved. However, the basic process of cellular response is similar and differences in disease causes are easily appreciated.

Appropriate management (nutritional and environment requirements etc.) of pet birds is not totally known. The percentage of critical patients and mortality will be greater in companion-bird practice until management of individual species is understood.

Practitioners must realize that there are numerous conditions that can be diagnosed by necropsy alone, probably in less time than it takes to prepare a carcass for submission to a diagnostic laboratory. This might be the case where the caged bird has been a household pet and infectious disease would be unlikely. If the bird was recently imported or purchased from a dealer or breeder, it would be advisable to submit the cadaver to a diagnostic laboratory.

Diagnosing disease in aviaries and flocks by necropsy of several birds is made easier by the advantage of readily available material (birds) for laboratory procedures (not so in expensive cage or household pet birds) and the ease with which a necropsy can be carried out. Collection of adequate material for laboratory studies facilitates an accurate diagnosis.

COLLECTION, STORAGE, AND TRANSPORT OF SPECIMENS

Necropsies on autolyzed or frozen birds are usually unrewarding and unproductive for laboratory procedures. Dead birds preserve better when the plumage has been thoroughly soaked with water and detergent. That decreases the insulating capability of plumage and allows body heat to escape rapidly. If shipping is necessary, place the wetted bird in a plastic bag surrounded by another plastic bag or container containing ice that can be sealed, place the bag in a styrofoam box with adequate paper or filler material, and send to the diagnostic laboratory. Two major points to remember are to wet the plumage as soon after death as possible to cool the carcass, and to use the fastest means of transportation to the laboratory. Placing dry dead birds in a refrigerator is inadequate, because plumage insulation retains body heat and autolysis continues. *Never* freeze a bird or pack it in dry ice. The only exception would be if there is likely to be a long delay before necropsy can be carried out or if the bird is submitted for microbiological, especially virological, studies.

Shipping Live Birds — Diagnosing flock problems requires looking at more than one bird; six are preferred for flock representation. Specimens should be selected to include one healthy bird, three coming down with clinical signs typical of the flock problem, and two birds with advanced illness. Fresh dead birds are desirable for acute diseases of low prevalence. Select these birds so that an accurate picture of the flock problem is represented.

To ship birds, use a 30-dozen egg case with holes in it for ventilation adequate for the season of the year. In the summer, cut numerous holes, in winter only a few. The bottom of the egg case should be reinforced with three filler flats; these will absorb moisture associated with droppings. The egg case will accommodate six adult birds, three to a side. Secure with at least two strong rope ties to facilitate handling and prevent birds from escaping. Additionally, surrounding the case with filament tape will increase security. Birds in such a container may be shipped air freight, but bus companies won't accept them for transportation. Do not include feed and water when shipping or taking birds to the laboratory in person. The only reason for submitting feed is for toxicological studies. In this case, put the feed in a separate container and submit with the necessary forms completed. Containers must be properly identified at all times.

COLLECTION OF BLOOD SAMPLES

Blood specimens may be taken at necropsy (immediately after the bird is killed) via the heart or from femoral vessels after the leg is disarticulated. In diagnostic flock problems, certain serologic procedures may need to be repeated. Therefore, it may be desirable to repeat blood collections by holding birds for additional laboratory tests. In some situations, paired blood samples collected several days apart to determine a rising or falling antibody titer to a disease (e.g., Newcastle) may be advisable. Repeated blood samples may be taken from the jugular vein, basilar (wing) vein, or by heart puncture.

Jugular Vein — The right jugular vein is best for venipuncture in all birds including Passeriformes (finches), Columbiformes (pigeons, doves), Psittaciformes (parakeets), and Galliformes (chickens, turkeys). There is no left jugular vein in birds, only a left internal carotid artery. In most birds, the right jugular vein is situated subcutaneously in the right lateral cervical region, dorsolaterally adjacent to the esophagus or

slightly to the right of the dorsal median line. The right jugular vein can be seen through the skin by parting the feathers and exposing the skin surface in the cervical region. That is facilitated by wetting the skin and feathers before parting them.

Hold the bird lightly in the palm of the left hand (right-handed operator) with the back of the bird adjacent to the palm. The head of the bird is gently held between the index and middle fingers with pressure on the mandible and rotated laterally downwards. The thumb rests against the junction of the right cervical region with the thorax. Slight pressure by the thumb causes vein distension. Do *not* compress the trachea by improper positioning.

For venipuncture of small birds, use disposable 1 cc tuberculin syringes with 25 gauge, ⅝ inch needles. Do not use excess vacuum, since this can collapse the vessel wall. The volume of blood to be withdrawn should be predetermined (approximately 1 ml/100 g is maximum).

Saphenous Vein — Used when bleeding ducks with the point of collection near the hock joint.

Basilar (Wing) Vein — Venipuncture of the basilar vein is the simplest method for obtaining blood from turkeys, chickens, pigeons, and most fowl under field conditions. This is especially true if the bird is to be returned to the flock. Hold the bird in lateral recumbancy and remove feathers by plucking from underneath the wing in the carpal area. The vein will be seen lying in the depression between the biceps brachialis and triceps humeralis muscles. The vein is more easily seen if the skin is dampened with 70% alcohol or a colorless disinfectant. Extend the wings dorsally over the back and grip wings firmly in area of wing web with left hand. Then insert 19 or 20 gauge ¾″ needle opposite to the flow of blood. Another method is to extend the wing and insert the 19 or 20 gauge needle under the tendon (as it passes over the joint to anchor the needle) towards the point of bifurcation of the wing vein. Blood may also be collected puncturing the wing vein with a lancet or scalpel blade and letting blood drip into a vial. Because of the thin muscular wall of this vein, hematomas are a common sequel.

Cardiac Puncture — Before attempting to bleed live birds via cardiac puncture, it is advisable to practice on freshly killed birds to learn exactly where and at what angle to insert the needle: (1) thoracic inlet or ventral (anteroposteriorly), (2) lateral, and (3) anteromedial. It takes a little experience to become familiar with the anatomy.

1. With the anterior or thoracic inlet approach, the bird should be held on its back with its keel up. The crop and its contents are pressed to one side with the index finger as the needle is guided into the ventral angle of the inlet. After penetrating the inlet, the needle is directed horizontally and posteriorly along the midline until the heart is pierced, at the same time maintaining a slight negative pressure on the syringe handle after entry into the thoracic cavity.
2. In the lateral approach, form an imaginary vertical line at the anterior end at right angle with the keel, then palpate for heartbeats and insert the needle with a slight negative pressure on the syringe handle until blood enters the chamber. One may insert the needle straight in under the floating rib.
3. In the anteromedial approach, puncture of the heart is made between the sternum and metasternum (in a mature chicken) about an inch above and posterior to the point of the keel.

For a right-handed person, place the chicken on the edge of a table with its left side and left wing down the side of the table. Place the right wing down over left wing and lean against the wings and table. This leaves both hands free and keeps the bird secure. Locate the anterior end of the keel bone (cranial process of the sternal crest) and from this point go posteriorly for 1 inch and dorsally 1 inch (more or less, depending on size of the bird) and insert the needle in the notch between the lateral process and metasternum. Direct the needle (18 gauge 2-3 inch needle) at an angle of 45 degrees towards the left shoulder joint, putting a slight negative pressure on syringe.

The size and length of the needle required for heart and venipuncture will depend on the size of the bird: for young chicks and poults, a 1¼-inch, 20-gauge needle; for mature chickens, a 2-inch, 20-gauge needle; and mature turkeys may require larger needles.

In very small birds, a blood sample sufficient for making a blood smear can be obtained by trimming a claw a little short. One may lancet the comb to obtain blood for a smear. In dead birds, aspirate blood from the heart.

PREPARING BLOOD SAMPLES FOR SEROLOGICAL EXAMINATION

1. Collect blood in clean 2 cc or 10 cc tubes depending on amount of serum needed.
2. Do not fill the tube more than *one-half full.*

3. Cap or cover with tape—do *not* use corks.
4. Lay the tube on its side until the blood clots. An occasional sample may require a long time to clot, especially turkey blood. Adding a drop of tissue extract (20% embryo w/o head/legs—use SPF embryos) will hasten clotting.
5. Keep warm (85°-90°) for 2 to 4 hours (on side). This hastens syneresis. A fresh sample should never be refrigerated immediately after collection.
6. Refrigerate overnight to maximize serum separation.
7. Decant serum into separate tube for testing or submission to the laboratory.
8. Serum samples in poor condition (red or cloudy) are not satisfactory for serological testing.

The number of blood samples required depends upon the disease. For determining Newcastle or *Mycoplasma* status in large flocks (above 10,000), 20 samples are preferred. For other diseases, 4 to 6 samples may be sufficient. However, in small flocks, testing the entire flock may be advantageous.

LABORATORY PROCEDURES

Obtaining adequate and desirable tissue samples from dead birds to carry out microbiologic, serologic, histopathologic, or animal inoculation tests is imperative.

SEROLOGY

Serological monitoring is important for detecting the presence or absence of antibodies to infectious agents (bacteria and viruses) in poultry flocks and aviaries. Antibodies provide evidence of the immune status (vaccination program) or disease status (acute or chronic) of the flock. Serological testing is the most rapid and economical method for supporting disease diagnoses. Detection of serum antibodies in birds following a disease outbreak or vaccination gives reasonable assurance that a certain disease occurred or that the vaccination procedure had an effect. Another reason for serological testing is detection and quantitation of maternal antibody for determining timing of early vaccination procedures.

Serological procedures are either qualitative or quantitative. The agar gel precipitin (AGP) test is an example of a qualitative technique. There are several disease agents for which AGP test procedures are available. This type of test, however, only confirms the presence or absence of a particular antibody. Examples of quantitative serological tests include virus neutralization (VN) using eggs or cell culture, hemagglutination inhibition (HI), and ELISA (enzyme-linked immunosorbent assay). Virus neutralization is a good technique, but takes 3-7 days to obtain results, and bacterial contamination frequently is a problem when serum samples are not collected aseptically.

Bacterial and viral agents that hemagglutinate lend themselves to the HI test. This is an excellent procedure that can be performed quickly and economically. The problem is that all agents do not hemagglutinate.

ELISA has received considerable attention and techniques are being developed for almost every pathogen of animal and man, including a variety of poultry pathogens. ELISA is convenient, reliable, fast, highly sensitive, and results have compared favorably with other assay procedures such as virus neutralization. It appears to be the test of the future—the major reasons being the rapid turn-around time once a test is established, and the ability to incorporate computer-assisted analysis and data management as part of the actual test procedure. The computer may report results graphically in the form of a histogram or bar graph for easy interpretation. This holds great promise for those who depend heavily on serological testing as the basis for both disease diagnosis and vaccination monitoring. ELISA offers a single system instead of multiple or diverse systems such as HI for Newcastle disease, VN for bronchitis, or AGP for adenoviruses. The ELISA test requires relatively small amounts of reagents and serum or blood, which makes it ideally suited for profiling day-old chicks or pet birds. The test is specifically sensitive and can be performed in hours and maybe, in the future, in minutes, possibly while the pet or flock owner is still in the veterinarian's office. This would result in a specific diagnosis of disease and, if treatable, instant dispensing of the appropriate drugs.

A battery of tests can be run on the same serum sample for diseases and pathogens, such as *Mycoplasma gallisepticum* (MG), *Mycoplasma synovial* (MS), Newcastle, bronchitis, Gumboro, reoviruses, avian encephalomyelitis (AE), etc. A wide variety of serologic tests needs to be performed to obtain a complete picture of all disease-causing agents that may be confronting a flock manager. Serological evaluation of a disease problem combined with gross pathology can probably make individual diagnosis nearly 100% accurate.

Other diagnostic procedures such as virus isolation and histopathology are less economical to perform and time-consuming. Virus isolation is usually a slow, tedious process that often leads to negative results.

The following procedures during the course of necropsy may be helpful for checking for some commonly encountered diseases. They are not intended as definitive diagnostic methods and are not all inconclusive.

Wet Mount Smears — Wet mount smears or Giemsa stained smears of mucosal scrapings can be made for protozoal parasites and are very good for studying morphology. Examination of wet mount scrapings on slides of plaques or necrotic foci (digested by addition of 20% sodium or potassium hydroxide) under high power magnification can easily be done for the presence of mold hyphae. Darkfield or phase illumination of fresh bile wet mounts for the presence of campylobacters can be done.

Blood Smears — Blood samples are useful for estimating the degree of anemia by determining packed cell volume (PCV) and hemoglobin (Hb). Available plasma or serum can be utilized for analyses of serum protein and electrolytes or antibody determinations. Blood samples for PCV, Hb, and smear preparations should be collected in containers containing EDTA or heparin. Heparin containers are most suitable for blood electrolyte analysis. Organisms are markedly concentrated in the buffy coat following centrifugation. Make fresh blood smears and air-dry for staining by Giemsa's, Gram's, Wright's methods, etc.

Tissue Smears — In sinusitis, make thin smears of nasal or sinus exudate for staining by Giemsa's, Gram's, or methylene blue methods. Inoculate appropriate media for culture of suspected organisms.

Sear and incise the surface of an abscess and inoculate culture media with the extracted material, using a sterile inoculating loop or swab. Make smears from the abscess on clean glass slides (dilute with a drop of water if the material is too thick), air-dry and heat fix, and make Gram's, acid-fast, or any other stain as desired.

DIAGNOSTIC CYTOLOGY

Cytology is excellent for evaluating and interpretating gross lesions and may suggest alternate diagnostic procedures. This provides better disease definition with recommendation for a more specific therapeutic regimen. Frequently in cytology, the etiologic agent causing the lesion may be identified. Interpretation of a slide may direct efforts towards specific type of culturing (aerobic, anaerobic or fungal). Cytology does

not give information concerning cellular architecture or invasiveness of a lesion, its information is cellular. The results of cytological evaluation are immediate and are as accurate as the specimen taken. Reports on sampling preparations, general principles of interpretation and cytologic evaluation of specific organs are available in the literature.

METHODS OF EUTHANASIA

Frequently, sick birds from an aviary or flock are presented for diagnosis. This presents a problem as how to best euthanatize them, because no single method is satisfactory under all circumstances. Several options are available, and one has to be aware of the veterinarian's image and protocol standards encouraged by animal welfare groups.

Gas Cannister Method—Bird is confined in a container appropriate for its size. An overdose of a gaseous anesthetic is administered until the bird is dead. For small caged birds, a cottonball is saturated with ether or chloroform and placed in the container (bell jar) with the bird. For larger birds, a cylinder of CO_2 with a tube connecting the cylinder to the chamber is the method of choice. The larger chamber may be a small garbage can with the tube entrance into the lid. The container may accommodate one or several birds at one time. Gasing with halothane is more expensive, but more pleasant for bird and owner. The bird may be bled out by severing the femoral vessels, thus preventing blood spilling into the abdominal cavity when removing the organs.

Injection Method—The injection of a barbituate intracardically, intravenously (0.3 ml/kg) into the wing vein, intramuscularly, intraperitoneally, or orally is a rapid method of euthanasia. Minimum dosages should be used to prevent artifact changes in tissues, because they interfere when histopathologic studies are done. An injection of T-61 (0.05 ml/kg) can be used. Lay bird on its back, open skin, reflect legs, cut femoral vessels, and collect blood for serology in tube. Allow bird to bleed out without creating an artefact in the lungs. Sciatic nerve can be checked along with leg muscles before making cut for exsanguination. Injection of 10-25 cc air directly into the heart chamber or wing veins is considered fast and lethal. This is done with a syringe and a 1½-inch, 18-gauge needle inserted through the thoracic inlet into the heart. An air embolism created via intravenous administration of air would appear to be aesthetically undesirable in view of violent convulsions that may occur prior to death.

Disarticulating Cervical Vertebrae — This is a common, simple, and fast method for killing birds (chickens — all ages, turkeys — poults, game birds) of all ages. The legs are held in the left hand with the palm upward, the wings are extended so that the left hand can grasp the primary wing feathers and hold them adjacent to the legs, the head is grasped by the right hand and placed in the palm using the thumb as a fulcrum on the back of the neck close to the head and index and adjacent fingers on the lower side of the mandible as a lever. This forms a ring surrounding the head at the atlanto-cervical joint. The left arm is held close to the body and extended. Extend the right hand, and as the bird is pulled taut, by simultaneous pressure of the right and left arms, the right wrist is flexed quickly so that the skull is bent backward and the neck is dislocated. A sudden release of tension is noted by the operator. Holding both wings prevents flapping and stirring of dust and feathers. If done properly, the skin is not broken and the head is not torn off. A subcutaneous cuff hematoma will appear at the level of disarticulation because the blood vessels are separated. Blood may run down the neck into lungs causing a hemorrhagic artefact. Bleeding out through the femoral vessels may be done.

The cervical vertebral column of small birds can be crushed between the handles of a pair of large scissors. The neck of a young chick can also be broken easily by pressing it firmly against a sharp table edge or by pinching between thumb and index finger.

Decapitation — Sudden decapitation with sharp scissors is satisfactory for young specimens. This prevents artifacts of blood from occurring in brain parenchyma. The exposed neck may be placed into a test tube to collect blood for serologic tests.

Electrocution — Electricity has been used for many years in one form or another and a 110 volt system is adequate for euthanasia of animals. It is quick and effective of all types of domestic animals and birds. To reduce the element of danger to a minimum, a heavy, rubber-insulated cord is used. This cord is plugged into a shock-proof switch having a red pilot light as an indicator. When the red pilot light is on, it indicates that the current is on; hence neither the terminals nor the animal should be touched until after the current is switched off. It is best to attach the terminals to the vent and comb before plugging the cord into the outlet. With this system, the bird rarely struggles and thus does not stir up dust or regurgitate crop contents. There is less danger of agonal hemorrhages occurring or of blood spilling when tissue specimens are desired. Wear rubber gloves and boots and avoid stainless steel tables or other conductive material.

NECROPSY PRECAUTIONS

Wetting birds with detergent solution keeps dandruff and loose dry feathers from creating a nuisance during necropsy. Extra precautions are advisable if there is reason to suspect that the birds are infected with a disease transmissible to man, e.g., chlamydiosis. The carcass and necropsy table surface should be thoroughly wetted with a detergent and disinfectant if the bird is a member of the parrot or pigeon family. The bird should be dipped in a 5% solution of Lysol® as a safeguard against psittacosis.

After the plumage is wetted with detergent and/or disinfectant, the bird may be placed in the center of a partially open plastic garbage bag. When the examination is completed, all tissues are already in the bag and ready for convenient disposal. Paper may be used to cover working surfaces and when the necropsy is completed, the paper is wrapped around the carcass and used as a means of disposal. Wearing gloves is recommended for any necropsy technique; however in the prosection of birds, they are not always worn since forceps and scissors are used for cutting and probing. Nevertheless, it would be wise to wear rubber gloves at all times while performing necropsies, since human health hazards are not always recognized at necropsy.

NECROPSY EQUIPMENT

The equipment includes shears for cutting bones, enterotome scissors for incising gut, and a knife for cutting skin and muscle. A scalpel and forceps are desirable in dissecting small caged birds. A supply of sterile syringes, needles, vials (blood chemistries, serology, etc.), petri dishes for collecting samples (tissues for bacteriology and virology), swabs, tissue specimen jars (histopathology), and whirl-pack containers should be available for anticipated laboratory procedures.

NECROPSY PROCEDURE

The procedure is written to accommodate both caged birds and poultry. It is important that necropsies be done in a consistently thorough and precise manner. Practice the procedure on a few cull birds to establish confidence regarding normal size, color, location of organs,

and sequence in which they can be removed with a minimum of disorientation and contamination for culturing. Bacteriological examination is probably the most important single ancillary procedure utilized in avian species to support a diagnosis.

EXTERNAL EXAMINATION

If mortality is occurring, both live and dead birds typical of the disease problem should be submitted. Restraining a live bird for external examination is done by holding the bird so that the keel is cradled in the palm of the hand with the index finger passing between the legs and the thumb and second finger passing outside the legs. The other hand is used to examine external structures.

Prior to performing the necropsy, observe the cadaver for body weight, color, plumage, and dehydration. For live birds, observe behavior. Note the prominence of the sternum (keel bone) and fullness of the pectoral muscles. The curvature of the keel bone is an important indicator of skeletal normality. Palpate the crop for fullness and type of feed.

Observe the plumage for moult and presence of external parasites. Check for discharges from orifices of the head (eye, nose, or ear).

Observe the skin for thickness, swellings, tumor-like lesions, and excessive keratosis. Check the head and feet (shanks) for discoloration, thickness, and dehydration.

INTERNAL EXAMINATION

Lay the bird on its back and wet the plumage with disinfectant and/or detergent with the table wetted down. In small caged birds the wings and legs may be taped to the table or small tacks may be used to pin the legs and wing to a cork necropsy board to prevent movement while removing organs. The use of a dissecting microscope is beneficial in caged bird necropsies.

1. Incise the loose skin between the medial surface of each thigh and the abdomen. Each leg is then grasped firmly in the area of the femur near the coxofemoral joint and rotated forwards, downwards and outwards until the head of the femur is broken free of its acetabular attachment. The cadaver will lie on its back on the table. A blood sample may be collected from the torn femoral vessels in the

acetabular region. Extend the skin incision laterally over the medial aspect of each leg to expose the muscles and stifle joint. Break the tibiotarsus and culture bone marrow.

2. A transverse V-shaped skin incision is made across the posterior abdomen ventral to the keel and above the pubis to join the two previous medial thigh incisions. Reflect the cut edge of the skin anteriorly over the breast and extend over the thoracic inlet to the posterior border of the mandible, thus exposing the breast muscles, keel, crop, esophagus, and trachea for examination. Lobes of thymic tissue are located along each side of the neck and are present as pink-gray lobules of firm lymphoid tissue from the thoracic inlet anteriorly. The vagus nerves lie along each side of the neck and are easily identified because of their normal cross striations.

   Check the crop wall that is normally transparent and thin.
3. An incision is made in the pectoral muscles on each side of the keel at the junction of the sternal and vertebral ribs. The cranial end of each incision should intersect the thoracic inlet at the midpoint of the clavicle and coracoid.
4. With scissors, make a transverse incision through the posterior part of the abdominal muscles midway between keel and vent. On each side, continue the incision anteriorly through a point at the junction of the sternal and vertebral ribs and cut midpoint through the coracoid and clavicle bones. With thumb and index finger inserted at the keel, lift the ventral posterior abdominal wall and keel in one piece and observe the viscera and air sacs *in situ*. With the viscera visible, culturing (with a loop or sterile swab) of the air sacs and organs is performed before any further manipulation of the organs. Tissues or organs may be collected aseptically and prepared for shipment to the laboratory. Intestinal cultures are taken on opening the intestine. If the bird was just killed, a blood sample may be collected intracardically at this point.
5. Locate the spleen by lifting the posterior surface of the liver from the proventriculus-gizzard junction. The spleen can be removed by freeing the left margin of the gizzard and reflecting it to the bird's right side. Examine the abdominal viscera and observe the serosa for fibrinous deposits.
6. Examine the pancreas located within the duodenal loop. Examine the proventriculus and ventriculus (gizzard). In seed eating birds, the proventriculus and gizzard are distinct organs. In carnivores, the proventriculus is large, and the gizzard appears as a sac and not

as a viable muscular organ. Transect the esophagus at the anterior border of the proventriculus and reflect the entire gastrointestinal tract posteriorly to the cloaca. If the cloaca remains attached to the vent, it serves as an anchor when the intestine is opened with an enterotome. Cut intestine close to the mesenteric attachment so that the entire alimentary tract can be laid out and examined. The small intestine is of relatively uniform diameter throughout its length. About mid-point in the small intestine, a small diverticulum, called Meckle's diverticulum (remanant of the yolk sac), is present. The ceca (if present) are attached to the distal small intestine. The large intestine and rectum are short, with no gross division.

7. With an enterotome, begin with a longitudinal incision in the proventriculus, and continue through the ventriculus, small intestine, ceca, colon, and rectum to cloaca.

   In carnivorous birds, a cast or pellet of undigested fur, etc. may be present in the ventriculus or gizzard.

   The mucosal contents of the proximal duodenum normally have a thick creamy to fluid consistency and should *not* be mistaken for exudate. In dead birds, autolysis of intestinal mucosa resembles digestive contents with a serosanguinous creamy consistency.

   Open the ceca and note small nodular elevations of lymphoid tissue (cecal tonsils) at the junction of the neck of ceca and small intestine. Normal cecal mucosa is smooth with mucosal folds on its surface and nodules of lymphoid tissue submucosally. The large intestine and rectum should be examined.

   Pull large intestine posteriorly (toward prosector) and examine the bursa of Fabricus located on the dorsal aspect of the cloaca. The bursa will have undergone atrophy in most normal birds at sexual maturity (at about 20 weeks in chickens).

8. Remove and examine the liver and spleen. Note changes in color, size, shape, and consistency. The liver is normally dull red-brown, creamy-yellow if fatty, and pale yellow in young birds under 1 week of age if still metabolizing yolk. A greenish tint is present with overproduction of bile pigments or stasis because of hepatopathies, intoxications, or metabolic diseases. Increased friability is present in a fatty liver.

   The gall bladder, if present, is enlarged in birds that have not eaten recently.

   The spleen in gallinaceous, raptor, and psittacine birds has a spherical shape, and in passeriformes (canaries), an enlongate cigar shape.

9. Examine the genitalia. In the immature or inactive ovary, only a small triangular shaped organ at the anterior extremity of the left kidney is present. A nodular surface indicates developing immature follicles. With increased ovarian activity, a mass of spherical globules appear in various sizes. Mature ova are large, yellow, and contain yellow yolk material, whereas immature ova are small and pale cream-gray. Raptorial birds, e.g., hawks and falcons, have two functional ovaries and oviducts in contrast to only a functional left ovary and oviduct in other birds.

    Open the left oviduct longitudinally. The remnants of the right oviduct may become fluid-filled and cystic.

    The testes are located along the midline adjacent to the anterior pole of the kidney. Presence of melanin is normal in many gallinaceous and passerine birds. In spermatogenesis, the color changes to gray or white.

    The adrenal glands, yellow and triangular, are located at the anterior pole of the kidney.
10. Examine the ureters and kidneys *in situ*. The kidneys are brown, elongated, and trilobuled, extending from behind the lungs to the cloaca. They are contained in a shallow body cavitation of the vertebral column and pelvic bones and project slightly into the abdominal cavity. Ureters are medially located near the midline of vertebral column. Precipitates of uric acid may be present within the ureters and kidneys.
11. Examine the pericardial sac for its transparency and quantity of fluid present. Excess opaque fluid should be aspirated and cultured. The size, shape, and color of the heart should be noted. Open the heart and follow the large vessels. In starvation, epicardial fat undergoes serous atrophy. Note any paleness, streaking, or nodules within the myocardium.
12. Examine the brachial plexuses and sciatic nerves. Normal peripheral nerves are glistening white, rather uniform in diameter and have a transverse striated pattern that is easily observed. The brachial plexus is located anterior to the first rib near the vertebral column. The extrapelvic sciatic nerve is exposed by careful separation of the adductor muscles by blunt dissection. The intrapelvic portion is exposed by removal of the overlying portion of the kidneys by blunt dissection.
13. Bone strength can be determined by placing the index and middle fingers under the bone and using the bone as a fulcrum. Place

pressure on the upper surface of the bone with the thumb and attempt to break it. The way the bone snaps or bends with pressure reveals information about the skeletal structure. The radius, ulna, tibia, or tarsometatarsus bones are the most useful for this technique.

With an osteotome, split one femur longitudinally and examine the bone marrow. In most adult birds, the marrow cavity contains adipose tissue. If marrow is active, it will consist of dark red-brown, gelatinous tissue.

Examine the epiphyseal growth plates at the junction between the head and body of the ribs. In immature birds, a sharp knife is adequate to shave the bone until the growth plate is observed. A saw may be required to expose the growth plate in adults.

The hock joint should be examined for abnormal shape and structure from misplacement of the tendons associated with various twisted leg syndromes.

14. Now examine the head, and with scissors cut through one lateral commissure of the mouth and examine the oral cavity. Check for plaques, necrotic ulcers and hemorrhage, and exudate.
15. Make a longitudinal incision in the esophagus and crop to where proventriculus was removed. Observe for mucus plaques, necrotic exudate, hemorrhages, and odor.
16. Make a longitudinal incision in the larynx, trachea, syrinx, and bronchi and extend into the lungs. Observe for exudates, mucus, parasites, and hemorrhage.
17. Examine the lungs by bluntly reflecting them with scissors medially from the rib cage. In the lungs observe for nodules and discoloration. Note curvature or flattening of the rib cage. Note amount of mineralization when the rib cage is cut by scissors.
18. Reflect the skin of the neck laterally (from initial skin incision) and examine the paired vagus nerves for normal striations. Checking the head by pulling the skin on the neck over the top of the head. The cranium is exposed and examined for signs of trauma.
19. With heavy scissors, remove the upper beak by a transverse cut near the eyes. This will allow inspection of the nasal cavities and expose the open anterior end of the infraorbital sinuses for signs of exudates.
20. Insert one blade of the scissors into the infraorbital sinus. Make a longitudinal lateral incision through the wall of each sinus and examine them. Culture the sinuses if indicated.

21. To examine the brain, disarticulate the head at the atlantoccipital junction and remove the lower mandible. Hold the head between the thumb and forefinger of one hand and make a transverse incision behind the eyes. Remove the calvarium by placing the tip of the scissors in the occipital foramen lateral to the brain stem, cut through the bone to the cranial cavity, cut around the base of the brain laterally and anteriorly to the transverse cut. The incision should pass just above the eye cavities. Lift the top of the calvarium to expose the brain parenchyma. By cutting the cranial nerves anterior to posterior, "peel" out the brain from the cranium and examine.

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CHAPTER 15

MANAGEMENT AND COMMON GROSS PATHOLOGICAL FINDINGS OF DIAGNOSTIC SIGNIFICANCE IN BIRDS

CLINICAL EXAMINATION

OBSERVE the bird for body weight, color, plumage, dehydration, and evaluate the bird's attitude by observing whether it is active, alert, or dull, droopy and listless. Abnormal movement of the head or mouth suggests a respiratory disease. Listen for rattling and gurgling sounds by holding the bird's head close to your ear.

Botulism causes a flaccid neck paralysis in chickens and ducks and bilateral leg paralysis in pheasants. Newcastle disease and thiamine deficiency may cause a star gazing or twisted neck, whereas encephalomalacia (vitamin E deficiency) may cause various other nervous signs. Marek's disease causes leg, neck, or wing paralysis. Rickets may cause birds to squat and walk with a stilted gait and have curvature of the keel bone. Tremors of the head, neck and sometimes body of a chicken that is unsteady and falling over are associated with avian encephalomyelitis.

In layer birds with a precipitous egg drop approaching zero in a few days may be considered pathognomonic for Newcastle disease. With infectious bronchitis, an outbreak of rales rapidly going through the flock with a heavy to moderate drop in production unaccompanied by mortality is usually observed.

MANAGEMENT FACTORS IN COMMERCIAL FLOCKS IN HEALTH AND DISEASE

Learn the normal profile of birds along with obtaining a good history. The more information available about history and environment, the

better the chance for a solution to the problem. Histories are sometimes biased because they include only situations, events, and signs that the caretaker, owner, service worker, or neighbor has observed and remembered, or care to report. Good avian producers know the feed and water consumption and egg production (if layers) at all times, but more important, they observe normal sounds and actions of the flock. When any of these are abnormal, interpret them as signs of abnormal health and evaluate noninfectious conditions *first.* Many so-called disease problems referred to laboratories for diagnosis are noninfectious, management problems such as beak trimming errors; consumption of litter and trash; feed and water deprivation; chilling or overheating of young birds; injury from rough handling, automatic equipment, or drug injection; electrical failures; cannibalism; smothering; overcrowding; poor arrangement of feeders, waterers, and ventilators; cheap low-quality feed ingredients; ingredients causing feed refusal; rodent and predator attacks; and improper vaccination procedures. Other management factors such as feed formulation and body weight; lighting program; routine medications and vaccinations; age; previous history of disease; farm location; and unusual weather or farm events and duration of signs, number sick, death loss, posture of carcass, and when and where they were found dead are important clues. This requires a systematic approach to be sure nothing is overlooked. The completeness of evaluation of management factors may make the difference between a correct diagnosis, no diagnosis, or a wrong diagnosis of the flock problem.

MANAGEMENT FACTORS IN SMALL AVIAN FLOCKS IN HEALTH AND DISEASE

The number of small farm flocks for home egg and meat production are increasing. Owners of small farm flock will depend upon their veterinary practitioner for help in managing health and disease in their flocks. Usually these owners have limited knowledge of poultry science. There has always been a substantial fancier's interest in maintaining so-called exotic breeds of chickens, pigeons (fancy and racing), and fighting cocks. Gamebird (pheasants, quail, and chukers) production is increasing and the industry is seeking more veterinary help.

The value of birds will range from a few cents to several hundred dollars. Sentimental feeling may or may not be a significant factor in the bird's value. Many management procedures are not at the state-of-art

level with what is generally practiced in the commercial avian industry. Interestingly, the health management of a small flock differs little from that of a large one; it just needs to be scaled down. Many times rations or treatments talked about at club meetings do not necessarily meet the health requirements of birds. By the time veterinary services are sought, the birds may be afflicted with a disease complex.

The best source of baby chicks or pullets for small flock owners seeking meat-type chickens or layers is a supplier for large commercial operations. Usually these birds are vaccinated against Marek's disease and are free of pullorum-typhoid, mycoplasma, and other infectious agents. Breeders of fancy exotic breeds should embark on systemic disease prevention programs, especially with show birds in pullorum-typhoid free flocks certified by the official state testing agency. It is necessary to have a clean flock before transporting birds interstate. Mycoplasmosis, *M. gallisepticum* and *M. synovae,* is a disease acquired through showing. It may be impossible to maintain mycoplasma-free flocks; therefore, a vaccination program may need to be implemented.

The purchaser of small chicks should have houses and equipment thoroughly clean and disinfected before orders are placed. Brooders should function properly so that overheating and chilling do not occur. Omphalitis is usually associated with poor sanitation and occurs within three to five days after birth. Starvation (starveouts) occur in five to seven days and may be a sequel to overheating, chilling, navel-ill, inadequate lighting, or improper feeding and watering arrangements. Starting young chickens on a slick surface rather than on recommended conventional litter frequently causes leg problems.

Chickens that fail to grow and appear to be doing poorly when they are six to eight weeks of age should be evaluated for characteristics of the breed and proper management. Light breeds (egg laying type) usually weigh 0.40 to 0.60 kg and heavy breeds (broiler type) 1.25 to 2.0 kg at six to eight weeks of age. Feed sold to small or backyard flock owners may be of marginal quality from being in storage for several months especially through hot summer months. Feed becomes rancid as fat soluble vitamins break down and the nutritional value is diminished. Inadvertently, layer feed may be fed to growing chicks or good quality feed is purchased but mixed with scratch grain that dilutes the nutritional value of the ration. These types of rations usually are the basis of many leg problems in meat-type or broiler chickens that prevent the birds from obtaining adequate food intake and further compounds the problem. Growing birds need a well-fortified ration of 22-24% protein

to achieve the necessary growth rate and diminish leg problems. A 20% protein chick starter can be mixed 50/50 with turkey or game bird starter to obtain a 22-24% protein ration. If this option is not available, a protein supplement such as fish meal may be used to fortify lower protein starter rations. Treatment with a water soluble vitamin-trace mineral mixture in the drinking water may avert leg problems if administered at the first signs of trouble. Abnormal moulting may occur in growing chickens, if the ration is inadequate in protein and vitamins. Young chickens go through a normal molting sequence prior to maturity.

In young birds, coccidiosis should be suspected if birds are showing general unthriftiness, lack of fleshing and anemia or have loose, watery droppings. When these general clinical signs appear and chickens are floor-reared, there is a good possibility that both coccidiosis and other intestinal parasites may be present. Confirmation by laboratory diagnosis is necessary. Most commercial feeds contain an anticoccidial drug to assist in preventing coccidiosis.

In small farm flocks that have quit laying, there are several points need to be considered. Hens need 14-15 hours of light stimulation to maintain egg production and most small farm flocks are not under artificial lights when the days are short. Day length starts decreasing from June 22 and continues until December 21. The ration may be inadequate because they are on a grain ration (because of cost) or a laying ration is fed, but birds have unlimited access to scratch grain and they fill up on scratch before they feed on the laying mash. Both situations contribute to a nutritionally inadequate ration. Diminished egg production will occur when birds are without feed and water. A partial molt (head and neck) may occur a few days later from this stress. At the end of a 10-12 month laying period, there is a natural molt and cessation of egg production.

Cannibalism occurs in small farm flock layers and begins with vent prolapse with subsequent pecking. This occurs in obese layers that are more prone to prolapse and predominantly on a grain diet high in energy but low in protein. Other causes of cannibalism include crowding, pelleted feed, disposition of chickens, etc.

In laying chickens from small flocks, there are numerous inquiries on external egg quality. Thin shelled eggs are frequently associated with hot weather, hens laying for eight months or longer, deficiencies in calcium, phosphorus or vitamin D. Abnormally shaped eggs may be due to Newcastle virus, bronchitis, or uterus abnormality from an earlier viral infection.

Vaccination programs should be developed in small farm flocks. Marek's vaccination must be administered to one-day-old chickens. Vaccination on the premises is recommended against Newcastle disease, infectious bronchitis and fowl pox. Laryngotracheitis vaccination is done only if the disease is prevalent in the area. If birds are shown at various shows, fowl pox and infectious laryngotracheitis vaccination should be done. Many disease outbreaks originate from the show circuit. The fancier and shower of birds should always be alerted to proper vaccination programs.

Veterinarians should avoid or handle with tact and caution nutritional deficiencies in chickens on commercial feed. Any type of spraying may cause adverse effects on birds (either meat or layers) and this problem may best be directed to a toxicological laboratory. Diseases that may be transmitted to chicks through egg or hatchery-related diseases (omphalitis, avian encephalomyelitis, etc.) should be evaluated completely, with laboratory assistance for a complete workup of the problem.

COMMON GROSS PATHOLOGY FINDINGS OF DIAGNOSTIC SIGNIFICANCE IN BIRDS

Poultry

Skin and Feathers

Examine the feathers and skin and check for lice, mites, trauma, sloughing (necrosis), thickening of skin or feather follicles (xanthomatosis, Marek's) and abnormal feather loss (moulting). The state of nutrition or hydration is noted by checking the keel bone (curvature is noted in Ca/P imbalance) and prominence that may be associated with debilitating disease, malabsorption, or lack of food intake.

In examination of the vent, white masses of lice eggs (mites) may be observed attached to the base of the feathers. A hyperemic mass protruding from the vent indicates prolapse of the oviduct commonly seen in pullets and old fat hens. This leads to cannibalism, partial evisceration and death. In chicks (usually under 1 week of age), a large mass of fecal material pasted over the vent is most likely due to adverse environment (chilling or overheating) or bacterial infection (colibacillosis, paracolon, or salmonellosis).

Feet and Legs

A thickening and fibrosis or hemorrhage of the tendon above the hock may indicate rupture of the gastrocnemius tendon. A soft fluctuating swelling in the tendon sheath may be the result of a tendosynovitis associated with bacterial diseases such as fowl cholera, pullorum disease, or infectious synovitis. Perosis is caused by a widening and flattening of the tarsal condyle with a medial displacement of the gastrocnemius tendon. This is associated with manganese and choline deficiency. Scaly leg mite causes a scaly roughened condition of the legs. Bumblefoot is a large abscess affecting the foot pad. Swelling of toes and joints with visible chalky deposits is gout. Crooked toes will result from birds being on slippery surfaces and curly-toe paralysis is observed with riboflavin deficiency. Plantar necrosis of feet is seen in birds that have been on floors cleaned with phenol compounds or those with buildup of litter on feet. Joints have a gray, mucoid exudate in infectious synovitis; it is more yellow in fowl cholera.

Head, Beak, Eyes, and Nasal Sinuses

In evaluating the head, examine the eyes for a roughened, ulcerated, corneal surface from ammonia burn. Birds with this condition have photophobia and will stand quietly with eyelids closed. Blind birds will stand with eyelids open. An irregular pupil or depigmentation of the iris (infiltration of lymphocytes) will cause pupillary constriction with Marek's disease. A blue-gray opacity of the lens (cataract) is a sequel of epidemic tremor. The presence of a cheesy exudate beneath eyelids may be due to vitamin A deficiency or aspergillosis. A turbidity of intraocular fluids (intraocular exudate) may be associated with aspergillosis, pasteurellosis (fowl cholera), salmonellosis, colibacillosis, and paracolon infection. Intraocular hemorrhage are found in hemorrhagic disease. Encrustations on the eyelids and dermatitis of the foot are observed in pantothenic acid deficiency. Ocular lacrimation, conjunctivitis, and swelling of the eyelids may be caused by fowl pox or laryngotracheitis. Facial swelling, nasal discharge, and fluid or exudate accumulation causes eyelids to stick together in coryza, chronic respiratory disease, fowl cholera, or aspergillosis. Scabs on wattles are due to fowl pox or trauma; swellings are caused by injury, chronic fowl cholera, or xanthomatosis; and a dry white powdery skin is due to favus mycosis. Swollen combs with black tips are caused by freezing and swellings or nodules are associated with Marek's disease. Cyanosis of the comb (darkened comb) is seen in systemic diseases with impairment of circulation. Scab formation at the commissure of the mouth indicates a dietary deficiency.

Oral Cavity, Esophagus and Crop

Cheesy exudates in the palatine cleft are present in fowl cholera and vitamin A deficiency. Pustular lesions present in the esophagus confirm vitamin A deficiency. Bilateral focal necrotic foci of the upper mouth are found in T_2-mycotoxicosis (*Fusarium* sp.). A crop lining containing soft curd-like material on the surface is indicative of *Candida albicans* infection. A thickened, almost hyperplastic crop wall with gray mucoid exudate is identified with *Capillaria* parasitism. Yellow necrotic masses in the pharynx, esophagus, and crop suggest trichomoniasis, which must be confirmed by microscopic examination. Necrotic foci in heart and liver may be a sequela to trichomoniasis. Diphtheritic patches in the oral region of the pharynx are associated with wet fowl pox.

Trachea, Lungs and Air Sacs

Laryngotracheitis produces a cheesy or hemorrhagic plug in the larynx and trachea and a yellow pseudomembrane in the trachea in chronic cases. Infectious bronchitis and Newcastle disease cause congestion and accumulation of mucus in the trachea. A yellow cast occluding the lower trachea (synrix) and bronchi is a frequent sequela to infectious bronchitis.

Diphtheritic mass occluding the larynx as a caseous core is found in wet fowl pox. Plaque-like lesions in trachea lining may be associated with eye and air sac lesions of aspergillosis. Gapeworms in trachea are red and surrounded by mucus.

Button-ulcer plaques and/or greenish mold on air sacs and mesentery indicate aspergillosis.

Pericardium and Heart

Fibrinous pericarditis is a sequal to chronic respiratory disease. Yellow nodular lesions are a common sequela to pullorum disease.

Alimentary Tract

Diffuse white foci in the gizzard muscle are seen in vitamin E deficiency and yellow nodular foci in pullorum disease. Nodular thickening with ulceration of the mucosa in the proventriculus is present in Marek's disease. Foreign bodies may cause erosions and perforations.

Nodules on the serosal surface of the intestine indicate coligranuloma (Hjarre's disease), tuberculosis, leukosis, or possible mucosal attachments of tapeworms. Scolices of flattened segmented tapeworms are

attached to the mucosa by suckers or hooks. Pinpoint hemorrhages, white foci, transverse white streaks, and ballooning of the intestine with flecks of blood in mucus indicates intestinal coccidiosis. Whitish transverse streaks in the duodenal mucosa and visible by serosal examination indicate *Eimera acervulina* infection. Blood in the ceca indicates cecal coccidiosis or hemorrhagic disease. Linear streaks, focal white foci, or yellow ulcers may indicate ulcerative enteritis. These ulcers may perforate the intestinal wall, causing peritonitis.

Yellow caseous cores in ceca may be associated with histomoniasis (blackhead) or coccidiosis, or in young birds, salmonella or paracolon infections. The presence of a small nematode in the ceca indicates *Heterakis sp.* Thickened white plaques are found in rectal mucosa in young birds with pullorum, typhoid, or paratyphoid infections. In turkeys, a distended intestine, darkened and filled with bloody material suggests hemorrhagic enteritis.

Liver and Spleen

Fatty liver syndrome in caged layers is characterized by a pale to yellowish friable and sometimes ruptured liver with abdominal blood clots. Tuberculosis and coligranulomas are characterized by yellow caseous nodules that "shell" out easily. Enlarged livers with necrotic foci are associated with *Salmonella,* paracolon, *Pasteurella,* and vibrionic infections. Additionally, a bronze sheen is observed in fowl typhoid. Usually, larger yellow necrotic foci are present with ulcerative enteritis. Large circumscribed necrotic areas with a concave center and peripheral concentric rings are found in blackhead with an accompanying typhylitis. Leukosis (including Marek's disease) is associated with a nodular or diffuse enlargement by the infiltration of lymphoid cells. Spleen, kidney, and liver are usually similarily involved.

In the spleen, hemorrhages, necrotic foci, and tumors may be found. Splenic enlargement may be caused by bacterial diseases such as pullorum, typhoid, ulcerative enteritis, and lymphomatosis.

Reproductive Tract

Angular, shrunken, discolored ova are indicative of pullorum; a gray, cauliflower appearance suggests leukosis; coagulated or "cooked ova" are sequela to egg yolk peritonitis often following fowl cholera; and a collapsed balloon appearance of yolk follicles indicates an abrupt or sudden departure from normal metabolism, frequently associated with acute stress such as dehydration or acute respiratory disease.

In the oviduct, impactions, tumor, or cysts may be present. The right oviduct (nonfunctional) remains vestigal but may become enlarged and cystic.

Kidneys

Lesions of the kidneys are frequently not observed because kidneys conform to the sacral depression and are easily overlooked. The color of normal kidneys is reddish-brown. Severe nephritis may be associated with gout (visceral and arthritic), dehydration, vitamin A deficiency, coccidiosis (goose), or poisonings. Beneath the kidneys lie the sciatic plexuses with nerve trunks in which abnormal nerves (loss of striation, yellow and swollen) indicate Marek's disease.

Brain and Peripheral Nerves

Encephalomalacia, a lesion of vitamin E deficiency, causes hemorrhage and edema of the cerebellum with flattening of the convolutions. With time, a yellow discoloration will be observed. Localized fowl cholera is indicated by yellow exudate in the ear canal and surrounding skull bones.

Cervical nerves, brachial plexuses and vagosympathetic trunks in the neck should be checked for enlargements, loss of striations, and whiteness that indicate Marek's.

Caged Birds

Skin

Fowl Pox—Characterized by dry, focal, scab-covered, wart-like lesions on unfeathered areas about the head (especially around eyelids), wings, and sometimes, the vent.

Trauma—Lacerations, wounds and scabs caused by fighting or hyperexcitability may be observed. Traumatic wounds, frequently seen on the back of the head, are caused by aggressive cagemates. Frequently, chronic skin lacerations on the back of the head expose the underlying skull, because the lacerated skin contracts with maturing fibrosis.

Traumatic lesions frequently occur on the carpal areas in birds housed in cages that are too small. Trauma to the cere and beak may occur as a result of birds flying into mirrors or glass windows.

Neoplasia and/or tumor-like lesions—Feather cysts, sometimes found in budgerigars and Norwich canaries, are nodules formed by cysts filled

with feather-like material. Feather cysts can occur anywhere on the body but are more frequent on the wings, a condition known as *hypopteronosis cystica.* Feather cysts are frequently seen in Macaws.

Subcutaneous nodules over boney structures may be lesions of polyhyperostosis.

White to yellow subcutaneous nodules on the legs and around joints are found in gout.

Lipomas, fibromas, and their malignant counterparts are common neoplasms in budgerigars. Lipomas commonly occur on the sternum of budgerigars where they are frequently traumatized and tend to ulcerate. In the ventrolateral aspect of the neck at the thoracic inlet are compatible with hyperplastic goiter or the less common thyroglossal duct cyst.

Skin abscesses — Relatively rare except when associated with skin lipomas in budgerigars. Not frequently associated with joints.

Cnemidocoptes pilae — Associated with proliferative crusts on legs, feet, and face with chronic dermatitis around the vent in budgerigars and parakeets.

Subcutaneous emphysema — May result from rupture of air sacs or from fracture of communicating pneumatic bones. Localized subcutaneous emphysema around the dorsum of the head and neck may be due to involvement of the cervicocephalic air sac system.

French moult — Loss of pigmentation and flight feathers that are ragged and have a worn appearance. The feather shafts are easily bent, and many tail and wing feathers will have fallen out. Occurs most commonly in fledgling budgerigars at about the time they leave the nest. Also, consider psittacine papovavirus infection, which causes abnormal feather development.

Feet and Legs

Bumblefoot — Trauma and/or irritation of the ball of the foot with invasion of bacteria (usually *Staphylococcus aureus*) into subcutaneous tissues resulting in cellulitis. Synovitis and arthritis are frequent sequela with either local abscess formation or extension dorsally to cause cellulitis of the tarsometatarsal region and osteomyelitis with some cases terminating in septicemia. Improper perches, claws caught in cracks of wire netting, use of sandpaper on cage floors or perches, and disinfectant or chemical residues initiate the problem.

In scaly leg syndrome, the skin of the tarsometatarsal region and foot is covered with thickened hyperplastic scaly epidermal debris. It is found

especially in budgerigars and canaries with *Cnemidocoptes* sp. mites. Tassel foot is a name given to thick masses of hyperplastic epidermis on the feet and digits of small passerine birds such as canaries and finches with *Cnemidocoptes* sp. infestations.

Pox virus infections may be characterized by focal nodule formation of the skin of feet and lower legs and in some passerines, proliferative lesions can appear similar to neoplasia.

Tight leg bands or other entangled objects (rubber bands, string, etc.) will cause edema and possibly gangrenous necrosis by obstructing the blood supply.

Articular gout is evident from chalky material within the distended joints and synovial sheaths.

Arthritis and swollen wing joints are frequently indicative of salmonellosis. The synovial fluid is clear at first, and later becomes grayish and purulent.

Dermatitis on the plantar surface of the toes may be indicative of hypovitaminosis B.

Beak, Eyes and Nasal Sinuses

Deformed Beak — Associated with cnemidocoptic mite infestation at the base of the beak, resulting in an overgrowth of the upper beak. Improper wear or a beak composed of soft, crumbly, poorly formed keratin is found in sulphur-crested cockatoos. Psittacine beak and feather disease is characterized by loss of feathers, abnormally shaped feathers and overgrowth and irregularity of the surface of the beak. The disease occurs in a number of psittacine species including sulphur-crested cockatoos, lovebirds, budgerigars, and galahs.

Congenital malformations are associated with abnormalities of bone growth in the skull. Local neoplasia may cause abnormal bone development.

Cere of budgerigars may show abnormal pigmentation especially brown hypertrophy.

Conjunctival redness and/or watery secretions indicates conjunctivitis from viral, chlamydial, mycoplasmal, or bacterial infections.

Blood in the anterior chamber of the eye can be associated with trauma or hemorrhagic disease.

Panophthalmitis septicemic diseases (e.g., salmonellosis) have fibrin or purulent exudate in the anterior chamber of the eye.

Protrusion of the eye may be due to intraorbital space occupying lesions of hemorrhage (trauma) or neoplasia (lymphosarcoma or extension of brain tumor).

Erosions and crusts of epithelium around the eyes and mouth may be associated with pox.

Accumulation of serous, mucoid, or caseous exudate with excessive congestion of the mucosal linings of the nasal and infraorbital cavities (sinusitis) may be a sequel to mycoplasma, haemophilus, chlamydiosis, or other bacterial infections in psittacine birds. Sinusitis and blepharitis may also be a feature of pox in canaries. In canary pox, secondary viremia may result in typical skin lesions that may be well developed and involve the mouth and entrance to the larynx.

Oral Cavity, Esophagus and Crop

Avian pox virus infections result in a diphtheritic membrane tightly adherent to the mucosa, with a hemorrhagic ulcerated submucosa upon removal. These yellowish-brown plaques may be found on the hard palate, under the tongue, and infrequently on the epiglottis.

Hypovitaminosis A may be observed as a whiteness of the epithelium of the choanal cleft and pharynx in parrots.

Mycotic infections of the crop are seen when the crop mucosa is excessively convoluted, roughened, and thickened, with whitish, flocculent, mucus, cheesy exudate loosely adherent to the epithelium. Candidiasis, trichomoniasis, and capillariasis all may produce this lesion.

Trachea, Lungs, and Air Sacs

Ammonia vapors or excessive dust will cause the mucosa to be excessively moist, glistening with accumulations of serous and/or mucous exudate.

Hemorrhages or excessive congestion of the upper trachea are present in birds killed by "breaking the neck."

Tracheal mites will cause seromucoid tracheitis, seen as small brownish masses with a hand lens.

Mycosis is evident as small focal plaques near the syrinx.

Air sacs—Presence of froth, mucus, or fibrin on membranes along with caseous material suggests different stages of air sac inflammation probably from mycoplasmal, bacterial, chlamydiae, or fungal infections. A black to green, velvety appearance suggests aspergillosis.

Helminths, parasites, and mites can be present or embedded in air sacs with little or no significant inflammatory reaction present.

Birds that have drowned, died from botulism or were given barbiturates intraperitoneally may have excess fluid present in the air sacs. Barbiturates may precipitate on or in air sac membranes with focal deposits of white mineralized plaques as artifacts.

Pericardium and Heart

Distended pericardial sac with blood because of cardiac tamponade can result from cardiac blood sampling; if clear pericardial fluid suggests heart failure. A fibrinous fluid in pericardium is associated with chlamydiosis.

Septicemic diseases (e.g., colibacillosis) result in a fibrinous pericarditis.

Visceral gout is seen as a dull, gray-white, crystalline film of urates over the pericardial surface.

Intracardiac injection with barbiturates may result in deposits of white, mineralized plaques on the pericardial surfaces.

One or both ventricles may be dilated or have undergone myocardial hypertrophy in chronic cardiac failure.

Arteriosclerotic or atherosclerotic lesions may occur in the wall of the aorta, especially in older parrots.

Alimentary Tract

Ballooning, flaccidity, dilation, and obstruction indicate localized alterations in smooth muscle tone, resulting from inflammation.

Thin layer of granular to dull, gray-white film deposits on the serosal surface is observed in visceral gout.

Focal to diffuse white spots, bars, and/or associated petechiae in small intestine represent lesions in the intestinal wall visible through the serosal surface and suggest ulcerative enteritis or coccidiosis.

Small focal nodules suggesting granuloma formation in the intestinal wall may be the result of colibacillosis, tuberculosis, or certain nematodes and cestodes projecting their heads deeply into the intestinal wall.

Excess accumulations of mucoid exudate along with flecks of hemorrhage and flaccidity or dilation are primarily associated with coccidial infections or capillariasis.

Mucosal congestion of the duodenum is frequently present in bacterial diseases such as salmonellosis, pasteurellosis and also may be seen in viral diseases.

Tapeworms and intestinal flukes include numerous species and are the commonest helminths of insectivorous birds. *Dilepis undula,* a tapeworm, is an important contributory cause of death in hand reared nestlings of the thrush family.

Diffuse congestion and hemorrhage may occur in the proventriculus by Newcastle disease.

Splanchnic neuropathy (proventricular dilatation syndrome) is present in psittacine birds with an extremely dilated proventriculus that becomes thin-walled. Most often seen in newly acquired macaws that have been released from quarantine within two months. Seeds are retained in proventriculus, with passage of undigested seeds in droppings. Pectoral muscle atrophy with little or no body fat and the ventriculus may or may not exhibit dilation with an occasional flaccid crop.

Liver and Spleen

Pericapsular coating with a fibrinous film is seen in septicemic diseases and congestive heart failure.

Visceral gout results in a dull, gray film of precipitated uric acid being deposited perihepatically.

Chlamydiosis has a foamy, fibrinous, perihepatic exudate.

Pacheco virus disease (parrots) and *Salmonella,* and *Pasteurella* infections have pinpoint, multifocal yellow necrotic foci in the liver.

Size and Color—Pale liver because of fatty infiltration is common in obese budgerigars. In other debilitated birds and birds affected with aflatoxin, a fatty liver may be present. Shrunken, fibrosed, deformed liver because of congestive heart failure or chronic hepatitis is commonly observed in parrots and budgerigars.

Pale yellow liver is common in young birds (less than 1 week of age) because of metabolizing of yolk.

Psittacosis is characterized by a swollen, congested liver with rounded edges of the lobes in psittacines; if the spleen is enlarged, it is more definitive. Congested and enlarged spleens may be observed in septicemias and bacteremias, but psittacosis should *always* be suspected.

Neoplasia occurs as focal nodules that are soft and gray in the spleen and liver.

A pale, enlarged liver with a somewhat waxy, cut surface is suggestive of amyloidosis.

Spleen is congested and enlarged in septicemic diseases such as chlamydiosis. In leukosis, it is enlarged and contains white-gray infiltrations or nodular accumulations of lymphoid tissue.

Peritoneum

Fibrinous peritonitis is associated with septicemia or extension of localized inflammation because of perforated ulcers (ulcerative enteritis) of the small intestine or gizzard. Egg material in abdominal cavity from rupturing ova because of stress associated from disease or management may be observed in the mesentery of peritoneum as a small nodule of brown inspissated material implanted or free in the abdominal cavity.

Reproductive Tract

Ovaries — Degeneration, rupture, or involution of follicles may result from stress associated with acute environmental or nutritional changes or systemic infectious diseases.

Follicular rupture because of *Salmonella pullorum* results in yolk peritonitis.

Remnants of the right oviduct may remain and become filled with fluid resembling a large cyst.

Impaction of the oviduct is frequently seen in cage birds and associated with an ascending infection or segmental hypoplasia resulting in internal laying.

Ovarian and oviductal neoplasms may result in implants of neoplastic tissue on the serosa of organs and on the peritoneum.

Kidneys

Intoxications, metabolic disorders, and some viral infections may cause discoloration, swelling of parenchyma (becoming pale), and bulging out of the body cavitation into abdomen. Swollen convoluted tubules with white precipitates of uric acid are found in the kidney or in the anterior portion of a dilated ureter.

Visceral gout may or may not involve the kidney.

Large white polycystic nephroblastomas are common primary tumors of the kidneys in caged birds.

Renal adenocarcinomas are relatively common in budgerigars. In budgerigars, the tumor may invade the intermuscular and subcutaneous areas of the lumbar vertebral column. Most renal adenocarcinomas project into the abdominal cavity.

Nephritis and nephrosis are characterized by yellow, hemorrhagic, swollen, white streaks, possibly urates.

Brain and Peripheral Nerves

Trauma of the head may result in hemorrhage in cranial bones.

Meningitis is usually characterized by diffuse congestion of the brain surface and a fibrinopurulent exudate.

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CHAPTER 16

DISPOSAL OF DEAD ANIMALS AND BIRDS

DISPOSAL of dead animals is the responsibility of owners. Animals must be disposed of in an acceptable manner usually within 24 hours after death. In some states, a violation of this statute is a misdemeanor. Three important factors to keep in mind in disposing of dead animals: (1) disease should not be spread; (2) a public nuisance should not be created; and (3) toxic chemicals should be kept out of the food chain.

Placing of dead animals in streams or near public roads is considered to be a public nuisance. In disposing of large animals, the size of the cadaver usually discourages digging a hole for burial. The use of chemicals or burning is discouraged because of cost. There are many approved methods of disposal including: (1) by a licensed disposal plant with trucks usually dispatched to the farm to pick up the dead animals; (2) by burying with all parts of the animal covered by at least four feet of earth; (3) by burning, usually outside city limits if there is no violation of local county ordinances; and (4) any other method approved by the state veterinarian.

HEALTH ASPECTS OF DISPOSAL

There is danger in spreading disease from dead animals to man or to other domestic and wild animals. More than 150 infectious diseases are naturally transmitted between vertebrate animals and man. Chances of transmitting an infectious agent vary with different types of disease. Uncertainty as to cause of death often leads to careless handling of cadavers. The safest course is to dispose of dead animals by one of the methods approved by state laws, which vary for each state. In disposing of dead animals, it is best to treat the animal as though it died of an infectious agent.

If animals and birds feed on dead animals, they may contract disease or they may carry and disseminate infectious agents over a wide area. Wild animals eating dead infected animals may carry viruses in fecal droppings for two weeks or more. Research work on transmissible gastroenteritis of pigs (TGE) has proven that the virus will live in the intestine and droppings of starlings for 17 hours. Diseases that can spread by the fecal route include blackleg, anthrax, hog cholera, TGE, salmonellosis, etc. The carriers might be dogs, foxes, coyotes, opposums, racoons, buzzards and other animals and birds eating dead flesh. Flies feeding on or developed from eggs in dead carcass are capable of transmitting infectious agents.

Water drainage from dead animals or from the area they were allowed to decompose may be responsible for the spread of infectious agents. Outbreaks of leptospirosis, hog cholera, etc., have occurred on farms downstream from infected premises.

Veterinarians are frequently called to necropsy dead animals. Responsibility of putting examined organs back in the abdominal and thoracic cavities and suturing the skin together (where knife slits are made and then wire or twine used to pull the skin together) rests with the veterinarian.

Animals dying in the summer cause less problems associated with contamination because rapid deterioration destroys infectious agents. Whereas, dead animals that lay around in winter are eaten by predators over a period of a week or two, so infectious agents that survive well in cool temperatures may be spread over the area. It is not wise to leave dead animals laying out in back pastures or timber. This is especially true now that we know more about diseases such as sarcocystis. We overlook many cases of animals that do poorly because of a heavy infection of sarcocystis. Some undiagnosed bovine and ovine abortion cases may also be related to this problem.

The health aspects of disposal in general, transporting dead animals, disposal at rendering plants, by burning, by burial or in a disposal pit, and in sanitary land fills will be discussed. These are all options open for disposal of dead animals, keeping in mind that we must allow veterinarians access to do a necropsy on many of these animals so that diagnoses may be made for proper treatment of in-contact animals and for disease surveillance.

DISPOSAL OF DEAD ANIMALS

In the next few years, disposing of dead animals will become more difficult. The reason for this appears to be that fewer and fewer land

fill areas, especially in metropolitan areas, are available for such use. Ordinances in many municipalities also forbid the use of land fills for disposal of dead animals. This is due to the frequent presence of rodents and insects in land fills that can carry disease to surrounding residential areas. Rendering plants can be used, but because of public opinion, this is not always feasible. They are, however, the main source of disposal of large animals in many areas of the country. In recent years, the air pollution situation has made it uneconomical for local animal hospitals to remodel or build state certified crematories. To meet such regulations, the costs are estimated to vary from $15,000 to $40,000. For individual veterinary practitioners, the cost is prohibitive for building a facility to use only for disposal of dead animals and waste.

Sanitary Land Fills

Sanitary land fills provide a method for final disposal of solid wastes on land, whereby the solid waste is not burned, but covered daily with soil. Local city or county ordinances may determine whether or not such land fills may be used for disposing of dead animals. There are several chemicals that will speed breakdown of body tissue and act as disinfectants against infectious agents. There is no one disinfectant effective against all pathogens. Chemicals may not be practical to use on large animals because of the quantity required and cost.

Burning

Burning animals is difficult and in most cases is limited to baby pigs, young chicks, and poults. It is too time-consuming and costly to burn large animals, because the carcasses must be completely burned to white ash. Burning cadavers, whether in an incinerator or open pit, creates obnoxious odors. This should be considered before investing in an incinerator, especially if you live near a residential area. Commercial incinerators may be used. These incinerators use oil, natural gas, or L.P. gas; they vary in quality, and must meet heavy duty specifications and be capable of withstanding high temperatures. Before incinerators are installed, ordinances must be checked so as to avoid legal difficulties. With anticipated fuel costs, the cost of operating an incinerator prohibits its installation and use by veterinary clinics and hospitals.

Burning dead animals on brush piles using old tires, etc. is not recommended. This method will generally produce a charred, half consumed carcass that attracts dogs or wild animals and is a potential source for spreading disease.

Rendering Plant

The preferred place for disposal of dead animals is at a rendering or disposal plant. Such plants are properly equipped to handle dead animals in an efficient and safe manner. These plants are usually licensed and supervised by the state veterinarian. Unfortunately, rendering plants are not located in strategically desired places for livestock producers to dispose of animals and for this reason, other methods may be necessary such as burning, burial, disposal pit, and land fills.

Burial or Disposal Pit

Livestock producers may be faced with disposal of dead animals on their own land, when there are no local disposal plant or sanitary land fills available. A suitable burial site, proper materials, and necessary equipment are important. All dead animals should be properly disposed of so that wild animals will not be able to spread disease from the cadavers. Select a site to which dead animals may be moved with the equipment available on the ranch. Transporting on public roads or across property under the control of others will generally be subject to restrictions (See Statutes on Transportation of Dead Animals in your state). The site should be some distance away from neighboring buildings, public roadways, and property boundries, where it is less likely to be objectionable to people who are using adjacent property. This will usually pose no problem in larger, ranch-type operations.

The topography should be such that the water table will remain below the deepest part of the planned excavation. The site should have good surface and natural drainage that should be away from any excavation or able to be diverted away. Avoid location up-slope from water sources—wells, springs, or streams.

After considering the above points, decide whether to bury or dispose of animals in a pit. Factors pertaining to burial include: availability of equipment, excavations on short notice to complete burials soon after death, large drained or undrained excavations being prepared in advance of needs so that availability of excavating equipment is not

critical, and covering dead animals in either drained or undrained excavations, which requires that suitable equipment is available without delay. Waiting for hired units or detaching or attaching difficult-to-change, tractor-mounted equipment may delay burial beyond the time allowed by law. The entire carcass should be buried at least 4 feet below the surface and covered with 4 feet of earth. Even though surface water is diverted away, carcasses should be covered to avoid water ponding in the excavation. Undrained excavations must be long and trenchlike and the dead animals placed at the high end and covered so as not to impound water.

A backhoe may be used to excavate a rectangular-shaped pit with vertical side walls. The pit may be excavated larger at the top to slope the walls and reduce the tendency for cave-ins. The depth of the pit may be determined by maximum depth the back hole can dig, caving tendency of earth walls, ground water level, and pit capacity. It is not necessary to require a pit drain; however, it is more important to keep water out of the pit than provide the drain. Pits in earth that readily cave in may be walled with concrete blocks or pressured treated wood. A pit cover may be made of concrete, pressure treated wood, steel, or other building materials. The cover should be water tight to prevent rain from entering the pit, restrict access of flies and predators to the pit, confine most of the odor, provide convenient access for dropping dead animals into the pit, and be constructed so it may be skidded from old to new pits.

In disposing of dead animals, the public is more aware today because of the disposal methods of certain toxic chemical wastes in the past. State and federal laws are changing and becoming more restrictive on how dead animals may be disposed of associated with infectious or chemical products in their bodies. The principal person who will be asked to render opinion will in all probability be the veterinarian, who with the Public Health office, will be making decisions on proper animal disposal. This means veterinarians (maybe the local practitioner) must be aware of state and federal statutes regarding animal disposal. Rendering companies (in some states) called to pick up animals are now required to get a diagnosis by a veterinarian as to cause of death if more than one animal is to be picked up at one time. The whole idea is to keep poisons, such as lead or biphenols, out of the food chain. In the future, this may present a real dilemma, if an animal should die from ingesting a toxic chemical and could not be sent to a rendering plant. It may not be able to incinerate the animal because certain products may be released into the atmosphere and burial may or may not be acceptable because of certain ordinances.

LIVESTOCK WASTE DISPOSAL REGULATIONS — THE VETERINARIAN'S ROLE

In time, it can be expected that all states will have regulatory programs dealing with livestock waste disposal. Cooperation with governments in their animal waste control programs will be necessary. When regulations are administered judiciously and by people with a background and understanding of the problems of livestock production, compliance should be satisfactory and resentment kept to a low level.

In the excitement created by proposals to control disposition of animal waste through regulations, the existence of the common law theory of nuisance is sometimes forgotten. In view of the increased importance of the common law nuisance theory as applied to livestock operations and the imminence of further federal and state regulations affecting livestock producers, it is incumbent on veterinarians to think in terms of the zoonotic and environmental impact of operations with which they are involved, and to advise their clients along lines that will enable them to stay out of trouble with their neighbors and government agencies and to stay in business. Large animal practitioners' livelihood depends on their client staying in business.

In case after case, livestock operations, particularly those involving swine and poultry, have been brought to a halt through the issuance of injunctions. Odor, noise, and water pollution have been major factors influencing the courts, and dust, unsightliness, and the harboring of pests have also had some weight.

With the growth of cities and concentration of residences in subdivisions and sometimes in areas several miles from the city, the defense that "I was here first" has less and less application. This is sometimes called the "coming to" theory, the gist being that if you choose to come to my area and are made uncomfortable by the operation I am carrying on, it is your own fault. This theory still has some potency when there is only one or possibly even a small number of plaintiffs. The courts simply refuse to use it when large numbers of people are involved and the development of needed residential areas is impeded.

It is important to be cognizant of agenda items on meetings of Pollution Control Boards when hearings are held on proposed animal waste regulations. The need for regulations should be recognized, but when promulgated, the rules should be reasonable so as not to cause shifts in livestock producing communities to communities that have less rigid requirements. Veterinarians in mixed or large animal practices should be particularly concerned.

INCINERATORS

Incinerators are a method by which veterinarians may dispose of animals that have been necropsied. A well planned incinerator room can be maintained at the same high level of cleanliness as any other area in the hospital complex. The coordination of efforts by the manufacturer, veterinarian, and architect can make an incinerator a creditable and useful adjunct piece of laboratory equipment.

Determination of an incinerator design depends on the physical and chemical properties of the material to be destroyed. Waste from veterinary pathological processes may consist of whole or partial cadavers of animals, organs, dressings, and volumes of cage cleanings (if the hospital wastes are to be disposed of in this manner).

Animal refuse is composed of water, fat, proteins, and bones. When the cadaver is exposed to heat, a definite sequence in the destructive process takes place. Hair and fur flash off and external charring begins immediately. As the temperature of the cadaver increases, it reaches a point where the fat is rendered soluble and runs freely from the cadaver. At incinerating temperature, liquid fat burns rapidly. In many cases, the burning of the fat and the elevated temperature of the furnace cause fatty material to vaporize faster than the volume of the furnace will permit combustion. For this reason, a separate combustion chamber at an elevated temperature following the primary ignition chamber is utilized. In the second chamber, time and space are provided for complete oxidation before waste gases reach the chimmey. While the fat is rendering, dehydration is progressing, since carcasses are high in moisture content. Dehydration consumes a major portion of the energy cost involved in operating an incinerator. Tissues char locally while this goes on, somewhat protected by the release of steam and the large quantities of latent heat absorbed in this process. When dehydration is completed, the charred residue, essentially carbon, burns rapidly, much in the manner of charcoal. Bones at high temperature lose the organic binders that give form and structure to their basic calcareous composition. Upon slight disturbances, the bones disperse into powder.

Temperatures vary from 1500 to 1800°F and it is a general rule, that all gases leaving the process must have been heated to at least 1350°F. At this temperature, all organic substance causing smoke or odor are dissociated and combine readily with oxygen, if it is present, forming water and carbon dioxide principally, with smaller amounts of oxidized products of nitrogen and sulfur.

While the above statements are limited to incineration, individual veterinarians will be faced with a choice of burial, collection by local services, or taking the cadavers to the nearest municipal incinerator. All of these methods depend on the local ordinances and the desire of the veterinarian to carry out his own disposal procedures. Under many conditions, a strong point in favor of incineration is that it makes available a definite and immediate method of destruction. One limiting factor in incineration is the cost incurred in building a heavy duty unit that can incinerate the wide range of materials usually is present in an animal hospital operation. Regardless of the type of incinerator, it must be remembered that the basic process of pathological combustion described above is unalterable. That is, fats still render and burn, dehydration must be completed, and odors are destroyed only by temperatures in excess of 1350°F in effluent gases.

BIRD CARCASS DISPOSAL

Carcasses of birds that die from disease agents remain a source of infection for penmates and other poultry on the same or other farms. Sick birds with discharges from body openings should be euthanatized and removed from the premises. Some birds may be taken to a reference laboratory and utilized for diagnostic purposes; others can be disposed of by one of several methods to prevent disseminating disease. The logistics of disposal is usually not a serious problem for aviaries, game bird farms, small farm flocks and most commercial (egg layers or broilers) operations. In the event of a major disaster (e.g., velogenic Newcastle disease or enzootic avian influenza), total depopulation of an area is usually coordinated by a government agency. In any size operation, whether losses are severe or normal expected mortalities, all carcasses should be disposed of by one of the following methods to prevent disease dissemination.

Incineration or Burning

This is the surest way of destroying infectious material. There are many smokeless, odorless incinerators available commercially for disposal of carcasses. They are expensive to buy and costly to operate. Be sure to purchase one or build one that meets city or county code specifications and does not create undesirable air pollution. Many small animal hospitals have incineration available and this is an excellent way to dispose of cage birds.

Burial

Severe losses create a serious disposal problem. A deep hole may be dug and the carcasses buried so that predators or dogs cannot get to them. Using a backhoe, dig a deep narrow trench, where each day's collection can be deposited and covered until the trench is filled.

Pit or Tank

For small losses and normal attrition, a decomposition pit can be used. In building it, precautions should be taken that it is located where it will not contaminate drinking water supplies, where the roof or walls will not cave in, and where predators or dogs cannot dig into it or flies or other insects can gain entry. The pit cover should be sealed with tar paper or plastic and be strong enough to hold a soil overlay of at least 12 inches.

An electrically-heated septic tank for disposal of dead birds and waste products from large poultry farms and processing plants has been developed by the Agricultural Research Service, USDA, in cooperation with the Maine and Connecticut Agricultural Experiment Stations. The carcasses and/or waste products are digested in the heated septic tank. Heat (37.8°C) and mesophilic bacteria digest all but bones within two weeks. Neutralizing the mass at intervals with lime and adding hot water further accelerate the rate of decomposition.

Rendering

Freshly dead poultry, like livestock, can be rendered into fertilizer or other products. Commercial or contract haulers of dead carcasses should have the cans steamed and sterilized before leaving them on farms for collection of the dead bird carcasses. Take precautionary measures so that the haulers do not introduce disease from another premises.

In summary, veterinarians may be brought into making recommendations for animal or bird disposal, whether by burial, incineration, or by local rendering services. Be sure to check local and state codes before making recommendations.

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CHAPTER 17

MALPRACTICE IN VETERINARY MEDICINE

MALPRACTICE constitutes a real threat to members of all healing professions. Doctors of medicine were the first and still are the primary targets. This litigious plague has spread to involve veterinary medicine, particularly practitioners. Veterinarians would be wise to recognize this problem and its potential significance and to act for their own protection. There is no malpractice Santa Claus; the malpractice problem will be solved only by your own affirmative professional and purposeful efforts.

The law requires that every person act with due or ordinary care toward his client. Failure to do so constitutes negligence. Ordinary care means the degree of care commonly possessed and exercised by an ordinary prudent person in the conduct of his own affairs. Each professional must meet accepted standards in carrying out their professional activities. The standard is always what the ordinary, reputable licensed practitioner, in the same field of practice, in the same or similar locality, would do and what he would refrain from doing and caring for in a similar case. The failure of a practitioner to meet accepted standards of practice, with injury resulting to his patient, constitutes professional negligence; this is malpractice.

Whenever a veterinary practitioner undertakes to render professional services, the law burdens him with certain obligations. The practitioner must possess the degree of skill and knowledge commonly possessed by other reputable licensed practitioners in the locality; he must exercise the degree of care, attention, diligence, and judgment commonly exercised by other reputable practitioners in the profession in the care of similar cases. Accepted methods of treatment must be

utilized, not wandering into fields of experimentation, and he must have been authorized to administer the care and treatment. These obligations constitute the legal responsibilities of the veterinary practitioner.

No greater degree of skill and care is required of a practitioner in diagnosis than that in treatment of disease. No inference of negligence arises from the mere failure to make a correct diagnosis. It must be further established that failure to make a diagnosis was due to failure on the part of the practitioner to possess or to exercise the required degree of skill or care or diligence.

The use of a consultant provides a great degree of protection to the veterinary practitioner. Malpractice suits are rarely lost where it is shown that an independent consultant was in contact with the case during what is established to have been the critical period. It should be repeated again, that in the final analysis, actionable negligence of malpractice consists of the practitioner doing something he should not do or omitting something he should do, measured against what the standard, reputable practitioner would do or would refrain from doing in the care of a similar case. Whether a practitioner is to receive a fee or is to render his service gratuitously in no way affects his legal responsibilities. Specifically, in *Varney vs. Peecome* (Nebraska) the court said that a veterinarian, in the absence of a special contract, engages to use such reasonable skill, diligence, and attention as may be ordinarily expected of persons of that profession; and that he does not undertake to use the highest degree of skill or an extraordinary amount of diligence.

A practitioner is not an insurer of results unless he makes himself one by a special agreement or undertaking. It is unwise to promise too much or to guarantee a cure for any particular case. In *Lyford vs. Martin* (Minnesota), a veterinarian was sued on the allegation that he had agreed to effect a cure.

By a special contract, a practitioner may limit or he may extend his legal obligations. He may agree to treat a case only for a particular condition, or at a particular place, or for a limited time. On the other hand, it has been pointed out that he may broaden his obligation and his liability by specially contracting to effect a certain result. In the latter event, if he fails to produce the warranted result, he becomes liable on his contract and, in defense, it would be immaterial that he had exercised the highest degree of skill and care.

Most malpractice claims brought against members of a healing profession are not honestly justifiable. Moreover, the targets of these actions are not exclusively or generally charlatans or quacks. On the contrary,

more than half of the defendants are found to be, in their respected fields of practice, above average in experience, reputation, and ability. This is understandable, because the practitioner who has most to lose will likely be the easiest to shake down. He is the one most likely to be hurt by publicity, who is ready to buy his peace, and to make a settlement in order to conclude the matter quickly.

It is clear that no practitioner can be justly accused of malpractice if he cares for his patient with meticulous attention according to the requirements of good veterinary medicine. However, no course of conduct on his part can safeguard him from an unjustifiable malpractice claim or suit. Whenever there is completion of professional services rendered with a less than perfect end result, a potential malpractice claim exists. The poor result plus unfounded suggestion that the treatment administered was a failure, equals another malpractice claim. There may be little that can be done to remove one of the factors such as poor results from the equation of malpractice, but it should be possible to eliminate the factor of criticism of reasonable skill, diligence, and care.

A very important potential for malpractice claims is that of criticism. Criticizing a colleague is almost invariably the first step in the thinking that is necessary for bringing about a malpractice suit. More than 75% of all malpractice cases are precipitated by criticism by one practitioner of the work or the result obtained by another practitioner. To criticize the work or the result obtained by another practitioner until and unless we are in possession of *all* the facts of the particular case is unethical.

Consent for surgery or for other treatment must always be obtained. The legal owner is the usual source of proper consent for work on an animal. It is recommended that a written consent for euthanasia always be obtained, especially with pets and companion animals.

The statute of limitations provides a time after which specified actions cannot be brought. Most legal actions must be brought within one year after the alleged neglient act or omission occurred. The statute provides a longer time during which actions on contracts such as a suit to collect a professional bill may be brought. It should be born in mind, therefore, that in some instances, waiting a certain time is advisable before suing for a bill.

A practitioner is liable for his own acts and for the acts of a partner or an employee, in so far as such acts occur within the scope of the partnership or of employment. Care should be exercised in selection of assistants and the delegation of duties to them.

Statements should not be made to owners or to anyone else that may be interpreted as an acknowledgement of fault on your part of the services rendered. No claim or threat of claim should be discussed with anyone until legal advise has been taken; and advise should be immediately obtained when a threatening situation presents itself.

Ordinary malpractice suits must be established in court by means of expert testimony. Lay witnesses may testify concerning things that were actually done, but as to the propriety of those things, the expert is the only legimate witness. What constitutes good practice, either in diagnosis or in treatment, is a question for expert witnesses and can be determined only by their testimony. As to matters that are within common knowledge and understanding of laymen, no expert testimony is necessary or required.

In a typical malpractice case, veterinarians serving as expert witnesses may be on opposite sides in the law suit. Obviously, they generally disagree in their expressed opinions. Otherwise, there may have been no suit in the first place. Something must have been found by the plaintiff to condemn. The expert on both sides are expected to deal with the issues fairly and honestly. Nothing should be asked of the expert for the defense except truthful and unbiased testimony. If one (as an expert witness) is to be a benefit to the defendant, one must create the impression of impartiality, which one should strive to possess. The expert witness called to testify in a malpractice suit generally has no first hand knowledge of the facts of the case. He is usually called upon to express an opinion in reply to hypothetical questions.

The vast majority of malpractice suits can be avoided by scrupulous attention to the requirements of good practice, by good record keeping, and by an equally scrupulous concern for the reputation of fellow practitioners. This offers a remedy to the shocking and viscous malpractice situation that has crept into veterinary practitioners lives. All possible safeguards and precautions must be employed and must be put into effect against unjust malpractice accusations.

In today's society, litigation involving virtually anything is becoming more and more common. The veterinary profession is no exception, and with the advent of special interest organizations such as animal rights groups, human-companion animal bond groups, environmental groups, and other similar organizations, there will be an increase in the frequency of litigation related to problems in veterinary medicine. Hence, veterinarians, regardless of which facet of the profession they are involved in, should acquaint themselves with the general types of cases

that potentially may involve litigation. Means of identifying such problem areas should be considered, and procedural policies should be implemented to preclude the embarrassment of public replay of incomplete records, diagnostic aids, and quick judgements. Secondly, such an approach offers the foundation of simply conducting good medicine and laboratory practices for which every veterinarian, regardless of specialty, should strive.

It is vital for veterinarians to remember that each time a client is accepted, legal obligations as a professional come into play. If at any time, the client believes that he has suffered a loss because of incompetence, the legal system provides a vehicle for him to present his dispute to an impartial factfinder and, assuming he meets his burden of proof, he may recover significant sums to compensate him for his loss.

Statutory theories of liability include acts involving medical practice, communicable diseases, and use of narcotic substances. As our society becomes more bureaucratic, the parameters of professional life are bound to become increasingly constricted. As a professional, you must remember that you are held accountable for knowing the contents of *all statutes* concerned with the practice of veterinary medicine.

Unique factors exist in veterinary diagnostic medicine that tend to complicate forensic studies and testimony. First, significant parts of the history and clinical signs are usually missing because of the failure of the animal owner to observe or interpret these events. An animal found dead usually is translated into a "sudden death." Secondly, tissue samples are often submitted in lieu of the entire animal; these samples may be inappropriate for necessary evaluations or otherwise may have lost integrity because of decomposition or unsuitable sample handling methods. (Any diagnostician can recount tales of receiving a feed sample, liver, and heart from a valuable race horse that demonstrated neurologic signs for a few days and then "died suddenly". An insurance company is not interested in the lesion observed; it wants the "cause of death".) A third and understated factor is economics. Most diagnostic services are paid for by the owner of the animal and also are usually subsidized by taxpayers. The resources are not available to pursue every investigation with all of the tests that might add to a definitive diagnosis.

When asked to educate the court and/or state an opinion on material with which he had no previous involvement, the veterinarian is, essentially, at the mercy of the attorney who requested his assistance. How does the diagnostician prepare, particularly when direction from the attorney is poor? How active should the diagnostician and the laboratory be in the development of a case for litigation?

Diagnostic medicine is a science of interpretation. It depends upon the quality of the specimens submitted, on the economic feasibility of pursuit, and upon varying degrees of subjective interpretation to arrive at a conclusion. How does the diagnostician convey this element of interpretive probability or, in other words, what constitutes a "reasonable degree of medical certainty"?

GUIDELINES FOR PRACTITIONERS

Maintain professional standards — The following standards are taken from the Principles of Veterinary Medical Ethics, American Veterinary Medical Association. First, a veterinarian must conduct a relationship to the public, colleagues, and patients, and allied professions so as to merit their full confidence and respect. Second, although a veterinarian may choose whom he/she will serve, once he/she has undertaken the care of a patient, he/she must not neglect it. In an emergency, he/she must render service to the best of one's ability. Third, a veterinarian must not employ professional knowledge or dispense services under terms or conditions that tend to interfere with the free exercise of judgment and skill, or tend to cause a deterioration of the quality of veterinary medical. Fourth, a veterinarian must strive continually to improve veterinary knowledge and skill, making available to colleagues the benefit of professional accomplishments, and must seek, through consultation, assistance of others when it appears that the quality of service may be enhanced. Fifth, the veterinary profession must safeguard the public and itself against veterinarians deficient in moral character or professional competence. Veterinarians must observe all laws, must uphold the honor and dignity of the profession, and accept its self-imposed discipline. Sixth, the responsibilities of the veterinary profession extend not only to the patient but also to society. The health of the community and the patient is of paramount concern.

Provide competent service — Competent service involves following a professional routine that includes, as a minimum, the following steps: gather the facts, formulate hypotheses, analyze data, make tentative diagnoses, seek advice and counsel where needed, explain treatment alternatives to the client, establish the treatment plan, supervise the treatment, and report/explain the results to the client.

Document services — An important area neglected by many veterinarians is documentation of services. It is vitally important that veterinarians

understand that a large and important segment of their work is intangible and, therefore, may slip by "unnoticed" until it is placed under legal scrutiny. Therefore, perceive your work as divisible into the two elements; tangible (physical treatment and care of the animal) and intangible (diagnosis, analysis of options, selection of a mode of treatment, and treatment application. Each element of routine and complex veterinary cases must be documented carefully. Therefore, the "Case Management Flow Sheet" is essential for completeness.

Call your attorney immediately when one of your cases appears adversarial. Call earlier rather than later and always be open to compromise. Consider that no one person can be expert in all areas of veterinary medicine. Also remember that no one wins a lawsuit. Frequently, the stakes are cut down the middle and one seldom wins everything he asks for.

Playing your cards as a defendant involves a subtle combination of using and applying your own professional skills to assist your attorney and, at the same time, being open to his advice about the legal merits of your case. You must have a strong working partnership with the attorney you have selected. This role contrasts sharply with your position as an expert witness. While you may have appeared many times as an expert witness in cases brought against other professionals, when you are the defendant your professional expertise is being questioned. You are no longer an expert in an unimpeachable sense of the word; your expertise has become suspect.

GUIDELINES FOR DIAGNOSTICIANS

Diagnosticians may appear in the courtroom, either on a voluntary or involuntary basis. It is hoped that most will appear voluntarily after negotiating a substantial fee for their services. Sometime fee agreements break down. Furthermore, in many states expert witness fees must be approved by the presiding judge. It is always possible, even if you do not obtain the retainer you requested, that you may be compelled to testify as a lay witness about the factual aspects of your work.

The second essential idea to remember as you prepare to appear in court as an expert witness is that you are an advisor for the jury and, in the absence of a jury, your primary role is to advise the judge. In many courtrooms, the expert medical witness is considered to be a quasi-dignitary. He can often get special considerations for purposes of arranging his court appearance time to fit his schedule.

One experienced medical witness explained his approach to medical testimony as follows: "When I'm on the stand, I think of the jury as a patient. I go into the medical problem involved, and I explain it just as I would with a patient. And I do my best to get across the truth, just as I would to a patient. It's as simple as that."

GUIDELINES FOR WORKING WITH ATTORNEYS

The following guidelines will assist you in maintaining a congenial and mutually satisfying relationship with the supporting attorney and will assist you in standing your ground against opposing attorneys.

First, educate yourself thoroughly about the legal issues. Second, study the scientific issues. Third, clarify the objectives of "your" attorney (sympathetic attorney). Fourth, stand firm on your scientific conclusions when you are confident of them (Don't accept the role of an expert witness unless you are absolutely confident of your scientific conclusions). Fifth, point out strengths and weaknesses in the case to the sympathetic attorney as you discover them. Sixth, be candid with all parties concerned, in and out of court.

GUIDELINES FOR PREPARING EXPERT TESTIMONY

Preparation of expert testimony begins with your commitment to be knowledgeable about your role as an expert witness, as well as the specific scientific issues involved in each case. The following guidelines are suggested. First, be sure you are acting within your area of expertise. Second, obtain all relevant diagnostic records prepared by other veterinarians. Third, review all laboratory findings. Fourth, collect all potential resources, including leading commentaries on both sides of the scientific issues involved. Fifth, reread the leading authorities in the particular scientific dispute, if any. Sixth, perform any laboratory or clinical verifications needed to give you hands-on experience with the critical verifications. Seventh, write your report succinctly and candidly. Eighth, prepare visual aids for the courtroom. Ninth, consult with your peers to discuss your conclusions on the controversial aspects of your work.

GUIDELINES FOR PRESENTING EXPERT TESTIMONY

Every expert should have a courtroom procedure that he follows during the week prior to his court appearance. It would be particularly helpful for you to understand the mechanics of litigation. The following guidelines should also be considered: First, know the facts in your dispute like the back of your hand. Second, be prepared to demonstrate your confidence in the hypotheses, analyses, and conclusions that you present in your report. Third, when on the witness stand, answer only the questions asked. Be especially sure that you can define the scientific words (words of art) that you use in the process of explaining your conclusions. Fourth, recognize the areas in your presentation where you are vulnerable and have answers ready for the inevitable questions designed to probe your vulnerabilities thoughtout. Fifth, remember you can always say, "I don't know," when you are uncertain of the correct answer. Sixth, always be candid.

RULES OF EVIDENCE FOR EXPERT WITNESSES

The Rules of Evidence are exclusionary, designed to shield the jury from confusing or prejudicial information. When the trial is before a judge (a bench trial), these rules are significantly less important.

The following is a summary of the rules that bear on the role of expert witnesses.

1. Laymen may only testify on facts. Lay testimony is limited to items of personal knowledge or observation. You will be subject to this rule if you are called to testify on facts only.
2. Experts may testify on facts and opinions. The judge determines the scope of your expertise. Experts may testify on items on which they do not have personal knowledge.
3. Only sworn witnesses, lay and expert, may testify (submit evidence). Attorneys do not testify (they ask questions and argue). Questions presented by attorneys are not evidence. The answers given by lay and expert witnesses to these questions are evidence.
4. When written documents are submitted as evidence, only the original is admissible, when it is available. Always be prepared to produce the original document, if asked.
5. All testimony is subject to criticism, clarification, and verification (cross examination) by opposing attorneys.

Attorneys are given wide latitude in the sequence of questioning and in their style of examination. You must be resilient enough to brush off emotional ploys discussed previously in this paper and to maintain your composure while appearing as an expert witness. Unless the judge directs otherwise, all questions asked by a lawyer must be answered. (Remember, "I don't know" and "I don't understand the question" are acceptable answers.) Expert witnesses are only as good as the foundation (the scientific methods) used to produce their observations! Be prepared to explain your methodology and the reasons why you selected it. The factfinder (the judge or jury) is free to believe all, part, or none of the testimony of lay or expert witnesses. The factfinder is the ultimate authority in the case.

This chapter is included to enlighten and bring out information that may be useful in the conduct of a veterinary practice and help to reduce the trauma of litigation.

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APPENDIX ONE

STAINING PROCEDURE FOR ROUTINE HEMATOLOGY AND CYTOLOGY STAINS

I. Wright's Stain

Staining solutions*:

1. Wright's stain—Dissolve 0.1 gm powdered Wright's stain into 60 ml of absolute methanol. Allow the solution to stand in a tightly sealed brown bottle for one to two weeks. Filter the solution before using.
2. Wright's buffer—Dissolve 3.80 Na_2HPO_4 (Dibasic Sodium Phosphate) and 5.47 gm KH_2PO_4 (Monobasic Potassium Phosphate) into 500 ml of distilled water. Bring total volume to 1,000 ml with distilled water.

*Wright's stain and buffer may be purchased already prepared from the manufacturer through suppliers, such as Fisher Scientific Co.,[1] American Scientific Products[2] and Harleco.[3]

Staining procedure:

1. Flood air dried smear with Wright's stain and allow to stand for one to three minutes.
2. Add an equal amount of Wright's buffer and mix by gently blowing on the slide until a metallic green sheen forms on the surface. Allow to stand for two to six minutes (the exact time must be determined for each batch of stain).
3. Gently rinse the stain from the slide using tap water or distilled water and a wash bottle or beaker.
4. Prop up the slide and allow it to air dry.

Staining results:

The erythrocytes should have a yellowish-red cytoplasm with a dark violet nucleus in properly stained slides. Heterophils have a pale to dark violet nucleus and a colorless cytoplasm (if nontoxic) with red-orange, rod-shaped granules. Eosinophils have a pale to dark violet nucleus, pale blue cytoplasm,

[1]Fisher Scientific Company, 711 Forbes Ave., Pittsburgh, Pa 15219.
[2]American Scientific Products, Division of American Hospital Supply Corporation, McGraw Park, Il 60085.
[3]Harleco, 480 Democrat Rd., Gibbstown, New Jersey 08027.

and red-orange, round cytoplasmic granules. Basophils have dark purple granules. Lymphocytes have a dark purple nucleus with a pale blue cytoplasm. Thrombocytes have a dark purple nucleus and colorless cytoplasm that often contains red granules.

II. Wright-Giemsa Stain

Staining solutions:

1. Wright's stain may be modified by adding Giemsa stain. Several solutions are available from various commercial sources (e.g., Fisher Scientific Co. and American Scientific Products).
2. A recommended procedure is to dissolve 300 mg of powdered Wright's stain and 30 gm powdered Giemsa stain into 100 ml of absolute methanol. Allow the solution to age for one to two days. Filter the solution and use as indicated for Wright's staining procedure.

Staining results:

The results are similar to Wright's stain alone except the cell nuclei become reddish purple instead of violet.

III. Giemsa Stain

Staining solution:

1. Dissolve 0.5 gm powdered Giemsa stain into 33 ml of glycerin by allowing the solution to stand at 55° to 60°C for one to two hours.
2. Add 33 ml methyl alcohol and allow to stand for 24 hours.
3. Prepare the staining solution by adding 0.67 ml (30 drops) of the Giemsa solution to 30 ml of distilled water.

Staining procedure:

1. Fix dried smears in absolute methyl alcohol for two to seven minutes and allow to air dry.
2. Flood the prepared smears with the staining solution and allow to stand for 15 to 40 minutes.
3. Rinse the slide distilled water and allow to air dry.

Staining results:

The purchase of a commercially prepared Giemsa stain is recommended to avoid the difficulties often encountered when preparing the Giemsa stain. Properly prepared Giemsa stain should provide results similar to Wright's stain except the cell nuclei become reddish purple instead of violet.

IV. Quick (Stat) Stains

Staining solutions:

The quik stains are commercially prepared staining sets. The commonly used stains are:

(1) Diff Quik® stain set
American Scientific Products
Division of American Hospital Supply Corporation
McGraw Park, IL 60085

(2) Hema-Tek® stain set
Ames Division, Miles Laboratory, Inc.
P.O. Box 70, Elkhart, IN 46515

Staining procedure:

1. Dip the air dried slide into the methanolic fixative solution for five one second dips and allow the excess to drain.
2. Dip the alcohol fixed slide into Solution I (Diff Quik's® buffered Eosin Y Solution) for five one second dips and drain off the excess.
3. Dip the slide into Solution II (Diff Quik's® buffered solution of methylene blue and Azure A dyes) for five one second dips and drain off the excess.
4. Rinse the slide with distilled or de-ionized water (i.e., five one second dips) and allow the slide to air dry.

The staining procedure can be modified according to the desired staining effects or thickness of the smear. Increasing the number of dips in solutions I or II will intensify the overall staining of the smear. A paler stain is obtained by fewer dips (a minimum of three dips is required). Eosinophilic staining is enhanced by increasing the number of dips in Solution I and basophilic staining is increased by additional dips in solution II. Staining for coccidial organisms requires a longer staining procedure than the 15-second procedure, but less than 3 minutes. Coccidial merozoites are crescent-shaped and have blue staining with a central red nuclear body.

Staining results:

The staining qualities of the smears are similar to those of Wright-Giemsa stain.

V. New Methylene Blue Stain

Indications:

New methylene blue stain is used for reticulocyte staining and counting procedures. It is also used as a stain to complement Wright's stain when examining cytology specimens. It helps to demonstrate yeast and fungal organisms in scrapings.

Staining solution:

Dissolve 0.5 gm powdered new methylene blue into a solution containing 99.0 ml of 0.85 percent saline and 1.0 ml of 40 percent formalin. The stain solution is filtered and stored in a brown bottle.

Staining procedure:

1. Reticulocyte stain—Mix equal parts of whole blood and stain in a small test tube and allow to stand for 15 to 20 minutes. A standard blood smear is prepared and allowed to air dry.
2. Cytology stain—new methylene blue stain is used as a wet mount on a dried smear. A small drop of stain is applied to an air dried smear and a coverslip is placed over the smear for microscopic examination.

Staining results:

New methylene blue does not stain hemoglobin, therefore erythrocytes have a colorless cytoplasm, distinct cytoplasmic border, and purple nucleus. Most erythrocytes contain a variable amount of reticulum that appears as blue

cytoplasmic precipitate or clumps. Granulocytes have purple nuclei and pale blue cytoplasm. The cytoplasmic granules may not stain with new methylene blue stain. New methylene blue stain provides a more distinctive chromatin and nucleolar appearance to nuclei than alcohol based stains, such as Wright's stain. Since new methylene blue stain is water soluble, it can be used to demonstrate fibrin, lipid droplets, and fungal hyphae which either dissolve or do not stain well with alcohol based stains.

APPENDIX TWO
STAINING PROCEDURE FOR SPECIAL STAINS

I. Acid-Fast Stain

Staining solutions:

1. Ziehl-Neelsen carbolfuchsin—Dissolve 3 gm basic fuchsin in 100 ml of 95 percent ethyl alcohol. Prepare a five percent phenol solution by dissolving five grams of phenol in 100 ml distilled water. The Ziehl-Neelsen carbolfuchsin is prepared by mixing ten ml of alcoholic basic fuchsin and 90 ml of five percent phenol and allowed to stand for 24 hours. The solution is filtered prior to use.
2. Acid alcohol—Mix 2.0 ml of concentrated hydrochloric acid and 98.0 ml of 95 percent ethyl alcohol (decolorizing agent).
3. Methylene blue—A saturated solution of methylene blue is prepared by adding 1.5 gm of powdered methylene blue to 100 ml of 95 percent ethyl alcohol. Slowly add the alcohol to dissolve the powder. Thirty ml of the saturated alcoholic solution of methylene blue is added to 100.0 ml of distilled water and 0.1 ml of 10 percent potassium hydroxide. This solution is filtered and diluted 1:20 with distilled water to prepare the final methylene blue counter stain (counterstain).

Staining procedure:

1. The smear is allowed to air dry and then gently heat fixed (see Gram's stain procedure).
2. Cover slide with Ziehl-Neelsen carbolfuchsin and steam gently using a water bath for three to five minutes.
3. Rinse in tap water.
4. Decolorize with acid alcohol until little red color remains visible to the unaided eye.
5. Rinse in tap water.
6. Counterstain with aqueous methylene blue solution for five to twenty seconds, depending upon the thickness of the smear.
7. Rinse in tap water and allow to dry.

Staining results:

Acid fast positive organisms (i.e., tubercle bacilli) appear distinctly red whereas other bacteria, leukocytes, and debris appears blue. Cryptosporidia stain red with a blue background.

II. Gram's Stain

Staining solutions:

1. Crystal violet stain—Dissolve 2.0 gm powdered Crystal violet into 20 ml of 95 percent ethyl alcohol and mix with 80 ml of 1.0 percent aqueous ammonium oxalate. The solution is stable and can be stored for months.
2. Gram's iodine—Dissolve 1.0 gm of iodine (I_2) and 2.0 gm of potassium iodide (KI) into 300 ml distilled water. Prepare fresh every three weeks.
3. Ethyl alcohol (95 percent)
4. Safranin stain—Dissolve 0.25 gm of Safranin 0 into 10 ml of 95 percent ethyl alcohol. This solution is added to 100 ml of distilled water.

Staining procedure:

1. Make a thin smear of the clinical material. Allow to air dry, then fix the film by quickly passing the slide through a Bunsen burner flame several times.
2. Flood the smear with crystal violet for one minute.
3. Pour off the crystal violet and rinse the slide with Gram's iodine. Allow the smear to remain covered with iodine for one minute.
4. Rinse the slide with water and shake off the excess.
5. Decolorize with (95%) ethanol until the liquid flows colorless from the slide (about 10 seconds). Wash with water.
6. Flood the smear with Safranin for 30 seconds to one minute. Wash the slide with water for one to five seconds and blot dry.

Staining results:

A satisfactorily stained smear will show Gram positive organisms as deep violet and Gram negative organisms as red. The Gram stain may be affected by the nature of the material on the smear. Smears often vary in thickness on the slide and excessive decolorization may occur in very thin areas causing Gram positive organisms to appear Gram negative. Likewise, thicker areas may be poorly decolorized causing Gram negative organisms to appear Gram positive. Because Gram staining has a varied technic, the procedure requires practice before the cytologist gains confidence in the stain.

III. Macchiavello's Stain

Staining solutions:

1. Basic fuchsin stain—A 0.1 M phosphate buffer (pH 7.3 - 7.4) is prepared by adding 80 ml of 0.1 M anhydrous dibasic sodium phosphate (Na_2HPO_4) to 20 ml of 0.1 M monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$). Dissolve 0.25 gm of basic fuchsin chloride into 100 ml of the 0.1 M phosphate buffer to make the stain. The 0.25 percent stain solution should be prepared fresh with each day of use and filtered prior to staining of the slide.
2. Citric acid solution—Dissolve 0.5 gm citric acid into 100 ml of distilled water. The 0.5 percent citric acid solution should be made fresh when mold growth occurs.
3. Methylene blue stain—A stock solution is prepared by adding 1.0 gm of methylene blue chloride to 10 ml of 95 percent ethyl alcohol and slowly

adding 100 ml of distilled water and 5 ml of phenol (melted crystals). A working stain solution is made by diluting the stock solution 1:10 with distilled water.

Staining procedure:

1. Air dried smears are heat fixed (see Gram's stain procedure).
2. The slide is flooded with the basic fuchsin stain and allowed to stain for five minutes.
3. The slide is quickly washed in tap water and dipped one to ten times in the citric acid solution (one to three seconds).
4. Rinse the slide in tap water.
5. The slide is counterstained with the methylene blue stain for 20 to 30 seconds.
6. The slide is washed in tap water and allowed to air dry.

Staining results:

In a properly prepared slide, the chlamydial elementary bodies (200-300 μm (stain red and the larger initial bodies (900-1,000 μm) stain blue. Some smears contain nonchlamydial particles that stain red making the interpretation difficult. Heterophil and eosinophil granules frequently stain red. Mycoplasma colonies may resemble chlamydia. Excessive decolorization with citric acid may decolorize the elementary bodies making them appear blue. Therefore, the citric acid decolorization step may be omitted or shortened in some smears.

IV. Modified Gimenez Stain

Staining solutions:

1. Carbol basic fuchsin -
   a. Stock solution—Dissolve 10 gm of basic fuchsin in 100 ml of ethyl alcohol. Add 250 ml of 4.0 percent phenol solution to 100 ml of the basic fuchsin—ethanol solution. Finally, add 650 ml of distilled water and incubate the solution at 37°C for 48 hours.
   b. Buffer solution—Dissolve 53.61 gm $Na_2HPO_4 \cdot 7H_2O$ (Dibasic sodium phosphate) into 1000 ml distilled water. Dissolve 27.60 gm $NaH_2PO_4 \cdot 7H_2O$ (Monobasic sodium phosphate) into 1000 ml distilled water. The buffer solution is prepared by adding 3.5 ml of the monobasic sodium phosphate solution (0.2 M) and 15.5 ml of the Dibasic sodium phosphate solution (0.2 m) to 19.0 ml of distilled water.
   c. Working carbol basic fuchsin solution—Mix 4.0 ml of the carbol fuchsin stock solution with 10 ml of the buffer solution and filter twice before using. Filtration is required to minimize the amount of red stain precipitate that may interfere with the staining results.
2. Malachite green—Dissolve 0.8 gm powdered malachite green into 100 ml distilled water.

Staining procedure:

1. The smear is allowed to air dry and then gently heat fixed (see Gram's stain procedure).

2. Cover slide with working solution of carbol fuchsin and allow to stand for one to two minutes.
3. Rinse in tap water.
4. Cover slide with Malachite green solution for six to nine seconds.
5. Rinse in tap water and recover slide with Malachite green solution for an additional six to nine seconds.
6. Wash in tap water and allow to air dry.

Staining results:

Chlamydial inclusions are circular and stain red against a blue-green cellular background. Chlamydia may be found intracellular and extracellular.

V. Victoria Blue 4-R Stain

Indications:

Stains treponemes and other bacteria in porcine colonic mucosal smears.

Staining solution:

Dissolve 0.5 gm of Victoria blue 4-R powder in 100 ml of water to make a 0.5% Victoria blue 4-R solution.

Staining procedure:

Make smears and air dry. Place smears on staining rack and flood with 0.5% Victoria blue solution for 15 to 30 minutes. Rinse smears, dry and examine.

Staining results:

Treponemes and other bacteria stain blue. Use Victoria blue designated 4-R. This stain is simple to use and difficult to overstain.

VI. Sudan III or Sudan IV Stains

Staining solution:

Dissolve 0.7 gm. Sudan III in 100 ml. propylene glycol.
Filter solution before use.

Staining procedure:

Sudan stains are used as wet mount stains. A small drop of stain is placed on the smear and a coverslip is added.

Because of the difficulty of preparing stains solutions, it is advised that commercially prepared Sudan stains be purchased.

VII. Staining results:

Fat droplets or globules stain a red-orange color.

Procedure for Clearing Parasites for Examination

1. Fix in 70% ETOH for at least 1 hour

Lactophenol Solution Preportion for clearing
2 parts Glycerine
1 part Lactic acid
1 part Liquified Phenol
1 part Water

2. Place several drops of lactophenol solution on parasite, then coverslip specimen.

VIII. Procedure to Differentiate Carotenes From Bilirubin (Rimington Phase Test)

Procedure:

A piece of fat is minced and shaken with ether and water. Carotenes which are soluble in ether will rise to the top. A yellow top layer indicates carotene. Conjugated bilirubin (diglucuronide) is water soluble and will color the lower layer yellow.

Comments:

Icterus cannot always be diagnosed (horses and dairy cattle) on gross examination alone because carotenes circulating in the plasma are deposited in fat producing yellow tissues.

IX. 10% Neutral Buffered Formalin

Indication

A general fixative suitable for routine processing of mammallian and avian tissues.

Reagents

37 to 40% formaldehyde solution	100.0 ml
Distilled water	900.0 ml
Sodium phosphate monobasic	4.0 gm
Sodium phosphate dibasic (anhydrous)	6.5 gm

Procedure

Premix the phosphate buffers in hot water and allow to dissolve before adding remaining water and formaldehyde solution. Add the formaldehyde after solution has cooled to minimize vapors. Invert container several times to ensure mixing. Mix and use in well-ventilated areas only.

Comments

Even though this formalin is buffered, the pH will decrease with time, causing formation of acid hematin pigments in tissues that are engorged with blood.

Reference

Luna, L.G. (ed.): Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. Edition 3. New York, McGraw-Hill Book Co., 1968, p. 3.

APPENDIX THREE
A SIMPLIFIED FLOW CHART FOR ISOLATION OF BACTERIAL PATHOGENS

Table 1.

GRAM-NEGATIVE MICROORGANISMS

Growth on MacConkey Agar

Rapid and Profuse Color

Red or pink — Colorless

Branch #1
A. Escherichia
B. Klebsiella
C. Enterobacter
*

TSI (H_2S)

\+ —

Branch #2
D. Salmonella
E. Proteus
**

TSI (Acid Butt)

\+ —

Branch #3
E. Proteus
F. Aeromonas
G. Serratia
***

Branch #4
H. Pseudomonas
I. Acinetobacter

Poor or Delayed TSI (Acid Butt)

\+ —

Branch #5
J. Pasteurella
K. Actinobacillus
L. Yersinia

Branch #6
M. Bordetella
I. Acinetobacter

None TSI (Acid Butt)

\+ —

Branch #7
J. Pasteurella
K. Actinobacillus

Branch #8
N. Moraxella
O. Brucella
I. Acintetobacter

Enteric Gram-negative organisms sometimes give atypical biochemical reactions. These are indicated below: *, **, ***

* Serratia (pigmented), Citrobacter and Arizona (Paracolons) may appear in this branch

** Edwardsiella, Citrobacter, and Arizona (Paracolons) may appear in this branch

*** Escherichia, Klebsiella, Enterobacter (rare isolates), Shigella (rare from animals), and Citrobacter (Paracolons) may appear in this branch

This diagnostic flow chart modified from Bailie and Coles, Am Ani Hosp, Scient Proc, (April 20-25, 1975):481-512.

Genera of Gram-Negative Organisms Possibly Isolated

A. *Escherichia coli*
   1. Colonies
      a. Usually red on MacConkey's
      b. May be colorless (rare)
      c. May be hemolytic on blood agar
   2. Organisms
      a. Gram-negative
      b. Small rods to cocco-bacilli
      c. Arranged singly
      d. Usually motile
   3. Differentiation—see Branch #1 Reactions

B. *Klebsiella* (rarely isolated from small animals)
   1. Colonies
      a. Usually red-pink on MacConkey's
      b. May be colorless
      c. Very wet consistency
   2. Organisms
      a. Gram-negative
      b. Small rods to cocco-bacilli
      c. Arranged singly
      d. Capsule present
      e. Non-motile
   3. Differentiation—see Branch #1 Reactions

C. *Enterobacter* (rarely isolated from small animals)
   1. Colonies
      a. Usually pink on MacConkey agar
      b. May be white and opaque to colorless
      c. May be wet in consistency
   2. Organisms
      a. Gram-negative
      b. Small rods to cocco-bacilli
      c. Arranged singly
      d. Capsule may be present
   3. Differentiation—see Branch #1 Reactions

Branch #1 Reactions

	Indole	Urea
Escherichia	+	-
Klebsiella	-	+
Enterobacter	-	-

See footnote*, Appendix Table 1

D. *Salmonella* spp
   1. Colonies
      a. Colorless on MacConkey's agar
      b. Rare strains are pink-red
   2. Organisms
      a. Gram-negative
      b. Small rods
      c. Arranged singly
   3. Differentiation—see Branch #2 Reactions

E. *Proteus vulgaris* and *mirabilis*
   1. Colonies
      a. Colorless on MacConkey's agar
      b. Irregular filmy edges
      c. Swarms on blood agar
      d. Characteristic fetid odor
   2. Organisms
      a. Gram-negative
      b. Small rods
      c. Arranged singly
   3. Differentiation—see Branch #2 Reactions

Branch #2 Reactions

	Indole	Urea
Salmonella	–	–
Proteus	±	+

See footnote**, Appendix Table 1

E. *Proteus morganii* and *rettgeri*
   1. Colonies—colorless on MacConkey's agar
   2. Organisms
      a. Gram-negative
      b. Small rods
      c. Arranged singly
   3. Differentiation—see Branch #3 Reactions

F. Aeromonas (rare in small animals; found in fish and rabbits)
   1. Colonies—colorless on MacConkey's agar
   2. Organisms
      a. Gram-negative
      b. Rapidly motile
   3. Differentiation—see Branch #3 Reactions

G. *Serratia* (rare in small animals, related to *Enterobacter*)
   1. Colonies
      a. Usually colorless on MacConkey's agar
      b. May be red pigmented, especially at room temperature

2. Organisms
   a. Gram-negative
   b. Motile
3. Differentiation—see Branch #3 Reactions

Branch #3 Reactions

	Indole	Urea	Oxidase
Proteus	+	+/–	–
Aeromonas	+	–	+
Serratia	–	–	–

See footnote***, Appendix Table 1

H. *Pseudomonas* (commonly isolated from ears)
   1. Colonies
      a. Colorless to pigmented (green, blue, brown) on MacConkey's agar
      b. Characteristic fruity odor
      c. Wide zone, complete hemolysis (may be delayed)
   2. Organisms
      a. Gram-negative
      b. Arranged singly
      c. Motile
   3. Differentiation—see Branch #4 Reactions

I. *Acinetobacter (Herellea)* (rarely isolated)
   1. Colonies—opaque on MacConkey's agar
   2. Organisms
      a. Gram-variable—some Gram-positive; some Gram-negative
      b. May be rods or cocci
      c. Often arranged in pairs
   3. Differentiation—see Branch #4 Reactions

Branch #4 Reactions

Oxidase	Motility	
Pseudomonas	+	+
Acinetobacter	–	–

J. *Pasteurella hemolytica*
   1. Colonies
      a. Small and usually red on MacConkey's
      b. Narrow zone of complete hemolysis on blood agar
   2. Organisms
      a. Very small cocco-bacilli
      b. May stain bi-polar
   3. Differentiation—see Branch #5 Reactions

K. *Actinobacillus* spp
   1. Colonies
      a. Small and usually red if grown on MacConkey's
      b. One species, *A. suis,* is hemolytic
   2. Organisms
      a. Small to pleomorphic rods
      b. May show bipolar staining
   3. Differentiation—see below Branch #5 Reactions

L. *Yersinia (Pasteurella) pestis* and *pseudotuberculosis*
   1. Colonies
      a. Small and colorless on MacConkey's agar
      b. Possible slight incomplete hemolysis on blood agar
   2. Organisms
      a. Short rods to filaments
      b. May show bipolar staining
   3. Differentiation—see Branch #5 Reactions

Branch #5 Reactions

	Urea	Oxidase
Pasteurella hemolytica	-	+
Actinobacillus	+	+
Yersinia	+	-

M. *Bordetella bronchiseptica* (common in upper respiratory tract of dog)
   1. Colonies
      a. Colorless, opaque colonies on MacConkey's agar
      b. May produce hemolysis on blood agar
   2. Organisms
      a. Small cocco-bacilli
      b. May show bipolar staining
   3. Differentiation—see Branch #6 Reactions

I. *Acinetobacter (Herellea* or *Mima)* (rarely isolated - See J.)

Branch #6 Reactions

	Urea	Oxidase
Bordetella	+	+
Acinetobacter	-	-

J. *Pasteurella (multocida, gallinarum,* and *pneumotropica)* (commonly to rarely isolated from small animals)
   1. Colonies—smooth to mucoid on blood agar
   2. Organisms
      a. Small cocco-bacilli
      b. May show bipolar staining
   3. Differentiation—see Branch #7 Reactions

K. *Actinobacillus* (See L.)

Branch #7 Reactions

	Indole	Urea
P. multocida	+	–
P. gallinarum	–	–
P. pneumotropica	+	+
Actinobacillus	–	+

N. *Moraxella* (rarely isolated from small animals)
   1. Colonies—small and may produce narrow zone, complete hemolysis on blood agar
   2. Organisms—bacilli arranged in pairs
   3. Differentiation—see Branch #8 Reactions

O. *Brucella canis*
   1. Colonies—small and smooth on blood agar
   2. Organisms very tiny cocco-bacilli
   3. Differentiation—see Branch #8 Reactions

I. *Acinetobacter* (See J.)

Branch #8 Reactions

	Urea	Oxidase
Moraxella	–	+
Brucella canis	+ +	+
Acinetobacter	–	–

P. Footnotes *, **, and *** (Appendix Table 1)
   1. *Citrobacter, Arizona, Edwardsiella,* (paracolons) and *Shigella*
      a. Rarely isolated in small animals
      b. Enteric species of bacteria
      c. Colonies and organisms similar to other Enterobacteria

Footnote Reactions

	H_2S	Indole	Urea	Citrate
Citrobacter	±	±	±	+
Arizona	+	–	–	+
Edwardsiella	+	+	–	–
Shigella	–	±	–	–

Appendix Table 2.

SELECTED TYPICAL REACTIONS OF GRAM-NEGATIVE BACTERIA

	TSI	*H_2S*	*Indole*	*Urea*	*Oxidase*	*Citrate*	*Motility*
Branch #1							
A. *Escherichia*	A/A	–	+	–	–	–	+
B. *Klebsiella*	A/A	–	–	+	–	+	–
C. *Enterobacter*	A-Alk/A	–	–	–	–	+	+
Branch #2							
D. *Salmonella*	Alk/A	+	–	–	–	±	+
E. *Proteus*	Alk/A	+	±	+	–	±	+ +
Branch #3							
E. *Proteus*	Alk/A	–	+	+	–	±	+
F. *Aeromonas*	A/A	–	+	–	+	±	+
G. *Providence*	Alk/A	–	+	–	–	+	+
H. *Serratia*	Alk-A/A	–	–	–	–	+	+
Branch #4							
I. *Pseudomonas*	Alk/NC	–	–	–	+	+	+
J. *Acinetobacter*	Alk/NC-Alk	–	–	–	–	–	–
Branch #5							
K. *Pasteurella hemolytica*	A/A	–	–	–	+	–	–
L. *Actinobacillus*	A/A	–	–	+	+	–	–
M. *Yersinia*	Alk/A	–	–	+	–	–	±
Branch #6							
N. *Bordetella*	Alk/NC	–	–	+	+	+	+
J. *Acinetobacter*	Alk/NC-Alk	–	–	–	±	±	–
Branch #7							
K. *P. multocida*	A/A	–	+	–	+	–	–
P. gallinarum	A/A	–	–	–	+	–	–
P. pneumotropica	A/A	–	+	+	+	–	–
L. *Actinobacillus*	A/A	–	–	+	+	–	–
Branch #8							
O. *Moraxella*	Alk/NC	–	–	–	+	±	–
P. *Brucella*	Alk/NC	–	–	+ +	+	–	–
J. *Acinetobacter*	Alk/NC-Alk	–	–	–	±	±	–
Footnotes (Table 1)							
Citrobacter	Alk/A	±	±	±	–	+	+
Arizona	Alk/A	+	–	–	–	+	+
Edwardsiella	Alk/A	+	+	–	–	–	+
Shigella	Alk/A	–	±	–	–	–	–

Appendix Table 3.

GRAM-POSITIVE MICROORGANISMS

Hemolysis on Blood Agar

Complete		*Incomplete or None*	
Cocci or Small Rods		*Large Rods or Filaments*	
Catalase		Branching	
+	−	+	−
A. Staphylococcus B. Corynebacterium C. Listeria	D. Streptococcus B. Corynebacterium	E. *Streptomyces* sp.	F. *Bacillus* sp.

Cocci or Short Rods		Long Rods or Filaments	
Catalase		Branching	
+	−	+	−
A. Staphylococcus A. *Micrococcus* sp. B. Corynebacterium F. *Bacillus* sp. G. *Nocardia* sp.	D. *Streptococcus* spp H. Erysipelothrix I. Lactobacillus	E. *Streptomyces* sp. G. Nocardia	F. *Bacillus* sp. H. Erysipelothrix I. Lactobacillus

Genera of Gram-Positive Organisms Possibly Isolated (See Appendix Table 3)

A. *Staphylococcus and Micrococcus*
   1. Colonies
      a. White to yellow
      b. Circular and smooth
      c. Hemolytic or nonhemolytic, possibly double zone
   2. Organisms
      a. Gram-positive cocci
      b. Arranged in clusters or packets
   3. Differentiation

	Hemolytic	Acid Butt TSI	Coagulase
Staphylococcus aureus or *S. intermedius*	+ (−)	+	+
Staphylococcus epidermidis	−	+	−
Micrococcus sp.	−	−	−

B. *Corynebacterium* sp. (rarely isolated from small animals)
   1. Colonies—variable

2. Organisms
   a. Gram-positive, small rods to cocco-bacilli
   b. Arranged in Chinese letters or palisades
3. Differentiation

	Hemolytic	Consistency	Casein	Urea	Catalase
C. pyogenes	+	dry	+	-	-
C. pseudotuberculosis	+	crumbly	-	+	+
C. renale	-	dry	-	+ +	+
C. equi	-	wet	-	-	+

4. Other organisms of similar morphology occasionally isolated are not classifiable, referred to as diphtheroids

C. *Listeria monocytogenes* (rarely isolated from small animals)
1. Colonies
   a. Complete narrow zone hemolysis
   b. Pale white
   c. Tiny
2. Organisms
   a. Gram-positive small rods
   b. Arranged in palisades or individually
3. Differentiation—only likely small Gram-positive rod motile at 25°C. Umbrella pattern in motility medium

D. *Streptococcus* spp
1. Colonies
   a. Transparent to pale white
   b. Smooth and glistening to rough
   c. Most tiny (*S. zooepidemicus* moderately large)
   d. Hemolysis variable, complete (beta), incomplete (alpha), or non (gamma)
2. Organisms
   a. Gram-positive cocci
   b. Arranged in chains (seen better from broth)
3. Differentiation
   a. Catalase negative
   b. Hemolysis
      (1) Beta—likely pathogen
      (2) Alpha—questionable pathogen
      (3) Gamma (non)—most likely not a pathogen
   c. Growth in 6.5% NaCl, fecal *Streptococcus* (Lancefield group D)

E. *Streptomyces sp.* (isolated as a contaminant, rarely associated with disease, confused with *Nocardia*)
1. Colonies
   a. White to gray
   b. Adherent to agar
   c. Very rough

d. Odor of dry soil
e. Hemolytic or nonhemolytic

2. Organisms
a. Gram-positive
b. Filaments to long rods
c. Branching
d. May be beaded

3. Differentiation—produce clearing of casein agar

F. *Bacillus sp.* (usually isolated as a contaminant; most important pathogen, *B. anthracis*

1. Colonies
a. White to gray
b. Smooth or rough
c. Hemolytic or nonhemolytic

2. Organisms
a. Gram-positive—large rods
b. Spore-former

3. Differentiation—*B. anthracis* String of Pearls test positive, all others negative
a. Make one streak of suspect on Mueller-Hinton agar plate
b. Place coverslip on streak
c. Place 10 unit Penicillin disc on streak next to coverslip
d. Incubate 2-4 hours
e. Chains of spherical cells will be observed under high dry of microscope

G. *Nocardia*

1. Colonies
a. After 3-5 days incubation
b. Gray to brown
c. Dry
d. Adherent or pit into agar

2. Organisms
a. Gram-positive
b. Modified acid fast—decolorize with 1% H_2SO_4
c. Filaments to small rods
d. Beaded
e. May see branching

3. Differentiation—most strains do not clear casein agar

H. *Erysipelothrix rhusiopathiae* (uncommonly isolated from small animals)

1. Colonies
a. Tiny on blood agar after 48 hours
b. Narrow zone of incomplete hemolysis

2. Organisms
a. Gram-positive
b. Thin rods of variable length

3. Differentiation—produces A/A + H_2S in TSI agar slant (only Gram-positive rod to do this)

I. *Lactobacillus sp.* (isolated as part of normal flora)
   1. Colonies
      a. Tiny after 48 hours (Streptococci-like)
      b. May have narrow zone of incomplete hemolysis
   2. Organisms
      a. Gram-positive
      b. Short rods, often in chains to filaments
   3. Differentiation
      a. Catalase negative
      b. H_2S negative in TSI

APPENDIX FOUR
PHOTOGRAPHY AND KNIFE SHARPENING

PHOTOGRAPHY is a technological development that if utilized properly during necropsy will give the best possible record of lesions from macroscopic specimens, even better than a written description that frequently may be inadequate. Fresh specimens cannot await the convenience of a photographer. The only solution is to have photographic facilities readily available for the prosector to use to take suitable pictures.

Actually photographic records supplement and complement, but do not supplant, verbal discription of lesions or a particular anatomic finding. Words used by an individual to describe the morphology of injury or disease are usually not as graphic or compelling as a well made color photograph of the subject or lesion in question. There are times when photographs are considered ancillary evidence in recording gunshot or traumatic injuries in deaths with medical legal implications, in which finding the bullet is essential or trauma must be identified as inflicted by humans or predators. It is imperative to establish evidence so that insurance adjusters can make the right decisions regarding claims.

Photographs together with data from various cases will be of interest when accumulated and can be of benefit to professional groups or individuals when utilized for teaching or for continuing education in various regional programs.

Official aids are particularly important when one is developing herd health programs with a client. Photographs graphically demonstrate the lesions to clients and can be more effective than words in describing the effect that such lesions have on the host.

Establishing the time-of-death may have a direct bearing on determining the cause and manner of death in cases with medicolegal significance. Photographic records are of a major importance in such cases to document the appearance of a body before transient or irreplaceable evidence is destroyed during necropsy. Photography may play a major role in recording accurately the appearance of details that may slip one's mind in description. This may lead to a solution relative to diagnosis. Photography, therefore, has many facets and numerous uses to veterinarians in practice. Records today are invaluable and must be well documented with all the facts, because of potential malpractice claims that are being frequently made against veterinary practitioners. Photographs also can be used for more effective presentations at meetings.

There are a few simple items that must be taken into consideration for photography. The principle aim must be simplicity and rapidity of camera operation because time is of the essence for busy veterinary practitioners. Today, automatic cameras meet the above criteria. Therefore, photography can be simple and easy, with minimum interruption and delay during necropsy.

SELECTION AND SHARPENING OF KNIVES

A sharp knife is as important to the prosector as a scapel is to the surgeon. Putting on a good show during necropsy with a sharp knife is important and it behooves us to get this point across with some dispatch. It is logical to assume that the owner and others viewing a necropsy will judge the prosector on his expertise in opening the carcass and removing organs. If the owner happens to have a sharper knife in his pocket than is being used by the prosector and if the owner can open a carcass with greater expertise, it will be obvious to everyone watching that a pumpkin head has been hired to do the necropsy. The difference between an artist at work in necropsy and a dolt is a sharp knife.

Clients may not understand necropsy procedure and pathologic changes but be assured they understand the skill of a professional performing a systematic necropsy.

Sharpening

Knives can be sharpened or honed on a carborundum stone. This can be wet with either water or oil, but it is preferable to use water or shampoo rather than oil. Keep the pores of the carborundum stone open by cleaning with liquid that floats off the refuse of steel particles. Sharpen on the coarse side and when the bevel is correct, turn to the fine side to finish the honing process. A more convenient hone is a dry stone shaped like a steel. This has a laboratory grit of 672 grain and the finer the grit, the finer the edge that the hone can put on the knife.

To sharpen a knife with the correct bevel, place it flat on the stone or hone and then raise the back or heel of the knife ¼ of an inch to give a 20° bevel and then pull/push against the cutting edge. If the knife is very blunt, use a flat stone or hone, rest the end of the hone on a table and then apply pressure to the knife, holding it as described before.

Do not confuse a hone with a steel. A hone is responsible for "invisible" teeth formation or the sharpening process. You do not sharpen on a steel but only true the edge by pulling the tiny invisible "teeth" into position in the same plane. If the hone is used continuously without using the steel, the knife will be worn away quickly. It should not be necessary with ordinary use to sharpen the knife on the hone more than once per day. However, this is dependent on whether the knife has come in contact with grit or dirt from skinning the animal or attempting to cut bony structures. Frequently, only steeling the knife is necessary if the knife is not too dull. If the knife is dull, heavy pressure will have to be applied when using a hone to

develop the "teeth" of the knife. However, no pressure is used when the knife is laid on the steel, and only its own weight rests on the steel to true the teeth. Again, with the steel, draw the knife against the cutting edge.

Arkansas Stone. This is a small stone and it is desirable not to oil it as it then ceases to be a pocket stone. Use it dry and use the stone on the knife rather than drawing the knife over the stone. Arkansas stones come in 6″ and 8″ and are soft and hard. The hard variety is desirable for instruments and needles. It should be kept clean by shampooing or using liquid soap.

High carbon steel is porous and substances such as blood get into the pores and discolor the steel and these discolorations will remain for life. The new stainless steels or alloy steels are used in necropsy knives and these are a little tougher than the high-carbon steel but harder to sharpen.

It is not possible to tell a good knife merely by observation, since during the processing the steel is heat-treated and Rockwell tested to check its hardness.

Recommended Equipment for Veterinarians. Ten inch or 14″ hones are probably the best to use. Hones should be kept clean by degreasing and removing steel particles with a household cleanser such as "Bonami® " or "Dutch® " cleanser. A polished-glass smooth steel is desirable.

For home use, use a fine hone and do not use the steel at all. Scissors can be ground on the flat hone by resting them on their bevels and pulling the hone along the hone 4-5 times.

Serrated blades cannot be sharpened but have to be retooled. This is as expensive as buying a new knife. However, scalloped edge knives can be sharpened on the hand hone by resting the beveled side on the hone and pulling very slowly over the hone. It should be necessary to sharpen these knives only every few years.

Certain individuals can't or won't take the time necessary to learn to sharpen a knife. There is no reason whatever that these individuals go through their professional years using a dull knife. All they have to do is make contact with a local butcher or some local handyman to keep them supplied with razor sharp knives. There is no difference whatever between hiring someone to sharpen knives and hiring a pharmacist to formulate your drugs. There is absolutely no excuse for prosectors to try to do a necropsy with a dull knife. Probably more necropsies would be done in the field, if practitioners had a sharp knife with them.

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