TECHNICAL BULLETIN

VETERINARY NECROPSY PROTOCOL FOR MILITARY WORKING DOGS AND PATHOLOGY SPECIMEN SUBMISSION GUIDELINES

HEADQUARTERS, DEPARTMENT OF THE ARMY
24 MAY 2001

TECHNICAL BULLETIN
No. MED 283

HEADQUARTERS
DEPARTMENT OF THE ARMY
Washington, DC, *24 May 2001*

VETERINARY NECROPSY PROTOCOL FOR MILITARY WORKING DOGS AND PATHOLOGY SPECIMEN SUBMISSION GUIDELINES

You can help to improve this bulletin. If you find any mistakes or have a recommendation to improve procedures, please let us know. Mail a memorandum or DA Form 2028 (Recommended Changes to Publications and Blank Forms) directly to DODVSA, 5109 Leesburg Pike, Falls Church, VA 22041-3258.

Approved for public release; distribution is unlimited.

*This bulletin supersedes TB MED 283/AFR 163-10, 23 February 1979.

List of Tables

List of Figures

CHAPTER 1
INTRODUCTION

1–1. Scope and purpose

a. This medical bulletin provides necessary technical and administrative guidance for U.S. Army Veterinary Corps officers, animal care specialists, and contract civilian veterinarians involved in the necropsy (postmortem examination) of military working dogs (MWDs). It supplies guidance on—

(1) Preparing for the MWD necropsy.

(2) Necropsy procedures and techniques.

(3) Collection and handling of histologic, cytologic, microbiologic and toxicologic specimens.

(4) Submission of specimens.

b. Disease processes and mechanisms, and causes of death are considered vital information by the Department of Defense (DOD) MWD program. The purpose of performing necropsies is to gather information to—

(1) Determine the cause of death.

(2) Discover the incidence of disease.

(3) Ascertain the effects of disease.

(4) Determine the range of disease manifestations.

(5) Study the effects of the environment.

(6) Provide additional retrospective diagnostic information.

c. All MWDs that die or are euthanized will undergo a complete necropsy as soon after death as possible. Ideally, a necropsy will be initiated within 1 hour of death. The necropsy will be performed in accordance with AR 40-905/SECNAVINST 6401.1A/ AFI 48-131, paragraph 5–2, and this technical bulletin. *All tissues collected from MWDs will be submitted to the Armed Forces Institute of Pathology (AFIP) for evaluation and archiving.*

d. According to AR 40-905/SECNAVINST 6401.1A/ AFI 48-131, paragraph 5–2, necropsy of other Government-owned animals is at the discretion of the installation commander and the attending veterinarian.

e. The medical information gathered from each MWD is collected in a DOD database. The information in this database will be used to improve the health, performance and well-being of MWDs; manage the DOD MWD program; and monitor MWDs as possible sentinels for human disease.

1–2. References

Related publications, prescribed and referenced forms, and a selected bibliography are listed in appendix A.

1–3. Explanation of abbreviations and terms

The glossary contains a list of abbreviations and terms used in this publication.

CHAPTER 2

PLANNING FACTORS AND REQUIREMENTS FOR AN MWD NECROPSY

2–1. Instruments, equipment and supplies

Examples of instruments, equipment and supplies routinely used for an MWD necropsy are listed below. Sources are included for less common items.

a. Necropsy table (or 1 to 2 dental or exam tables); downdraft necropsy table, TBJ Incorporated, 1671 Orchard Drive, Chambersburg, PA 17201.

b. Electric hair clippers with assorted blades.

c. Euthanasia solution.

d. Syringes and syringe needles of various sizes.

e. Sterile specimen cups.

f. Clot or serum (red-top) and EDTA (purple-top) vacuum blood tubes.

g. Laboratory request forms from the local military hospital's clinical laboratory. Refer to AR 40-905/SECNAVINST 6401.1A/AFI 48-131, table 6–2. Do not use local forms for submission of tissues to the AFIP.

h. Tissue trimming knives and double-edged blades; Baxter Scientific, 8855 McGaw Road, Columbia, MD 21045.

i. Very sharp 6-inch necropsy knife, sharpening stone, oil and sharpening steel; National Logistic Services, LLC (NLS-Animal Health), 11407 Cronhill Drive, Owings Mills, MD 21117.

j. Surgical-quality curved and straight scissors, tissue forceps and hemostats.

k. Scalpel blades and handles of various sizes, #4 and #8 scalpel handles and #22 and #60 scalpel blades; National Logistic Services, LLC (NLS-Animal Health), 11407 Cronhill Drive, Owings Mills, MD 21117, and dissecting scalpel handle and No. 62 dissecting blades; Miles Inc. Diagnostics Division, Elkhart, IN 46515.

l. Biosafety sharps container.

m. Rongeurs and bone cutting shears; National Logistic Services, LLC (NLS-Animal Health), 11407 Cronhill Drive, Owings Mills, MD 21117.

n. Heavy bone chisel, Virchow's skull breaker or similar prying device; Thermo Shandon, 171 Industry Drive, Pittsburgh, PA 15275.

o. Cork-surface or polyethylene dissecting boards; Thermo Shandon, 171 Industry Drive, Pittsburgh, PA 15275.

p. Electric cast/bone cutting saw with large sectioned blade or spinal column blade; Thermo Shandon, 171 Industry Drive, Pittsburgh, PA 15275.

q. Heavy-duty plastic bags (biohazard or garbage).

r. Large lopping shears; any local hardware store or garden center.

s. Physiologic saline for rinsing tissues; about 1 to 2 gallons (4 to 8 liters).

t. 10 percent neutral buffered formalin solution, about 2 to 3 gallons (8 to 12 liters) for each necropsy; Columbia Diagnostic, 8001 Research Way, Springfield, VA.

u. Wide-mouth plastic specimen jars with watertight, screw top lids; Thomas Scientific, PO Box 99, Swedesboro, NJ 08085-0099.

v. Gauze (surgical sponges) or fabric tissue specimen bags for specimen segregation; Millhiser, Incorporated, PO Box 24099, Richmond, VA 23244.

w. Large tissue histology cassettes for submitting small samples; Baxter Health Corporation, 3651 Birchwood, Waukegan, IL 60085.

x. Plastic sealing wrap; Thomas Scientific, PO Box 99, Swedesboro, NJ 08085-0099.

y. Sealable polyethylene specimen bags; Thomas Scientific, PO Box 99, Swedesboro, NJ 08085-0099, or Read Plastics, 12331 Wilkens Avenue, Rockville, MD 20743.

z. Heat sealable heavy-duty plastic storage bags (bags, .005 millimeter, National Stock Number 8135-00-890-1842/46) or resealable plastic bags; Kapak Corporation, 5305 Parkdale Drive, Minneapolis, MN 55416-1681.

aa. Indelible ink markers available in most exchanges and office supply stores, ink pens, pencils and water-resistant paper.

ab. Vacuum/heat sealer machine; Rennco Inc., 23721 M-60 West, Homer, MI 49245, or Read Plastics, 12331 Wilkens Avenue, Rockville, MD 20743.

ac. Disposable sterile suture removal packs or similar sterilized surgical instruments (for collecting specimens for bacterial culture).

ad. Bacterial culture transport swabs, sterile Petri dishes and blood culture vials.

ae. Formaldehyde respirator and cartridge respirators for formaldehyde vapors; Fischer Scientific, 711 Forbes Avenue, Pittsburgh, PA, 15219-4785, or Lab Safety Supply, Inc., PO Box 1368, Janesville, WI 53547-1368.

af. Frosted-end microscope slides.

ag. Protective eye wear (especially important when cutting bone and working with formalin); AO Safety Protective Goggles, Aearo Company, 90 Mechanic Street, Southbridge, MA 01550.

ah. Surgical masks and surgical caps.

ai. Protective plastic or rubber aprons and operating room shoe covers; The Butler Company, 30 Vantage Point Drive, Rochester, NY 14625.

aj. Surgical "scrub" garments.

ak. Rubber surgery or vinyl examination gloves.

al. 100 percent nitrile gloves, 4 mils thick (impermeable to formalin), (#7005M medium gloves, #7005L large gloves, #6005XL extra large gloves); Best Manufacturing Company, Menlo, GA 30731.

am. Hematology and cytology stain; EM Science Harleco, 480 Democrat Road, Gibbstown, NJ 08027, or VWR Scientific, PO Box 626, Bridgeport, NJ 08014.

an. DD Form 1743 (Death Certificate of Military Dog).

ao. DD Form 1626 (Veterinary Necropsy Report).

2–2. Utilization of enlisted personnel

As a component of skill level training, enlisted personnel holding Military Occupational Skill 91T, Animal Care Specialist, should be familiar with STP 8-91T14-SM-TG, Task 081-891-1067, Assist with the Collection of Necropsy Tissue of a Military Working Dog. The MWD necropsy is a valuable training experience that should be provided to all Veterinary Services personnel. Discussions on euthanasia, normal anatomy and surgical landmarks are just a few examples of the training information that can come from an MWD necropsy. Food Inspection Specialists can share in this training by providing valuable assistance as information recorders, photographers, specimen collectors and more. The MWD necropsy can be a valuable team-building exercise.

2–3. Preservation of the carcass

If a necropsy cannot be performed within *1 hour* of the time of death, refrigerate the carcass. *Do not freeze the carcass or the collected tissue specimens (including formalin-fixed tissue).*

2–4. Military hospital coordination

MWD expenses are paid with Defense Health Program (DHP) funds. If a military hospital is nearby, necropsy equipment and supplies may be readily accessible. The hospital's morgue may be used as a temporary refrigerated holding facility and may be an excellent location for conducting the necropsy. If any support from the local military hospital's pathology section is anticipated, prior coordination is recommended.

2–5. MWD medical records

Review of the MWD medical record is a prerequisite to an intelligently planned postmortem examination. After the necropsy, medical records (including the DD Form 1626 and DD Form 1743) and radiographs of deceased MWDs must be sent to: Central Records Repository, DOD Military Working Dog Veterinary Service, ATTN: MCVS-MWD, Bldg. 7595, 1219 Knight Street, Lackland Air Force Base, TX 78236-5631. See paragraph 2–10i for additional details.

2–6. Antemortem laboratory tests

a. Collect antemortem blood and urine specimens for routine analysis as listed below. Collect peripheral blood in 10.0 ml red-top and 7.0 ml purple-top (EDTA anticoagulant) tubes. Collect a voided urine sample if possible; otherwise, urine may be obtained at necropsy by cystocentesis. Annotate the method of collection on the urinalysis laboratory request form.

b. The blood tests listed in table 2–1 are the minimum required.

c. The urine tests shown in table 2–2 are the minimum required.

2–7. Euthanasia of MWDs

a. MWDs are euthanized, based on the clinical judgment of a veterinarian, because of debilitating and incurable diseases. Euthanasia must be carried out according to American Veterinary Medical Association Guidelines. The accountable unit must authorize euthanasia of an MWD. Verify the identification of the MWD presented for euthanasia. Euthanize the MWD with a licensed euthanasia solution approved for intravenous use. *Never administer intravenous euthanasia solution by any route other than a peripheral vein.*

b. Complete DD Form 1743 to include the reason for euthanasia or cause of death and file in the animal's medical record (AF Form 2110A (Health Record) or DD Form 2344 (Veterinary Treatment Record)).

Table 2–1. Antemortem blood tests for a necropsy

Alanine aminotransferase (ALT) (previously SGPT)
Albumin
Alkaline phosphatase
Aspartate aminotransferase (AST) (previously SGOT)
Blood urea nitrogen
Calcium
Chloride
Creatine phosphokinase (CK or CPK)
Creatinine
Differential white blood cell count
Glucose
Hemoglobin
Packed cell volume
Phosphorus
Platelet count
Potassium
Sodium
Total bilirubin
Total CO_2 (carbon dioxide)
Total red blood cell count
Total serum protein
Total white blood cell count

Table 2–2. Antemortem urine tests for a necropsy

Bilirubin
Glucose
Ketones
Microscopic sediment examination
Occult blood
pH
Protein
Specific gravity
Urobilinogen

c. Euthanasia without a stated reason is not to be listed as the cause of death. When euthanasia is performed, list the medical reason (for example, renal failure, congestive heart failure, degenerative joint disease or splenic hemangiosarcoma).

d. Annotate the circumstances leading to or surrounding the death/euthanasia and necropsy on an SF 600 (Health Record—Chronological Record of Medical Care) in the animal's medical record (AF Form 2110A or DD Form 2344). Significant necropsy findings may also be annotated.

2–8. Necropsy report

a. Review the guidance for completing DD Form 1626 in appendix B. The report must convey accurate, reliable, and objective information on the necropsy. Complete all blocks on the form.

b. The original copy of DD Form 1626 will be filed in the animal's medical record (AF Form 2110A or DD Form 2344) for forwarding to the Central Records Repository, DOD Military Working Dog Veterinary Service, according to paragraph 2–5. *A copy of the DD Form 1626, DD Form 1743, and DD Form 2619 (Military Working Dog Master Problem List) will be included with tissue specimens sent to AFIP for diagnostic services according to the instructions in paragraph 4–2.*

2–9. Specimen collection

This paragraph applies to techniques other than histology. (See para 2–10 for histology specimens.)

a. General information. The prosector must be prepared to collect and submit to the appropriate laboratory a variety of specimens for microbiological agent culture and identification, cytologic evaluation, and toxicologic analysis. These diagnostic procedures require that specimens be collected separately and by different techniques than those for histologic examination (para 2–10). Determination of the specific laboratory's submission requirements for clinical pathology may require prior coordination. Regardless of the type of specimen collected, all specimen containers must be labeled with the MWD's name and alphanumeric tattoo identification. Attach the results of all diagnostic tests from the necropsy to DD Form 1626.

b. Microbiology.

(1) Microbiologic samples should be taken as soon as possible during the necropsy.

(2) Use sterile instruments to cut away contaminated overlying and adjacent surfaces. Collect representative specimens with additional sterile instruments or culture swabs. (The forceps and scissors found in the disposable sterilized suture removal packs can be used.)

(3) Submit collected specimens promptly. If a delay in submission is anticipated, rapidly refrigerate the specimens. For virology, storage of specimens in an ultra-low temperature freezer (-70 degrees Celsius) is recommended. The local military hospital may have an ultra-low temperature freezer. Do not send microbiologic cultures on dry ice. Dry ice can form carbonic acid, which may sterilize the sample.

c. Cytology.

(1) *General information.*

(a) Cytologic specimens are collected and prepared in a variety of ways.

(b) Have at hand numerous pre-cleaned, frosted-end glass microscope slides and paper towels. Label the microscope slides with the name, tattoo number of the MWD, and site of specimen collection (for example, leg tumor, bone marrow, or renal abscess, etc.).

(c) Thoroughly air-dry the cytologic specimen before methanol fixation and staining with a Wright's or modified-Wright's stain.

(d) If methanol fixation is not available, unfixed, air-dried specimens may also be submitted. Submit at least two unstained slides of each lesion.

(e) Never prepare, store or ship cytology specimens with formalin, as formalin fumes will cause cytological artifacts.

(2) *Solid tissue specimens.*

(a) Solid specimens (organs, neoplasms, etc.) are best examined by the impression smear technique.

(b) Dissect the specimen to about 1 square centimeter (cm).

(c) Repeatedly blot the cut surface of the specimen on a paper towel until almost no moisture is evident.

(d) Quickly and lightly touch the blotted surface of the tissue specimen to the microscope slide.

(e) Again blot the tissue on a paper towel before making each additional impression on the microscope slide.

(3) *Semisolid tissue specimens.*

(a) Semisolid specimens (needle aspirates, bone marrow, etc.) can be prepared by the "squash" preparation technique.

(b) Place the specimen on one slide and very gently place a second slide, frosted side down, on top.

(c) Using only the weight of the top slide, quickly pull the slides away from each other, smearing the specimen between them. This results in two preparations.

(4) *Fluid tissue specimens.*

(a) Preparation. Fluid specimens (peripheral blood, needle aspirates, bone marrow, etc.) can be prepared using several techniques.

(b) Centrifuge. Specimens with a low cell count may be centrifuged and the pellet and a drop of supernatant transferred to a glass slide for smear preparation.

(c) Blood smear technique. Place a drop of the fluid specimen on a microscope slide. Using a second slide held at a 45-degree angle, back into the drop, then gently slide it forward smearing the specimen. The

ideal preparation will have a thin (monolayer) "feathered edge." In some cases, examination of a smear prepared from the buffy coat of a microhematocrit tube will be an essential test in the complete necropsy (a suspected ehrlichiosis case, for example).

(d) Paint brush technique. This is a good technique for bone marrow. Using a clean, dry, camel hair brush, lightly touch the specimen, then gently paint it onto a microscope slide. Several passes can be made on the same slide to produce a thin (monolayer) preparation.

(e) Swab technique. Fluid specimens can be collected on a sterile swab then gently rolled on a microscope slide. Several passes can be made on the same slide to produce a thin (monolayer) preparation.

(5) *Formalin exposure.* **Avoid formalin exposure!** Never expose cytologic preparations to formalin or formalin fumes because artifacts (distortion) will result.

(6) *Number and type of specimens.* Always prepare and submit at least two stained and two unstained specimens. (See para 5–2.) Do not send cytologic preparations in the same package with formalin-fixed tissue specimens.

(7) *Cytology slide shipping.* Submit cytologic preparations in a slide box or plastic slide holder to maintain separation of slides and prevent breakage.

d. Toxicology. Specimens for toxicologic analyses are not routinely collected, but when collected, these general recommendations should be followed.

(1) Collect at least 6.0 cubic cm (3.0 x 2.0 x 1.0 cm) of kidney, lung, liver and fat. Also collect samples of vitreous humor, bile and urine. Place these specimens in an ultra-low temperature freezer (-70 degrees Celsius) as soon as possible.

(2) If ingestion of a toxic agent is suspected, also collect and freeze stomach contents (approximately 60.0 ml).

(3) If immediate access to an ultra-low temperature freezer is not available, use a conventional freezer, then transfer to an ultra-low temperature freezer as soon as possible. The local military hospital may have an ultra-low temperature freezer.

e. Photography. Photography can be a valuable addition to the necropsy by conveying the prosector's observations to the histopathologist. Photographs taken with digital, 35mm, and self-developing cameras, and videotape are all appropriate formats for submission with the necropsy report.

2–10. Collection and fixation of tissue specimens for histologic examination

WARNING

Formalin is a suspected human carcinogen requiring special protective equipment. Follow the precautions in the applicable Material Safety Data Sheet. (See app A for suggested sources.) Handle formalin under a fume hood whenever possible. Goggles and portable respirators approved for formalin provide a safe and effective alternative to a fume hood. Portable formalin respirators and filter cartridges are available from several sources (para 2–1ae).

a. Collect representative tissue specimens from all normal and abnormal organs and other tissues as listed in appendix C. *All tissue specimens collected for formalin fixation will be no more than 0.5 cm (about 1/4-inch) thick.* For routine histopathology, a specimen of approximately the size (area) of a nickel is sufficient. Use sharp, surgical-quality cutting instruments and tissue trimming knives. It is not necessary to separately label all submitted tissues, as most tissues are identifiable after formalin fixation. However, small tissues, tissues with a similar appearance (salivary gland, lymph nodes, etc.), tissues requiring left and right identification, and other tissues requiring specific identification should be separately labeled in cloth tissue specimen bags or other types of labeled containers.

b. Fix tissue specimens collected for histologic examination in 10 percent neutral buffered formalin (hereafter referred to as formalin). The use of commercially prepared formalin is highly recommended. Completely submerge tissue specimens in a volume of formalin equal to 10 times the volume of the specimen (10:1). Rigid, wide-mouth, plastic containers with watertight (screw-on) lids and 2 to 3 gallons (approximately 12 liters) of formalin are needed for each necropsy. Use small specimen containers: tissue cassettes, cloth tissue bags, etc. (tea bags are a useful substitute), for identification or protection of individual tissues. To allow proper fixation and avoid artifact, tissues placed in cassettes should not be compressed or pinched when the cassette lid is closed. All specimen containers must be labeled with the MWD's name and tattoo number.

c. Agitate specimen containers periodically during the initial 24 hours of fixation. Change all formalin after this 24-hour period.

d. All tissues, except the brain, should be properly fixed after 48 hours in formalin (assuming periodic agitation and fresh formalin at 24 hours).

e. Since the brain is soft and friable it should not be cut prior to complete fixation. Submit the brain intact. Sagittal bisection is permissible only if part of the brain is needed for laboratory testing, for example, to test for rabies. To prevent deformation of the intact or sagittally bisected brain, it may be suspended on a large sheet of gauze that is fastened around the top of a sealed container. After changing the formalin at 24 hours, an additional 7 to 10 days is usually required for the brain to become firm enough to cut or ship.

f. Bone specimens should be thoroughly cleaned of all soft tissue not associated with a disease process. Specimens of bone lesions (osteosarcoma for example)

should be transected or have repeated linear incisions made at 1/4- to 1/2-inch intervals with a bone cutting saw to facilitate tissue fixation. The military hospital pathology laboratory may maintain a band saw for such purposes. Radiographs of bone lesions must be forwarded to the AFIP with the bone specimen.

g. Eyes are among the first organs to be examined and collected during the necropsy. The internal ocular structures are relatively fragile and precautions must be taken during fixation and preparation of the eye specimen for shipment. The eye must remain intact during fixation and shipment. Fix the eyes in a volume of formalin 20 times the volume of tissue (20:1). A small amount of formalin (approximately 0.25 ml) can be injected into the vitreous humor. To prevent distortion and damage to ocular structures, use formalin-saturated gauze to wrap and pack each eye in rigid plastic containers for shipment.

h. Commercially prepared formalin (para 2–1) is highly recommended because improperly buffered solutions cause the deposition of acid hematin pigment in tissues, which hinders histopathologic interpretation. If commercially prepared formalin cannot be obtained in bulk quantities, it can be prepared using the following formula:

(1) Formaldehyde, 37 to 40 percent: 100 ml.
(2) Distilled water: 900 ml.
(3) Sodium phosphate, monobasic: 4.0 gram.
(4) Sodium phosphate, dibasic (anhydrous): 6.5 gram.

i. All necropsy tissues and the necropsy report with laboratory results will be sent to the AFIP as soon after the necropsy as possible. (See chap 5.) Records and radiographs should be held for no more than 120 days following the postmortem examination. Contact the Department of Veterinary Pathology, AFIP, at DSN 662-2600, 1-202-782-2600, or e-mail: afipvet@afip.osd.mil and request the status of any case awaiting the final report for more than 120 days. Records and radiographs should then be sent to the DOD Military Working Dog Veterinary Service, Lackland Air Force Base, TX. (See para 2–5.)

CHAPTER 3

PROCEDURAL OUTLINE FOR AN MWD NECROPSY AND COLLECTION OF TISSUES FOR HISTOLOGIC EXAMINATION

3–1. Introduction

a. This chapter describes the recommended necropsy protocol for the military working dog. Regardless of the protocol used, the necropsy must be thorough and complete, and the submitted report must convey reliable and objective information.

b. Accurately describe all abnormalities on DD Form 1626. Collect samples of all abnormalities. Collect and label samples of all tissues as described in this protocol (see para 2–10) and as listed on DD Form 1626 and in appendix C. Use the abbreviation "NGLR" (no gross lesions recognized) or an equivalent notation as appropriate.

c. Use only physiologic saline to rinse tissues during the necropsy. Tissue specimens are sensitive to hydrostatic and osmotic pressures. Rinsing specimens with tap water will cause histomorphologic artifacts.

d. Lymph node examination is an important part of the necropsy. All lymph nodes that drain an area of inflammation or neoplasia, or that appear abnormal will be submitted for histopathology. The medial retropharyngeal, axillary, tracheobronchial, mesenteric and iliac lymph nodes will always be examined and a specimen of each submitted for histopathologic examination. Identify (label) all submitted lymph nodes.

3–2. External examination

Carefully examine all external structures and surfaces and record the following: weight, nutritional condition (obese, lean, cachetic, etc.), state of rigidity, extent of postmortem decomposition, the condition of the hair coat, skin, visible mucous membranes, eyes and bodily orifices. Note and describe tattoos, scars, cutaneous tumors, bony malformations, discharges and surgical incisions, etc.

3–3. Eyes

Clamp eyelids together with tissue forceps. Applying traction, use a scalpel to make an incision through the eyelids around the orbit. With a curved scissors, dissect the eyes from the orbits by cutting extraocular muscles, connective tissue attachments and optic nerve. Examine and collect lacrimal gland tissue. Check for eyelid and retrobulbar lesions. For later identification, leave eyelids attached to the left globe and remove them from the right (leave left, remove right). Remove skeletal muscle from the surface of the sclera to facilitate fixation. A small amount of formalin (0.25 ml) can be injected into the vitreous humor to aid in fixation.

3–4. Ventral midline incision; thoracic and pelvic limbs

a. With the carcass in dorsal recumbency, make a ventral midline incision that extends from the mandibular symphysis to the pubis. In males, cut the skin on each side of and reflect the external genitalia caudally. Dorsally reflect the skin from the abdomen, thorax and cervical regions and examine the exposed tissues. Note the amount and color of the subcutaneous adipose tissue. Starting near the sternum, carefully transect the muscles between the scapula and thorax, to allow the thoracic limbs to lay flat and maintain the carcass in a steady position.

b. Locate and examine the brachial plexus in the axillary space. Locate, examine and, if indicated, collect a specimen of the superficial cervical (pre-scapular) lymph nodes. These are embedded in the areolar connective tissue cranial to the shoulder, deep to the omotransversarius and brachiocephalicus muscles. Locate, examine and collect a specimen of the axillary lymph nodes, which lie dorsal to the deep pectoral muscles. Examine the inguinal lymph nodes, if apparent. These are located just cranial to the pubis in the fat over the inguinal canal.

c. Collect skin, mammary gland (if evident), and testes/epididymides at this point. (See para 3–9*e*(6).)

3–5. Hip Joint

a. Transect and remove the soft tissues (gracilis, adductor, pectineus and semimembranosus muscles) surrounding the hip joints to prepare for examination of the joint spaces. Examine and collect a representative specimen of skeletal muscle. Use a scalpel to incise the joint capsule at its femoral attachment. With forceps, carefully lift the flap of capsule to expose the joint space. Note the character of the joint fluid.

b. If joint fluid is abnormal, a cytological examination should be performed at this time. This can be done using a sterile swab to collect synovial fluid. Insert the swab into uncontaminated joint fluid and then gently roll it on a clean microscope slide. Air dry and stain as described in paragraph 2–9.

c. Using a scalpel, transect the ligament of the head of the femur (round ligament). Remove the head of the femur from the acetabulum to further expose the articular surfaces for examination. Collect any abnormal tissues.

3–6. Bone marrow

a. Specimens. For optimum results, bone marrow

specimens should be collected early in the necropsy. Femur, rib, sternum and vertebra are readily accessible sites for collection of histologic and cytologic red bone marrow specimens.

b. Femur. Red bone marrow may be collected from different locations on the femur.

(1) Using a bone saw, remove the head of the femur by cutting through the femoral neck. Collect red bone marrow from the femoral neck with a clean, dry, camel hair brush, then gently paint the material onto a microscope slide (see para 2–9c) for cytologic evaluation. Submit the femoral head in formalin for histologic evaluation.

(2) Alternatively, use bone cutting shears to remove a short segment of the shaft of the femur. Remove a specimen of red bone marrow (if present). Make impression smears using the technique described in paragraph 2–9c. Place the remaining red bone marrow specimen in a labeled cassette and fix in formalin for histologic evaluation.

c. Rib. Remove a short segment of rib. Using rongeurs, lightly squeeze near the cut end of the rib. Collect exposed marrow with a clean, dry, camel hair brush, then gently paint the material onto a microscope slide (see para 2–9c) for cytologic evaluation. Submit the entire section of rib in formalin for histologic evaluation. This technique may also be used with other flat bones.

3–7. Thyroid and parathyroid glands

Carefully remove the muscles ventral to the trachea by transecting them at the thoracic inlet and bluntly dissecting them toward the head. This will expose the right and left lobes of the thyroid gland and the adjacent parathyroid glands. They are located lateral to the first five to eight tracheal rings. Examine and collect the thyroid and parathyroid glands.

3–8. Salivary glands and lymph nodes

Locate and examine the mandibular, parotid and sublingual salivary glands, and mandibular lymph nodes. Collect a representative specimen of mandibular salivary gland and, if indicated, a mandibular lymph node. Ensure that collected salivary glands and lymph nodes are specifically identified in separately labeled cloth tissue specimen bags or other types of labeled containers.

3–9. Body cavities

Methodically examine all viscera in situ and observe for correct organ size and position. Record any abnormalities in abdominal or thoracic fluid characteristics.

a. Abdomen and adrenal glands.

(1) To completely expose the abdominal viscera, make a ventral midline incision through the abdominal wall from the xyphoid process to the pubis. From the anterior end of this incision, cut laterally along the posterior margin of the last ribs. From the posterior end of the incision, cut laterally just anterior to the pubis. Cystocentesis may be performed at this point for urinalysis.

(2) Shift the abdominal viscera to expose the adrenal glands adjacent to each kidney. Careful dissection of the fat overlying the kidneys and adrenal glands may be necessary. Note that the phrenicoabdominal veins cross each gland. Examine and collect both adrenal glands. Partially section them to examine the medulla and cortex. Submit adrenal glands in separately labeled cloth bags or tissue cassettes.

b. Thorax.

(1) Verify negative intrathoracic pressure by making a small stab incision through the tendinous portion of the diaphragm. Listen carefully for the influx of air and watch for relaxation of the diaphragm. If septicemia is suspected, aseptically collect heart blood in a sterile syringe and transfer it to a blood culture media vial.

(2) Free the diaphragm from its thoracic attachments. Remove the ventral half of the rib cage. To do this, incise the muscles on one side from the thoracic inlet to the last rib. Using bone cutting shears, cut the ribs along this incision. Examine the mediastinum, pericardium and diaphragm. Remove and collect the thymus or thymic remnants from the cranial mediastinum. Cut the muscles and ribs on the opposite side (the jagged cut ends of the remaining ribs may be covered with enough layers of damp gauze sponge to prevent injury to the prosector's hands and forearms). Examine the parietal and visceral pleura. Examine the ribs and intercostal muscles. If bone marrow has not been previously collected, take a sample of rib or sternebra. Prepare a cytologic specimen as described in paragraph 3–6 and then submit the collected bone specimen in formalin. Annotate the sample site on the DD Form 1626.

c. Thoracic viscera.

(1) Examine the viscera in situ and then remove, using the following procedure. The resultant "block" of thoracic viscera should include tongue, larynx, trachea, esophagus, heart, aorta and lungs. Expose the oral cavity by incising the muscular attachments on the mandibles from the symphysis to each ramus. For better access, it may be necessary to split the mandible at the symphysis with bone cutting shears. Examine the oral cavity, teeth, pharynx and larynx. Examine and collect the tonsils on each side of the base of the tongue.

(2) Cut the hyoid bones on each side by cutting through any articulation. Transect the posterior pharynx near the base of the tongue. Using blunt dissection and ventral and posterior traction on the tongue, remove the viscera from the cervical region and thorax. Record any lesions encountered in this process.

(3) Transect the esophagus, aorta and vena cava at the level of the diaphragm. The distal esophagus may be ligated or clamped prior to transection to prevent spillage of gastric contents. Place the thoracic viscera

on a large cutting board. Examine the tongue and collect a representative specimen. Using blunt-tipped scissors, open the esophagus along its entire length. Examine the mucosal surface and muscular tunic and collect a representative specimen. Strip the esophagus away from the thoracic viscera. Examine the medial retropharyngeal lymph nodes and collect a representative specimen. They are located near the dorsolateral surface of the previously transected pharynx. Examine and collect a representative specimen of the muscular diaphragm.

d. Pelvic canal. Locate the obturator foramina on the pelvis. Place the tip of bone cutting shears into one foramen and cut the pubis (caudal) and ischium (cranial). Repeat this on the opposite side. By blunt and sharp dissection, remove the freed section of bone to expose the pelvic canal.

e. Urogenital tract.

(1) The urinary and genital organs can be removed separately or together.

(2) Examine the perineum. Incise the skin and subcutaneous tissues around the external genitalia.

(3) Free both kidneys from attachment sites. Using ventral traction on the kidneys, bluntly dissect the ureters and excise at the urinary bladder. On both kidneys, incise and reflect the renal capsule to the hilus and examine the ureter and renal vasculature. Section the kidneys to the level of the renal pelvis, cutting the left kidney longitudinally and the right kidney transversely (for later identification). Extend the renal pelvic incisions into each ureter, observing for abnormalities. Serially section the kidneys and collect representative specimens from both (no more than 0.5 cm thick) that include the renal papillae. Collect a representative section of ureter. If toxicity is suspected, a minimum of 6.0 cubic cm of kidney should be frozen for later toxicologic analysis.

(4) Remove remaining urogenital organs. Record ovariohysterectomy or orchiectomy, as applicable, on the DD Form 1626.

(5) Examine the urinary bladder, urethra and prostate gland (as applicable). Using scissors, open the bladder and urethra along their ventral aspects to the os penis (male) or external urethral meatus (female). Closely examine the urethra and trigone of the urinary bladder for neoplasia, uroliths or other lesions. Collect representative specimens of urinary bladder, urethra and prostate gland (as applicable).

(6) For male genitalia, examine, collect, label and submit both testes and epididymides in entirety with a segment of each spermatic cord. For proper fixation, serially section the testes: left, longitudinally; right, transversely.

(7) For female genitalia, use scissors to open the vulva, vestibule, vagina, cervix and uterus to the oviducts. Examine and collect representative specimens of these structures and the ovaries.

f. Digestive tract.

(1) Examine the anus and rectum. Incise the skin and subcutaneous tissues around the anus.

(2) Using the following method, remove the abdominal viscera. Cut the dorsal attachments of the diaphragm. Begin removal of the digestive system and spleen by blunt and sharp dissection of the mesentery close to its point of attachment at the dorsal body wall. This will keep the mesenteric lymph nodes attached to their associated intestinal segments.

(3) Continue the dissection of the abdominal viscera through the pelvic canal. Remove the entire digestive tract and spleen to include the anal sacs and associated glands. Place this "block" of tissue on a cutting board for further dissection.

3–10. Vertebral column

Examine the ventral surface of the vertebral column and record abnormalities.

3–11. Iliac lymph nodes and abdominal aorta

Examine iliac lymph nodes and collect a representative specimen. They are located ventral to the fifth, sixth, or seventh lumbar vertebrae near the bifurcation of the aorta. Open the abdominal aorta, beginning at the level of the diaphragm and extending the incision into the iliac arteries. Observe and record any lesions. Collect a representative specimen near the bifurcation.

3–12. Heart, great vessels and pericardial sac

Check for abnormal fluid accumulation in the pericardial sac. Incise and reflect the pericardium. Examine the epicardium, coronary arteries, base of the heart and great vessels. Use the following method to carefully dissect the heart and great vessels. (See fig 3–1.) Following dissection and examination, the entire heart will be submitted in formalin.

a. Right heart. Using a sharp knife or scissors cut across the right auricle, opening the right atrium. Remove clotted blood from the atrium and examine the right atrioventricular (tricuspid) valve. Using a curved scissors, incise the tricuspid valve and ventricular wall along the interventricular septum to the apex of the heart. Continue the cut around the apex to the pulmonary valve. Transect the pulmonary valve. Examine and cut the papillary muscles and chordae tendineae. Remove clotted blood and rinse with physiological saline. Examine the endocardium of the right atrium and ventricle, the tricuspid and pulmonary valves and the exposed myocardium.

b. Pulmonary artery. Continue the initial cut into the pulmonary artery. Follow the branches of the pulmonary arteries into the lungs and examine for thrombi, heartworms and other lesions. Be careful not to cut

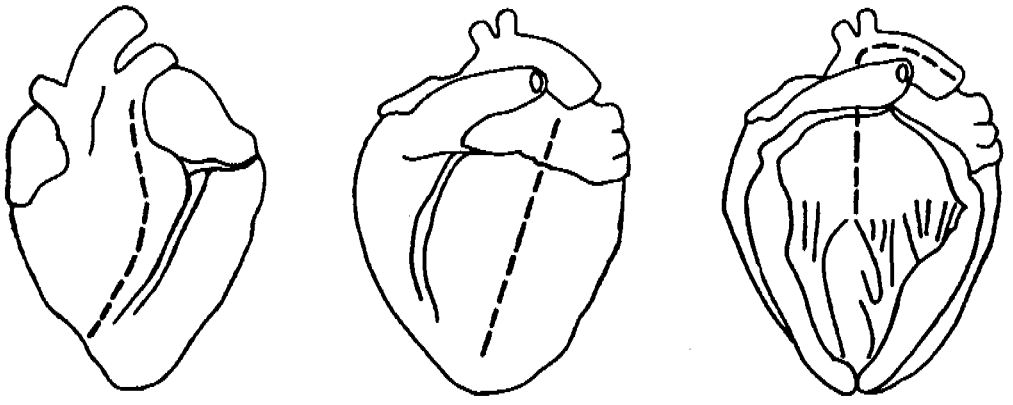

Figure 3–1. Views of the dissection of the heart: right ventricle (left), left ventricle (middle), and aortic outflow (right).

the airways of the right cranial lung lobe. (See para 3–13*b*.) Collect representative specimens as needed.

c. Left heart. Using scissors, open the left auricle and atrium. Insert a sharp knife through the mitral value and out the apex of the heart. Cutting outward, transect the mitral valve and ventricular wall. Remove clotted blood, rinse with physiologic saline and carefully examine the exposed valve leaflets, endocardium and myocardium. Place blunt tipped scissors under the leaflet of the mitral valve lying against the interventricular septum. Cut toward the base of the heart across the mitral and aortic valves.

d. Aorta. Open the transverse and descending aorta. The heart may now be removed from the lungs. Examine the aortic valve leaflets, the coronary artery ostia, the sub- and post-aortic valve regions, and the remaining aorta for constrictions, dilatations, patent ductus arteriosus and other abnormalities.

e. Fixation of heart. To enhance fixation, prop open the ventricular walls with pieces of tongue depressor. Repeated incisions in the thicker myocardium will also improve fixation. Immerse the entire heart in a sufficient volume of formalin (para 2–10).

3–13. Respiratory system

a. Trachea and tracheobronchial lymph nodes. Transect the cricoid cartilage. Use scissors to cut along the membranous border to open the trachea from the larynx to the level of the primary bronchi. Do NOT dissect the right cranial lung lobe. Examine the mucosal surface and collect representative specimens of trachea and larynx. Collect a specimen of tracheobronchial (hilar) lymph node located at the bifurcation of the trachea.

b. Lungs. Do not dissect the right cranial lung lobe. Carefully palpate all lung lobes for abnormalities. Isolate the right, cranial lung lobe for perfusion. Perfusion with formalin may be performed in situ or with the lobe removed. To perfuse in situ, insert a catheter and ligate the bronchus. Alternatively, remove the lobe and suspend with forceps by the bronchus. Gently fill the airways with formalin using a large syringe (in situ) or a squeeze bottle (lobe removed). Fill the lobe to its full inspiratory volume. Submit the entire lobe in formalin. The remaining lung lobes should be dissected by opening all major bronchi. Collect representative specimens, as indicated. Other lobes or even the entire lung may be perfused.

c. Remainder. After placing the lung specimens in formalin, cover any floating sections with formalin-soaked gauze to prevent drying.

3–14. Liver and pancreas

a. Return to the block of digestive organs previously removed (para 3–9*f*). Locate, examine and collect a representative specimen of pancreas with an attached segment of mesentery and duodenum. Avoiding cutting the bile duct on its course through the wall of the duodenum.

b. Open the duodenum to expose the major and minor duodenal papillae. Verify the patency of the bile duct by simultaneously applying gentle pressure to the gallbladder, while observing for expulsion of bile on the mucosal surface of the duodenum at the major papilla. Open the gallbladder and examine the mucosal surface. Collect a representative specimen.

c. Remove the liver from the abdominal block. Open the portal vein and examine for thrombosis. Serially section the liver at 1.0 cm intervals. Examine and record abnormalities. Collect a representative specimen of liver (no more than 0.5 cm thick). If toxicity is suspected, collect and freeze bile and a minimum of 6.0 cubic cm of liver tissue.

3–15. Spleen

Remove the spleen from the abdominal block, and serially section at 1.0 cm intervals. Examine, and record abnormalities. Collect a representative specimen.

3–16. Omentum and mesentery

Examine the omentum (still attached to the stomach) and the mesentery and mesenteric lymph nodes. Collect

at least one mesenteric lymph node. Use a labeled tissue cassette or specimen bag to maintain the identity of the collected lymph node(s).

3–17. Gastrointestinal tract

a. Dissect the mesentery away from the gut close to the serosa. This allows the intestines to be laid out straight on the table, making them easier to open.

b. Using blunt-tipped scissors, open the distal esophagus and the stomach along its greater curvature, through the pylorus to the duodenum. Open the stomach by lifting the free edge of the incision. A quick flipping action on the edge of the stomach (or intestine) using the point of the scissors or forceps will often separate the gut contents from the mucosa, avoiding the need to wipe or wash the mucosal surface. Examine the contents and mucosal surface. Record findings. If toxicity is suspected, collect and freeze stomach contents at this time (approximately 60.0 ml). Collect representative specimens. Avoid rinsing the mucosa with water or scraping it with dissection instruments, as this can cause histomorphologic artifacts. If rinsing is required, use physiologic saline or formalin.

c. Continue the cut from the duodenum to the rectum. Open the intestine, noting contents and any lesions. Collect approximately 6.0 cm long segments of duodenum, jejunum, ileum, ileocecocolic junction, colon, cecum and rectum. Alternatively, specimens of intestine may be collected by resecting unopened segments (approximately 10.0 cm), gently flushing the lumen with physiologic saline, ligating each end, then gently distending the segment with formalin using a syringe and needle. Collect anus and perianal skin and associated glands.

3–18. Joints

a. Musculoskeletal disease is a primary cause for removal of dogs from the MWD program. If a joint abnormality is detected clinically or at necropsy, submit a representative specimen of the joint.

b. The hip joint was previously examined (para 3–5). Carefully open the stifle, shoulder and elbow joints as described below. Examine for abnormal synovial fluid; ruptured, stretched, or frayed ligaments; eroded or ulcerated articular cartilage; thickened joint capsules; osteophyte formation; proliferative or thickened synovium and other changes. Open other joints if indicated by clinical history. Record any abnormalities. Collect samples for culture and histopathologic examination as indicated.

(1) *Stifle joint.* Remove the skin overlying the stifle joint. Locate and examine the popliteal lymph node. Transect the muscles and tendons just proximal to the stifle and the tendons just distal to the patella. Connect the medial ends of these incisions across the stifle joint. Carefully reflect the incised tissue, including the patella, exposing the joint space. Culture the joint fluid and collect cytology specimens, if indicated. Transect the cruciate ligaments and examine the articular surfaces.

(2) *Shoulder joint.* Remove the skin overlying the shoulder joint. Cut the muscles, tendons and joint capsule around the lateral portion of the proximal humerus just distal to the acromion and supraglenoid tubercle. Rotate the humeral head away from the glenoid cavity to expose the joint.

(3) *Elbow.* Remove the skin overlying the elbow. Maximally flex the joint. Transect the muscles, tendons and joint capsule over the elbow by making a medial to lateral U-shaped incision over the caudal aspect of the humerus parallel to the ulna. Rotate the foreleg to expose the articular surfaces. Examine the articular surfaces and sample joint fluid, if necessary.

3–19. Brain

To remove the head, hyperextend the neck and cut the attachments between the atlas and the occipital bone exposing the atlanto-occipital joint and dura mater. If indicated, aseptically collect cerebrospinal fluid. Transect the cervical spinal cord, remaining musculature and skin to fully disarticulate the head.

a. Make a dorsal midline incision from the nose to the foramen magnum.

b. Reflect the skin ventrally. Transect and collect a specimen of horizontal ear canal. Remove the temporal muscles from the cranium to at least the level of the zygomatic arch.

c. Use a bone saw to make three cuts through the skull. Take precautions to avoid cutting into the brain. The first cut is made transversely at the anterior limit of the cranial cavity, slightly posterior to the zygomatic process of the maxilla. Rotate the cranium to one side and connect the end of the transverse cut with the foramen magnum. Repeat on the opposite side. See figure 3–2.

d. Use a Virchow's skull breaker, bone chisel or similar instrument in the first (transverse) incision, to pry off the calvarium. Examine the internal surface of the calvarium and exposed sinuses.

e. If necessary, remove the tentorium osseum with rongeurs. Transect the olfactory lobes. Elevate the rostrum and carefully transect the cranial nerves and pituitary stalk, freeing the brain.

f. Immerse the whole brain in formalin (para 2–10). Bisect the brain only if tissue is required for immediate laboratory testing, such as for rabies.

3–20. Pituitary gland

Incise the dura mater surrounding the sella turcica to free the pituitary gland. Remove it carefully and place in a labeled tissue cassette.

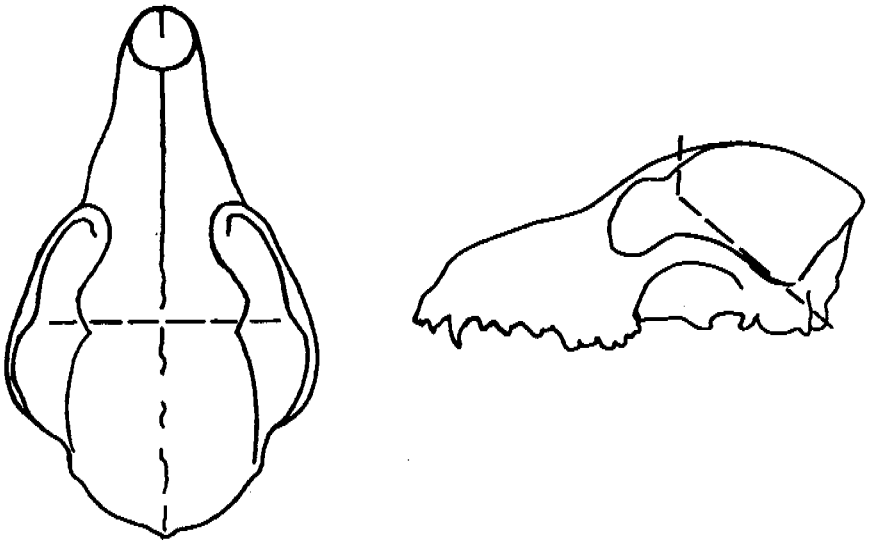

Figure 3–2. Removal of the brain

3–21. Nasal cavity and sinuses

Make a complete transverse cut across the frontal and maxillary bones rostral to the orbits. Examine the exposed nasal cavity and sinuses. Alternatively, a wedge of bone may be removed to expose these spaces. If indicated, submit a representative specimen.

3–22. Spinal cord

a. Remove the skin remaining on the carcass and examine dorsal subcutis and musculature.

b. Remove the epaxial muscles.

c. Exposing the spinal cord requires cutting through the cervical, thoracic and lumbar vertebral arches at different angles. Removing the thoracic section first allows visualization of the correct placement of the saw blade for the subsequent removal of the cervical and lumbar sections. If the cuts through the vertebral arches are made at the correct angles, there will be an immediate loss of resistance as the saw blade enters the spinal canal. Progressing from cervical to sacral vertebrae, the position of the saw blade changes from vertical (parallel to spinous processes) to horizontal (perpendicular to spinous processes). Do not cut into the spinal cord upon entering the canal. Additional tips for each vertebral section follow. (Refer to fig 3–3.)

(1) *Thoracic vertebrae.* Transect the spinous processes of the thoracic vertebrae at their bases. This will allow cutting through the thoracic vertebral arches at the proper angle. Cut through the thoracic vertebral arches adjacent to the remnants of the spinous processes at approximately a 45-degree angle. Make a transverse cut at the level of the C7-T1 articulation and a second cut at the level of the T13-L1 articulation (do not cut the spinal cord). Remove the freed vertebral arch segments to expose the spinal cord.

(2) *Lumbar vertebrae.* Cut through the lumbar vertebral arches immediately dorsal to the transverse processes at a 90-degree angle (perpendicular to the spinous processes). Make a transverse cut at the lumbosacral junction. Remove the lumbar vertebral arches to expose the spinal cord.

(3) *Cervical vertebrae.* Cut through the cervical vertebral arches midway between the spinous process-

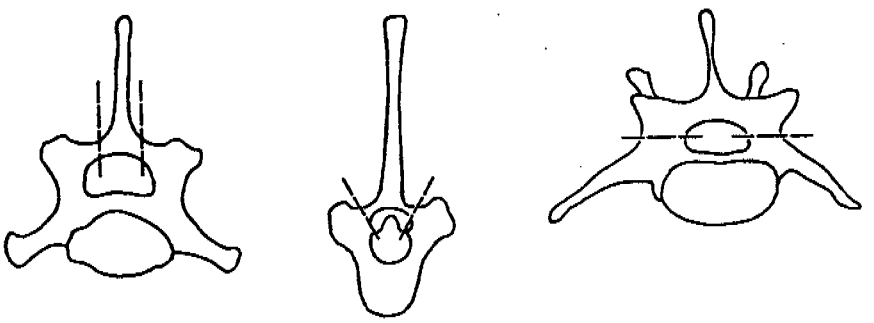

Figure 3–3. Removal of the spinal cord: cervical vertebra (left), thoracic vertebra (middle), and lumbar vertebra (right).

es and the transverse processes at a 0-degree angle (parallel to the spinous processes). Remove the freed vertebral arch segments to expose the spinal cord.

(4) *Sacral vertebrae.* If lesions are suspected in the cauda equina, cutting between the intermediate and lateral sacral crests can expose the sacral vertebral canal.

d. Beginning at the atlas, grasp the dura mater with tissue forceps and apply gentle dorsal traction. Using a scalpel or small scissors cut the nerve roots as far laterally into the intervertebral foramina as possible and remove the spinal cord from the canal. Examine the dura mater and spinal nerve roots.

e. Using small scissors, carefully cut and reflect the dura mater along the dorsal midline for the full length of the spinal cord. This facilitates examination and fixation of the spinal cord. Pay special attention to the cervical and lumbar intumescences. Record any abnormalities. Examine the vertebral canal for herniated disk material and other lesions. Place a suture through the dura mater (or use some other form of identification) to mark suspect areas of the spinal cord requiring the attention of the histopathologist. (Annotate on the DD Form 1626.)

f. Immerse the spinal cord and the attached dura mater in formalin.

3–23. Peripheral nerves

a. Radial nerve. Remove the skin from the brachium. Transect the tendon of the lateral head of the triceps muscle at its insertion on the ulna. Using blunt and sharp dissection, reflect to expose the radial nerve, which lies caudal to the brachialis muscle. Remove 3.0 to 4.0 cm of the radial nerve. Attach the ends of the nerve to a wooden tongue depressor or index card with a stapler. Be careful not to stretch the nerve. Label the tongue depressor or index card (in pencil) with the identity of the attached nerve.

b. Sciatic nerve (ischiatic). Remove the skin over the hindlimb. Incise the fascia lata to the stifle. Bluntly dissect between the biceps femoris and the vastus lateralis muscles to expose the sciatic nerve. Remove a 3.0 to 4.0 cm segment of the sciatic nerve and attach to a tongue depressor or index card. Label the tongue depressor or index card (in pencil) with the identity of the attached nerve.

CHAPTER 4

AFIP DIAGNOSTIC SERVICES AND PATHOLOGY SUPPORT FOR MWDS AND OTHER ANIMALS

4–1. General information

a. The Department of Veterinary Pathology, AFIP, offers no-cost diagnostic services for all government-owned animals, and privately-owned animals belonging to individuals who are authorized to receive DOD veterinary services. AFIP also offers a second opinion consultation service for military and civilian pathologists.

b. Euthanasia of privately-owned animals must be carried out according to AR 40-905/SECNAVINST 6401.1A/AFI 48-131, paragraph 3–4, and will be documented on DD Form 1745 (Animal Euthanasia). File this form in the animal's medical record (AF Form 2110A or DD Form 2344). DD Form 1626 may be used as needed.

c. Diagnostic materials accepted by the AFIP include formalin-fixed tissue specimens, paraffin-embedded tissue specimens, stained histologic sections, cytologic preparations, radiographs and photographs.

d. See appendix A for a complete list of referenced forms and sources.

4–2. Surgical biopsy and cytology diagnostic material

a. Fix tissues as previously described (see para 2–10) and prepare for shipment. (See chap 5.)

b. Complete one copy of DD Form 2834 (Veterinary Consultation Request) and forward with case material. Include complete signalment (identification, species, breed, sex and age). Include pertinent clinical information. Do NOT use an SF 515 (Medical Record—Tissue Examination) in place of DD Form 2834, as important demographic and clinical information may be omitted. Include copies of the forms mentioned in paragraph 2–8*b*.

c. Include a telephone number, fax number (commercial and DSN) and e-mail address. Be prepared to provide follow-up clinical information.

4–3. Case priorities and turn-around-times

a. Surgical biopsy and cytology cases. The Department of Veterinary Pathology provides consultation on surgical biopsies and cytologies. Consultation results are faxed to the contributor as soon as possible, usually within 2 to 10 days after receipt of the case material. The pathology report will also be mailed.

b. Necropsy cases. Consultation results for necropsy cases require several weeks to complete after receipt of the case material. The consultation letter will be faxed and mailed. Special situations justify a faster case turn-around-time. If this is the case, consult with the Department of Veterinary Pathology prior to submission of the necropsy materials. See contact information below.

4–4. Additional information

Direct any questions about AFIP services to the Chief, Diagnostic Services Branch, Department of Veterinary Pathology at the AFIP at DSN 662-2600; COMM 202-782-2600; COMM Fax 202-782-9150; DSN Fax 662-9150; or e-mail <afipvet@afip.osd.mil>. AFIP maintains a home page on the World Wide Web at http://www.afip.org/.

CHAPTER 5

SHIPMENT OF SPECIMENS TO THE AFIP

5–1. Packaging specimens

WARNING

Formalin is a suspected human carcinogen requiring special protective equipment. Follow the precautions in the applicable Material Safety Data Sheet. (See app A for suggested sources.) Handle formalin under a fume hood whenever possible. Goggles and portable respirators approved for formalin provide a safe and effective alternative to a fume hood. Portable formalin respirators and filter cartridges are available from several sources (para 2–1*ae*).

a. Tissue fixation must be according to paragraph 2–10.

b. Exposure of personnel to formalin when packaging tissues for shipment can be minimized by using the local military hospital's pathology laboratory services (fume hoods, vacuum sealers, etc.) to the maximum extent possible.

c. Remove adequately fixed tissue specimens from formalin (after 24 to 48 hours). Wrap tissue specimens in formalin-saturated gauze sponges or place tissue in formalin-soaked cloth tissue bags. Bone specimens with sharp edges must be adequately padded with formalin-saturated gauze to prevent perforation of plastic specimen bags. Pack formalin-fixed eyes in rigid specimen containers to prevent distortion of ocular structures.

d. Double-bag the tissues. Seal the wrapped tissue specimens in vacuum/heat-sealable heavy-duty plastic specimen bags, if available. Seal the first bag in a second bag. Each individual specimen container must be clearly labeled with the MWD's name and tattoo identification and, if required, the tissue identity or collection site. An index card labeled with this information in pencil or indelible ink can be included in each bag. Alternatively, the bags can be directly labeled using an indelible ink marker. Note that many types of ink are removed by formalin vapors.

e. Alternate methods of packaging wrapped tissue specimens include—

(1) Plastic specimen jars with screw-on lids, *not snap-on lids.* Seal the lid with several layers of plastic sealing wrap.

(2) Double- or triple-bagging using heavy-duty resealable plastic bags. To avoid bursting during shipment, remove as much air and formalin as possible.

f. Do not ship tissues immersed in formalin. Ship fixed tissues wrapped in formalin-saturated gauze in sealed unbreakable containers.

g. Protect the DD Form 1626 by using sealed plastic specimen bags. Remember regular ballpoint ink will disappear when exposed to formalin. Pack the DD Form 1626 with the specimen containers.

5–2. Shipping specimens

a. Correct packaging of specimen containers is essential to prevent breakage and the environmental contamination and tissue specimen dehydration that could result. Ship containers in a sturdy cardboard box lined with *two* heavy-duty plastic bags. Use adequate packing materials in sufficient amounts to ensure that specimen containers remain stable during shipment and do not contact the walls of the box. Close each plastic bag securely and seal the box with strapping or shipping tape.

b. Use cardboard or plastic microscope slide mailers to send cytology specimens. Remember, never prepare, store, or ship cytology specimens with formalin. (See para 2–9*c*.)

c. Complete DD Form 2834 and forward with case material.

d. Do NOT submit an SF 515 in place of DD Form 2834, as important demographic and clinical information may be omitted.

e. Include a telephone number, fax number (commercial and DSN) and e-mail address. Be prepared to provide follow-up clinical information.

f. For submission of MWD diagnostic materials to the AFIP, address the package to:

Regular Mail:
Armed Forces Institute of Pathology
ATTN: AFIP-RRR (Receiving and Accessions)
Bldg 54, Room G071
Washington, DC 20306-6000

Couriers:
Armed Forces Institute of Pathology
ATTN: Receiving and Accessions Division
6825 16th Street, NW
Washington, DC 20306-6000

5–3. Additional information

Direct any questions about specimen shipment to the Chief, Diagnostic Services Branch, Department of Veterinary Pathology at the AFIP at DSN 662-2600; COMM 202-782-2600; COMM Fax 202-782-9150; DSN Fax 662-9150; or e-mail <afipvet@afip.osd.mil>. AFIP maintains a home page on the World Wide Web at http://www.afip.org/.

APPENDIX A
REFERENCES

A–1. Related Publications

AR 40-905/SECNAVINST 6401.1A/AFI 48-131
Veterinary Health Services

Material Safety Data Sheet for Formalin
Material Safety Data Sheets may be purchased from several commercial vendors, or may be accessed from several web sites, including the following:
http://msds.pdc.cornell.edu/msdssrch.asp
http://hazard.com/msds
http://www.msdsonline.com/

STP 8-91T14-SM-TG
Soldier's Manual, Skill Levels 1/2/3/4 and Trainer's Guide, MOS 91T, Animal Care Specialist

2000 Report of the AVMA Panel on Euthanasia
This publication is available from:
http://www.avma.org/resources/euthanasia.pdf

A–2. Prescribed Forms

DD Form 2834
Veterinary Consultation Request. (Available on the OSD website, http://web1.whs.osd.mil/diorhome.htm).

A–3. Referenced Forms

AF Form 2110A
Health Record. (Initiated by AF personnel).

DD Form 1626
Veterinary Necropsy Report. (Available on the OSD website, http://web1.whs.osd.mil/diorhome.htm).

DD Form 1743
Death Certificate of Military Dog. (Available on the OSD website, http://web1.whs.osd.mil/diorhome.htm).

DD Form 1745
Animal Euthanasia. (Available on the OSD website, http://web1.whs.osd.mil/diorhome.htm).

DD Form 2344
Veterinary Treatment Record. (Available through normal forms supply channels).

DD Form 2619
Military Working Dog Master Problem List. (Available on the Army Electronic Library (AEL) CD-ROM and the OSD website, http://web1.whs.osd.mil/diorhome.htm).

SF 515
Medical Record—Tissue Examination. (Available through normal forms supply channels and the Army Electronic Library (AEL) CD-ROM).

SF 600
Health Record—Chronological Record of Medical Care. (Available through normal forms supply channels and on the Army Electronic Library (AEL) CD-ROM).

A–4. Selected Bibliography

Alden CL. 1981. *Color Atlas for Small Animal Necropsy.* Bonner Springs (KA): Veterinary Medicine Publishing Company.

Arrington JB, Mills B, Prophet EB, Sobin LH, editors. 1992. *Laboratory Methods in Histotechnology.* Washington, D.C.: American Registry of Pathology.

Banks WJ. 1993. *Applied Veterinary Histology.* 3rd ed. Chicago: Mosby Year Book.

Buergelt CD, Gross TL. 1982 Jun. Gross Postmortem Examination of Small Animals Part I: Introduction, Instrumentation, and Evisceration of the Carcass. *The Compendium on Continuing Education for the Practicing Veterinarian* 4(6):473–9.

Buergelt CD, Gross TL. 1982 Jul. Gross Postmortem Examination of Small Animals Part II: Organ Dissection. *The Compendium on Continuing Education for the Practicing Veterinarian* 4(7):550–558.

Cowell RL, Meinkoth JH, Tyler RD. 1999. *Diagnostic Cytology and Hematology of the Dog and Cat.* 2nd ed. St. Louis: Mosby, Inc.

De Lahunta A. 1983. *Veterinary Neuroanatomy and Clinical Neurology.* 2nd ed. Philadelphia: W.B. Saunders Company.

Dodd DC, King JM, Newson ME, Roth L. 1999. *The Necropsy Book.* Gurnee (IL): Charles Louis Davis, DVM Foundation Publisher.

Duncan RJ, Mahaffey EA, Prasse KW. 1994. *Veterinary Laboratory Medicine.* 3rd ed. Ames: Iowa State University Press.

Evans HE, De Lahunta A. 1996. *Miller's Guide to the Dissection of the Dog.* 4th ed. Philadelphia: W.B. Saunders Company.

Evans HE. 1993. *Miller's Anatomy of the Dog.* 3rd ed. Philadelphia: W.B. Saunders Company.

Getty R. 1975. *The Anatomy of the Domestic Animals.* 5th ed. Philadelphia: W.B. Saunders Company.

Jubb KVF, Kennedy PC, Palmer N, editors. 1993. *Pathology of Domestic Animals.* 4th ed. New York: Academic Press, Inc.

Popesko, Peter. 1984. *Atlas of Topographical Anatomy of the Domestic Animals.* 4th ed. Philadelphia: W.B. Saunders Company.

APPENDIX B

GUIDE TO COMPLETING DD FORM 1626 (VETERINARY NECROPSY REPORT)

All blocks on this form must be completed. The combination of these instructions and those on the DD Form 1626 may be used as a guide for performing a necropsy and recording the results. DD Form 1626 may be printed or photocopied without the instruction pages to minimize the length.

a. Section I—Administrative data.

(1) *Part A—Contributor's data.* Blocks 1-7: Name and address of the reporting veterinary unit. Enter the rank, full name and title of the contributing Veterinary Corps officer and the military unit to which that officer is assigned. Station address, telephone number, fax number and e-mail address are all important information.

(2) *Part B—Animal identification and related data.*

(a) Blocks 8 through 16: Animal identification. Complete all information blocks.

(b) Block 17: Euthanized. Check yes or no. If yes, specify the brand name or the active agent of the euthanasia product, the amount given and the site of intravenous administration.

(c) Block 18: Cause of death. Concisely state the most likely cause of death. The medical reason(s) for death is (are) critical information. If the animal was euthanized, state why. Examples of reasons for euthanasia include chronic degenerative joint disease, neoplasia and congestive heart failure. *Do not list "euthanasia" as the cause of death.*

(d) Block 19: Name and address of unit accountable for animal. Enter the military unit that is accountable for the MWD. Include the complete military unit designation and station address.

(e) Blocks 20-23: Complete all information.

(f) Blocks 24: Materials forwarded. List categories such as tissues, culture results, radiographs and gross photographs.

b. Section II—Clinical and pathological data.

(1) *Block 25: Clinical abstract.* This abstract addresses the recent pertinent clinical information regarding this case, as well as previous significant medical history. Include illnesses and recent treatments, laboratory and radiologic findings, surgery, wounds, fractures, immunizations, working environment and recent temporary duty assignments. Attach a copy of DD Form 1743 and DD Form 2619.

(2) *Block 26: Clinical diagnoses.* Concisely list the clinical diagnoses that pertain to the death of the animal.

(3) *Block 27: Gross necropsy diagnoses.* List all necropsy diagnoses (interpretations).

(4) *Blocks 28-31: Ancillary procedures.* List any ancillary materials and procedures that are included with the submission: gross photographs (block 28); results of microbiologic culture and identification (block 29); results from blood tests, urinalysis and cytologic preparations (block 30); and radiographs (block 31).

(5) *Block 32: Remarks.* This section can be used either for any additional information that supplements this report, or as a continuation of another information block.

c. Section III—Gross Findings (Instructions and Diagrams).

(1) Use each block for a systematic examination and reporting process. Describe lesions in detail or enter "NGLR" (no gross lesions recognized) or an equivalent notation as appropriate. Use the anatomic diagrams to indicate location, distribution and relative size of lesions.

(2) If needed, use a blank page to continue gross descriptions. Preface each continuation with the organ or tissue identification.

d. Section IV—Tissue checklist.

APPENDIX C
LIST OF TISSUE SPECIMENS COLLECTED FOR HISTOLOGIC EXAMINATION

C–1. Two different lists of the tissues to be collected during the necropsy are provided in this appendix. In figure C–1, the tissues are in alphabetical order. In figure C–2, they are listed in the order in which they are encountered in this necropsy protocol. (DD Form 1626 is also in this necropsy protocol order.)

C–2. Tissue specimens are usually cut at a maximum thickness of 0.5 cm; however, some specimens, such as the heart, are left intact. Do not place tissue specimens into containers that are too small. Tissues placed in cassettes should not be compressed or pinched when the cassette lid is closed. Do not allow tissues to freeze.

C–3. Do not ship cytologic specimens with formalin fixed tissue. Formalin will cause distortion of cellular architecture making evaluation of the cytologic specimen difficult.

MWD Name

MWD Tattoo

Adrenal glands (left and right)

Anus/perianal area/anal sac

Aorta, abdominal (specify other site)

Axillary lymph node(s) (left or right)[1]

Bone (rib, sternebra; specify other site)

Bone marrow

Brain, cerebrum, cerebellum, brain stem (submit intact)

Cecum

Colon

Diaphragm (muscular portion)

Duodenum

Ear canal

Esophagus

Eyes (left and right)

Gallbladder

Haired skin (specify site)

Heart, entire organ, opened

Ileocecocolic junction

Ileum

Figure C–1. Tissue list—alphabetical order (1 of 3)

Iliac lymph node(s)[1]

Jejunum

Kidneys (left and right)

Lacrimal gland

Liver

Lung

Mammary tissue (if applicable)

Mandibular salivary gland

Medial retropharyngeal lymph node(s) (left or right)[1]

Mesenteric lymph node(s)[1]

Ovaries (left and right)

Pancreas

Peripheral nerve (specify: sciatic, radial, or other nerve)

Pituitary gland[1]

Prostate gland

Rectum

Skeletal muscle

Spinal cord (intact preferred or specify and identify segments)

Spleen

Stomach

Testes (with epididymides and spermatic cords) (left and right)

Figure C–1. Tissue list—alphabetical order (2 of 3)

Thymus/thymic remnants[1]

Thyroid gland and parathyroid glands[1]

Tongue

Tonsils (left or right)[1]

Trachea

Tracheobronchial lymph node(s)[1]

Ureters

Urethra

Urinary bladder

Uterus; cervix; vagina

Other specimens:

Other lymph nodes (specify)[1]

Other salivary glands (specify)

Tumors/neoplasms (describe site)[1]

[1] Submit in labeled containers/cassettes.

Figure C–1. Tissue list—alphabetical order (3 of 3)

MWD Name

MWD Tattoo

Eyes (left/right)

Lacrimal gland

Axillary lymph nodes (left/right)[1]

Haired skin (specify site)

Mammary tissue (if applicable)

Skeletal muscle

Bone marrow

Thyroid gland and parathyroid glands[1]

Mandibular salivary glands (left/right)

Adrenal glands (left/right)

Thymus/thymic remnants[1]

Bone (rib, sternebra; specify other site)

Tonsils (left/right)[1]

Tongue

Esophagus

Medial retropharyngeal lymph nodes (left/right)[1]

Diaphragm (muscular portion)

Kidneys (left/right)

Figure C–2. Tissue list—descriptive order (1 of 3)

Ureters

Urinary bladder

Urethra

Prostate gland

Testes (with epididymides and spermatic cords) (left/right)

Uterus; cervix; vagina

Ovaries (left/right)

Iliac lymph nodes (left/right)[1]

Aorta, abdominal (specify other site)

Heart (entire organ, opened)

Trachea

Tracheobronchial lymph nodes (left/right)[1]

Lung

Pancreas

Liver

Gallbladder

Spleen

Mesenteric lymph nodes[1]

Stomach

Duodenum

Jejunum

Figure C–2. Tissue list—descriptive order (2 of 3)

Ileum

Ileocecocolic junction

Cecum

Colon

Rectum

Anus/perianal area/anal sac

Ear canal

Brain, cerebrum, cerebellum, brain stem (submit intact)

Pituitary gland[1]

Spinal cord

Peripheral nerve (specify)

Other specimens:

Other lymph nodes (specify)[1]

Other salivary glands (specify)

Tumors/neoplasms (describe site)[1]

Bones/joints

[1] Submit in labeled containers/cassettes.

Figure C–2. Tissue list—descriptive order (3 of 3)

GLOSSARY

Section I
Abbreviations

AFI
Air Force Instruction

AFIP
Armed Forces Institute of Pathology

cm
centimeter(s)

DOD
Department of Defense

DODVSA
Department of Defense Veterinary Service Activity

DSN
Defense Switching Network

ml
milliliter(s)

MWD
military working dog

NGLR
no gross lesions recognized

SECNAVINST
Secretary of the Navy Instruction

Section II
Terms

Government-owned animal
An animal that is owned, maintained, or managed by a military or Federal agency or activity.

Necropsy
The postmortem examination of an animal.

Privately-owned animal
An animal owned and maintained by an individual who is authorized DOD veterinary services.

Veterinary Corps officer
A commissioned officer in the Army Veterinary Corps.

INDEX

Topics are identified by paragraph number.

By Order of the Secretary of the Army:

ERIC K. SHINSEKI
General, United States Army
Chief of Staff

Official:

JOEL B. HUDSON
Administrative Assistant to the
Secretary of the Army

DISTRIBUTION:
To be distributed in accordance with Initial Distribution Number (IDN) 342384, requirements for TB MED 283.