TECHNICAL NOTE

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Microscopic Diagnosis from Frozen Canine Tissues

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ABSTRACT: Frozen tissues were studied microscopically to determine their value for diagnostic purposes. Sections were taken from lung, liver, kidney, small intestine, and brain of ten diseased dogs that died or were euthanatized. Some tissues were frozen, held for two or seven days, and then formalin-fixed. Tissues that were formalin-fixed immediately served as controls. Freezing changes such as transudate, cell shrinkage, fractures, hemolysis, and hematin formation were a nuisance, but usually did not prevent making a diagnosis. Viral inclusions, microfilaria, fibrosis, and intestinal bacteria remained distinct.

KEYWORDS: pathology and biology, freezing, tissues (biology), microtomy, frozen sections, tissue preservation

Although pathologists would prefer to evaluate well-fixed tissue, there are times when tissues or cadavers have been frozen and a diagnosis is still expected. In a previous paper [*1*], we evaluated the effects of freezing on histological parameters. In this report, the ability to make a diagnosis from frozen tissue is evaluated.

Materials and Methods

Organ samples were collected from ten clinical cases of dogs submitted to the Kansas State University Veterinary Hospital for evaluation; two died, eight were euthanatized. Dogs were necropsied no longer than 8 h after death. The following organs were sampled:

lung—right apical lobe, approximately 1 cm in thickness;
liver—right lobe, approximately 1 cm in thickness;
kidney—right, transverse sections, 1 cm in thickness;
jejunum—cranial, approximately 3 cm long;
brain—right hemisphere, transverse sections, 1 cm in thickness; and
other—sections were taken from organs that had obvious gross lesions.

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Three samples were collected from each organ and were assigned randomly to one of the following treatments:

(1) immediate fixation in 10% buffered formalin at room temperature (control);
(2) frozen to −18°C and stored at the same temperature for two days, followed by fixation in 10% buffered formalin at room temperature; and
(3) frozen to −18°C and stored at the same temperature for seven days, followed by fixation in 10% buffered formalin at room temperature.

After fixation, the tissues were trimmed, embedded, sectioned, and stained with hematoxylin and eosin. One slide was made for each sample and treatment; 15 slides per dog. Microscopic diagnoses and freezing changes were recorded.

Results

Freezing Changes

Major changes for the organs studied were as follows:

lung—an accumulation of transudate in the alveoli, bronchioles, and bronchi; inconsistent loss of cilia; septal cell shrinkage; hemolysis; and hematin formation;
liver—shrunken, pale-staining hepatocytes; distended, transudate-filled sinusoids; hemolysis; hematin formation;
kidney—transudate in capsular spaces, vacuolation and loss of tubular epithelium, increased numbers of casts, hemolysis and transudate accumulation in the interstitium, and hematin formation;
small intestine—increased mucosal autolysis, and fractures in the submucosa and tunica muscularis; and
brain—large fractures in both the white and gray matter, increased eosinophilia of neurons, and meningeal separation.

Tissues frozen for seven days had more transudate, more fractures, and greater sinusoidal dilatation. Renal tubular epithelium had more vacuoles and greater detachment from basement membranes. Tubular lumens had more casts.

Pathological Changes in Frozen Tissue

The following are brief descriptions of microscopic, morphological changes for each dog.

Dog 1—pink, round, viral intranuclear/intracytoplasmic inclusions characteristic for distemper in the renal pelvis and bronchial epithelium (Fig. 1).
Dog 2—a chemodectoma that had been located cranial to the heart (Fig. 2), fat emboli in the liver.
Dog 3—microfilaria of *Dirofilaria immitis* (heartworms) in blood vessels in the lung, liver, and kidney (Fig. 3).
Dog 4—chronic glomerulonephritis characterized by shrunken glomeruli and thickened capsular membranes (Fig. 4).
Dog 5—no significant changes in the five routine tissues examined.
Dog 6—bile duct proliferation.
Dog 7—no significant changes.
Dog 8—thrombi in the lung.
Dog 9—clumps of bacteria on the mucosa of the small intestine (Fig. 5).
Dog 10—no significant changes.

Pathological changes were the same in tissue frozen for seven days or for two days.

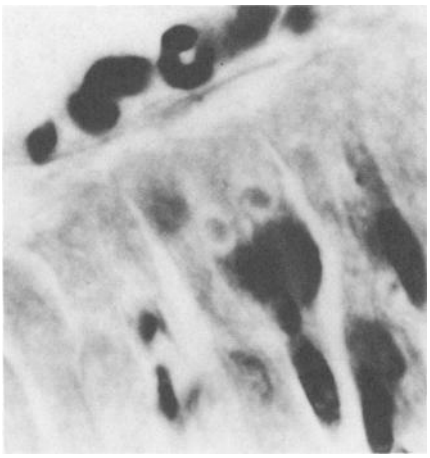

FIG. 1—*Intracytoplasmic viral inclusion bodies (canine distemper) in bronchial epithelium. Frozen seven days; H&E stain (× 1000).*

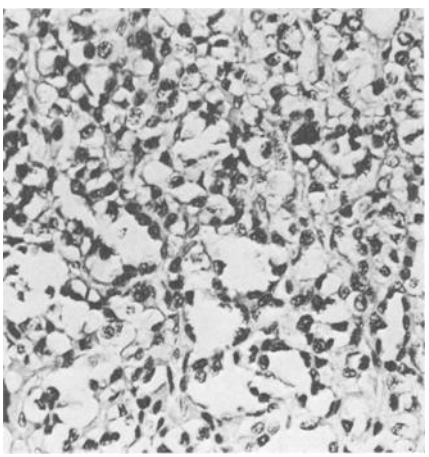

FIG. 2—*Chemodectoma with several well-preserved neoplastic cells along capillaries. Frozen seven days; H&E stain (× 200).*

Discussion

Major microscopic changes caused by freezing were accumulation of transudate, cell shrinkage, fractures, hemolysis, and hematin formation. These changes have been discussed previously [*1,2*].

Pathological changes that remained unchanged in frozen tissues were viral inclusion bodies, fibrosis, microfilaria, bile duct proliferation, and bacterial enteritis. The chemodectoma was more difficult to recognize in frozen tissue than in controls. However, the pattern remained: lobules divided by connective tissue strands and neoplastic cells distributed along capillaries.

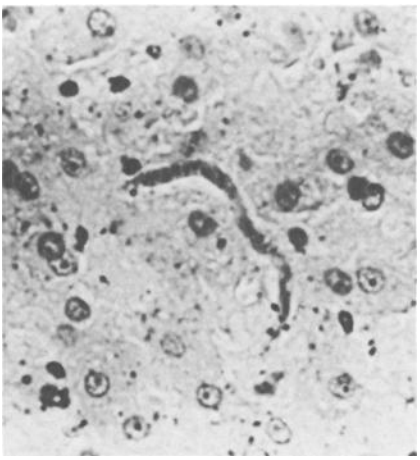

FIG. 3—*Microfilaria of heartworms* (Dirofilaria immitis) *in liver. Hematin in background. Frozen seven days; H&E stain (× 400).*

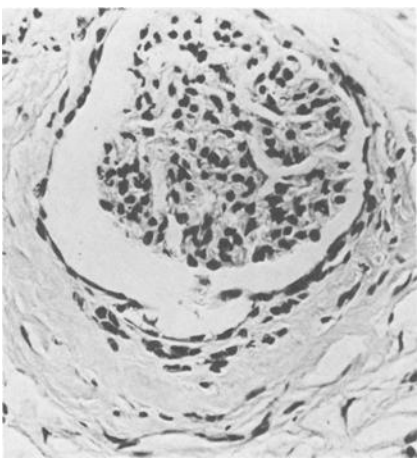

FIG. 4—*Capsular fibrosis and shrunken glomerulus. Frozen seven days; H&E stain (× 400).*

Since the first ten dogs available for necropsy were used, some dogs had neither interesting diseases nor microscopic changes. Dog 5 had a prostatic carcinoma without metastasis. Since the entire prostate was fixed in formalin, frozen tissue was not evaluated. Dog 8 had endocardiosis, a valvular fibrosis limited to dogs, which was diagnosed grossly without need for microscopic evaluation.

In this study, there were two instances in which diagnoses were missed, when compared to controls. In Dog 1, inclusions were not seen in astrocytes, but even in well-fixed tissue they are often hard to find. Inclusions were seen in the lung and kidney of that dog. Control slides from Dog 8 had a hemangiosarcoma of the spleen, but in frozen tissue hemolysis and endothelial cell shrinkage made the diagnosis impossible.

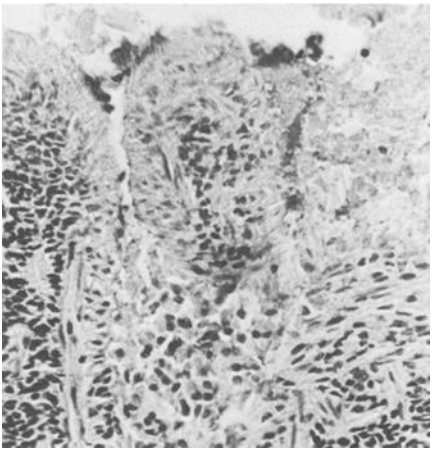

FIG. 5—*Bacteria on the mucosal surface of the small intestine. Frozen seven days; H&E stain (× 200).*

Although freezing damage such as hemolysis, transudate accumulation, and hematin occurred, it did not prevent making most microscopic diagnoses. Circulatory disturbances and subtle changes, such as anoxia, would be difficult, or impossible to recognize. However, most diagnoses can be made from frozen tissue; viral inclusions, parasites, bacteria, and fibrosis remained distinct.

References

[*1*] Baraibar, M. A. and Schoning, P., "Effects of Freezing and Frozen Storage on Histological Characteristics of Canine Tissues," *Journal of Forensic Sciences*, Vol. 30, No. 2, April 1985, pp. 439–447.

[*2*] Thompson, S. W. and Luna, L. G., *An Atlas of Artifacts*, Charles C Thomas, Springfield, IL, 1978.

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