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Selective Bronchial Catheterization and
Bronchial Brush Biopsy in the Dog

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SELECTIVE BRONCHIAL CATHETERIZATION AND BRONCHIAL BRUSH BIOPSY IN THE DOG

By

Gary Wayne Thayer

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ABSTRACT

SELECTIVE BRONCHIAL CATHETERIZATION AND
BRONCHIAL BRUSH BIOPSY IN THE DOG

By

Gary Wayne Thayer

Principle, lobar and segmental bronchi were selectively catheterized via the cricothyroid membrane or endotracheal tube using a polyethylene catheter. Cytologic specimens were collected using nylon and stainless steel bronchial brushes attached to flexible guide wires. Cytologic preparations made by touching or smearing the brush on the slides were compared.

Inflammatory disease was created in 1 lung of a group of dogs. Bronchial brush biopsies were compared by evaluating the relative number of inflammatory cells seen in the cytologic specimens from the control lung, the affected lung, and bronchial washings. The procedure was performed on 19 dogs with spontaneous respiratory tract disease.

Selective bronchial catheterization via the endotracheal tube was found to be the method of choice. Nylon bronchial brushes with cytologic specimens made by the smearing technique were superior in the dog. The bronchial brush biopsy was shown to be a selective procedure in identifying an area of pathology within the lungs.

Fifteen of 19 (79%) dogs with spontaneous respiratory tract disease had bronchial brush biopsies consistent with the final diagnosis.

This work is dedicated to my wife and parents for their inspiration, encouragement, and sacrifices that I might obtain my career goals as a veterinarian.

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INTRODUCTION

Bronchopulmonary disease accounts for a significant number of the medical problems seen in small animal practice. At Michigan State University's Small Animal Clinic, 465 canine patients admitted during the years 1970 through 1975 had clinical lower respiratory tract disease. Until the late 1960's, the veterinary clinician had few aids with which to examine the lower respiratory tract. To diagnose, prescribe and monitor therapy, he had to rely on history and the physical examination. In addition, diagnostic aids including radiography and basic laboratory examinations such as complete blood counts, fecal examination, microfilaria check, and evaluation of fluid obtained by thoracocentesis, (in the event pleural fluid was present) were also utilized. Thoracotomy was the only means of visualizing the tissues of the lower respiratory tract and obtaining biopsy specimens.

To allow more specific evaluation of the lower respiratory tract without risk of thoracotomy, bronchoscopy and bronchial washing were developed. These techniques visualized the airways for collection of cytologic and culture specimens from the dog and cat (O'Brien, 1970). Various percutaneous needle biopsy techniques for obtaining lung and pleural tissues for histopathologic evaluation were also described (Reif, 1974). In addition, transtracheal aspiration for collection of cytologic and cultural specimens from the lower respiratory tracts of dogs and cats

was introduced (Creighton and Wilkens, 1974a). The development of the flexible fiberoptic bronchoscope made possible the visualization and sampling of more peripheral lung tissue from known locations.

The development of more sophisticated diagnostic equipment and techniques made possible advances in diagnostic capabilities and the understanding of lower respiratory tract diseases. None of these techniques, however, allowed collection of biopsy material from small diameter bronchi. In 1964, a technique was described for use in human patients that allowed selective catheterization of specific bronchi with the aid of an image intensified fluoroscope, and the collection of cytologic samples from the peripheral bronchial tree by the use of a nylon brush attached to a flexible wire that could be passed through the catheter (Hattori, 1964). This procedure has been subsequently modified and improved, and is now used in hospitals throughout the United States.

The objectives of this work are to describe and evaluate the technique of selective catheterization of bronchi in the dog and the collection of cytological specimens from the peripheral bronchial tree using a bronchial brush.

More specific aims of the project are to:

1. Evaluate procedures for the selective catheterization of primary, secondary, and tertiary bronchi using an image intensified fluoroscope.

2. Collect cytologic specimens by means of the bronchial brush through the preplaced catheter.

3. Document the amount of trauma at the biopsy site and post-biopsy complications for both nylon and stainless steel brushes.

4. Evaluate methods of preparation of the biopsy specimens for cytologic evaluation.

5. Evaluate the cytologic specimens obtained with nylon and steel brushes with regards to the number of cells obtained and cellular morphology.

6. Evaluate the cytologic specimens obtained by bronchial washings and nylon bronchial brushings with regards to the number of cells obtained and cellular morphology.

7. Evaluate the selectivity and sensitivity of the nylon bronchial brush in the detection of experimental inflammatory disease.

8. Apply and evaluate the techniques of selective bronchial catheterization and bronchial brush biopsy to clinical patients with respiratory disease.

LITERATURE REVIEW

History of Bronchial Brushing

Selective bronchial catheterization was first described in France (Metras, 1947). The first description of this procedure in the American literature was in 1964; in it the first description of the technique of bronchial brush biopsy appeared (Hattori, 1964). In 1966, the use of arterial catheters for the purpose of selective bronchography was described (Fennessy, 1966). One year later, this technique was described in conjunction with bronchial brush biopsy (Fennessy, 1967). In recent years, the procedures have been used extensively for the diagnosis of lower respiratory disease in medical centers throughout the United States (Backus, 1971; Bean, et al., 1968; Bibbo, et al., 1973; Fennessy, et al., 1973; Murphy and Komorowski, 1974; Penido, et al., 1972; Rockoff, 1974; Skitarelic and von Haam, 1974; Smith and Warrock, 1972; Solomon, et al., 1974; Willson and Eskridge, 1970; Zavala, et al., 1972).

Indications for Bronchial Brushing

Many authors have reported the usefulness of selective bronchial catheterization and bronchial brush biopsy in the diagnosis of solitary peripheral pulmonary lesions that cannot be visualized by bronchoscopy (Fennessy, et al., 1970; Fennessy, et al., 1973; Fennessy and Kittle, 1973; Firestone, 1973; Hattori, et al., 1964; Janower and Richard, 1971;

Rockoff, 1974). Recently, this technique has been successful in the diagnosis of diffuse lung disease (Fennessy and Kittle, 1973; Janower and Richard, 1971) and has been used to drain lung abscesses (Rockoff, 1974). In addition, bronchial brushes can be used to obtain cultures from the lower respiratory tract for bacteria, (Backus, 1971; Bibbo, et al., 1973; Fennessy, et al., 1973; Fennessy and Kittle, 1973; Murphy and Komorowski, 1974; Rockoff, 1974), viruses (Fennessy, et al., 1973; Fennessy and Kittle, 1973), and fungi (Fennessy and Kittle, 1973). Two authors have found selective bronchial catheterization and bronchial brush biopsy useful in the diagnosis of infectious lung disease, secondary to immunosuppressive therapy in transplant patients (Fennessy, et al., 1973; Fennessy and Kittle, 1973).

Contraindications for Bronchial Brushing

In the human patient, contraindications to this technique include recent severe hemoptysis, suspected vascular lesions, thrombocytopenia, or other bleeding disorders, and dyspnea at rest (Fennessy, et al., 1973; Fennessy and Kittle, 1973). These authors emphasize that the contraindications should be ignored if the need for a definitive diagnosis is imperative to the well-being of the patient.

Applied Gross Anatomy

The right lung of the dog has four lobes, cranial, middle, caudal, and intermediate lobes. The left lung, being smaller than the right, has only two lobes, the cranial and caudal lobes. The bronchial tree begins as the trachea bifurcates at the level of the fifth intercostal space, just to the right of the midline and dorsal to the base of the heart.

The right principle bronchus begins at the tracheal bifurcation and enters the dorsal aspect of the hilus of the lung. The right cranial lobar bronchus leaves the principle bronchus just distal to the hilus from its lateral aspect and bends cranially. The right cranial lobar bronchus then gives off a large bronchus just distal to the hilus from its dorsal aspect, the caudal segmental bronchus, which ventilates the caudal bronchopulmonary segment of the cranial lobe. The right cranial bronchus continues cranioventrally as the cranial segmental bronchus and ventilates the cranial bronchopulmonary segment.

After the exit of the cranial lobar bronchus, the right principle bronchus gives off the right middle lobar bronchus from its ventrolateral aspect. Shortly after its beginning, the middle lobar bronchus gives off a large bronchus from its lateral aspect which ventilates the dorsal bronchopulmonary segment of the middle lobe; it is named the dorsal segmental bronchus. The middle lobar bronchus continues as the ventral segmental bronchus, which ventilates the ventral bronchopulmonary segment of the right middle lobe.

After giving off the right middle lobar bronchus, the principle bronchus gives off the intermediate lobar bronchus from its ventral medial wall. The intermediate lobar bronchus passes caudad and divides into the dorsal and ventral segmental bronchi. These bronchi ventilate the dorsal and ventral bronchopulmonary segments of the intermediate lobe respectively.

Following the exit of the intermediate lobar bronchus, the right principle bronchus continues as the caudal lobar bronchus and gives off two

bronchi from its ventrolateral aspect. The first of these is the ventral basal bronchopulmonary segment of the caudal lobe. The second ventrolateral bronchus is named the lateral segmental basal; it ventilates the lateral basal bronchopulmonary segment.

From its dorsal surface, the caudal lobar bronchus gives off two bronchi, the cranial dorsal segmental bronchus and the caudal dorsal bronchopulmonary segment and caudal dorsal bronchopulmonary segment of the caudal lobe respectively. The caudal lobar bronchus continues as the dorsal basal segmental bronchus ventilating the dorsal basal bronchopulmonary segment of the caudal lobes.

The left principle bronchus enters the hilus of the left lung and gives off the left cranial lobar bronchus from its lateral aspect, which divides into the cranial segmental bronchus and the caudal segmental bronchus. These bronchi ventilate the cranial bronchopulmonary and caudal bronchopulmonary segments of the left cranial lobe respectively. The left principle bronchus continues as the left caudal lobar bronchus and branches as in the right caudal lobe.

The segmental bronchi in all lobes branch into subsegmental bronchi and terminate in bronchioles described under microscopic anatomy (Hare, 1975).

Applied Microscopic Anatomy

The mucous membrane of bronchi are arranged in longitudinal folds and are lined by pseudostratified columnar ciliated epithelium with goblet cells on a prominent basement membrane. The epithelium changes to a simple columnar ciliated epithelium with goblet cells as the bronchi

decrease in size.

The lamina propria consists of a thin subepithelial layer composed of loose connective tissue with few elastic fibers, many leukocytes, and a prominent capillary network. Just beneath the connective tissue is a thick layer of longitudinally oriented elastic fibers arranged in bundles in the larger bronchi and having a spiral course in smaller bronchi.

The muscularis mucosa is composed of smooth muscle circularly arranged in large bronchi and tends to be spirally arranged in smaller bronchi. Elastic tendons connect the smooth muscle cells of the muscularis mucosa to the elastic fibers of the lamina propria and submucosa.

The submucosa is primarily loose connective tissue containing many elastic fibers, blood vessels, and the bulk of the simple cuboidal tubuloacinar mucous glands. These glands decrease in number as the size of the bronchi decrease and are absent in the smallest bronchi of the dog.

The bronchi are surrounded by a fibroelastic cartilage layer consisting of hyaline and elastic cartilage plates interconnected by dense irregular elastic tissue. Hyaline cartilage predominates in the larger bronchi, while in the smaller bronchi elastic cartilage is more prevalent. The adventitia surrounds the fibroelastic layer and consists of elastic fibers and loose connective tissue with a few tubular acinar glands in the larger bronchi.

The terminal bronchi branch dichotomously giving rise to primary bronchioles which continue to branch to secondary, tertiary and respiratory bronchioles. The mucous membranes of the primary and secondary bronchioles appear histologically in longitudinal folds which decrease in height toward the end of the secondary bronchioles. The lumen is lined by

simple columnar epithelium in the primary and secondary bronchioles and simple cubiodal epithelium in the tertiary and respiratory bronchioles. The number of goblet cells and ciliated cells decrease as the bronchioles get smaller and are rare beyond the secondary bronchioles. The lamina propria is composed of loose connective tissue with many lymphocytes and elastic fibers which decrease in number toward the respiratory bronchiole. The muscularis mucosa consists of a few layers of spirally wound, smooth muscle, which are reduced to one layer and to occasional cells in the respiratory bronchioles. The adventitial layer is composed of loose connective tissue with many elastic fibers that are continuous with those of the surrounding lung tissues. Respiratory bronchioles are distinguished from tertiary bronchioles by having direct communication with alveoli. In the dog, the respiratory bronchiole represents the typical distal airway because the tertiary bronchiole is very short.

The alveolar ducts extend distad to the respiratory bronchiole and open directly into alveoli. They are lined by simple squamous or simple cuboidal epithelium identical to that of the alveoli. Sparse amounts of elastic connective tissue fibers and occasional muscle cells are found beneath the epithelium.

Large sac-like structures terminate the alveolar ducts made up of alveolar septa. Many alveoli open into these structures; they are called alveolar sacs. The alveoli are lined by squamous alveolar epithelial cells (type I cells). Interspaced between these cells are cuboidal epithelial cells (type II cells) that are thought to produce surfactant. A third cell type is the alveolar marcophage (dust cell) (Dellman, et al., 1976).

Technique of Selective Bronchial Catheterization and Bronchial Brushing

Selective bronchial catheterization and bronchial brush biopsy are accomplished in the human patient by one of two techniques after the affected area of the lung has been identified by radiography. The radiopaque polyethylene catheter is introduced into the trachea by passage through natural airways, either the nose (Backus, 1971; Bean, et al., 1968; Bibbo, et al., 1973; Fennessy, 1966; Fennessy, 1967; Fennessy, 1968; Fennessy, et al., 1970; Genoe, 1974; Janower and Richard, 1971; Penido, et al., 1972; Pierce and Jacobs, 1969; Willson and Eskridge, 1970; Zavala, et al., 1972) or mouth (Fennessy, et al., 1970; Willson and Eskridge, 1970). In the alternative technique, catheterization is accomplished by the passage of the catheter through the cricothyroid membrane (Rockoff, 1974). Anesthesia for both techniques is accomplished by premedication with atropine, a narcotic or non-narcotic tranquilizer, and local anesthesia of the respiratory tract (Backus, 1971; Fennessey, 1966; Fennessy, 1967; Fennessy, 1968; Fennessy, et al., 1970; Janower and Richard, 1971; Penido, et al., 1972; Rockoff, 1974; Willson and Eskridge, 1970).

The catheter is directed to the desired bronchus under fluoroscopic control. Some clinicians prefer to preshape the catheter to facilitate entry of the diseased bronchus (Bibbo, et al., 1973; Fennessy, 1966; Fennessy, 1967; Janower and Richard, 1971; Rockoff, 1974; Smith and Warrack, 1972). Introduction of this preshaped catheter can be facilitated if a guide wire is used (Backus, 1971; Bean, et al., 1968; Fennessy, 1967; Fennessy, 1968; Fennessy, et al., 1970; Genoe, 1974; Zavala, et al., 1972). Other authors use a two catheter system, the larger being preshaped to the

lobar bronchus and the smaller or inside catheter being straight. A controllable guide wire is used to guide the catheter to the lesion (Willson and Eskridge, 1970). Some clinicians have developed guided catheter systems in which the shape of the catheter can be changed mechanically while inside the bronchial tree (Pierce and Jacobs, 1969; Zavala, et al., 1972). After placement of the catheter, radiographs are taken in multiple views to insure the desired area of lung has been catheterized. A small nylon brush is passed through the catheter and into the lesion (Backus, 1971; Bean, et al., 1968; Bibbo, et al., 1973; Fennessy, 1967; Fennessy, 1968; Fennessy, et al., 1970; Fennessy, et al., 1973; Fennessy and Kittle, 1973; Genoe, 1974; Hattori, et al., 1964; Janower and Richard, 1971; Rockoff, 1974; Willson and Eskridge, 1970). Steel brushes are recommended when the catheter is against the lesion (Backus, 1971; Bibbo, et al., 1973; Fennessy, et al., 1970) or in diffuse lung disease (Fennessy, 1968; Fennessy, 1974; Fennessy and Kittle, 1973). The brushes may be deflectable, meaning that the brush tip can be deflected independently of the catheter (Bibbo, et al., 1973; Fennessy and Kittle, 1973; Fennessy, et al., 1973; Genoe, 1974; Hattori, et al., 1964; Willson and Eskridge, 1970) or of the non-deflectable variety (Bean, et al., 1968; Fennessy, 1968; Janower and Richard, 1971). One article described the use of an acrylic sponge in place of the brush (Smith and Warrack, 1972). After brushing the lesion vigorously, the brush is removed from the catheter and slides are prepared for microscopic evaluation.

Many authors have combined selective bronchial catheterization and bronchial brush biopsy with bronchial washing for cytology and culture,

Fennessy, et al., 1970; Genoe, 1974), forceps biopsy (Fennessy, 1968), selective bronchography (Bean, et al., 1968; Fennessy, 1968; Genoe, 1974; Hattori, et al., 1964; Janower and Richard, 1971; Penido, et al., 1972), or a combination of all four techniques using the same catheter (Fennessy, 1967; Fennessy, et al., 1973; Fennessy and Kittle, 1973; Rockoff, 1974). Some authors have reported submitting the actual brushes for bacterial culture (Backus, 1971; Bibbo, et al., 1973; Fennessy, 1967; Fennessy, 1968; Fennessy, et al., 1970; Fennessy, et al., 1973; Fennessy and Kittle, 1973; Murphy and Komorowski, 1974; Rockoff, 1974) or viral culture (Fennessy, et al., 1973; Fennessy and Kittle, 1973).

Slides and histologic specimens are prepared by various methods, depending on the laboratory. Slides are made directly from the nylon brush and fixed in 95% ethyl alcohol (Bean, et al., 1968; Bibbo, et al., 1973; Fennessy, 1967; Fennessy, 1968; Fennessy, et al., 1970; Fennessy, et al., 1973; Fennessy and Kittle, 1973; Hattori, et al., 1964; Janower and Richard, 1971; Murphy and Komorowski, 1974; Skitarelic and Worthamm, 1974; Solomon, et al., 1974; Zavala, et al., 1972). Some workers submit air dried specimens, along with the alcohol-fixed slides, for bacterial staining (Bean, et al., 1968; Fennessy, et al., 1973; Fennessy and Kittle, 1973). Wet preparations are most often stained by the papanicolaou method (Bibbo, et al., 1973; Hattori, et al., 1965; Murphy and Komorowski, 1974; Skitarelic and von Hamm, 1974; Zavala, et al., 1972). Steel brushes are placed in 10% formalin, and the tissue adherent to the brush is teased off with a forceps after the tissue is fixed. The tissue is then collected, embedded in a paraffin-block, sectioned and stained by routine hemotoxylin

and eosin techniques (Bibbo, et al., 1973; Fennessy, et al., 1970; Fennessy, et al., 1973; Fennessy and Kittle, 1973).

One author suggests soaking the brushes in saline and spinning down the cellular material to be passed through a millipore filter to collect the cells for staining (Zavala, et al., 1972). The clinician using the sponge submits the sponge to be embedded, sectioned, and stained with hemotoxylin and eosin (Smith and Warrick, 1972).

Complications of Bronchial Brushing

Several minor complications have been reported in the human including slight febrile reactions (Fennessy, 1967; Fennessy, 1968; Fennessy, et al., 1970; Janower and Richard, 1971), blood tinged sputum (Fennessy, et al., 1970), and slight epistaxis due to catheter trauma (Fennessy, 1967; Fennessy, 1968; Janower and Richard, 1971). More serious complications include pneumothorax, which has not resulted in deaths (Fennessy and Kittle, 1973; Fennessy, et al., 1973; Janower and Richard, 1971; Rockoff, 1974), gross hemoptysis (Bean, et al., 1968), and hemorrhage, which has resulted in the death of two patients (Fennessy and Kittle, 1973; Fennessy, et al., 1973).

When the trachea is approached through the cricothyroid membrane, about one-third of the patients will develop subcutaneous emphysema that resolves spontaneously in 24 to 48 hours (Rockoff, 1974). Massive hemorrhage occurred when an anomalous right cricothyroid artery, which crossed the cricothyroid membrane, was lacerated with the needle and pushed into the tracheal lumen drowning the patient (Shillaci, et al., 1976).

Some clinicians report loosing the tip of a brush in the bronchial tree. These were retrieved by forceps inserted through the catheter (Fennessy, 1968; Fennessy, et al., 1970; Fennessy and Kittle, 1973). Septicemia, pneumonia, and lidocaine reactions have been reported (Fennessy and Kittle, 1973).

Cytology Obtained From the Lower Respiratory Tract of Animals

Roszel described the use of cytology in the diagnosis of disease of several organ systems of animals. Included in this series were five cases of respiratory neoplasia of which 2 were tumors of the lower respiratory tract with neoplastic cells in bronchial washings (Roszel, 1967).

Creighton and Wilkins (1974a) described ciliated columnar to cuboidal epithelial cells in aspirates from normal and abnormal dogs. Reactive hyperplastic epithelial cells (histiocytes) were seen in chronic inflammatory processes. Neoplastic cells were observed in some cases where cancer was suspected. Many types of inflammatory cells including neutrophils, eosinophils, lymphocytes, plasma cells, and mast cells were observed. Also, macrophages were seen. Foreign material such as bacteria, parasitic microfilaria, fungal elements, and anthracitic pigments were observed. Viral inclusion bodies were identified in 2 dogs (Creighton and Wilkins, 1974a).

In another paper evaluating the transtracheal aspiration biopsy on 75 canine patients, Creighton and Wilkins (1974b) defined eight cellular patterns they felt to be indicative of specific types of neoplastic or inflammatory disease. The cytology was compared with the other data available on these patients. They concluded that cytology is useful in further

defining diseases of the lower respiratory tract, but should be used only in conjunction with other diagnostic techniques (Creighton and Wilkins, 1974b).

One author reported that the greatest percentage of cells, in bronchial wash solutions from normal dogs, were either macrophages or epithelial cells. Neutrophils averaged 1.3% of the cells seen in bronchial washings from normal dogs; with lymphocytes, basophils and mast cells also being observed (Patterson, et al., 1974).

Washings obtained through a rigid bronchoscope, from patients which were eventually confirmed to have primary pulmonary neoplasia, contained neoplastic cells. These tumors included squamous cell carcinoma, adenocarcinoma, mixed squamous and adenocarcinoma, large and small cell undifferentiated carcinoma, and alveolar cell carcinoma. Aspirates from dogs with metastatic disease were not so rewarding because most of these tumors did not involve the tracheobronchial tree (O'Brien and Roszel, 1974).

A cellular pattern suggestive of canine distemper has been proposed and is characterized by intracytoplasmic inclusion bodies in the bronchial epithelial cells (O'Brien and Roszel, 1974). Chodosh, et al., suggests that in washings from human patients in which the predominant cells are eosinophils, a diagnosis of allergic bronchitis is appropriate and those in which neutrophils predominate (60-90%) are suggestive of an inflammatory disease. He also stated that the alveolar macrophage is a sensitive indicator of cellular response, and a paucity of these cells is commonly the first sign of acute inflammatory disease processes. A marked increase in these cells denotes that the cellular response is good and is usually followed by recovery (Chodosh, et al., 1970).

No reports of cytology obtained by bronchial brush biopsy were found from animal patients.

Results Obtained by Bronchial Brushing in Human Patients

The results of bronchial brush biopsies performed on human patients have been reported by several clinicians and are summarized in Tables 1, 2, and 3.

The accuracy of this technique in the diagnosis of primary pulmonary disease in the human patient has been reported as low as 62% and as high as 92%, with most clinicians reporting about 70% of the confirmed cases to have malignant cells in the bronchial brush biopsy. Tabulation of the data in Table 1 demonstrates that 73% of the patients with confirmed primary pulmonary neoplasia had malignant cells in the biopsy specimens.

Data on the effectiveness of this technique for diagnosis of metastatic lung disease is less abundant. Tabulation of the reports listed in Table 2 indicates that 51% of those patients biopsied that were later confirmed to have metastatic pulmonary disease had neoplastic cells in the bronchial brush biopsy.

Reports of bronchial brush biopsy as a diagnostic tool in non-neoplastic disease are few and difficult to interpret because in many cases, no definitive diagnosis is obtained. Tabulation of the data in Table 3 shows the bronchial brush biopsy to be helpful in the diagnosis of 16% of the confirmed cases of inflammatory disease. One clinician states that the most common radiographic diagnosis of pulmonary infiltrates is that of a non-specific inflammation that usually does not extend into the bronchial tree, making the results of bronchial brush biopsy unrewarding (Fennessy, 1968).

TABLE 1. BRONCHIAL BRUSH BIOPSY IN HUMAN PATIENTS WITH PRIMARY PULMONARY NEOPLASIA

Reference	Confirmed Primary Pulmonary Neoplasia	Neoplastic Cells in Biopsy	False Positives For Neoplasia
Hattori, et al., 1964	14	13 (92%)	0
Fennessy, et al., 1973	224	157 (70%)	9
Willson and Eskridge, 1970	44	35 (80%)	0
Solomon, et al., 1974	47	41 (87%)	0
Fennessy, 1967	37	23 (62%)	0
Murphy and Komorowski, 1974	20	13 (65%)	0
Fennessy, 1968	50	32 (64%)	0
Fennessy and Kittle, 1973	238	182 (76%)	0
Fennessy, et al., 1970	117	83 (71%)	0
Penido, et al., 1972	56	40 (71%)	1
Bean, et al., 1968	57	39 (68%)	0
Zavala, et al., 1972	73	57 (78%)	0
Genoe, 1974	23	16 (70%)	0
Smith and Warrack, 1972	27	18 (67%)	4
Skitarelic and von Haam, 1974	47	40 (85%)	0
	1074	789 (73%)	14

TABLE 2. BRONCHIAL BRUSH BIOPSY IN HUMAN PATIENTS WITH SECONDARY PULMONARY NEOPLASIA

Reference	Confirmed Metastatic Pulmonary Neoplasia	Neoplastic Cells in Biopsy
Fennessy, et al., 1973	30	16 (53%)
Willson and Eskridge, 1970	1	0 (0%)
Fennessy, 1967	6	2 (33%)
Murphy and Komorowski, 1974	2	2 (100%)
Fennessy, 1968	9	4 (44%)
Fennessy, et al., 1970	15	8 (53%)
	63	32 (51%)

TABLE 3. BRONCHIAL BRUSH BIOPSY IN HUMAN PATIENTS WITH CONFIRMED INFLAMMATORY DISEASE

Reference	Confirmed Inflammatory Disease	Brush Biopsy Aided Diagnosis[a]
Fennessy, 1967	50	4 (8%)
Murphy and Komorowski, 1974	15	5 (33%)
Fennessy, 1968	27	6 (22%)
	92	15 (16%)

[a]Cytology or culture of brush for virus, bacteria, or fungi was helpful in diagnosis.

Several clinicians comment that the most common cause for failure of this technique is improper catheter placement and failure to verify placement of the brush by radiographs in at least 2 views (Bibbo, et al., 1973; Fennessy, 1967; Fennessy, 1968). Another cause for failure is a sharp curve (Bibbo, et al., 1973) or stenosis (Skitarelic and von Haam, 1974) in the diseased bronchus making it impossible to catheterize. Also, placement of the brush in the necrotic center of a tumor produces a lack of neoplastic cells in the biopsy and a false negative result (Bibbo, et al., 1973). Bean emphasizes that the lesion must extend into the bronchus for the brush biopsy to be successful (Bean, et al., 1968).

One clinician suggests that the bronchial brush biopsy is most useful when the lesion is at the level of the second or third generation bronchi (Firestone, 1973), while another clinician emphasizes the usefulness of the technique in the diagnosis of more peripheral pulmonary lesions (Fennessy, et al., 1970).

It has been shown that bronchial brush biopsy increases the number of pulmonary cancer patients with neoplastic cells in their sputum. In one group of patients, 106 had neoplastic cells in their sputum after bronchial brush biopsy of 160 that were negative before the procedure (Fennessy and Kittle, 1973).

The cells obtained by bronchial brushing were well preserved and in sheets, while those obtained by washing showed some degree of degeneration and were isolated or in well defined clumps. This study suggested there is no advantage to performing a bronchial wash combined with a well performed bronchial brush (Bibbo, et al., 1973).

One clinician showed that in contrast to brushing, the diagnosis yield obtained by bronchial washing was small. In 46 bronchial washings, only 7 contained malignant cells, while 46 of these confirmed cases of pulmonary neoplasia had diagnostic bronchial brush biopsies (Solomon, et al., 1974).

Some authors report that controllable brush (deflectable tip) systems decrease the time necessary to perform the procedure and increase the range of lung field accessible by this technique (Willson and Eskridge, 1970).

Zavala states that a mobile catheter system increases the diagnostic accuracy of this technique by 10% (Zavala, et al., 1972).

Fennessy and his co-workers found that transtracheal brush or forceps biopsy were most successful in the diagnosis of primary pulmonary neoplasia and of less value in metastatic pulmonary disease. Also, it was of value in the diagnosis of opportunist pulmonary infections such as aspergillosis or Pneumocystis carinii when other diagnostic methods failed. They suggest that the lack of diagnostic biopsy in many cases of inflammatory disease may be due to previous antibiotic therapy (Fennessy, et al., 1973).

MATERIALS AND METHODS

Equipment for Selective Bronchial Catheterization and Bronchial Brushing

Selective bronchial catheterization and bronchial brush biopsy required the use of an image intensified fluoroscope. It was necessary to perform this technique under general anesthesia. The capability of inhalation anesthesia was not essential but was preferred, as usually dogs with respiratory disease are less than ideal anesthetic risks.

Bronchial catheters used with nylon brushes were made from 75cm lengths of .071 inch inside diameter and .093 inch outside diameter polyethylene tubing[a]. Stainless steel bronchial brushes required a larger catheter (.085 inch inside diameter and .110 inch outside diameter). The tip of the catheter to be inserted into the bronchial tree was curved approximately 65° at a distance of 1.5cm from the tip. This allowed easier entry into some of the second and third generation bronchi (Figure 1).

Two types of bronchial brushes were employed; a disposable 1.72mm diameter nylon brush[b] mounted on a 100cm deflectable tip guide wire (Figures 2 and 3), and a disposable 1.72mm diameter stainless steel bronchial brush[b] attached to a 100cm non-deflectable guide wire.

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[a]Formocath, Becton-Dickson, Rutherford, NJ.

[b]Mill-Rose Company, Mentor, OH.

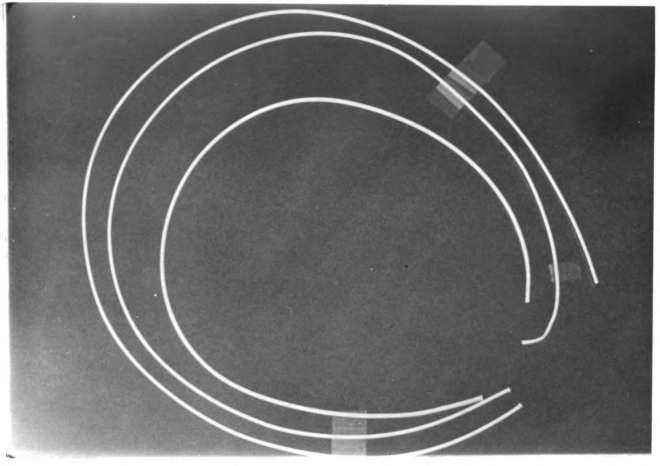

FIGURE 1. POLYETHYLENE CATHETERS USED IN SELECTIVE BRONCHIAL CATHETERIZATION FOR BRONCHIAL BRUSHING.

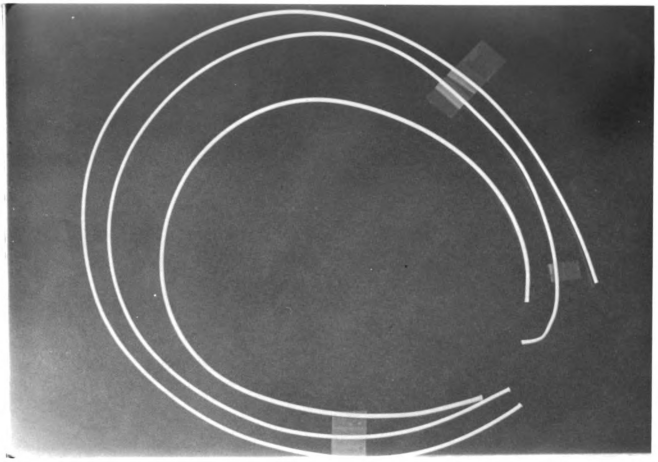

FIGURE 1. POLYETHYLENE CATHETERS USED IN SELECTIVE BRONCHIAL CATHETERIZATION FOR BRONCHIAL BRUSHING.

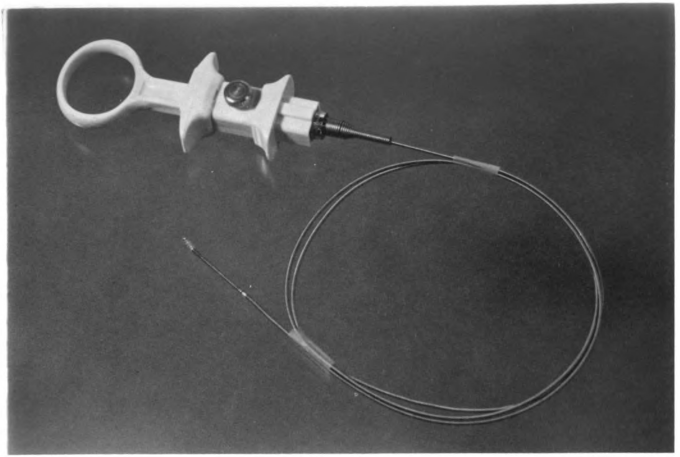

FIGURE 2. 1.72mm NYLON BRONCHIAL BRUSH MOUNTED ON A 100cm DEFLECTABLE TIP GUIDE WIRE.

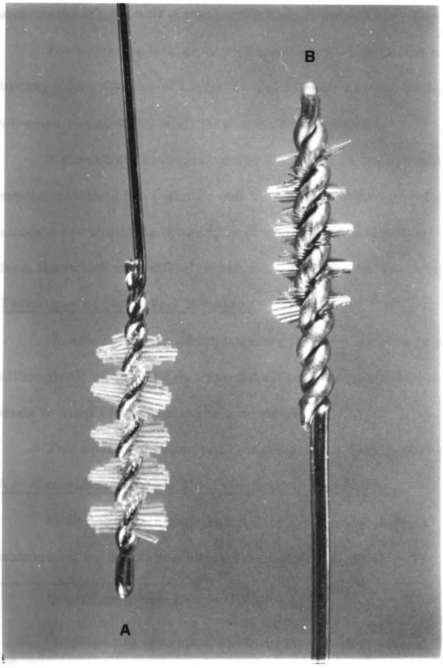

FIGURE 3. 1.72mm NYLON (A) AND STEEL (B) BRONCHIAL BRUSHES.

Access to the endotracheal tube while maintaining ventilatory support was accomplished by the use of a Norman Mask Elbow[c] (Figure 4) or a disposable swivel adapter[d] inserted in the anesthetic system between the endotracheal tube and the anesthesia machine delivery hoses.

For selective bronchial catheterization through the cricothyroid membrane, a No. 10 scalpel blade, 14 ga. 1½ inch needle, and a 100cm guide wire are required in addition to the above material.

A fenestrated surgical drape was used in both techniques to prevent contamination of the catheter and brushes. Several standard microscopic slides were available at the time of biopsy and slides were prepared directly from the brush immediately upon removal from the bronchus.

Technique of Bronchial Brushing

No attempt was made to perform this procedure in a sterile manner, although all equipment was sterilized between animals and an effort was made to keep contamination to a minimum.

Two routes of bronchial catheterization were performed and evaluated.

A. Catheterization via the cricothyroid membrane

Four dogs were anesthetized with thiamylal sodium[e], intubated and maintained by inhalation anesthesia using halothane[f]. The dogs were placed

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[c]North American Drager, Telford, PA.

[d]Olympus Corporation of America, New Hyde Park, NY.

[e]Surital, Park Davis, Co., Detroit, MI.

[f]Fluothane, Ayrest Laboratories Incorporated, New York, NY.

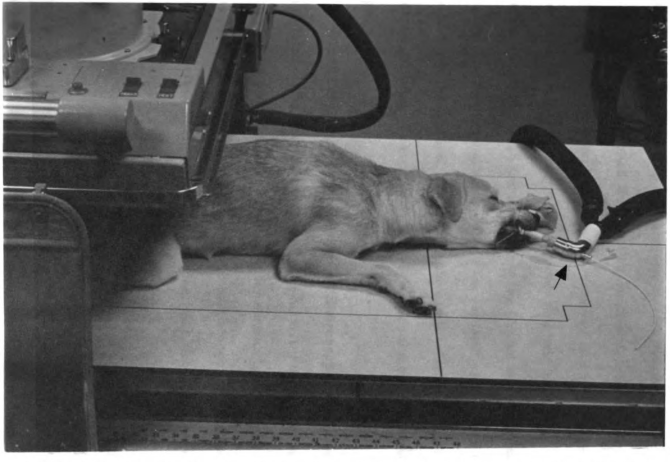

FIGURE 4. NORMAN MASK ELBOW (arrow) INSERTED IN THE ANESTHETIC SYSTEM WITH BRONCHIAL CATHETER INSERTED. THE DRAPE HAS BEEN REMOVED FOR PHOTOGRAPHIC ILLUSTRATION.

in dorsal recumbency and their laryngeal areas clipped and surgically prepared. A fenestrated surgical drape was placed over the dog and table to prevent contamination of the catheter and brushes. The cricothyroid membrane was palpated between the cricoid and thyroid cartilages of the larynx with a gloved finger and a small stab incision made over the membrane. The endotracheal tube was removed and a 14 gauge needle passed through the cricothyroid membrane and into the larynx. A guide wire was passed through the needle into the larynx and directed into the trachea. The needle was removed and the catheter placed over the guide wire and into the trachea, after which the guide wire was removed. The endotracheal tube was replaced and the dog positioned under the fluoroscope.

With the aid of the image intensified fluoroscope, the catheter was manipulated to the desired bronchus. The majority of the catheter manipulation was done with the dog in ventral recumbency. The right and left principle bronchi along with the caudal lobar bronchi of the caudal lobes were catheterized using a straight catheter. All other bronchi were catheterized with a curved catheter.

The right or left cranial lobar bronchi were catheterized by directing the catheter into the principle bronchus of the side desired and its tip placed beyond the orifices of the cranial lobar bronchi. The tip of the catheter was then directed towards the lateral wall of the bronchus and the catheter withdrawn until the tip entered the cranial lobar bronchus. The catheter was then directed either dorsal or ventral and advanced depending on the segmental bronchus desired (Figures 5, 6, 7, 8). To catheterize the

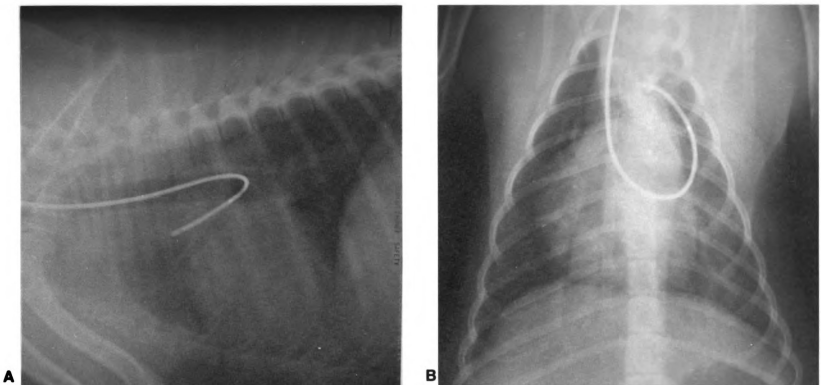

FIGURE 5. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 1 YEAR OLD BEAGLE DOG DEMONSTRATING SELECTIVE CATHETERIZATION OF THE CRANIAL SEGMENTAL BRONCHUS OF THE LEFT CRANIAL LOBE.

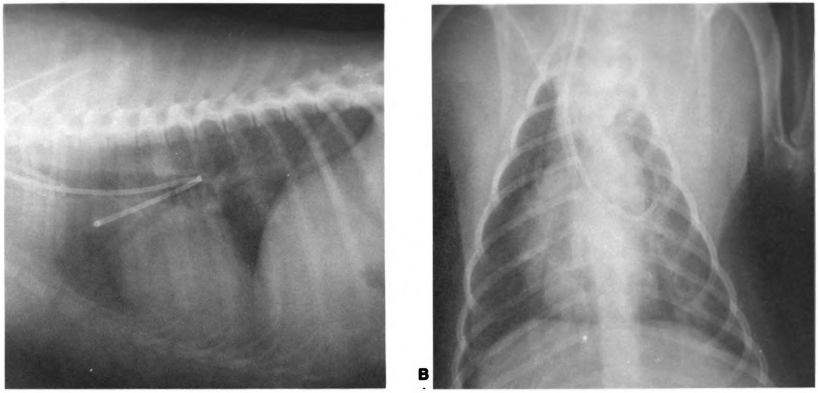

FIGURE 6. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 1 YEAR OLD BEAGLE DOG DEMONSTRATING SELECTIVE CATHETERIZATION OF THE CAUDAL SEGMENTAL BRONCHUS OF THE LEFT CRANIAL LOBE.

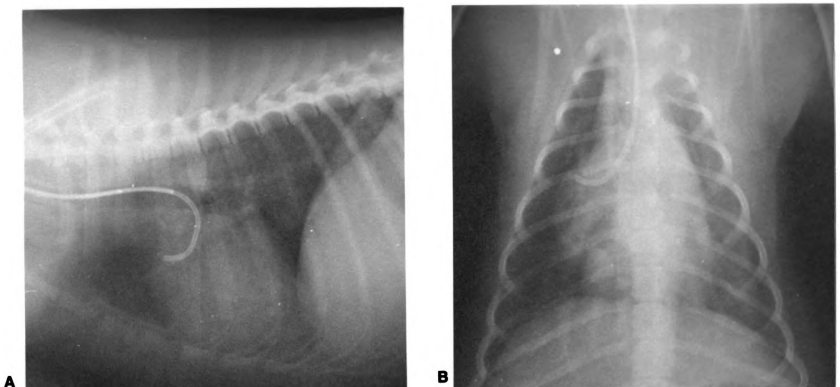

FIGURE 7. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 1 YEAR OLD BEAGLE DOG DEMONSTRATING SELECTIVE CATHETERIZATION OF THE CRANIAL SEGMENTAL BRONCHUS OF THE RIGHT CRANIAL LOBE.

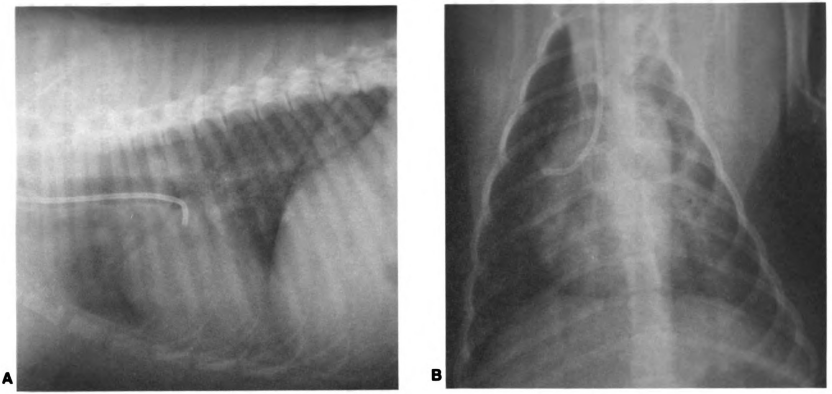

FIGURE 8. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 1 YEAR OLD BEAGLE DOG DEMONSTRATING SELECTIVE CATHETERIZATION OF THE CAUDAL SEGMENTAL BRONCHUS OF THE RIGHT CRANIAL LOBE.

right middle lobar bronchus, the tip of the catheter was directed down the right principle bronchus past the orifice of the right middle lobar bronchus. The tip was directed ventro-lateral and the catheter withdrawn until the tip entered the middle lobar bronchi. The catheter was then advanced into the ventral segmental bronchus (Figure 9). The intermediate lobar bronchus was catheterized by directing the catheter down the right principle bronchus with the tip directed ventro-medial until the intermediate lobar bronchus was entered. The catheter tip was then directed either dorsal or ventral (Figure 10) depending on the segmental bronchus desired. The animal was then positioned in lateral recumbency to insure proper placement of the catheter. The bronchial brush was passed through the catheter and into the bronchus and the biopsy collected by moving the brush vigorously over the bronchial wall. The brush was pulled back into the tip of the catheter to prevent contamination of the biopsy material during withdrawal. The catheter, along with the brush, was then withdrawn from the animal. Slides were prepared as described later. The entry into the trachea through the cricothyroid membrane was not sutured. The animals were observed for complications.

B. Catheterization via the endotracheal tube

The second method was to catheterize the bronchus through the endotracheal tube. Four dogs were anesthetized as previously described. An adapter was placed in the anesthetic system at the junction of the endotracheal tube and the hoses (Figure 4). A fenestrated surgical drape was then placed over the dog and table to protect the catheter and brushes from contamination. The catheter was introduced through the adapter, into the

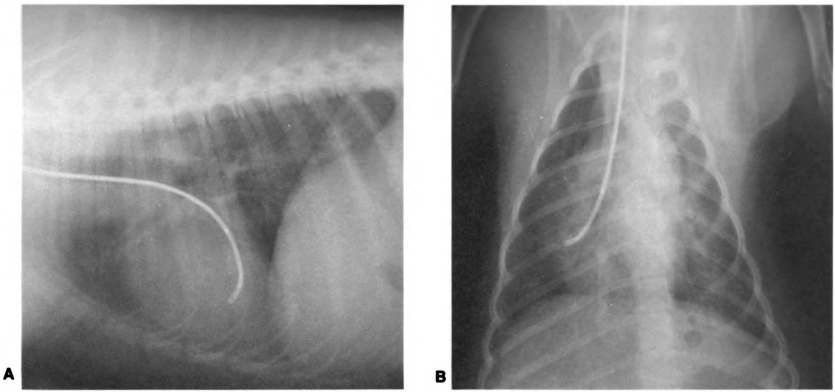

FIGURE 9. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 1 YEAR OLD BEAGLE DOG DEMONSTRATING SELECTIVE CATHETERIZATION OF THE VENTRAL SEGMENTAL BRONCHUS OF THE RIGHT MIDDLE LOBE.

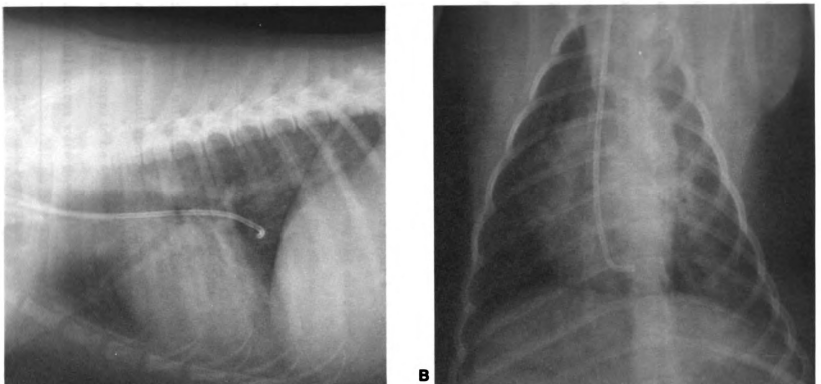

FIGURE 10. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 1 YEAR OLD BEAGLE DOG DEMONSTRATING SELECTIVE CATHETERIZATION OF THE VENTRAL SEGMENTAL BRONCHUS OF THE INTERMEDIATE LOBE.

endotracheal tube and positioned with the aid of the image intensified fluoroscope as was previously described. The animals were observed in two views to confirm catheter position. The brush was passed through the catheter and into the bronchus (Figure 11). The biopsy was taken by vigorously brushing the bronchial wall. The brush was then pulled back into the tip of the catheter to protect the biopsy while the brush and catheter were removed from the dog (Figure 12). Slides were prepared from the brush after it was pushed from the tip of the catheter (Figure 13). The dogs were allowed to recover from anesthesia and observed for complications.

Preparation of Slides for Cytologic Evaluation

Slides were prepared from nylon bronchial brushes using 2 different techniques. The touch technique was performed by touching the brush to the slide in several areas. The smear technique was performed by rolling the brush as it was pushed along the slide resulting in smearing of the biopsy material (Figure 14). The slides were air dried, stained with modified polychrome methylene blue[g], and cover slips applied.

Evaluation of Cytologic Preparations

Comparison of the cytology obtained by each technique was accomplished utilizing the criteria listed in Table 4. All slides were evaluated without knowledge of their sources. These criteria allowed numerical scores to be alloted to each slide for each area of interest. The numerical scores were then added for all slides from the same technique and

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[g]Hema-Tek Stain Pak, Ames Co., Division of Miles Labs, Inc., Elkhardt, IN.

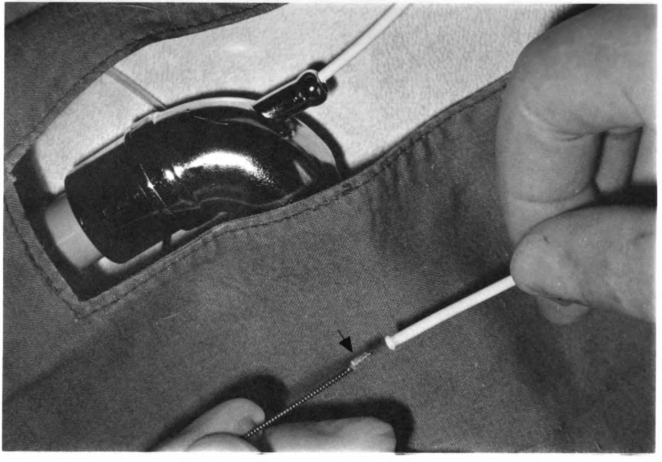


FIGURE 11. BRONCHIAL BRUSH (arrow) BEING INSERTED INTO THE CATHETER PREVIOUSLY THROUGH THE ENDOTRACHEAL TUBE VIA THE NORMAN MASK ELBOW.

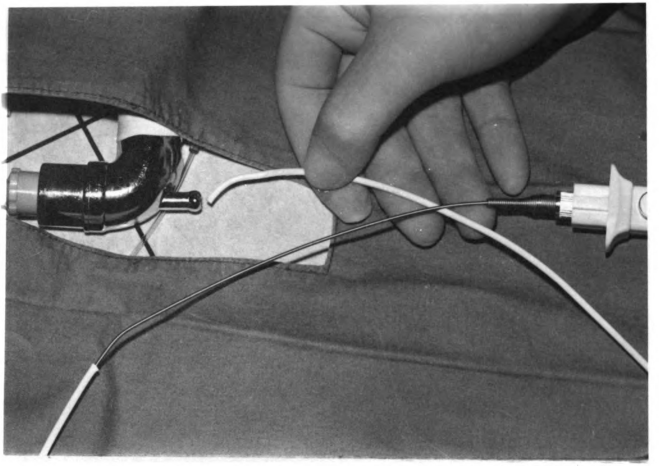


FIGURE 12. CATHETER WITH BRONCHIAL BRUSH INSIDE BEING REMOVED FROM THE DOG FOLLOWING BRUSHING OF A BRONCHUS.

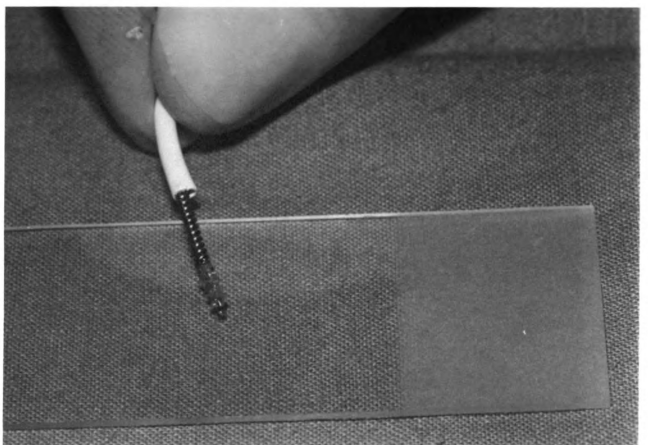


FIGURE 13. PREPARATION OF SLIDE WITH BRUSH PUSHED BEYOND THE TIP OF THE CATHETER.

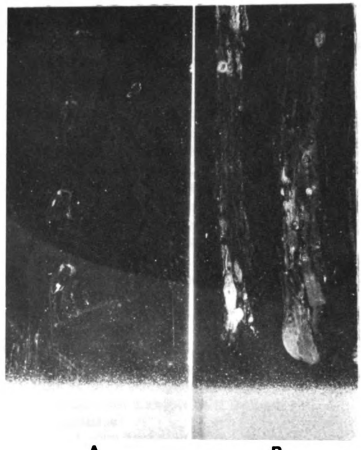


FIGURE 14. TOUCH (A) AND SMEAR (B) TECHNIQUES FOR PREPARATION OF BRONCHIAL BRUSH CYTOLOGIC PREPARATIONS.

TABLE 4. CRITERIA FOR EVALUATION OF CYTOLOGIC PREPARATIONS

		Numerical Score
Readable Fields With 4 X Objective:		
Excellent	- Approximately 10 Readable Fields	3
Adequate	- Approximately 5 Readable Fields	2
Poor	- 2 or Less Readable Fields	1
Hemorrhage:		
Excessive	- Number of RBC's Exceeds Nucleated Cells	1
Moderate	- Equal Number of RBC's and Nucleated Cells	2
Minimal	- Nucleated Cells Exceed RBC's	3
Nuclear Detail:		
Excellent	- Nuclei Stain Darkly Basophilic	3
	- Good Differential Staining of Nucleus	
	- Even Distribution of Nuclear Chromatin	
	- Nuclear Membranes Clearly Defined	
Adequate	- Nuclear Membranes Generally Intact	2
	- Nuclear Staining Less Basophilic	
	- Nucleoli Somewhat Prominent	
Poor	- Poorly Defined Nuclear Membranes	1
	- Bare Nuclei Present	
	- Cells Appear Nonviable With Predominant Nucleoli	
Cytoplasmic Detail		
Excellent	- Cytoplasmic Membranes Intact	3
	- Cilia Present on Many Cells	
	- Pink to Blue Background Stain	
	- Granulocytes Exhibit Well Defined Granules	
Adequate	- Cytoplasmic Membranes Generally Intact	2
	- Differential Staining is Fair (Lighter Background)	
	- Some Epithelial Cells Exhibit Cilia	
	- Granulocytic Granules Identifiable	
Poor	- Bare Nuclei	1
	- Cytoplasmic Membranes Not Intact	
	- Excessive Dispersion of Granulocytic Granules	

their means compared by application of the student 't' test. If the variance of the means were not equal, a modification of the test was used where no pooled variance term is used (Steel and Torrie, 1960). The level of significance was established at .05 (p 0.05) for all statistical analysis of data.

Evaluation of Nylon Bronchial Brushes vs. Steel Bronchial Brushes

Four dogs had biopsies performed with nylon brushes at 4 different anatomical locations within the bronchial tree. These dogs were killed 24, 48, 72, and 96 hours after biopsy. Gross and histologic examinations of the biopsy sites were performed. Four dogs were biopsied with steel brushes and the same observations made.

Ten slides were randomly chosen for comparison of the cytology from each of the 2 groups and were evaluated as previously described.

Evaluation of Nylon Bronchial Brushing vs. Bronchial Washing in Experimental Inflammatory Disease

Four clinically normal Beagles with normal thoracic radiographic patterns were used in this portion of the study. The dogs were anesthetized with pentobarbital sodium and endotracheal tubes inserted.

A bronchial wash was performed by instilling 10-20cc of normal saline into the bronchial tree through a catheter that had been passed through the endotracheal tube. Suction was applied to the catheter recovering as much of the saline from the bronchial tree as possible. The bronchial washings were prepared for cytological examination by centrifuging at 3,000 rpm for 7-10 minutes and removing the supernate by pippette. The sediment was smeared on glass slides and air dried for staining and application of cover slips as previously described.

Two bronchial brush biopsies were obtained from each of the right and left lungs using the endotracheal tube technique previously described. Slides were prepared using the smear technique and stained with modified polychrome methylene blue.

The right main bronchus was selectively catheterized and 6ml of 60% propyliodone suspended in peanut oil[h] was instilled in the right lung with the intent to create inflammatory disease (Myer, et al., 1974). Its distribution was recorded radiographically. The left lung served as a control.

The dogs were evaluated at daily intervals for 4 days. At each evaluation, the clinical status of the dogs was noted and thoracic radiographs taken. Two bronchial washings were performed and two bronchial brush biopsies from each lung were obtained. On the fourth day the dogs were killed. The lungs and bronchial tree were examined for gross lesions and tissues were collected for microscopic examination.

The bronchial brushings and bronchial washings were evaluated using the criteria in Table 4. The inflammatory response was evaluated in the bronchial washings and brushings according to the criteria in Table 5.

TABLE 5. CRITERIA FOR EVALUATION OF INFLAMMATORY RESPONSE IN BRONCHIAL CYTOLOGIC PREPARATIONS

	Numerical Score
Excessive - Number of Inflammatory Cells Exceeds Epithelial Cells	3
Moderate - Number of Inflammatory Cells Equal to Epithelial Cells	2
Minimal - Number of Epithelial Cells Exceeds Inflammatory Cells	1

[h]Dionosil Oily, Glaxo Laboratories, Ltd., Greenford, England.

The inflammatory response in the bronchial washings and brushings were compared to find differences in their ability to detect inflammatory disease. The cytologic preparations obtained by bronchial brushing were compared to the radiographic appearance and post-mortem findings in the lung of origin. Brush biopsies from affected and non-affected lungs in the same animal were compared to evaluate the selectivity of the bronchial brush biopsy. The cytologic preparations from both the bronchial brush biopsies and bronchial washings were compared to the clinical status and radiographic appearance of the thorax in each dog to measure the sensitivity of each method to detect sub-clinical inflammatory disease.

Evaluation of Bronchial Brushing in Dogs with Clinical Disease

Selective catheterization and nylon bronchial brush biopsy was performed in 19 clinical patients. Results of the bronchial brush biopsy were compared to clinical; endoscopic and radiogrpahic findings; hematology; gross pathological and histo-pathological findings when available.

RESULTS

Technique of Selective Bronchial Catheterization and Bronchial Brushing

Transtracheal catheterization via the cricothyroid membrane resulted in the following observations. While performing the technique on one dog, difficulty was encountered in passing the needle through the cricothyroid membrane and into the trachea. In 3 of 4 dogs, it was difficult to pass the catheter over the guide wire and into the trachea because of resistance by the tissues around the guide wire. In one dog, this became so time consuming, this approach was abandoned and the bronchi were catheterized through the endotracheal tube. One dog presented no difficulty during any part of the procedure. No difficulty was encountered in manipulating the catheter after it was in the trachea and the endotracheal tube replaced. Forty to 60 minutes were needed to perform catheterization by this technique.

One larynx was examined 24 hours after the catheterization revealing no significant gross lesions.

While performing the bronchial catheterization through the endotracheal tube, resistance was met when attempting to pass the catheter through the adapter and into the endotracheal tube in 2 dogs. This was overcome by disconnecting the adapter and passing the catheter through the adapter and into the endotracheal tube. Subsequently, the adapter was

reconnected to the endotracheal tube. Bronchial catheterization by this technique requires 20 to 30 minutes to complete.

The right and left main bronchi and the right middle lobar bronchus were the easiest to catheterize. The right and left cranial lobar bronchi were the most difficult to catheterize and required the curved tipped catheters. The bronchial brush could be passed to the periphery of all lobes.

Cytology obtained by selective bronchial catheterization and bronchial brush biopsy from normal dogs has as its primary cell type ciliated bronchial epithelial cells. Goblet cells were seen in fewer numbers. A few neutrophils were present in most fields, with lesser numbers of eosinophils and mast cells observed (Figure 15).

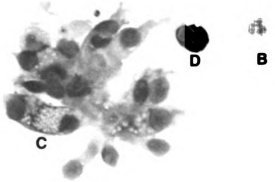
Preparation of Slides for Cytologic Evaluation

Technically, there was no difference in difficulty in preparing the slides by the touch or smear technique.

The results of evaluation of the cytologic preparations resulting from these techniques are presented in Table 6. No difference was seen in the amount of hemorrhage, nuclear detail, or cytoplasmic detail. A significantly greater number of readable fields was seen in the slides prepared by the smear technique.

Evaluation of Nylon Bronchial Brushes vs. Steel Bronchial Brushes

When using steel brushes, the need for a larger catheter resulted in more difficulty catheterizing the desired bronchus than with nylon bronchial brushes. Since steel brushes are more difficult to pass through the catheter and do not have a deflectable tip, collection of the biopsy is



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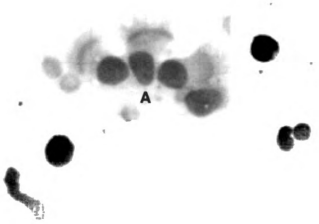

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FIGURE 15. CYTOLOGIC PREPARATIONS FROM A BRONCHIAL BRUSH BIOPSY OF A NORMAL DOG. CILIATED EPITHELIAL CELLS (A), NEUTROPHIL (B), GOBLET CELL (C), MAST CELL (D) CAN BE IDENTIFIED IN THESE PREPARATIONS MADE USING THE SMEAR TECHNIQUE. MODIFIED POLYCHROME METHYLENE BLUE STAIN; 450X (1), 720X (2).

TABLE 6. EVALUATION OF CYTOLOGIC PREPARATIONS OBTAINED BY THE TOUCH AND SMEAR TECHNIQUES

Slide	Readable Fields		Hemorrhage		Nuclear Detail		Cytoplasmic Detail	
	Touch	Smear	Touch	Smear	Touch	Smear	Touch	Smear
1	3	3	3	2	3	3	2	3
2	2	3	2	3	3	2	3	2
3	2	3	3	3	2	3	2	2
4	2	3	1	2	2	2	2	2
5	2	3	2	3	3	2	2	3
Mean	2.2[a]	3[a]	2.2	2.6	2.8	2.6	2.2	2.4
Standard Deviation	.44	0	.84	.55	.45	.54	.44	.55

[a]Significant Difference $p < 0.05$.

more difficult. If only a small specimen was collected by the steel brush, it was difficult to transfer this material to a slide.

The results of the comparisons of cytologic preparations obtained with nylon and steel bronchial brushes are presented in Table 7. There was significantly less hemorrhage with the nylon brushes, and therefore nucleated cells were more easily evaluated. The cytoplasmic detail was significantly better with the nylon brushes. No difference was seen in the number of readable fields or nuclear detail.

In the 4 dogs biopsied with nylon brushes and evaluated 24, 48, 72, and 96 hours after biopsy, no gross or histologic lesions were noted.

In the 4 dogs in which the biopsies were performed with steel brushes, 3 lesions were observed on the bronchial walls in the area the biopsy was obtained. Two lesions were seen in the dog evaluated 24 hours after the biopsy was performed. These lesions were abrasions of the bronchial wall .5cm in width and 1.5cm in length with blood clots filling the defects (Figure 16). Histologically these areas were denuded of epithelium and the basement membranes were ruptured. Blood clots were adhered to the mucosa and hemorrhage was present in the adjacent tissues. The third lesion was seen in the dog evaluated 96 hours after the biopsy was performed. The gross and histologic appearance of this lesion was essentially the same as the lesions in the other dog. There were no complications observed clinically in these 8 dogs at any time after the biopsy.

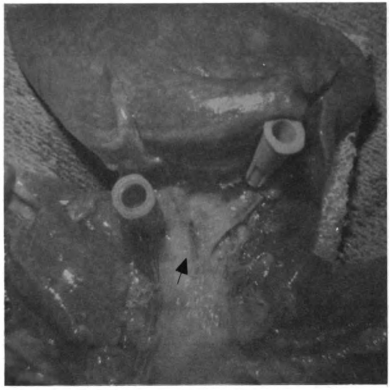

FIGURE 16. BRONCHUS OF A 1 YEAR OLD BEAGLE DOG 24 HOURS FOLLOWING BRONCHIAL BRUSH BIOPSY USING A STEEL BRONCHIAL BRUSH. THERE IS A DEEP ABRASION IN THE MUCOSAL LAYER OF THE BRONCHUS (arrow).

TABLE 7. EVALUATION OF CYTOLOGIC PREPARATIONS OBTAINED BY STEEL AND NYLON BRONCHIAL BRUSHES

Slide	Readable Fields		Hemorrhage		Nuclear Detail		Cytoplasmic Detail	
	Steel	Nylon	Steel	Nylon	Nylon	Steel	Steel	Nylon
1	3	3	2	3	3	3	2	3
2	3	3	1	2	2	2	1	3
3	2	3	1	3	2	2	1	2
4	3	3	2	2	2	3	1	3
5	2	3	1	2	1	2	2	2
6	2	3	3	3	2	3	2	2
7	3	3	1	3	3	2	1	2
8	3	3	2	3	2	2	1	3
9	2	3	2	3	2	2	1	2
10	3	3	1	3	2	2	1	2
Mean	2.4	3	1.6[a]	2.7[a]	2.1	2.4	1.3[b]	2.4[b]
Standard Deviation	.84	0	.69	.48	.56	.51	.40	.51

[a,b] Significant Difference $p < 0.05$.

Evaluation of Nylon Bronchial Brushing vs. Bronchial Washing in Experimental Inflammatory Disease

Dog 1

On day 0, the bronchogram revealed contrast media present in the right lung with only a small amount of residual contrast media present in the trachea (Figure 17).

On day 1, there were no abnormalities on the physical examination. Radiographically, an increased interstitial density was present throughout the right lung. An alveolar pattern was present in the right middle lobe characterized by air bronchograms. No abnormalities were observed in the left lung.

On day 2, the dog's temperature was 103.2°F and a productive cough was observed. Radiographically, the left lung appeared normal. The alveolar pattern was still present in the right middle lobe and the right cranial lobe. The interstitial density in the right caudal lobe had decreased.

By day 3, the temperature returned to normal and the cough was improved. The radiographic appearance of the thorax was similar to that noted on day 2.

Post-mortem examination revealed consolidation of the caudal portion of the right cranial lobe, the right middle lobe, and the cranial portion of the right caudal lobe (Figure 18). No gross lesions were seen in the left lung. On histologic examination, the affected areas were characterized by bronchial and alveolar exudate, which consisted primarily of neutrophils, with a significant increase in the number of macrophages. Vacuoles of various sizes were present in the macrophages and were attributed to the

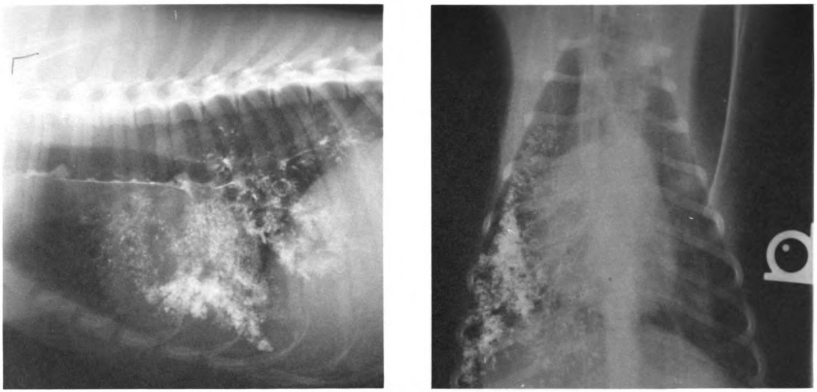

FIGURE 17. SELECTIVE BRONCHOGRAM OF A 1 YEAR OLD BEAGLE DOG. SIX ml OF 60% PROPYLIODONE SUSPENDED IN PEANUT OIL WAS DEPOSITED IN THE BRONCHI TO THE RIGHT CRANIAL, MIDDLE, INTERMEDIATE, AND CAUDAL LOBES. NOTICE THAT NO CONTRAST MEDIA IS PRESENT IN THE LEFT LUNG.

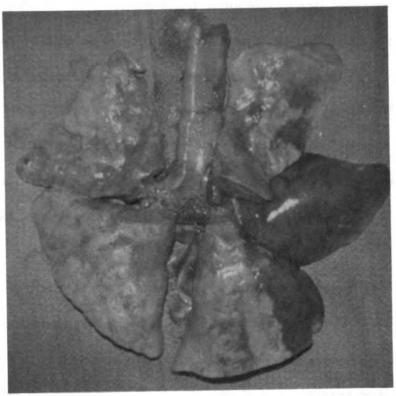

FIGURE 18. POST-MORTEM APPEARANCE WITH PARTIAL CONSOLIDATION OF THE RIGHT LOBES OF A DOG'S LUNGS 72 HOURS AFTER BRONCHOGRAPHY OF THE RIGHT LUNG LOBES WITH 6ml OF PROPYLIODONE SUSPENDED IN PEANUT OIL.

peanut oil present in the contrast media. Other areas of the lung and bronchial tree were normal.

Cytologic findings are listed in Table 8. Consistent with the radiographic and post-mortem findings, significantly more inflammatory changes were seen in the biopsies from the right lung than the left. There was no significant difference between the right bronchial brush biopsies and the bronchial wash cytologies in contrast to a significant difference between the left bronchial brush biopsies and the bronchial washings.

The cytologic preparations from affected areas consisted primarily of neutrophils. Many macrophages were seen as well as occasional mast cells and eosinophils (Figure 19). Bronchial epithelial cells were present in fewer numbers in bronchial biopsies from normal dogs.

Dog 2

On day 0, the bronchogram revealed contrast media in all lobes of the right lung and a small residual amount in the trachea.

On day 1, the physical examination was characterized by crackles ausculated over the right lung field. The body temperature was normal. Radiographically, there were increased bronchial and interstitial densities of the right lung. An alveolar pattern characterized by air bronchograms was present in the right middle lobe.

By day 2, abnormal lung sounds were reduced in intensity. Bronchial and interstitial densities were decreased in the right lung when evaluated radiographically. The abnormal density in the right middle lobe was reduced. The caudal part of the right cranial lobe showed evidence of an alveolar pattern.

FIGURE 19. CYTOLOGIC PREPARATION OF A BRONCHIAL BRUSH BIOPSY FROM LUNG 72 HOURS AFTER 6ml OF PROPYLIODONE SUSPENDED IN PEANUT OIL WAS INJECTED INTO ITS PRINCIPLE BRONCHUS. RED BLOOD CELLS(A), RUPTURED MACROPHAGES (B), NEUTROPHIL (C), EOSINOPHIL (D) WERE IDENTIFIED IN THIS PREPARATION. MODIFIED POLYCHROME METHYLENE BLUE STAIN; 720X.

TABLE 8. INFLAMMATORY RESPONSE OBSERVED WITH BRONCHIAL WASHING AND BRONCHIAL BRUSHING WITH INFLAMMATORY DISEASE

	Dog 1			Dog 2			Dog 3			Dog 4		
Day	Bronchial Wash	Right Brush	Left Brush	Bronchial Wash	Right Brush	Left Brush	Bronchial Wash	Right Brush	Left Brush	Bronchial Wash	Right Brush	Left Brush
0	1	1	1	1	1	1	1[e]	1	1	1[e]	1	1
	2	1	1	1	1	1	1[e]	1	1	1	1	1
1	1	1	1	3	2	1	1	1	2	1[e]	1	1
	3	2	1	3	3	1	2	1	2	3	2	2
2	2	3	1	1	2	1	2	2	2	1[e]	1	1
	2	3	2	2	3	1	3	2	3	3	1	3
3	2	3	1	2	3	2	1[e]	1	1	1[e]	3	1
	2	2	1	3	3	2	2	2	1	1[e]	3	1
Mean	1.9[a]	2.0[b]	1.1[a,b]	2[c]	2.3[d]	1.3[c,d]	1.6	1.4	1.8	1.5	1.6	1.4
Standard Deviation	.64	.90	.35	.90	.89	.46	.74	.52	.88	.92	.92	.74

[a,b,c,d] Significant Difference $p < 0.05$.

[e] No cells present.

On day 3, the lung sounds were normal. Bronchial and alveolar densities in the right lung had further resolved, and the alveolar patterns were no longer evident. Post-mortem examination revealed areas of consolidation in the caudal part of the right cranial lobe, the right middle lobe, and the cranial part of the right caudal lobes. In affected areas, there were histologic changes similar to that described in dog 1. Histologic examination of the left lung revealed a neutrophilic exudate in some of the major airways, while the lung parenchyma was normal.

The evaluation of the cytologic preparations are listed in Table 8. There was a significant difference between the amount of inflammatory change in the right and left bronchial trees as indicated by the bronchial brush biopsies. There was a significant difference between the left bronchial brushing and the bronchial washing, with the bronchial brush biopsies from the left bronchial tree again containing fewer inflammatory cells. No significant difference was present between the right bronchial brush biopsies and the bronchial washings. On day 3, in the left bronchial brushing, a moderate amount of inflammation was present, which correlated with the histologic finding of a neutrophilic exudate in the major airways of the left lung.

Dog 3

On day 0, the bronchogram revealed a substantial amount of contrast media in the trachea. No abnormalities were seen radiographically or on physical examination on day 1.

On day 2, the dog had a temperature of 103.5°F, increased respiratory rate, and mild dyspnea. An increase in interstitial and peribronchial densities were noted radiographically throughout both lung fields. The right middle lobe displayed an alveolar pattern characterized by air bronchograms.

By day 3, the temperature returned to normal and the breathing appeared normal. Radiographically, there was no change in the interstitial or peribronchial densities from day 2. There was reduced involvement of the right middle lobe. Alveolar densities were evident in the right cranial lobe. Post-mortem examination revealed consolidation of both lungs, the right lung being more uniformly affected than the left. The right cranial and middle lobes were the most severely affected. Histologically, the appearance of the affected areas was as described in dog 1. There were more extensive changes in the right lung than in the left.

The evaluation of the cytologic preparations from this dog are listed in Table 8. No significant differences were seen between bronchial brush biopsies from the left and right lungs. This reflected the bilateral involvement as documented on the radiographic and post-mortem examinations. There were no cells present in 3 of the cytologic preparations made from the bronchial washings from this dog.

Dog 4

On day 0, the bronchogram revealed that the right lung contained contrast media, however, a substantial amount was also present in the trachea.

On day 1, a fever of 103°F was present. Radiographically, the interstitial density of both lung fields was increased, and there was alveolarized contrast material in the caudal portions of the left cranial lobe. An alveolar pattern was present in the right cranial lobe.

During bronchial catheterization, the catheter penetrated a bronchus and the visceral pleura, and immediate dyspnea resulted. Radiography indicated a left hemi-pneumothorax. No special therapy was instituted. The dog was observed closely for the next 12 hours during which time the dyspnea resolved.

On day 2, the temperature was normal. Wheezes were heard over the dependent area of the right lung field. Radiographically, pneumothorax was still present, but the amount of pleural air appeared to be reduced. An overall increase in density was seen in both lung fields. Interpretation of the increased density in the left lung field was difficult because of the pneumothorax. The alveolar pattern of the right cranial lobe had resolved. An alveolar pattern was now present in the right middle lobe. On day 3, the wheezes were still present in the dependent areas of the right lung field. The pneumothorax was still present as judged radiographically. An increase in interstitial and bronchial densities was present. Areas of alveolar density were seen in the right cranial and right middle lobes.

Post-mortem examination revealed consolidation of caudal portions of the right cranial lobe, the right middle lobe, and the cranial portions of the right caudal lobe. The hilar region of the lobes of the left lung was less affected. A healing puncture wound on the lateral surface of the left cranial lobe was identified. Histologically, the inflammatory change seen in

the previous dogs was present in the affected areas of the right lung. Sections from the peripheral regions of the left lung were normal.

The evaluation of the cytologic preparations from this dog are shown in Table 8. No significant difference was seen between any of the biopsies or washings. Five washings contained no cells. Three false negative washings were obtained from this dog.

The bronchial washings and nylon bronchial brushings from these 4 dogs were compared by the criteria described in Table 4. The results of the evaluations of cytologic preparations obtained from bronchial brushings and bronchial washings are shown in Table 9. There were significantly more readable fields present in the bronchial brush biopsies as compared to the bronchial washings. Less hemorrhage was present in the bronchial washings. The nuclear and cytoplasmic detail was significantly better in the bronchial brushings than in the bronchial washings.

Evaluation of Bronchial Brushings in Dogs With Clinical Disease

Listed in Tables 10 and 11 are the pertinent data from clinical patients that underwent selective bronchial catheterization and bronchial brush biopsy. The cytological findings obtained by bronchial brush biopsy were evaluated in light of the other data available on these patients.

Data from dogs with confirmed neoplastic disease is shown in Table 10. Case 2 was primary lung neoplasia; cases 1, 3, 4, and 5 were secondary neoplastic disease of the lung.

Neoplastic cells were not present in the bronchial brush biopsy from the case with primary pulmonary neoplasia. The lack of neoplastic cells in

TABLE 9. EVALUATION OF CYTOLOGIC PREPARATIONS: BRONCHIAL BRUSHINGS VS. BRONCHIAL WASHINGS

	Readable Fields		Hemorrhage		Nuclear Detail		Cytoplasmic Detail	
Slide	Washing	Brushing	Washing	Brushing	Washing	Brushing	Washing	Brushing
1	3	3	3	3	2	2	2	2
2	2	2	3	2	2	3	1	2
3	3	3	3	3	1	1	1	3
4	1	3	3	1	2	2	1	3
5	1	2	3	3	2	2	2	2
6	3	3	3	3	1	2	1	2
7	3	3	3	2	2	3	1	3
8	2	3	3	1	2	2	1	2
9	3	2	3	3	2	2	1	2
10	1	3	3	3	1	3	2	3
11	3	3	2	3	2	3	1	2
12	2	3	3	2	1	2	1	2
13	3	1	3	1	2	2	1	2
14	1	3	3	3	1	3	1	3
15	2	3	3	3	1	2	2	2
16	3	3	3	2	2	2	2	2
17	1	2	3	3	1	2	1	1
18	1	3	3	3	3	3	1	2
19	3	2	3	3	1	2	1	2
20	3	3	3	2	2	2	1	3
21	1	3	3	3	1	2	1	2
22	2	2	3	3	1	3	1	2
23	3	3	3	2	2	2	2	3
24	1	2	3	3	1	2	1	3
25	2	2	3	3	1	2	1	2
26	1	3	3	3	2	3	1	3
27	2	3	3	2	1	1	1	2
28	3	3	3	2	1	2	1	3
29	1	3	3	3	1	3	1	2
30	1	2	3	2	2	2	2	2
31	2	3	3	2	1	2	1	3
32	3	3	3	3	1	3	1	1
Mean	2.1[a]	2.6[a]	2.96[b]	2.5[b]	1.55[c]	2.25[c]	1.22[d]	2.28[d]
Standard Deviation	.88	.54	.18	.71	.57	.56	.42	.58

[a,b,c,d] Significant Difference $p < 0.05$.

TABLE 10. PERTINENT DATA OF CLINICAL PATIENTS WITH NEOPLASTIC DISEASE EVALUATED BY BRONCHIAL BRUSH BIOPSY

Histological Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
1. Pulmonary metastasis in an 11 year old Poodle	-Swelling of elbow. -No respiratory signs.	-Increase in overall pulmonary density with many small round densities throughout.	-Normal epithelial cells.	-Post-mortem examination revealed an adenocarcinoma of the gastrointestinal tract with metastatic lesions of the lungs, liver, lymph nodes, pancreas, and bone.
2. Bronchiolar carcinoma in a 7 year old mix breed	-Dyspnea. -Muffled cardiac and respiratory sounds.	-Pleural fluid. -Coin lesion in right lobe.	-Mild purulent necrotic exudate.	-No bronchus involved grossly. Lesions about 2.5cm in diameter. Thoracocentesis revealed a pyogranulamatous exudate. -Mesothelioma present in pleural cavity.
3. Pulmonary metastasis 2^{o} pneumonia in an 8 year old, male Labrador Retriever	-Moist productive cough. -Inspiratory and expiratory dyspnea.	-Increase in density of the right cranial lobe while the right caudal had a mottled appearance. -Some pleural fluid present.	-Mucopurulent with large cocci. -Large round cells with very prominent nucleoli formed in clusters; thought to be neoplastic cells.	-Bacterial cultures negative. -Post-mortem examination revealed a thyroid adenocarcinoma with pulmonary metastasis.

TABLE 10 (cont'd.)

Histological Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
4. Adenocarcinoma in an 11 year old Irish Setter	-Fever. -Masses in mammary glands. -Cough. -Crackles. -Wheezes.	-Consolidation of right caudal lobe with air bronchograms.	-Many epithelial cells and a few neutrophils.	-Post-mortem examination revealed that about 75% of the lung fields were involved. -Tumor nodules were also present in the mammary glands.
5. Adenocarcinoma in a 10 year old Irish Setter	-Dyspnea at rest. -No lung sounds over right lung.	-Increased bronchial density throughout lung fields along with well circumscribed round densities in the left caudal lobe.	-Many carcinoma cells observed along with increased number of neutrophils.	-Post-mortem examination revealed adenocarcinoma involving the lungs, heart, pancreas, and intestine.

TABLE 11. PERTINENT DATA OF CLINICAL PATIENTS WITH NON-NEOPLASTIC DISEASE EVALUATED BY BRONCHIAL BRUSH BIOPSY

Clinical Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
1. Chronic bronchitis in a 4 year old Beagle	-Chronic dry unproductive cough. -Increased bronchial sounds. -Bronchoscopy revealed hyperemia of bronchial mucosa.	-Increased interstitial and bronchial densities. -Right heart enlargement; some enlargement and tortuosity of peripheral arterial structures.	-Mildly increased numbers of neutrophils seen with epithelial cells.	-Microfilaria negative and no peripheral eosinophila or basophilia present at this time. -Negative bacterial culture.
2. Chronic bronchitis in a 4 year old German Shorthair Pointer	-Chronic nonproductive dry cough. -Increased bronchial sounds.	-Generalized increase in interstitial and bronchial density (Figure 20)	-Large numbers of inflammatory cells, primarily neutrophils seen with epithelial cells (Figure 21).	-Mild eosinophilia and basophilia. -Fecal exam revealed Trichuris and Ancylostoma. -Microfilaria negative. -Bacterial culture negative. -Responded well to antibiotic and steroid therapy.

TABLE 11 (cont'd.)

Clinical Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
3. Bronchopneumonia in a 3 year old Doberman	-Chronic dry cough. -Mucopurulent nasal discharge.	-Right middle lobe contained strong increase in peribronchial density with air bronchograms present. -Also some increase in interstitial density. -The ventral portions of the right caudal and right intermediate lobes were involved to a lesser degree.	-Equal numbers of epithelial and inflammatory cells. -Predominent inflammatory cell was the neutrophil with a few eosinophils. -Bacteria were observed.	-Peripheral leukocytosis with an eosinophilia. -Negative bacteria culture.
4. Paralyzed vocal fold in a 9 year Labrador Retriever	-Dry chronic cough. -Abnormal bark. -Laryngoscopy revealed a paralyzed vocal fold.	-Normal lung fields.	-Primarily normal epithelial cells with occasional mucous strands with a few neutrophils.	
5. Dirofilaria immitis in a 4 year old Golden Retriever	-Chronic cough. -Exercise intolerence. -Inspiratory dyspnea. -Increased bronchial sounds.	-Enlarged right heart and pulmonary artery. -Enlarged and tortuous arterial structures.	-Normal epithelial cells.	-Microfilaria in peripheral circulation.

TABLE 11 (cont'd.)

Clinical Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
6. Pneumonia in a 3 year old Beagle	-Unilateral nasal discharge. -Moist productive cough.	-Wide spread increase in interstitial density. -Consolidation of right middle lobe.	-Large numbers of neutrophils with macrophages, lymphocytes and plasma cells.	-Culture was negative. -No response to antibiotics and died. -Mild leukocytosis with left shift. -Neoplasia cannot be ruled out on this animal.
7. Aspiration (lipid) pneumonia in a 1 year old Toy Poodle	-Chronic cough.	-Increased density of right middle lobe with air bronchograms present.	-Many normal epithelial cells with moderate numbers of inflammatory cells which were primarily mononuclear.	-Peripheral lymphocytes. -Lobectomy was done and histology of lobe revealed a mononuclear peribronchial infiltrate suggestive of lipid aspiration.

TABLE 11 (cont'd.)

Clinical Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
8. Chronic bronchitis in a 3 year old Irish Setter	-Increased expiratory effort. -Chronic cough. -Increased bronchial sounds. -Bronchoscopy revealed hyperemia of mucosa and mucopurulent exudate in bronchial lumen.	-Increased bronchial density and calcification of bronchial wall without involvement of interstitial tissue.	-Equal numbers of epithelial cells and inflammatory cells consisted of equal numbers of neutrophils. Some basophils were seen. The epithelial cells were pleomorphic.	-Peripheral leukocytosis with an eosinophilia and basophilia. -Bacterial culture revealed Klebsiella pneumonia which had a very narrow sensitivity pattern. -Minimal response to antibiotic or steroid therapy.
9. Chronic allergic bronchitis in a 3½ year old Australian Shepherd	-Chronic cough of 1 year duration. -Crackles.	-Increase in peribronchial density with some increase in interstitial density. -Bronchogram demonstrated wide-spread bronchiectasis.	-Good cellular detail. Significant increase in the number of eosinophils seen wuth a few neutrophils and mast cells.	

TABLE 11 (cont'd.)

Clinical Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
10. Interstitial pneumonia in a 7 year old Miniature Schnauzer	-Presented for atrophy and pain of facial muscles.	-Increased interstitial density with small nodular densities throughout lung fields.	-Predominent cell type was normal epithelial cells with a few neutrophils.	-Marked clearing of lung fields in 21 days of antibiotic therapy.
11. Chronic bronchitis in a 6 year old Dachshund	Dry unproductive cough of years duration. -Increased bronchial sounds. -Bronchoscopy revealed hyperemia of mucosa and some mucopurulent exudate.	-Increase in interstitial and bronchial density; not considered abnormal for a dog of this age.	-Increased numbers of neutrophils with with equal numbers of eosinophils.	-Streptococcus Klebsiella and Pasteurella multocida isolated from bronchial swab.
12. Parasitic bronchitis in a 2 year old Golden Retriever	-Cough of 8 months duration and intermittently productive.	-Significant increase in bronchial density with some increase in interstitial density.	-Mild increase in numbers of neutrophils and eosinophils with normal epithelial cells.	-Peripheral lymphocytosis, Capillaria ova observed in feces.

TABLE 11 (cont'd.)

Clinical Diagnosis	Physical Findings	Radiographic Findings	Bronchial Brush	Comments
13. Pulmonic infiltration and eosinophilia in a 5 year old Boxer	-Non-productive cough. -Dyspnea. -Increased bronchial sounds.	-Increased bronchial pattern and some increase in interstitial density.	-Significantly increased numbers of inflammatory cells with eosinophils being the most prominent with some neutrophils and mast cells.	-Peripheral eosinophilia and basophilia. -Dog was free of intestinal and cardiovascular parasites. -Responded well to steroid therapy.
14. Pulmonic infiltration and eosinophilia in a 3 year old German Shepherd	-Cough. -Intermittent dyspnea and cyanosis. -Wheezes.	-Increased bronchial and interstitial densities with many round nodular densities throughout lung fields (Figure 22).	-Significant increase in number of inflammatory cells with the eosinophil being the predominant cell type. -Mast cells observed (Figure 23).	-Marked leukocytosis with the predominant cell being the eosinophil with many in the band form. -Responded well to steroid therapy.

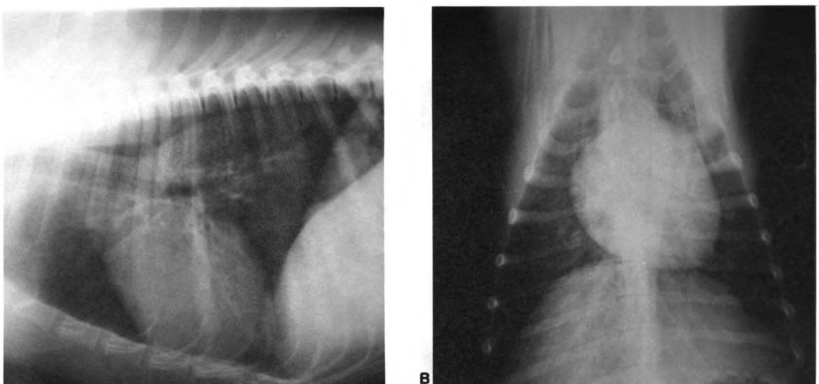

FIGURE 20. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 4 YEAR OLD GERMAN SHORTHAIR POINTER DOG WITH CHRONIC BRONCHITIS.

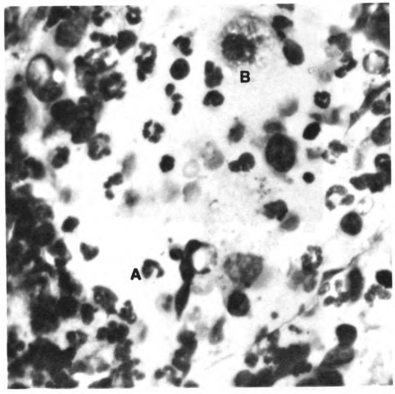

FIGURE 21. CYTOLOGIC PREPARATION FROM A BRONCHIAL BRUSH BIOPSY OF A DOG WITH BRONCHITIS; DEGENERATIVE NEUTROPHILS (A) AND MACROPHAGES (B) ARE PRIMARY CELL TYPES. MODIFIED POLYCHROME METHYLENE BLUE STAIN; 720X.

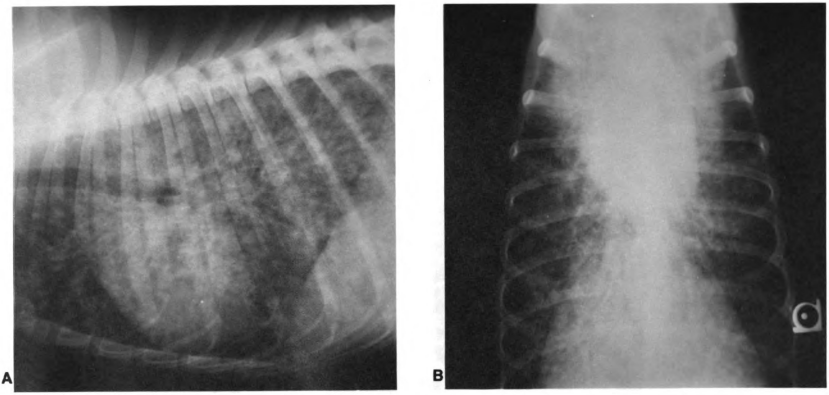

FIGURE 22. LATERAL (A) AND DORSO-VENTRAL (B) RADIOGRAPHS OF THE THORAX OF A 3 YEAR OLD GERMAN SHEPHERD DOG WITH PULMONIC INFILTRATION AND EOSINOPHILIA.

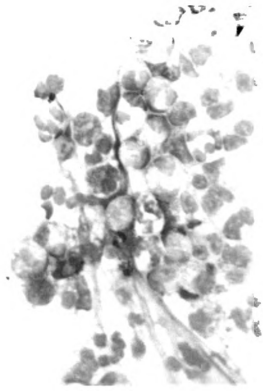

FIGURE 23. CYTOLOGIC PREPARATION FROM A BRONCHIAL BRUSH BIOPSY OF A 3 YEAR OLD GERMAN SHEPHERD DOG WITH PULMONIC INFILTRATION AND EOSINOPHILIA. THE CELLS ARE PREDOMINANTLY EOSINOPHILS. MODIFIED POLYCHROME METHYLENE BLUE STAIN; 720X.

the biopsy from this case could have resulted from improper catheter placement but post-mortem examination revealed no bronchi involved with the tumor.

Two of the cases of secondary neoplasia had neoplastic cells present in the biopsy samples.

One false negative biopsy was obtained in the cases of primary neoplastic disease and 2 false negative biopsies in those cases of metastatic disease.

Table 11 displays the pertinent data from patients with inflammatory disease. Evaluation of the bronchial brush biopsy in these cases is more difficult than those with neoplastic disease because no histological sections are available for a more definitive diagnosis. Fourteen cases of clinical inflammatory disease were biopsied by the bronchial brush technique. All 14 of these cases had cytologies that were consistent with the clinical diagnosis determined by evaluation of the data from physical examination and history, clinical pathology, radiography, and response to therapy. In 10 of the cases, the bronchial brush biopsy contributed to the diagnosis. These cases included 1 through 3, 6 through 9, 11, 13, and 14. As would be expected, all of these patients had disease problems which caused exudation into the bronchial tree. In the other cases studied, case 4 had a paralyzed vocal fold, and would not be expected to have any pulmonary pathology unless the larynx was impaired enough to caused aspiration of food and saliva. Case 5 had mild Dirofilaria immitis and would not be expected to produce bronchial exudation unless the disease was severe. Case 10 had interstitial pneumonia and one would not expect a bronchial

exudate unless it was severe. Capillaria aerophilia excretes its ova in the bronchial tree and a bronchial exudation might be expected. Since neither was found in case 12, it was considered to be a false negative result.

To summarize the results obtained in clinical patients undergoing bronchial brush biopsy, 15 or 79% of the biopsies were consistent with the final diagnosis. Twelve or 65% of the biopsies were of diagnostic value because the cytological pattern suggested a particular disease entity. Four false negatives were obtained.

DISCUSSION

Selective bronchial catheterization through the endotracheal tube does not require removal of the endotracheal tube, therefore assisted ventilation can be maintained throughout the procedure. No surgical scrub is necessary, thus reducing the time needed for the procedure. Multiple catheterizations are possible because the difficulty in passing the catheter over the guide wire and through the cricothyroid membrane is avoided. Manipulation of the patient under the fluoroscope is accomplished with less difficulty. These observations suggest that the preferred method is through the endotracheal tube rather than the cricothyroid membrane. A possible exception would be the patient in which the clinician suspects that the respiratory disease is caused by an infectious agent and other culture attempts have either been unsuccessful or several different organisms were present suggesting contamination by the upper airways. Catheterization through the cricothyroid membrane, rather than an endotracheal tube, would allow the upper airway to be bypassed and prevent contamination. The organisms cultured from a specimen obtained in this manner should more closely reflect of the flora of the lower respiratory tract.

It was clearly demonstrated that selective bronchial catheterization allows direct access to the peripheral portions of the cranial lobes; this has not previously been possible by other non-invasive diagnostic techniques in

the dog. The peripheral areas of other lobes were also accessible by this technique, further broadening its diagnostic capabilities.

The nylon bronchial brushes were shown to more useful in the dog than those constructed of stainless steel because they are easily manipulated, cause less trauma at the biopsy site, and the cytologic specimens obtained were superior by the criteria used in this study.

An exception would be a patient with a mass present in an easily accessible bronchial lumen. Use of a stainless steel brush in this situation might allow collection of a large enough volume of tissue for histologic examination. This type of lesion would most commonly be seen with a bronchogenic carcinoma, which tends to be found in the hilar area and is less disseminated than the bronchiolar alveolar carcinoma. Because the incidence of primary pulmonary neoplasia is low - 3.4 per 10,000 dogs (Cohen, et al., 1965) - and of the primary tumors, only 25% are of a type other than the bronchiolar alveolar carcinoma (Jubb and Kennedy, 1970), the number of cases where the stainless steel brush would be useful would be too few to justify recommending its use.

Smearing the specimens on the slides are shown to be superior to the touch technique because more readable microscopic fields resulted. No difference was seen in the amount of hemorrhage present on the slides, but when the specimens were smeared, the red cells were dispersed and the nucleated cells were more easily visualized; this adds support for choosing this technique.

The cytologic preparations produced by bronchial brush biopsy were shown to be more desirable than those obtained from bronchial washings.

It should be emphasized that 8 bronchial washings contained no cells for examination, and in no case was this true of the bronchial brush specimens. This would suggest that interpretable specimens will be more consistently obtained by bronchial brush biopsy.

When cellular degeneration is seen in a bronchial brush biopsy specimen, it is more probable that it is due to disease within the patient than sampling artifact that can be associated with cytologic techniques. This is substantiated by the nuclear and cytoplasmic detail being significantly better in bronchial brushing specimens compared to bronchial washing sepcimens. It follows that this technique should allow for more critical evaluation of cells for neoplastic changes.

An attempt was made to create inflammatory disease in 1 lung of 4 dogs using the other lung as a control. In 2 of these dogs, radiographic findings, post-mortem findings, and the cytologies from the bronchial brushings revealed that inflammatory lung disease was present in both lungs. This was probably due to aspiration into the left lung of contrast media, which had been inadvertently placed in the trachea.

The radiographic and post-mortem examinations of the other 2 dogs confirmed that pulmonary disease had been created only in the right lung. The cytologies from the bronchial brushings were consistent with inflammatory disease of only that right lung except for 3 specimens; 2 in dog 2 and 1 in dog 1. The post-mortem examination of dog 2 confirmed the presence of inflammatory exudate in the bronchial lumen with normal lung parenchyma. This suggested that false positives can occur when airways from unaffected areas contain aspirated exudate from other affected airways. It can be

concluded that the bronchial brush biopsy is a selective technique and that false positives are possible emphasizing the cytologic findings should be evaluated in light of the other data available from a patient. No false negatives were seen suggesting that if inflammatory disease was present, it would be revealed by bronchial brush biopsy. This is in contrast to 3 false negatives that were obtained by bronchial washings.

Inflammatory cells in greater numbers than were present on control samples were observed in the cytologic specimens from dog 3 twenty-four hours before clinical signs or radiographic examination revealed any evidence of respiratory disease. This observation suggests that subclinical respiratory disease in the dog might be revealed by cytologic techniques, as is true in the horse (Beech, 1975). This could be of value in patients with a fever of undetermined origin or an unexplained leukocytosis. Bronchial cytology may reveal low grade inflammatory respiratory disease in some of these patients.

Selective bronchial catheterization and bronchial brush biopsy were developed primarily as a means of confirming suspected cases of pulmonary neoplasia in the human. During the period of this study, only 5 dogs with pulmonary neoplasia were presented that could be confirmed by post-mortem examination. One of these was primary pulmonary neoplasia and had no neoplastic cells in the cytologic preparation obtained by bronchial brush biopsy. This is not a large enough sample to compare to the results reported in man.

The diagnostic accuracy of the technique in cases of metastatic pulmonary disease in the dog was equal to that in the human; 50% of the

confirmed cases had neoplastic cells in the bronchial brush biopsy. The diagnostic yield in metastatic disease cannot be expected to be as high as in primary disease because metastatic sites usually start as tumor emboli attached to the endothelium of capillary or lymph vessels (Schmidt, 1903). From this beginning, the cells migrate to the perivascular interstitial tissue where they grow by expansion and compress surrounding tissues (Janower and Blennerharsett, 1971). The tumors will not be detected by bronchial brush biopsy until the eroded bronchus allows the cell to spread into the bronchial tree. In contrast, in the primary lung tumor, the cell of origin is likely to be in the bronchial wall, therefore the tumor would be accessible to the bronchial brush at a much earlier time in the tumor's progression. The 3 false negatives obtained in the dogs with neoplastic disease were false negatives in the sense that they did not reveal the lung pathology present but were not true false negatives in that they probably did reveal the true status of the bronchial wall at the time the biopsy was performed.

If selective catheterization and bronchial brush biopsy is to be a useful diagnostic tool in the dog, it must be of value in the differential diagnosis of inflammatory disease of the dog's respiratory system, since the incidence of primary pulmonary neoplasia is low. In the series of 14 cases of inflammatory disease in this study, only 1 false negative was present. The bronchial brush biopsy in this case may have reflected the status of the bronchial wall and is a false negative only in the sense that the disease process was not detected. The brush biopsy did not lead directly to a diagnosis in the other 13 cases, but in all cases where an abnormal bronchial exudate would be expected, the bronchial brush

biopsy revealed an abnormal exudate. The results of the technique in evaluation of inflammatory disease in the dog are encouraging and have established the basis for its continued investigation as a biopsy technique. Also, its potential as a culture technique should be investigated.

Complications endangering the well-being of the patient were not frequently encountered. One dog developed a pneumothorax as a result of the bronchial catheter penetrating the pleura of the left cranial lobe. The dog recovered with no specific therapy other than cage rest. No other complications were observed.

The procedure is safe in the dog if a few precautions are adhered to. First, the dog should be properly evaluated as an anesthetic risk. The content of this evaluation will depend on the age of the animal and its current medical problems. Second, the clinician should become familiar with the bronchial anatomy of the dog to avoid unnecessary manipulation of the catheter with possible trauma to the bronchial wall and prolonged anesthesia. Third, the clinician should become familiar with the instrumentation prior to the procedure to avoid prolonged anesthesia and to insure that adequate biopsies are collected. Fourth, the patient should be monitored closely during the procedure to allow for immediate recognition of anesthetic or other complications.

Selective bronchial catheterization and bronchial brush biopsy were of diagnostic value in 63% of the patients in which they were employed. This indicates that they should be considered as part of a diagnostic plan when primary pulmonary neoplasia or inflammatory disease with bronchial exudation is suspected. Because a fluoroscope equipped with image intensification

is necessary to perform this procedure, it probably cannot be employed by most general practices but should be a part of the capabilities of a referral center accepting patients with suspected pulmonary disease. It is not suggested that this technique replace other cytological techniques; its advantage is that it allows the clinician to identify the site from which a biopsy is taken. Therefore, collection of the biopsy from the diseased area of the lung can be assured.

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