SCANNING ELECTRON MICROSCOPY OF THE RESPIRATORY TRACT OF CATTLE WITH SHIPPING FEVER

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ABSTRACT

SCANNING ELECTRON MICROSCOPY OF THE RESPIRATORY
TRACT OF CATTLE WITH SHIPPING FEVER

By

Helen L. Davidson

Representative animals with characteristic signs of shipping fever were selected from 8 calves which had been purchased from farms or feedlots. The diagnosis at the time of necropsy varied from early bovine respiratory disease (BRD) to acute fibrinous pneumonia caused by *Pasteurella sp*. or other pathogenic organisms. Mucosal epithelium was obtained from 5 calves which had been sick from 3 days to approximately 2 weeks and from 2 normal calves.

The scanning electron microscope was used to study the respiratory mucosa of the nasopharynx, turbinate, trachea, bronchus, bronchioles and alveoli from calves with shipping fever and to compare the tissue with similar micrographs from control calves.

In most areas the normal respiratory mucosa consisted of ciliated cells interspaced with flat or columnar cells. The flat nonciliated cells were densely covered with microvilli. In the airways the tall cells had ciliated cells at the base. In the normal lung tissue the alveoli appeared thin walled with uniform air spaces.

In calves with early BRD associated with *Pasteurella sp.,* the respiratory mucosal cells appeared to be denuded of cilia and appeared swollen, which caused the tissue to appear uneven on the surface.

Microvilli were scarce and appeared as short plications. Cell borders were indistinct in some areas and separation of cells was seen in other sections. The bronchioles were filled with exudate and the alveolar walls had thickened.

In animals with bronchopneumonia caused by *Pasteurella sp.*, the mucosal cilia varied from long slender structures to short stubs. The majority of cells were swollen, giving an uneven appearance to the surface. Microvilli were evident but appeared fewer in number on the cells. Cell borders were visible in most of the sections. Alveolar walls appeared thickened and had smaller air spaces. Bronchioles were filled with exudate.

The calf with an early BRD associated with *Pasteurella sp.*, *Corynebacterium pyogenes* and parainfluenza-3 virus had a nasopharyngeal mucosa which was very swollen, and most of the cells had no cilia or microvilli. The cell borders were not visible.

The calf which died of acute fibrinous pneumonia had most of the mucosal cilia tangled or they were present as broken stubs. Some cells were nonciliated. The microvilli on each cell varied from moderate to none. The surface of many cells was swollen. Pitted and necrotic areas were evident. The lungs had lost their normal architectural structure. Leukocytes and fibrin strands filled the alveoli.

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By

Helen L. Davidson

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To my patient husband

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INTRODUCTION

Shipping fever in cattle causes major economic loss to beef and dairy farmers and feedlot operations in all parts of the world. In the United States the loss has been estimated at more than $255 million annually.

The exact cause of shipping fever is difficult to establish because of the numerous etiologic agents, the variety of clinical manifestations, and other variables which are involved. The three main factors now associated with shipping fever are environmental stress, viruses, and bacteria.

The scanning electron microscope (SEM) is an excellent instrument which can be used to study the surface structure of normal and abnormal biologic tissue. In this study, the instrument has been used to study changes which occurred in the mucosal epithelium from calves with varying degrees of clinical signs of shipping fever.

In this thesis the term calf/calves or cows/cattle are used interchangeably because this terminology was found in the literature. The terms generally refer to young animals ranging in age from 4 months to 12 months.

OBJECTIVES

The objectives of this research were to:

1. Collect tissue sections from the nasopharynx, trachea, bronchi, bronchioles, and alveoli from calves which had clinical signs of shipping fever and from normal control calves.

2. Prepare these sections for examination by use of the scanning electron microscope (SEM).

3. Describe the changes which occurred in the mucosal epithelium in shipping fever and compare them with normal tissue sections.

4. Establish a relationship between the changes and the severity of the clinical signs.

REVIEW OF LITERATURE

Normal Anatomy of the Respiratory Tract

The respiratory system consists of three parts: conducting airways, a respiratory exchange area, and a ventilating system. Their primary functions are to provide oxygen and to remove carbon dioxide from the blood stream.

The conducting airways are composed of the nasal cavity and sinuses, nasopharynx, larynx, trachea, bronchi, and bronchioles of the lungs. These areas moisten, warm, and filter the air before it reaches the lungs. The respiratory region, which is made up of the bronchioles, the alveolar ducts and sacs, and the pulmonary alveoli, is the area where gas exchange takes place. The ventilating system consists of the thoracic cage, intercostal muscles, diaphragm, and pulmonary elastic connective tissue, which expands and contracts to change the volume of the lungs.

Nasopharynx

The nasal part of the nasopharynx is lined with ciliated, pseudostratified, columnar epithelium with many goblet cells and secretory glands. The upper part of the pharynx has a transitional zone of ciliated stratified, columnar epithelium which gradually changes to a noncornified, stratified squamous epithelium as it reaches the oral cavity.

Turbinate

The turbinates are convoluted projections which divide the air passages into the principal air chambers of the nose. They provide a large surface area which gives the film greater contact with the air stream. The epithelium, with its many goblet and secretory cells, is a ciliated pseudostratified columnar surface with areas of transition to stratified squamous cells.

Trachea

The trachea is a cartilaginous tube extending from the larynx to the principal bronchi. Its four layers are mucosa, submucosa, muscle, and cartilage, and the supporting connective tissue. The mucosa has many longitudinal folds and is covered with ciliated pseudostratified columnar epithelium. Numerous goblet cells are scattered through the epithelium.

Bronchi

The tubular bronchi retain the epithelial character of the trachea for a short distance as they branch into each side of the lungs. As the tubes diminish in diameter, the epithelium changes from tall ciliated pseudostratified columnar layer to a lower pseudostratified ciliated and nonciliated layer. Bronchial glands and mucous cells which produce a surface coating for the airways are numerous.

Bronchioles

The branches from the bronchi diminish in diameter to become the smaller terminal and respiratory bronchioles. The bronchioles consist of a single layer of ciliated columnar or cuboidal cells with many goblet cells present at the division point of the bronchi and bronchioles. As these bronchioles become smaller, longitudinal folds become more pronounced and bronchial glands are absent. Nonciliated Clara cells may project into the lumen of the respiratory bronchioles. The cilia extend farther into the bronchioles than the secretory glands.

Alveoli

The lungs are made of air spaces called alveoli which begin as small air spaces and become progressively larger as they form into a lobe. In cattle, the left lung can be divided into the apical, cardiac, and diaphragmatic areas. The right lung, which is much larger than the left, is divided into an anterior and posterior apical, cardiac, diaphragmatic and intermediate portions. The alveoli are lined with a very thin layer of alveolar epithelium. The surfactant may be produced by the alveolar epithelium or pneumocytes (Macklin, 1954). These secretory pneumocytes have been classified into three types. Type I are flat epithelial cells. Type II are rounded cells with plications or microvilli (Greenwood and Holland, 1975; Nowell et al., 1972; Groniowski et al., 1972). Type III have been described as "brush cells" with short, blunt microvilli on the free edges. Pores of Kohn are visible in the interalveolar septa (Meyrick and Reid, 1968; Nowell and Tyler, 1971; Wang and Thurlbeck, 1970).

Foreign matter and pathogens are removed by phagocytosis and movement of the mucous layer which flows over the ciliated epithelium. The viscid outer layer of mucus traps the matter, while the beating of the cilia in the deeper serous layer moves the material toward the pharynx, where lymphoid tissue is abundant or where the foreign agent may be swallowed. The efficiency of this mucous layer depends upon the function of the secreting cells and glands, the volume and quality of the mucus, and the rate of ciliary beat. Anything which interferes with any of these functions will inhibit the efficiency of the defense mechanism against invasion of the respiratory tract (Robbins and Angel, 1971; Smith, Jones and Hunt, 1972).

Shipping Fever in Cattle

History

Shipping fever is a bovine respiratory disease which causes serious economic loss to the cattle industry throughout the world. This disease was first described by Bollinger in 1878 (cited by Hutyra et al., 1946), where it caused great losses among stags and wild hogs. In 1880, Louis Pasteur first described the bacterium which he cultured from sick cattle (cited by Hamdy et al., 1965). This bacterium could not be differentiated from the fowl cholera organism or from the agent which caused respiratory disease in many other species. This disease was recognized as infectious in 1881. In 1939, the name pasteurellosis was proposed because most of the organisms cultured from diseased cattle belonged to a single species (cited by Hamdy et al., 1965). Some investigators believed that the *Pasteurella* organism found in shipping fever infections in various animal species was the same organism. Others thought that the organisms were closely related but were a different species of *Pasteurella*. Investigators have been unable to produce the disease experimentally in calves by using *Pasteurella sp.* alone (Gale and Smith, 1958; Collier et al., 1960; Hamdy et al., 1963, 1964; Trapp et al., 1966; Hoerlein et al., 1957; Hetrick et al., 1963). *Pasteurella* organisms can be isolated from apparently normal cattle.

Many names have been used to describe this respiratory disease, such as transit fever, stockyard fever, pneumonic pasteurellosis, and the common shipping fever. Another term, hemorrhagic septicemia, is now reserved for septicemic pasteurellosis which is so prevalent in cattle, swine, and beasts of burden in Southern Asia (cited by Blood and Henderson, 1971).

Shipping fever is presently considered as part of a bovine respiratory complex caused by the interaction of viral and bacterial agents combined with stress (cited by Hamdy et al., 1965). The name "shipping fever" is misleading because it calls attention to the shipping period rather than to the respiratory disease which causes loss of cattle. The general name of Bovine Respiratory Disease Complex (BRD), which includes pneumonic conditions and shipping fever, is now preferred for respiratory diseases.

Epizootiology

Geographic location does not appear to play a major role in shipping fever. The disease is found in all parts of the world and all sections of the United States. It has been described in the United Kingdom, Southern Asia, Mexico (Martel et al., 1974), South Africa (Omar, 1966), Canada (Childs, 1946), Europe, South America, Australia, and the United States (Carroll, 1968; Hatch and Meyer, 1968).

There is no scientific evidence that cattle which are subjected to cold weather are more susceptible to shipping fever than those in warm climates. However, a combination of cold and wet weather may contribute to the problem. In a survey made in Colorado, 18.9% of more than 400,000 calves died from BRD. Approximately 3/4 of the 18.9% calves which died had shipping fever. The highest incidence was in the spring and winter. This increase in the spring and winter, when the weather is most variable, is attributed to the greater number of cattle which are moved at that time (Jensen et al., 1976). Cattle can normally withstand cold if they have not been subjected to fatigue, stress, and environmental changes (Schipper, 1966).

Incidence

The incidence of BRD is high in young calves which have been weaned and removed from their natural environment. Yearlings are less susceptible to BRD than younger animals. Many of these older animals have been found to have significant antibodies against viral agents (Abinanti et al., 1961; Hoerlein et al., 1959; Sweat, 1966; Rosenquist et al., 1970).

Shipping fever develops in calves which have been transported from farms or ranches where they are raised to feedlots where they are fattened. Many of the animals are transported very long distances in trucks and rail cars (Ward, 1973). The disease is found in calves which have been transported from summer grazing pastures to home farms or to fair exhibitions (Blood and Henderson, 1971).

Etiology

The exact cause of shipping fever is difficult to establish because of the number of etiologic agents, predisposing factors, and inconsistent manifestations in sick calves (The Merck Manual, 1973). The three main factors associated with shipping fever are viral agents, bacterial invasion, and environmental stress. Many agents have been incriminated (Gale and King, 1961; Collier et al., 1962; Trapp et al., 1966; Magood et al., 1969; Baldwin et al., 1967).

In major outbreaks of BRD, the three most prevalent viruses which have been isolated are known to cause: 1) infectious bovine rhinotracheitis (IBR), 2) parainfluenza-3 (PI-3), and 3) bovine viral diarrhea-mucosal disease (BVD) (Reisinger et al., 1959; Madin et al., 1965; Gale and King, 1961; Sweat et al., 1967). In addition, more than eight other viruses have been isolated which cause a less severe clinical picture. Some of these include adenovirus, enterovirus, reovirus and rhinovirus (cited by Hamdy et al., 1965).

Infectious Bovine Rhinotracheitis (IBR). IBR was reported in Colorado in 1950. It was originally described by Schroeder and Moys (1954) as a significant but mild respiratory disease in cattle which was occasionally accompanied by pneumonia. Maden and York isolated the virus in tissue culture in 1956. The first successful vaccine was marketed in 1957 (York and Schwarz, 1956).

This acute contagious infection of the respiratory tract is caused by a filtrable virus of the herpes group which can be cultured in bovine fetal kidney cells. It has been isolated as long as four months after recovery from the disease. The virus has also been found in the nasal discharge and trachea of apparently normal cattle (Schroeder and Moys, 1954). This is the same virus which can be recovered in cattle with conjunctivitis, encephalitis, mastitis, infected pustular vulvovaginitis (cited by Anspaugh, 1970), and from aborted fetuses (McFeely et al., 1968).

Table 1. Factors associated with shipping fever in cattle

Stress +	Nonbacterial Agents +	Bacterial Agents
Anxiety	IBR	*P. multocida*
Dehydration	PI-3	*P. haemolytica*
Fatigue	BVD	*Corynebacterium*
Surgery	Enterovirus	*Pseudomonas*
Hunger	Adenovirus	*Streptococcus*
Cold	Rhinovirus	*Staphylococcus*
Heat	Other viruses	*E. coli*
Dampness	PPLO	*Haemophilus*
Deworming	*Chlamydia*	
Vaccination		

Infectious bovine rhinotracheitis is transmitted by air, contact with secretion or exudate droplets, and contaminated articles. Feedlot morbidity may reach 100%, but mortality is usually less than 5% of the sick cattle. Young calves appear to be most susceptible (cited by Anspaugh, 1970).

The disease has a 5- to 7-day incubation period with a sudden onset of high temperature, anorexia, depression, and a clear nasal discharge which lasts a couple of days. There may be some lacrimation and coughing. The animal is sick 10 to 14 days if no complications occur. Bacterial complications may prolong the disease and cause a severe serofibrinous exudate in the sinuses, turbinates and trachea. Often a pseudodiphtheroid membrane forms. The exudate may become so profuse that the air passages are blocked and death occurs (The Merck Manual, 1973).

The gross lesions consist of an intense inflammatory reaction in the nasal passages, larynx, trachea, and bronchial tree with hemorrhages, edema, and serofibrinous exudate. A mild bronchopneumonia may affect the lungs (Smith, Jones and Hunt, 1972).

Diagnosis can be made by the clinical signs, characteristic lesions, viral inclusions in biopsy tissue, isolation of the virus in tissue culture, fluorescent antibody technique, or the indirect hemagglutination test. Cattle are treated with antibiotics to reduce secondary bacterial infections. Recovery from the disease gives permanent immunity. Passive immunity is passed from the dam to the offspring through the colostrum and lasts 2 to 4 months. Active immunity occurs 10 to 14 days after vaccination and may last up to 3 years (Anspaugh, 1970).

<u>Myxovirus Parainfluenza-3 (PI-3)</u>. This virus was described in cattle by Andrewes et al. (1955) and it is also prevalent in horses and sheep. Isolation and identification were made in 1958 (Reisinger et al., 1959). It is a myxovirus with a predilection for the respiratory tract.

The virus is acquired by direct contact with sick calves, from milk from infected dams, or by exposure *in utero* (Kawakami, 1967; cited by Wood, 1968). Cattle may carry the virus a long time before the disease becomes apparent. Many animals show no clinical signs (Hoerlein et al., 1961; Abinanti et al., 1960; Hamdy et al., 1963; Dawson, 1964). Hoerlein et al. (1961) found significant titers in 70% of cattle from 12 feedlots with a population of 1827 animals. Antibodies can be detected and measured by the hemagglutination inhibition test. A 4-fold increase in titer in paired sera indicates infection has been sustained (Roberts and Carter, 1976). Serologically, PI-3 is the most common respiratory virus in cattle (Kramer et al., 1963). Diagnosis is based on clinical signs, hemagglutination inhibition, virus neutralization, fluorescent antibody technique, and tissue culture isolation (Roberts and Carter, 1976).

Lesions include bronchiolitis, alveolitis, and pneumonia primarily in the apical and cardiac lobes of the lungs (Jolly and Ditchfield, 1965). Viral inclusion bodies are found in the nasal and bronchial epithelium early in the course of the disease (Burroughs, 1967).

Passive immunity is passed in colostrum to calves which may have equal or greater levels of antibodies than found in the dams. This immunity lasts from 6 to 8 months (Sweat, 1967). Vaccination of calves 30 days prior to weaning or at the time of weaning is recommended (Woods et al., 1967; Hamdy et al., 1965).

Bovine Virus Diarrhea-Mucosal Disease (BVD). In 1946, a contagious disease with gastrointestinal involvement was described in cattle in New York State (Olafson et al., 1946). Later a similar disease appeared in Canada (Childs, 1946) and in southern California (Howard, 1969). Reports of the disease in the United States and all parts of the world have appeared.

This viral disease is transmitted by direct contact with infected calves, by contaminated feed, water or other articles. In affected animals the morbidity is usually low but mortality is high. Diagnosis of the disease is difficult because the viral agent involved is difficult to identify. Virus isolation in bovine cell culture, virus neutralization, or hemagglutination-inhibition may be used to identify the virus. The fluorescent antibody technique is probably the best laboratory procedure.

The clinical symptoms are similar to IBR and shipping fever in the early stage of the disease. The severity of the disease is variable from mild or inapparent to severe or chronic (Prier, 1966). The acute signs include high temperature, severe hemorrhagic or catarrhal diarrhea, anorexia, dehydration, severe leukopenia, and depression. Ulcers are often found in the mouth and a mucous nasal exudate may be present (Olafson et al., 1946). Hemorrhages may be seen in the epicardium and vaginal mucosa (Ramsey and Chivers, 1953). Ulcerative lesions have an affinity for the mucosa of the upper respiratory tract and the intestinal mucosa (Olafson et al., 1946).

There is limited evidence that recovery from the disease gives permanent immunity. Active immunity is promoted by a vaccine which should not be given to pregnant animals.

Pasteurellosis. The *Pasteurella sp.*, primarily *Pasteurella multocida* and *Pasteurella haemolytica*, are the two main bacterial agents which have been closely associated with shipping fever. Other invaders which have been identified are species of *Mycoplasma*, *Corynebacterium*, *Haemophilus*, *Pseudomonas*, *Chlamydia*, *Staphylococcus*, *Streptococcus*, and *Escherichia coli* (cited by Rosner, 1971).

The *Pasteurella* organism is a gram-negative short rod bacterium which can be frequently recovered from respiratory tract of normal animals. These organisms are ubiquitous in animals and birds of the world. They can be primary contaminants, a secondary invader especially following a viral disease with mucosal damage, or they can be found as normal flora in normal cattle (Hoerlein et al., 1961). Infection occurs when cattle are introduced into new herds or feedlots. However, cases have been found in closed herds and stabled or pastured cows. Infection with *Pasteurella sp.* has been found to be higher in cattle affected with shipping fever than in normal cattle (Hoerlein et al., 1961; Hamdy and Trapp, 1967).

In shipping fever the organisms are usually secondary invaders which cause consolidation of the lungs. The bacterium can be recovered from the pneumonic area (Smith, Jones and Hunt, 1972). The organism may invade the lymphatic system and the blood, causing small hemorrhages on the mucosal surface of internal organs and in the skin. This is often referred to as hemorrhagic septicemia. *Pasteurella* are transmitted by animal secretions, infected dust, contaminated stables, railroad cars, and in feedlots.

Isolation of *Pasteurella sp.* and significant increases in hemagglutination titers from animals with shipping fever were described by Hamdy and Trapp (1967). Investigators have not been able to produce the classical signs of shipping fever with *Pasteurella sp.* alone (Hamdy et al., 1963, 1964). *Pasteurella multocida* has been associated with shipping fever for many years. *Pasteurella haemolytica* has been linked with the disease more recently (cited by Carter, 1954).

The *Pasteurella sp.* have many different antigenic components which have not yet been fully identified (cited by Rosner, 1971). Many strains of *Pasteurella multocida* and *Pasteurella haemolytica* have been identified with similar and different biochemical reactions (Prince and Smith, 1966; Saxena and Hoerlein, 1966; Jubb and Kennedy, 1970). The many variants of the *Pasteurella sp.* are grouped together bacteriologically since the precise biochemical differences are only slight. There is a need for more biochemical identification of the strains of these two species in association with BRD. This would help correlate the serotypes to the disease they produce and increase the validity of the research.

Stress. Young calves are subjected to many stress factors from the time they leave the farm to their arrival at the feedlots. The shock of leaving familiar surroundings, being weaned, eating strange feed, and the change in environmental conditions from ranch to mass assembly can cause fatigue, anxiety, and nervousness (Ward, 1973).

The cattle are hauled in close contact in trucks or railcars, which may not be cleaned or disinfected and may harbor pathogens. The jogging of the trucks in long hauls is tiring (Jensen, 1968). Extreme cold or heat may affect their metabolism. Water and feed may not be readily available (Schipper, 1972).

Once they arrive at the feedlot, they are subjected to even greater stress factors. They are unloaded and herded into strange pens with other cattle. They are not accustomed to the large watering troughs and more changes in feed. Often they refuse to eat or drink for several days which may cause digestive disturbances and dehydration. In addition, they are given vaccinations, antibiotics, and food supplements along with being branded, dewormed, and dipped for ectoparasites (Schipper, 1972).

These stress factors cause a weight loss in calves, which takes some time to regain even in healthy animals. If they are exposed to disease agents during the long haul or after arrival at the feedlot, the additional stress can cause serious illness when their resistance is low.

Transmission Studies of Shipping Fever in Calves

Many transmission studies have been performed on the interrelationship of stress, viral agents and *Pasteurella sp.* in calves. In a study of shipping fever under field conditions, healthy calves which contacted *Pasteurella multocida, Pasteurella haemolytica* and PI-3 developed clinical signs similar to those seen in shipping fever. Artificially exposed calves also developed the clinical signs, but to a lesser degree (Heddleston et al., 1962).

In 1963, Hamdy et al. were able to induce the most marked clinical signs of shipping fever in calves by exposing them to a combination of PI-3, stress, and *Pasteurella sp.*, but not with any of the agents alone. Again, in 1964, Hamdy et al. did a study with 40 calves and exposed them to PI-3, bovine enterovirus (BEV), IBR, psittacosis lymphogranuloma venereum (PLV), and stress in different combinations or singly. Signs of shipping fever were evident in 9 calves which had been infected with a combination of virus, *Pasteurella sp.* and stress. The viral agents alone did not produce clinical signs.

Trapp et al. (1966) exposed 34 calves to various combinations of physical stress, *Pasteurella sp.*, PI-3, and pleuropneumonia-like organisms (PPLO). Pneumonia was found in 13 calves which had been exposed to PI-3 and *Pasteurella sp.* The microscopic lesions were similar to those found in field cases of shipping fever. A mixed viral infection, *Pasteurella sp.*, and stress appeared to cause the most severe clinical signs. The severity of the disease transmitted appeared to depend on the combination of the agents involved, the number of agents involved, and the time period during the disease when different agents were involved.

Clinical Signs of Shipping Fever

Signs of respiratory disease and fever develop within 2 to 21 days after arrival at the feedlot. Reduced appetite and a tired appearance are the first signs. The mucous membranes become involved causing eyes to water and the appearance of a mucopurulent nasal discharge. The temperature may rise to 104 to 108 F. Rapid breathing, loss of weight, and a soft hacking cough develop. The course of the disease varies, with possible recovery in 3 to 7 days if the animal responds to treatment. This depends upon the extent of lung involvement, intensity of the inflammation and the nature of the primary disease. Chronic pneumonia may result in some cases. If the disease progresses, death may occur anytime up to 3 weeks.

Lesions Found in Shipping Fever

The lesions are found primarily in the respiratory organs and vary according to the severity of the infection, length of illness, stage of the infection, and the complications of a secondary infection. There is generalized edema, hyperemia, and hemorrhage of the upper respiratory tract. Mucopurulent exudate may fill the nasopharnyx and sinuses. In early stages, there may be a bronchopneumonia with congestion of the lung. Later, 1/3 to 2/3 of both lungs may be consolidated. Fibrinous exudate fills the alveoli. In chronic cases abscesses and fibrosis occur in the lungs. Resolution of the pneumonia is impossible due to the necrosis in the lungs and possibly the obstruction of the lymphatics. Changes may be evident in other body tissue and organs (Jubb and Kennedy, 1970; The Merck Manual, 1973; Jensen et al., 1976).

Control of BRD Including Shipping Fever

The great increase in BRD closely parallels the increase in the number of cattle which move from ranges to feedlots. This problem is related to the stress of weaning and shipping, the number of agents which complicate the control, and the poor management practices. Many methods of control and prevention have been recommended. A good preconditioning program by the farmer is important. This includes preweaning of calves 3 to 4 weeks before shipping. Gradual conditioning of animals to new feed and watering troughs is necessary (Schipper et al., 1973). Vaccinations against IBR, PI-3, and BVD should be given about 14 days before leaving the ranch because of the loss of maternal antibodies. Dehorning, deworming, and castrations would cause less stress if done at home (Ward, 1973; McLean, 1973; Hoerlein, 1970). Many producers feel that the cost of administering a preconditioning program is too high and are not convinced of its value. As a result, the calves are not prepared for the drastic changes which occur (Hoerlein, 1973).

The American Association of Bovine Practitioners has recommended the use of a preconditioning procedure before shipping which is documented and travels with the animal. This includes a) weaning the calf and feeding it back to its original weight, b) dehorning and castrating in time for complete healing, c) treating for ectoparasites, d) vaccinating against viral agents at least 3 weeks before shipping.

Another critical point is the time between leaving the ranch and the arrival at the feedlot. Incompetent help, long hauls, exposure to toxic exhaust, and overcrowded loads and handling points should be avoided to help eliminate fatigue (Schipper, 1972). Calves may not be rested or fed on long hauls and may not eat or drink for several days. A calf may lose about 10% of its body weight, which takes about 14 days for a normal animal to regain (Hoerlein, 1973).

Treatment

Good management and proper medical treatment are necessary at feedlots. It is important that cattle are handled as little as possible and excitement and overcrowded conditions at feedlots are avoided. The cattle should be given a week's rest before dehorning, castrating, branding and deworming (Schipper, 1966).

The 1968 Symposium on Immunity to Bovine Respiratory Disease Complex recommended the following procedure if a preconditioning program was not used:

1. Provide nutritious feed and fresh water.
2. Provide windbreaks and avoid close confinement to allow freedom of movement.
3. Vaccinate within 48 hours after arrival at the feedlots.
4. Don't induce abortions until 21 to 28 days after arrival.
5. Check pens closely for 30 days.
6. Isolate and treat sick calves immediately.

The Scanning Electron Microscope

History

The first work on the construction of the scanning electron microscope (SEM) originated in Germany between 1930 and 1938. Great Britain, France, and the United States were also doing experimental work on construction of a SEM. World War II delayed the actual engineering of the instrument until 1948. Cambridge University Engineering Department worked on an instrument which led to the first commercial unit in 1965 (cited by Nixon, 1969).

Mechanical Principle

A specimen mounted on a metal stub is placed in a vacuum chamber. An electron gun produces a beam of electrons which pass toward the anode. Two or more condenser lenses reduce the beam to less than 100 Å, which acts as a probe. The probe bombards primary electrons of high energy at the surface of the specimen. The specimen emits low energy secondary electrons or signals. These signals are collected, amplified, and then displayed on a cathode tube. A camera is attached to the instrument for making micrographs of the image (Black, 1970).

This instrument is used to examine and visualize the microtopography of any object. Difference in composition is easily seen as depressions produce dark images while elevations appear light. This variable surface lighting gives the object the three-dimensional contour. The depth of field in a SEM is 300 to 500 times that of an optical microscope at an equivalent magnification.

Another great advantage is the wide range of magnifications from 20 to 100,000 times which is possible. The study of detail can be done quickly by increasing the magnification of the field. The specimen is easily rotated or tilted, which affords rapid serial pictures at variable magnifications as they appear in the field (Behnke and Anderson, 1973).

MATERIALS AND METHODS

Animals

Eight calves with characteristic signs of shipping fever were purchased from farm herds and feedlots in Michigan, Kentucky, and North Dakota[a] during a period of one year. One of the calves died shortly after it arrived. The remaining calves were killed. Tissues were selected from 5 representative animals and were processed for SEM studies.

Control tissues were obtained from 2 apparently normal calves supplied by the Department of Animal Husbandry and the Dairy Research Farm at Michigan State University.

Collection and Preparation of Tissues

The nasopharyngeal tissue was removed from the posterior cavity approximately 5 cm from the palatal opening. The turbinate tissue was removed from approximately the middle of the structure. Tracheal tissue was taken about midway between the tongue and the lungs. Bronchial tissue was obtained approximately 4 cm from the double bifurcation going into the cardiac lobe of the lung. Blocks of tissue containing bronchioles and alveoli were removed from the area with the most acute pneumonic process. Normal lung tissue was

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obtained from the cardiac lobe of the lung of normal calves. The mucous membranes were left intact to prevent curling and tearing during fixation and dehydration of the tissues.

The sections were immediately placed in a solution of 3% glutaraldehyde in 0.05 M s-collidine buffer at pH 7.4 and fixed overnight. Each section was then washed 3 times for 10 minutes each with 0.3 M sucrose wash solution in 0.05 M s-collidine buffer at pH 7.4, giving a balanced osmolality of 400 milliosmoles.

A piece of tissue was trimmed to approximately 1 cm in diameter and mounted onto an aluminum disc with 10% gelatin.[b] The mounted tissue was kept in modified Karnovsky's fixative (Karnovsky, 1965) until all sections were trimmed, mounted, and dehydration procedures were started. A phosphate buffer (pH 7.2) replaced the cacodylate buffer and was prepared by mixing 78 ml of 0.2 M Na_2PO_4 and 28 ml 0.2 M KH_2PO_4.

Dehydration of the Tissues

The mounted tissue sections were dehydrated by placing them through a series of 25, 40, 60, 80, 95, 100 and 100% ethanol for 1 hour each followed by a final 100% ethanol dehydration for 12 hours. The ethanol was replaced with amyl acetate by placing the sections for 1 hour each in solutions containing 2 parts ethanol to 1 part amyl acetate, 1 part ethanol to 1 part amyl acetate, and 2 solutions of 100% amyl acetate.

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[b]Difco Lab, Detroit, MI.

Critical Point Drying

The sections were dried in a critical point drying apparatus according to the procedure of Anderson (1951). Before drying, each disc with the mounted tissue was first wrapped in lens paper to prevent foreign material contained in the CO_2 tank from being deposited on the tissue surface. Carbon dioxide at not less than 850 psi was released for 15 minutes into the bomb containing the wrapped specimens. This procedure was repeated until amyl acetate was not detected by smelling the escaping fumes.

The dried sections, mounted on aluminum discs, were stored under vacuum in a desiccator containing anhydrous calcium sulfate until the time of examination.

Scanning Electron Microscopic Technique

The aluminum discs on which the sections were mounted were glued to an aluminum stub with a conductive paste.[c] They were coated in a mini-coater[d] under 100 µ vacuum with 400 Å of gold palladium[e] to prevent charging from the electron beam. Five sections were mounted at the same time on a chuck mount and put into a vacuum chamber of the SEM. An accelerating voltage from 10 KV to 20 KV was used. The magnification factor ranged from x45 to x9000. Black and white micrographs were made of the representative sections with a Polaroid camera[f] attached to the SEM.

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[c]Tube Koat, G. C. Electronics Div., Hydrometals, Inc., Rockford, IL.

[d]Film-Vac., Englewood, NJ.

[e]Ladd Research Industries, Inc., Burlington, VT.

[f]Polaroid 4 x 5 Land Film, Type 55 P/N, Polaroid Camera, Polaroid Corp., Cambridge, MA.

Microbiologic Examination

Swabs taken from the nasal cavity and trachea, and samples of necropsied liver, kidney, lung, and intestine were cultured for microorganisms. The swabs were streaked on trypticase soy agar[g] containing 5% defibrinated bovine blood, and were then placed in Schaedler's semisolid agar.[h] Tissue samples collected at necropsy were inoculated directly onto the blood agar. After 24 hours of incubation at 37 C, the plates were examined for bacterial pathogens and potential pathogens.

Virologic Examination

Frozen tissues from the kidney, liver, lungs, and intestine were examined for viruses by using the direct immunofluorescence procedure. The antiviral serum was labeled with a fluorescent dye. The tissue sections were stained with the fluorescent antibody and then examined with a fluorescence microscope. Specific fluorescence indicates the presence of the virus or viral antigen.

Fecal Examination

Fecal specimens were examined for parasites and ova by the flotation method.

Histopathologic Examination

Tissues were collected from the trachea, turbinate, nasopharynx, kidneys, liver, lungs, spleen, heart, and intestines. The sections were fixed in 10% neutral buffered formalin which is made with sodium

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[g]BBL Co., Cockeysville, MD.

[h]BBL Co., Cockeysville, MD.

dibasic phosphate and sodium monobasic phosphate. After dehydration with alcohol and xylol, the sections were embedded in paraffin. The paraffin sections were cut at 6 μ and stained with hematoxylin and eosin according to the established procedures (Luna, 1968).

RESULTS AND DISCUSSION

Clinical signs of respiratory disease of varying degree were apparent in calves 1, 2 and 3 for approximately 2 weeks and in calves 4 and 6 for about 4 weeks. Calf 5 was ill for less than 5 days. Calf 7 was ill for less than 3 days. Calf 8 had clinical signs of respiratory disease of unknown duration.

Pasteurella multocida and/or *Pasteurella haemolytica* were isolated from all calves except calf 6. Some of the animals also had other potential pathogens present. These included *Corynebacterium pyogenes, Pseudomonas aeruginosa,* nonhemolytic *Escherichia coli* and other *Corynebacterium sp.*

Calf 6 was found to have IBR virus on the frozen tissue sections. In calf 8, PI-3 virus was isolated from the respiratory tissue.

Some of the calves had single or multiple parasitic infections. Parasitic ova which were identified included coccidia, strongyles, trichostrongyles and *Trichuris*.

Gross Lesions Observed at Necropsy

Gross lesions ranged from those typical for acute to lesions seen in chronic bronchopneumonia. Consolidation of the lungs ranged from slight to 85%. Abscesses were seen in some of the lungs and fibrinous exudate covered the nasal and tracheal mucosa of some of the calves. Other lesions included edema and hemorrhage of the abomasum and swollen mesenteric lymph nodes.

Table 2. Viral and bacterial agents isolated and final diagnosis

Calf	Illness	Virus	Bacteria	Diagnosis
1	approx. 2 wks	none	*P. haemolytica*	1) Bronchopneumonia due to *Pasteurella sp.* 2) Parasitism with strongyles
2	approx. 2 wks	none	*P. multocida* *C. pyogenes*	1) Bronchopneumonia due to *Pasteurella sp.* and *C. pyogenes* 2) Parasitism with strongyles 3) Chronic suppurative synovitis and arthritis
3	approx. 2 wks DIED	none	*P. haemolytica* *P. aeruginosa* *E. coli* (nonhem.)	1) Acute fibrinous pneumonia caused by *Pasteurella sp.* 2) Parasitism with stomach worms
4	in lot 4 wks	none	*Pasteurella sp.* *E. coli* (nonhem.)	1) Chronic suppurative pneumonia caused by *Pasteurella sp.*
5	less than 5 days	none	*P. multocida*	1) Mucopurulent rhinitis or early BRD probably caused by *Pasteurella sp.*
6	in lot 4 wks	IBR	*C. pyogenes*	1) Chronic suppurative pneumonia caused by *C. pyogenes*
7	less than 3 days	none	*P. haemolytica* *Corynebacterium sp.*	1) Bronchopneumonia associated with both bacteria
8	?	PI-3	*P. multocida* *P. haemolytica* *C. pyogenes*	1) Early BRD associated with *Pasteurella sp.* and PI-3

Observations by Scanning Electron Microscopy and Histopathologic Reports

Nasopharyngeal Mucosa

Examination of the nasopharyngeal mucosa from a control calf by use of the scanning electron microscope revealed uniform cells with distinct cell borders and a smooth, even cell surface. The nonciliated cells were covered with uniform-sized microvilli which appeared dense and evenly distributed on each cell. Goblet cells and secretory glands were not visible in this section of mucosa (Figure 1). The nasopharyngeal cells in Figure 2 from a sick calf were covered with groups of cilia.

The histologic appearance of the nasopharynx from control calf 10 was apparently normal. There were lymphocytic nodules in the posterior submucosa but acute inflammatory reactions were not present. The mucosa appeared to be intact. The second control calf (9) had essentially normal nasopharyngeal tissue. There were individual muscle fibers with hyalinization, vacuolization and loss of striation similar to that seen in nutritional myopathy.

The mucosal surface from the nasopharynx of calf 2, which had been sick for approximately 2 weeks, was swollen causing an uneven surface due to rounding of cells. Both ciliated and nonciliated cells were apparent. In some areas the cilia were degenerated and appeared as very short tufts or broken strands. The cell borders could be seen and microvilli were visible but appeared separated and less numerous. Necrotic areas, mucus and debris were present. Goblet cells and secretory glands were not seen (Figures 2 and 3).

A second nasopharyngeal mucosal specimen from calf 8, which had chronic suppurative pneumonia associated with *Corynebacterium pyogenes*

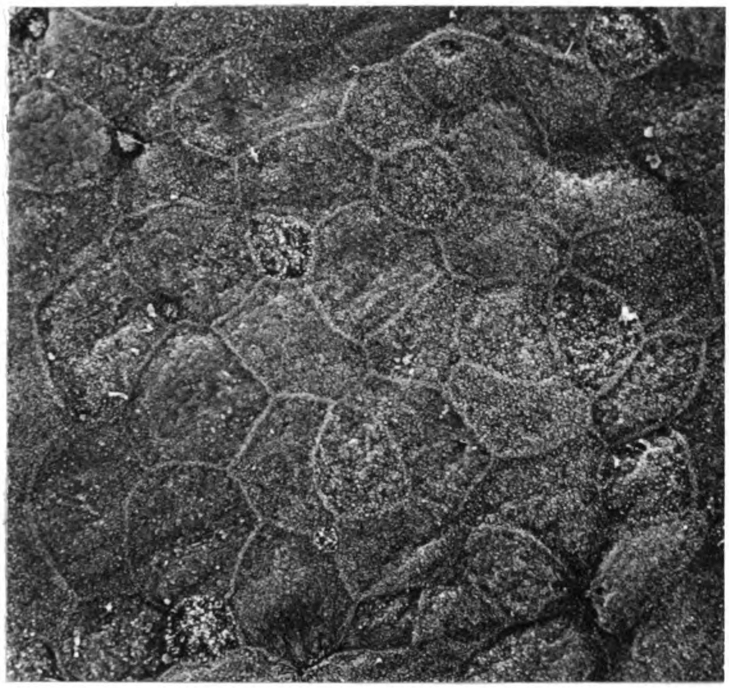

Figure 1. Nasopharyngeal mucosa from a normal calf. Cells are covered with microvilli. In this location ciliated cells were not present. In an area from a sick calf (Figure 2) ciliated cells formed part of the cell population. Cell borders are distinct and mucosal surface is relatively regular. Gold palladium coated. x 1600.

Figure 2. Nasopharyngeal mucosa from a sick calf. All cells are covered with microvilli. Some cells have long cilia (A) projecting from the surface, while other cells have broken or short stubs of cilia remaining (B). Cell surfaces appear to be bulging slightly more than in the control. Cell borders are less distinct. Areas of necrosis are visible. Gold palladium coated. x 1800.

Figure 3. Two cells appear necrotic in the nasopharyngeal mucosa of a sick calf. Cilia and microvilli are present on the adjoining cells. The microvilli are abundant but appear separated. Gold palladium coated. x 4500.

and IBR, appeared extremely swollen and bare. The surface appeared partially denuded of the epithelial layer. Some short tufts and remnants of cilia were scattered on the cells. A few microvilli-like stubs were faintly visible and cell borders were not distinct. A few leukocytes and globules of mucus were present (Figure 4). The virus of IBR was isolated from the lung tissue. *Pasteurella haemolytica* and *Corynebacterium pyogenes* were isolated from swabs taken from the nasal cavity.

Histopathologic examination revealed erosion of the epithelium leaving pit-like depressions. There was sloughing of epithelial cells and marked neutrophilic infiltration into and below the epithelium. Exudate, including mucus, cellular debris, and a few leukocytes, adhered to the surface in a few locations. Many plasma cells were seen in the subepithelium. There was a marked decrease in the depth and height of the epithelium to a single layer of cells in some areas.

Several SEM studies of normal tissue and changes which may occur in diseases of the respiratory tract epithelium have been performed by investigators who worked with human or animal tissue. Most of the investigations dealt with a single portion of the respiratory tract epithelium. An ultrastructural study of cilia during regeneration revealed that cilia formed as basal bodies which ascend and orient themselves at right angles to the surface of the cell in tracheal tissue (Hilding and Hilding, 1966). They found that PI-3 virus destroyed epithelium of organ culture by lysis. The viral maturation site could be identified among the microvilli at the time of maximal release of virions into the media.

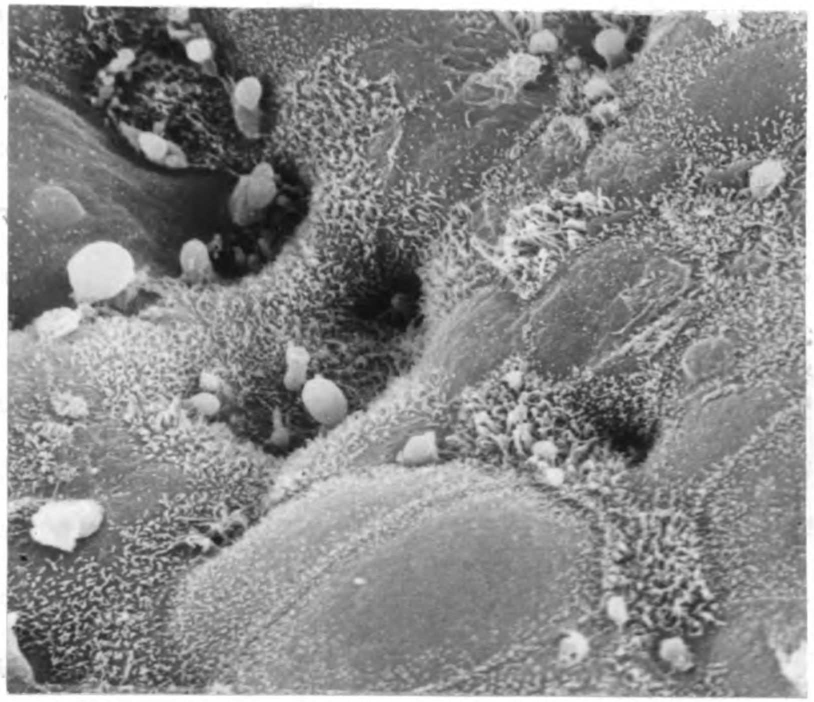

Figure 4. Nasopharyngeal mucosa from a sick calf. Cells are swollen causing an uneven appearance to the surface. All cilia are degenerated or lost and microvilli have been lost in some of the areas. Globules of mucus are lying on the surface. Openings of mucous glands are visible. Gold palladium coated. x 2000.

Horn (1966) isolated and cultivated the virus of the common cold in human epithelium and found it caused histopathologic changes in the epithelial cells. A SEM study by Dutta and Johnson (1974) found that cilia were destroyed *in vitro* and *in vivo* by viral bronchitis beginning on the 4th day following infection. By the 10th day, holes were seen in the surface, cilia were completely destroyed, and epithelial cells rounded up and sloughed off. A fibrinous exudate was visible along with microvilli-like structures. After 12 days the cilia began to reappear.

The nasopharyngeal mucosa from a sick calf (5) with early stage of BRD was denuded of cilia. Cells appeared swollen and individually separated causing them to protrude. Microvilli were not seen on the nonciliated cells. Leukocytes and globules of mucus were present on the surface (Figure 5). *Pasteurella multocida* was isolated from the nasal and tracheal swabs. A mucopurulent exudate had accumulated in the nasal passages. Histopathologic examination showed an accumulation of a few neutrophils in the lamina propria and submucosa at the bifurcation of the trachea. There was no indication of an exudative reaction in the lungs.

Turbinate

The turbinate mucosa from a normal animal consists of ciliated and nonciliated pseudostratified columnar cells with areas of transition to stratified squamous cells (Greep and Weiss, 1973). The micrographs from the control calf turbinate could not be used because it appeared too distorted, perhaps from mucoid film or overcoating of the tissue, for SEM examination.

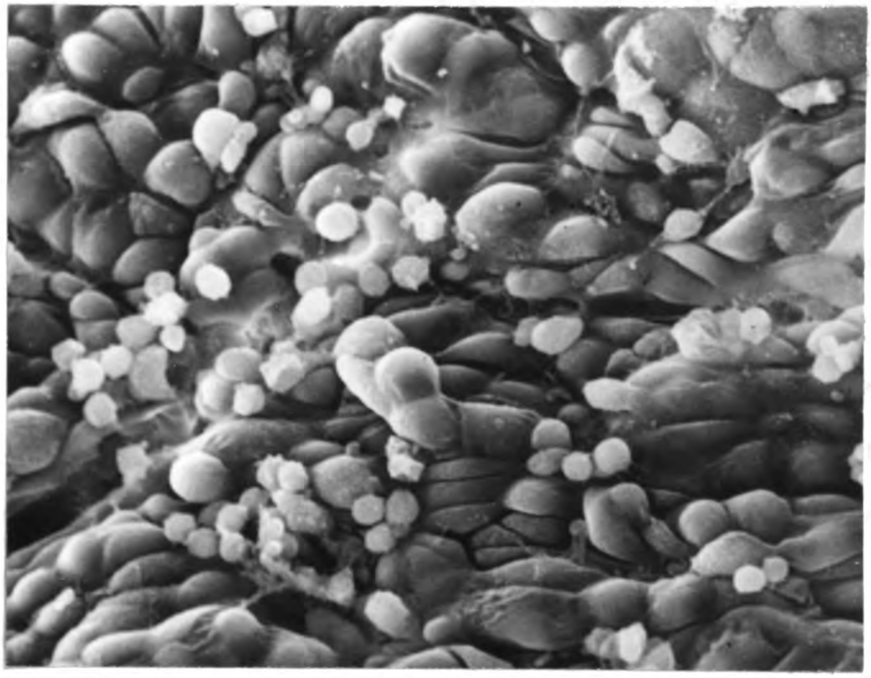

Figure 5. Nasopharyngeal mucosa from a sick calf. Cells are swollen and crowded, which gives a distorted appearance to the shape of the cells. A mucous film may also produce this distorted appearance. Cilia are absent. Microvilli are not visible. Mucous globules and a few leukocytes are lying on the mucosal surface. Gold palladium coated. x 500.

Scanning electron microscopic studies on normal rabbit olfactory cells by Barber and Boyd (1968) revealed good cell outline with a sparse number of ciliated cells with numerous groups of cilia which varied in length. The microvilli on the nonciliated cells were clearly seen.

The posterior nasal septum, anterior part of the superior nasal fossa, base of the turbinate, and nasopharynx investigated by Greenwood and Holland (1972) consisted mainly of ciliated cells with varying lengths of cilia on the same cell. The nonciliated cells of the nasal cavity were covered with microvilli which varied in length from cell to cell.

Histopathologic examination of the turbinate of the first control calf (9) showed lymphocytic nodules in the submucosa with variable numbers of lymphocytes cattered individually throughout the mucosa, but no indication of an inflammatory reaction was apparent. Evidence of erosion was not observed. The second control calf (10) appeared to have lymphocytic nodules in the submucosa of the posterior nasal pharynx, but acute inflammatory reaction was not seen and the mucosa appeared to remain intact. The sections were both within normal range.

The turbinate mucosa of calf 2 had been denuded of all the cilia. The microvilli appeared as short stubs on some of the cells, while other cells appeared to have an abundance of longer microvilli. The cell outlines were distinct, but some of the cells appeared rounded. There were areas of separation of cells and necrosis. A secretory gland was seen in the process of extruding mucus (Figure 6). The histopathologic examination did not include the turbinate tissue.

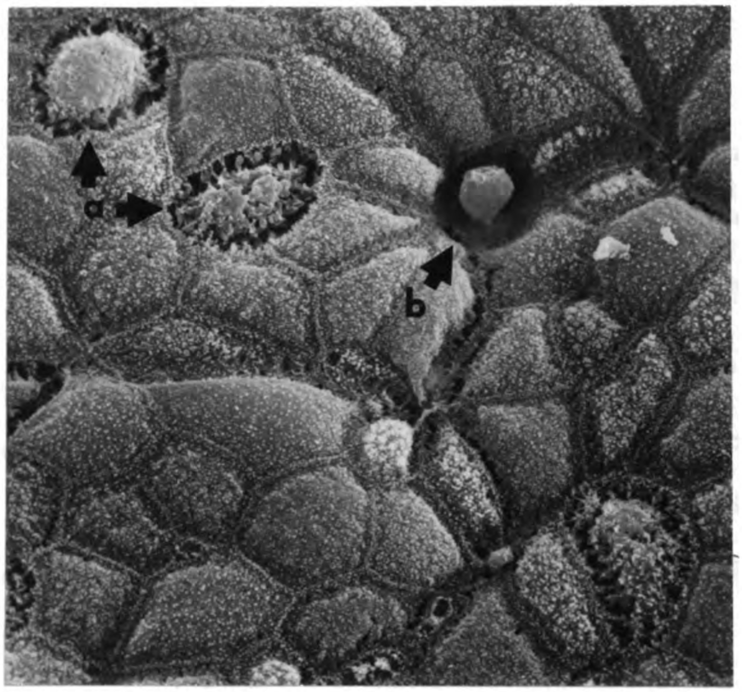

Figure 6. Mucosal surface of a turbinate from a sick calf. Cells appear slightly swollen. Cilia are not present, but microvilli are visible. Necrotic cells (A) and 1 mucous secreting cell (B) are visible. Gold palladium coated. x 1800.

Pasteurella multocida and *Corynebacterium pyogenes* were isolated from the nasal and tracheal swabs.

The turbinate of calf 3, which died, had a few cells with long cilia and many cells with matted and degenerated cilia in tufts. Areas of swelling and depressions were evident. Most of the cells were covered with microvilli of varying density and length. Microvilli outlined some of the cells with a thick outer ring but some of the cell borders were lost. Many of the cells appeared sunken, giving the surface a pitted appearance. Leukocytes and mucus were present (Figure 7).

Tracheal Mucosa

The mucosa from the trachea of a control calf was covered with long cilia. The cell borders or microvilli were not visible because of the dense cilia. Secretory glands were not visible (Figure 8). Numerous tracheal glands were normally present which are tubulo-alveolar and of mixed types (Greep and Weiss, 1973).

Microscopic examination of the trachea from a control calf (9) appeared to be within normal limits, except for a somewhat undulated surface. There were numerous lymphocytic nodules under the epithelium in the submucosa, but erosion or cellular infiltrations were not observed. Tracheal tissue of the control calf (10) appeared normal without evidence of erosion or inflammatory reaction.

A SEM study of mammalian trachea by Greenwood and Holland (1972) revealed groups of ciliated cells interspaced between polyhedral microvilli-covered cells. The intracellular junctions had compact arrangement of slightly longer microvilli. A study of normal rabbit trachea by Barber and Boyd (1968) revealed groups of cilia in each cell which grew in one direction toward the larynx.

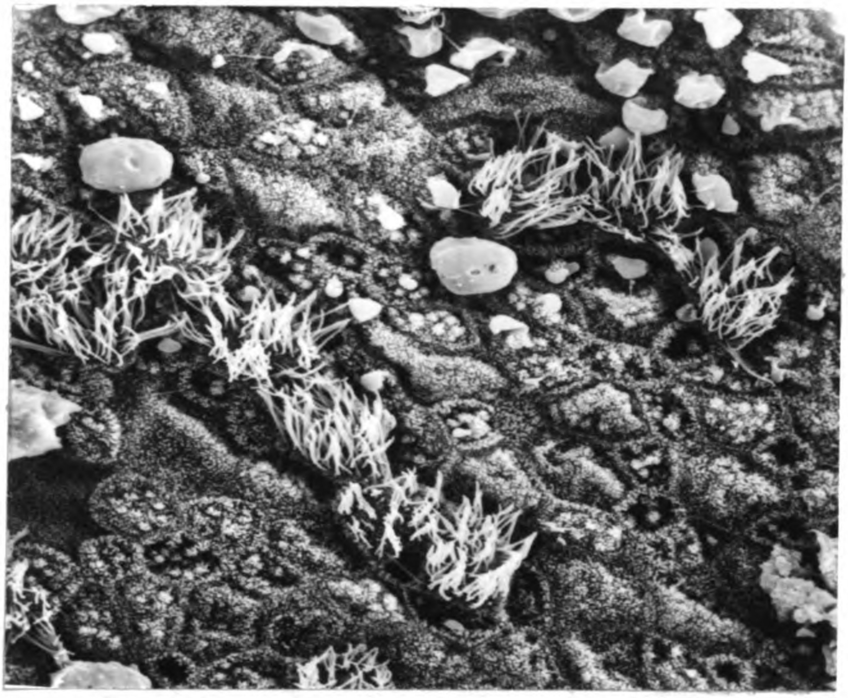

Figure 7. Mucosal surface of a turbinate from a sick calf. Cilia can be seen on a few of the cells. Some cilia appear to be broken, leaving only stubs of cilia. Other cilia appear to be tangled. A few mucous globules are lying on the surface. Gold palladium coated. x 2000.

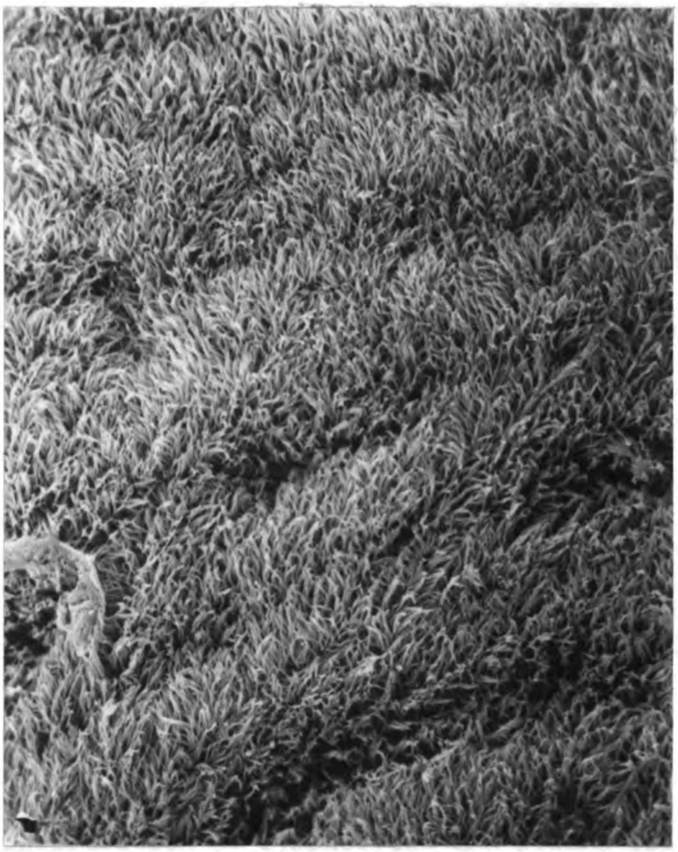

Figure 8. Tracheal mucosa from a normal animal. Cells are densely covered with cilia. Gold palladium coated. x 1000.

Severe changes appeared in the tracheal mucosa from the calf (3) which died. A few areas of mucosal surface had remnants of cilia left and only a few cells appeared to have microvilli. Most of the surface appeared bare, and the epithelium appeared to be destroyed. The remaining cells were swollen, leaving crypt-like separations of cells with uneven surface (Figure 9). *Pasteurella haemolytica* was isolated from the tracheal swab.

Most of the tracheal surface from a calf (2) with bronchopneumonia was covered with rounded and roughened epithelium. Cells with degenerated and matted cilia were scattered through the surface mucosa. Microvilli covered most of the nonciliated cells. Irregular and deep crypts separated areas of cells. Some of the cells appeared to be extruded. Exudate material was present (Figure 10). *Pasteurella multocida* and *Corynebacterium pyogenes* were isolated from tracheal swabs.

Microscopic lesions were typical of a chronic bronchitis characterized by infiltration of lymphocytes and plasma cells into the lamina propria and peribronchiolar proliferation of connective tissue.

A study conducted by Nelson (1974) found that IBR caused complete destruction of cilia and partially denuded the epithelial cell layer of the trachea in 3 infected calves. Reed and Boyde (1972), who cultured a rhinovirus in bovine tracheal epithelium, described a reduction in speed of ciliary action on the 4th day. By the 6th day only a few cells had degenerated cilia left. Extrusion and shedding of the epithelium was evident along with squamous metaplasia. A few microvilli were visible. When they used PI-3 virus, the loss of ciliary activity and cilia took 11 days. Cilia were destroyed and

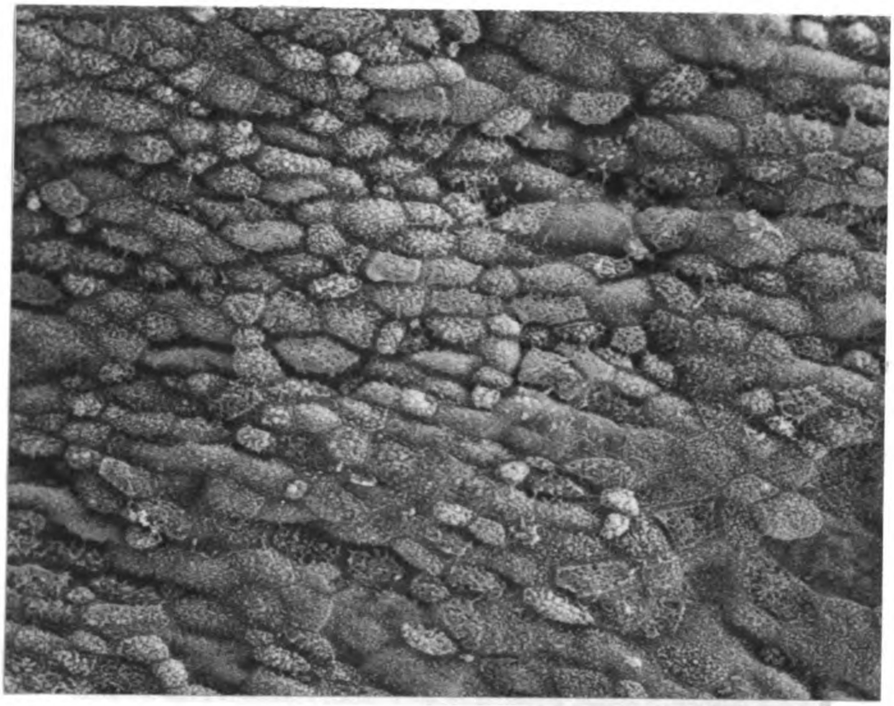

Figure 9. Tracheal mucosa from a sick calf. Rounded and roughened epithelium covers the surface. Suggested remnants of cilia are left. Microvilli are apparent on many of the cells. The cells appear to be pulling apart. Gold palladium coated. x 1000.

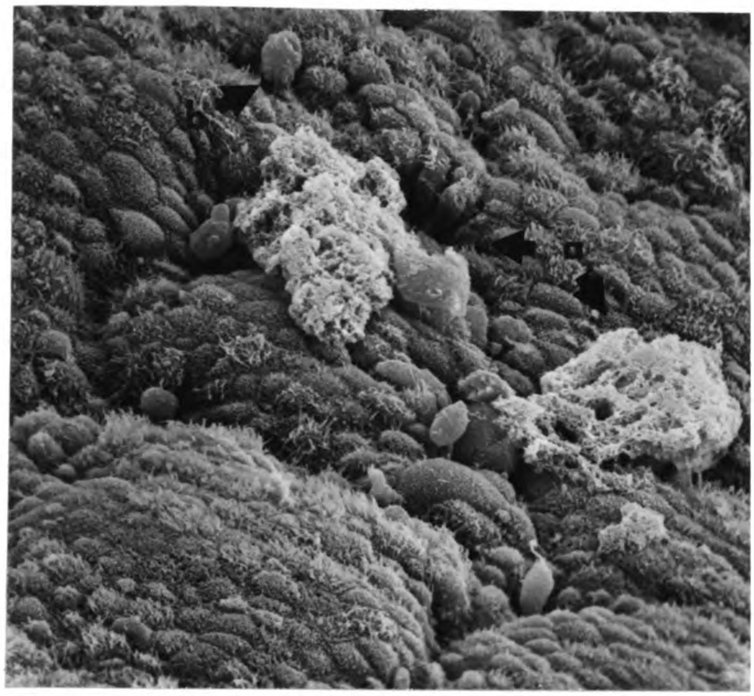

Figure 10. Tracheal epithelial cells from a sick calf. Cells are rounded with deep crypts separating areas of cells. Cells with degenerated and fragments of cilia give the surface a ragged appearance. Globules of mucus are present. Two masses of mucoid material (A) cover part of the membrane surface. One cell appears to be in the process of extrusion (B). Gold palladium coated. x 900.

mucus adhered to the base of the ciliated tufts which remained. Microvilli covered the nonciliated surfaces.

Bronchial Mucosa

Normal bronchial mucosa has lower columnar cells than seen in the tracheal mucosa. Ciliated and nonciliated cells are present (Greep and Weiss, 1973). The bronchial tissue of the control calf (9) was not satisfactory for SEM due to underprocessing during dehydration, or overcoating with palladium.

On histopathologic examination of the normal bronchus of control calf 10, the tissue appeared normal except for an undulating appearance of the epithelial layer with only a few lymphocytic nodules within the submucosa. The bronchus of control calf 9 appeared within normal limits except for a somewhat undulating surface with numerous lymphoid nodules with active lymphocytic follicles in the submucosa. The mucosa had globular projections into the lumen.

Severe changes were evident in the bronchus of the calf (3) which died with acute fibrinous pneumonia. Desquamation of a major portion of the surface mucosa and loss of cilia were apparent. Cell borders were not present, but atrophied microvilli could be seen. Pitted areas presented a rough appearance (Figure 11). Histopathologic examination revealed areas of necrosis as well as infiltration of large numbers of neutrophils into the bronchi.

Terminal Bronchiole Mucosa

The ciliated and nonciliated (Clara) cells were prominent in the terminal bronchioles of the control calf (10). The longitudinal folds of mucosa were pronounced in this small tubular structure (Figure 12). When the lung is inflated, these tubes are normally open for the

Figure 11. Mucosal surface of bronchus from a sick calf. Cells are nearly denuded of cilia. Cell borders are not distinct. Gold palladium coated. x 2000.

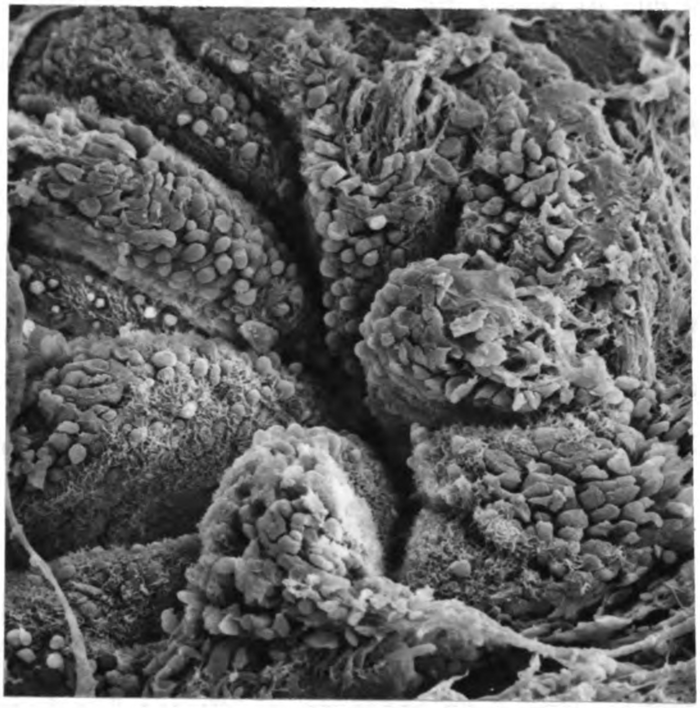

Figure 12. Section from the terminal bronchiole from a control animal with pronounced folds in the membrane. The surface contains ciliated and nonciliated cells and large, prominent, rounded cells. Gold palladium coated. x 400.

passage of air into the alveoli but are closed when the lung is deflated (Hughes et al., 1970). Histologically, the bronchioles of the control calf (10) were within normal range.

Ciliated and nonciliated cells of the bronchioles are described by Wang and Thurlbeck (1970) in a SEM study of lung tissue. Goblet cells may be present, but glands are not found in the bronchioles (Smith, Jones and Hunt, 1972).

The bronchiole of calf 5 with early BRD was filled with exudate. The walls were thickened and the inflammation extended into the peribronchiolar spaces, which are filled with exudate. Microscopic examination of the lung revealed focal areas of increased lymphoid tissue around some of the bronchi and bronchioles. The thickened alveolar walls seemed to center around bronchi and bronchioles, which had peribronchiolar lymphocytic hyperplasia. The lesions were relatively mild and limited.

Another bronchiole from a calf (2) with chronic bronchopneumonia was obliterated and the walls appeared to be continuous with the adjacent lung tissue. The walls of the alveoli in the area of the bronchiole were thickened and the air spaces varied in size (Figure 14).

Another terminal bronchiole removed from a different section of the lung from the same calf (2) was filled with exudate and had a thickened wall. The walls of the alveoli appeared thicker and many of the air spaces appeared smaller or collapsed. The lung sections may have been taken when the lungs were deflated or may be due to the method of preparation or excision of the tissue (Figure 15).

A higher magnification of the bronchiolar tissue (see Figure 15) had normal ciliated cells and cells with degenerated cilia. Microvilli

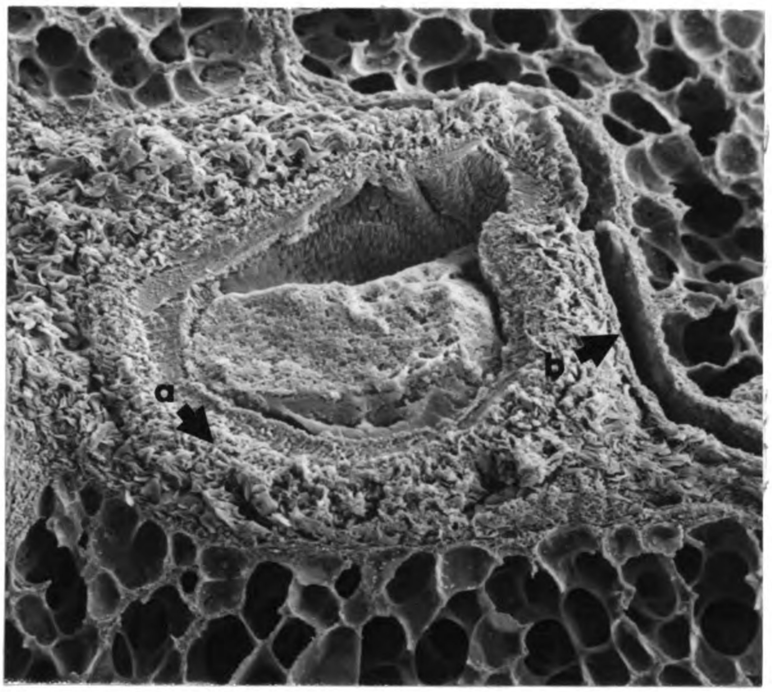

Figure 13. Terminal bronchiole from a sick calf. The small tubular airway has a plug blocking the structure. The peribronchiolar connective tissue appears to be filled with exudate (A). An interlobular connective tissue space is visible (B). Gold palladium coated. x 100.

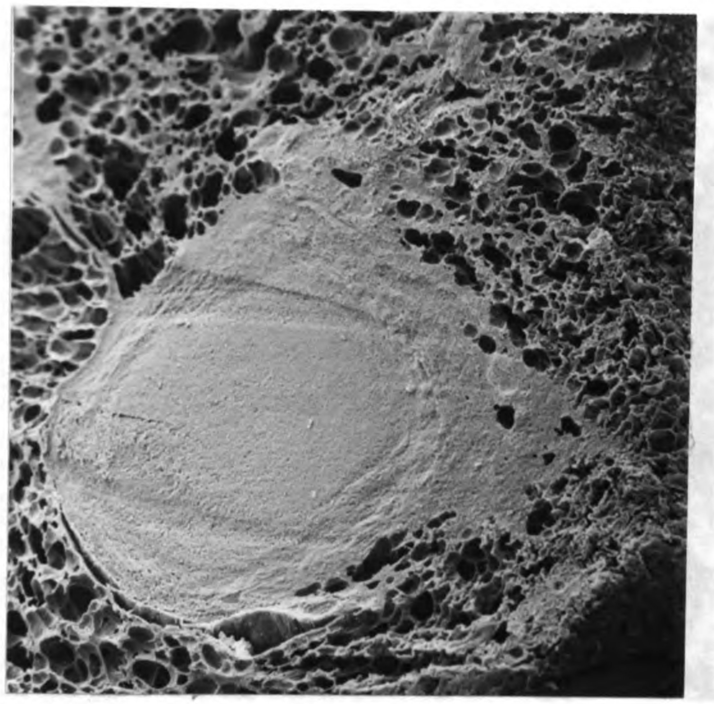

Figure 14. Terminal bronchiole from a sick animal. The tubular structure is filled with exudate. Inflammation and consolidation have extended to the adjacent pulmonary structure. Gold palladium coated. x 100.

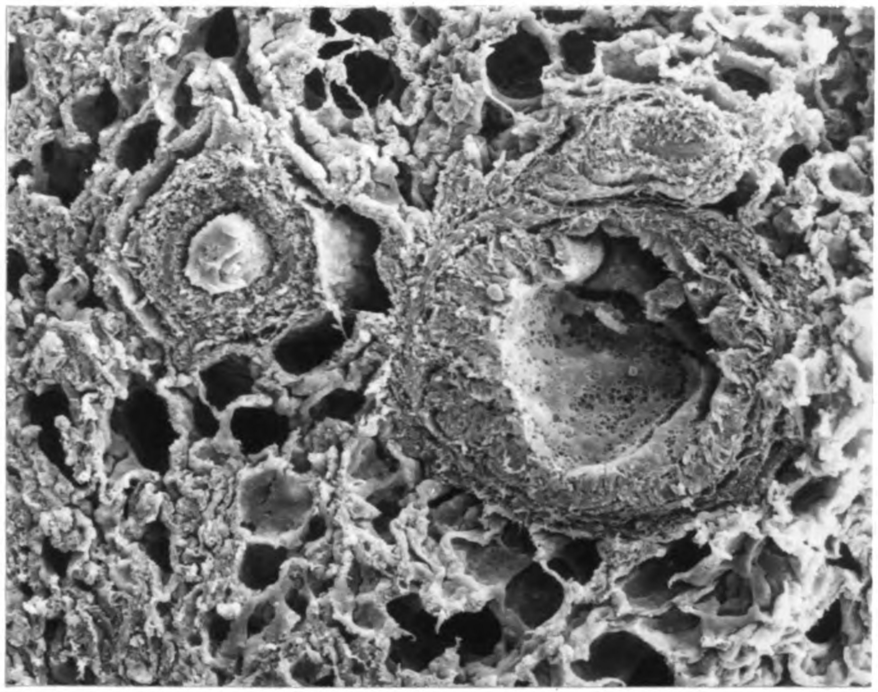

Figure 15. Terminal bronchiole from a sick calf. The tubular structure is lined with exudate. Both tubular structures appear thickened. The inflammation has caused the alveolar walls to appear thick and the air spaces small or obliterated. Gold palladium coated. x 100.

which varied in length could be seen on some of the nonciliated cells. Exudate adhered to the base of some cilia. Necrotic areas, cellular debris and exudate could be seen (Figure 16).

Histopathologic examination of the lungs revealed microscopic lesions of chronic bronchitis in addition to focal areas of necrosis and fibrin deposition both in the bronchi and in the alveolar spaces surrounding some of the bronchi and bronchioles.

The terminal bronchiole of the calf which died (3) had deep crypts which appeared to separate the cells. The cilia at the base of the tall nonciliated cells were tangled and broken. The cells appeared to be swollen individual structures unrelated to each other. One of the cells appeared to be extruding (Figure 17).

Microscopic examination revealed a severe acute fibrinous pneumonia with areas of necrosis as well as infiltration of large numbers of neutrophils into the bronchioles.

Alveoli

Normal alveoli from the control calf (10) had uniform air spaces within thin walls which were lined with a very thin layer of continuous alveolar epithelium. Pores of Kohn were visible in the walls (Figure 18). In dogs, pores of Kohn increase in number with an increase in age (Martin, 1966). A large common opening for many of the alveoli which may represent the alveolar sac could be seen (Figure 19).

On microscopic examination, normal lung tissue taken from a control calf (10) appeared within normal range.

A SEM study of alveoli (Nowell and Tyler, 1971) described the alveoli as deep polyhedrons with straight walls and uniform-sized air spaces. The thin-walled alveolar septa communicated through circular

Figure 16. Higher magnification of the mucous membrane of a terminal bronchiole from a sick calf. Some cells appear to be partially denuded of cilia. A few cells are necrotic, appearing as gaps in the surface. Some exudate is present adhering to the base of the cilia. Tangled remnants of cilia can be seen between the cells. Gold palladium coated. x 1750.

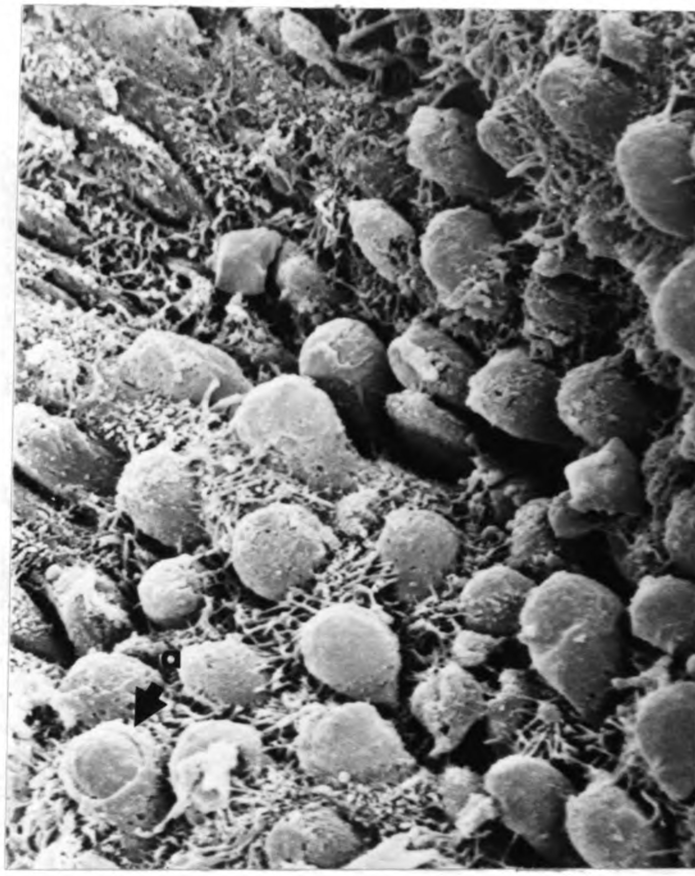

Figure 17. Epithelial cells of the terminal bronchiole from a sick calf. The cells are markedly swollen and appear as individual structures unrelated to each other. The tall cell surfaces appear bare. One of the cells (A) is being extruded. Gold palladium coated. x 2000.

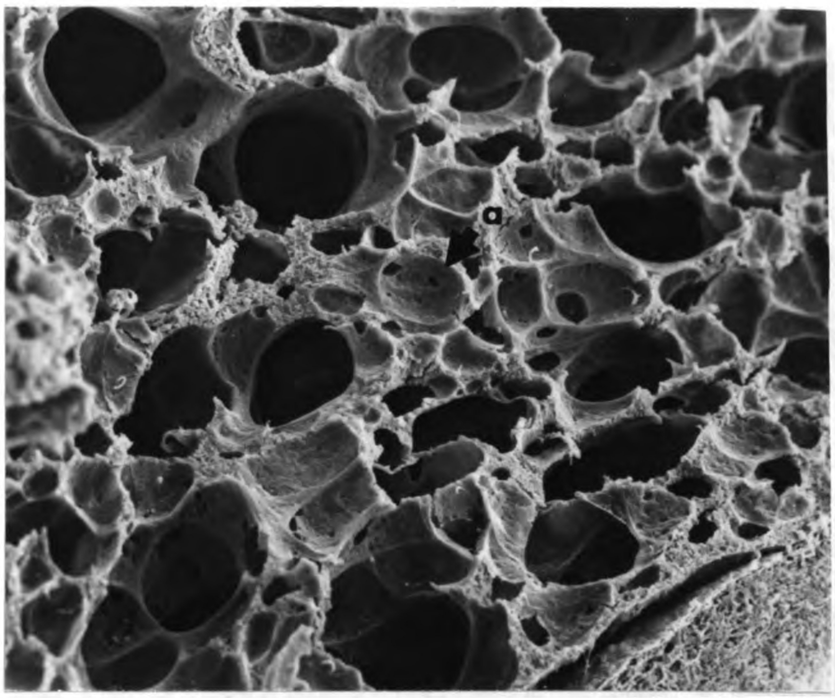

Figure 18. Alveoli from a normal calf. Alveoli are open and uniform. The walls are thin and regular. A few pores of Kohn (A) are visible. Gold palladium coated. x 100.

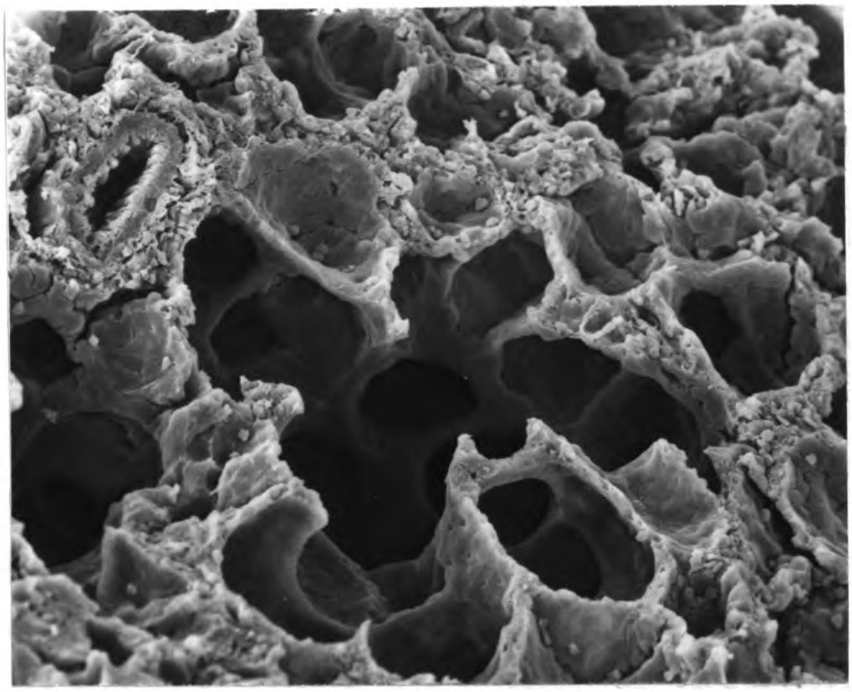

Figure 19. Higher magnification of the alveoli from a normal animal. Alveolar walls appear to have pores in their surfaces. Some alveoli communicate with adjacent alveoli. There appears to be a large common opening for many of the alveoli, which may represent the alveolar sac. Gold palladium coated. x 400.

alveolar pores with some of the alveoli opening directly from bronchioles. Groniowski et al. (1972) described distinct bumpy plications of the alveolar septa of the lungs from a dog. These plications were scanty when the walls were fully inflated and prominent when the alveolar walls were collapsed. Thin bridges connected the alveolar coat. Wang and Thurlbeck (1970) described many round elevations on the alveolar surface and fibrous strands connecting the alveoli in human lung tissue.

The air spaces in the lung tissue taken from a calf (2) which had chronic bronchopneumonia appeared variable in size because of the thickened alveolar walls. Inflammation had not extended to all parts of the lung, but a few slightly thickened alveolar walls were suggestive of an early inflammatory change (Figure 20).

Pasteurella multocida and *Corynebacterium pyogenes* were isolated from the lung tissue. Microscopic examination revealed focal areas of necrosis and fibrin deposition in the alveolar spaces. Other lesions of chronic bronchitis were characterized by infiltration of lymphocytes and plasma cells into the lamina propria.

In calf 5, which had an early BRD caused by *Pasteurella multocida*, the alveolar walls had thickened and had lost their structure in some areas. Other alveoli appeared ballooned, which may have been caused by the occluded bronchioles in the consolidated area (Figure 21).

Histopathologic examination showed no indication of exudative reaction in the lungs. Focal areas of atelectasis and focal areas of increased thickness of the alveolar wall due to infiltration with mononuclear type cells. The thickened alveolar walls seemed to center around the bronchi and bronchioles which had peribronchiolar lymphoid hyperplasia. The lesions were mild and limited in extent.

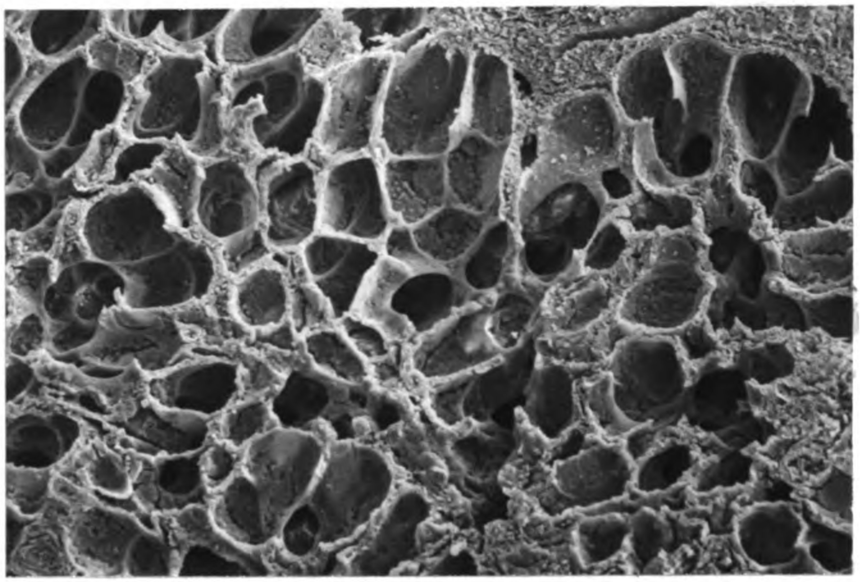

Figure 20. Alveoli from a sick animal taken from an area adjacent to pneumonic lesions. Inflammation has not extended to all parts of the lung, as indicated by the unaffected alveoli. There may be early inflammatory changes which are suggested by a few slightly thickened alveolar walls (compare with Figure 21). Gold palladium coated. x 180.

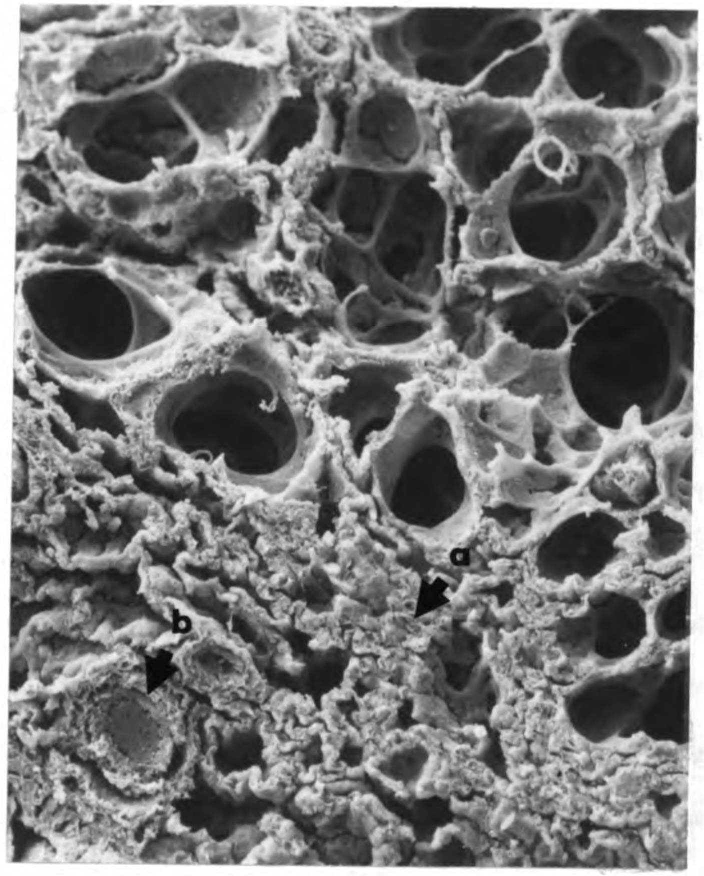

Figure 21. Alveoli from a sick animal. Some of the alveolar walls have thickened and other alveoli have collapsed (A). Adjacent alveoli appear unaffected. A small terminal bronchiole (B) is occluded (compare with Figure 20). Gold palladium coated. x 100.

The alveolar tissue taken from the area of consolidation in a calf (2) with chronic bronchopneumonia had most of the structural detail obliterated by thickened and collapsed alveolar walls (Figure 22).

An ultrastructural study of lung tissue from a calf with shipping fever revealed adenovirus type particles in the cells and degenerated organelles. Bronchial tissue revealed desquamation and degeneration of cells (Kim, 1975).

The alveolar tissue from a calf (3) with acute fibrinous pneumonia was filled with exudate containing large numbers of leukocytes. All the structural detail of the lung was obliterated (Figure 23). A higher magnification of the same area revealed alveoli which were densely packed with leukocytes interwoven with strands of fibrin throughout the area. Erythrocytes were absent (Figure 24).

Histopathologic examination revealed areas of necrosis and neutrophilic infiltration of the bronchi, bronchioles and surrounding alveolar spaces and accumulation of large quantitites of fibrin in the alveolar spaces.

In the cases of shipping fever which have been presented, a good correlation is apparent between the changes seen in respiratory mucosa by SEM and the results revealed by histopathologic, virologic, and bacteriologic examinations.

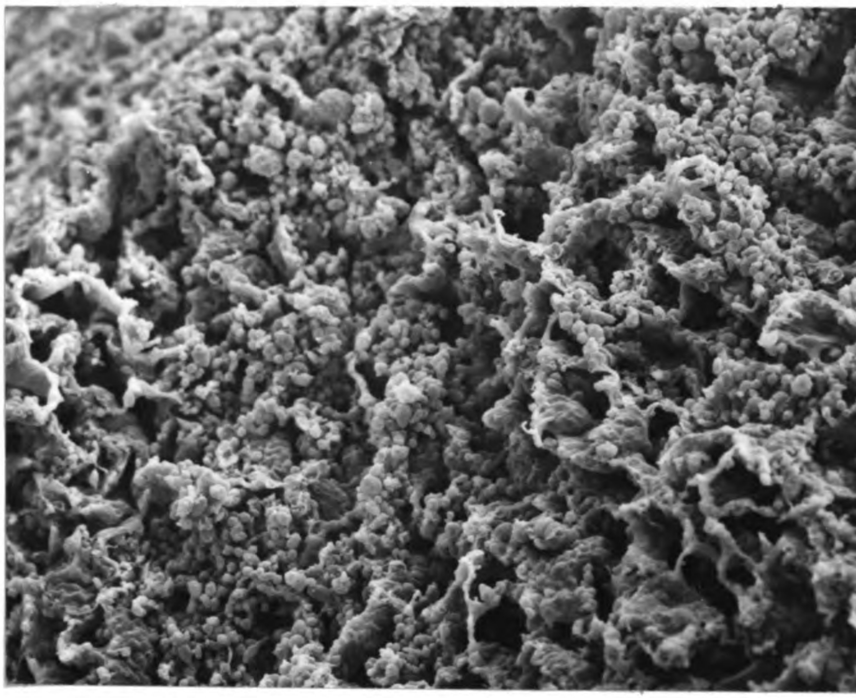

Figure 22. Consolidation of the lung in a calf with shipping fever. A few alveolar walls can be seen, but most of the structural details have been obliterated (compare with Figure 18). Gold palladium coated. x 180.

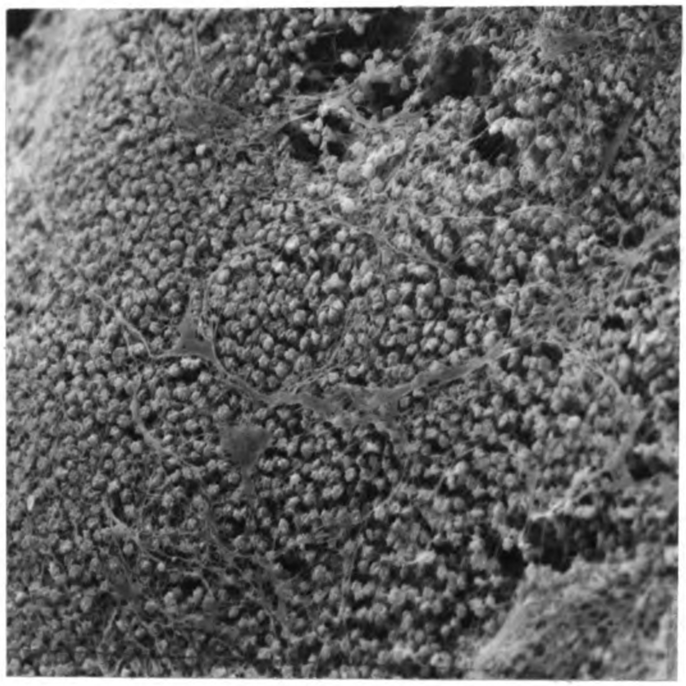

Figure 23. Exudate containing large numbers of leukocytes which obliterates all structural detail of the lung. There is total consolidation of this area (compare with Figure 18). Gold palladium coated. x 500.

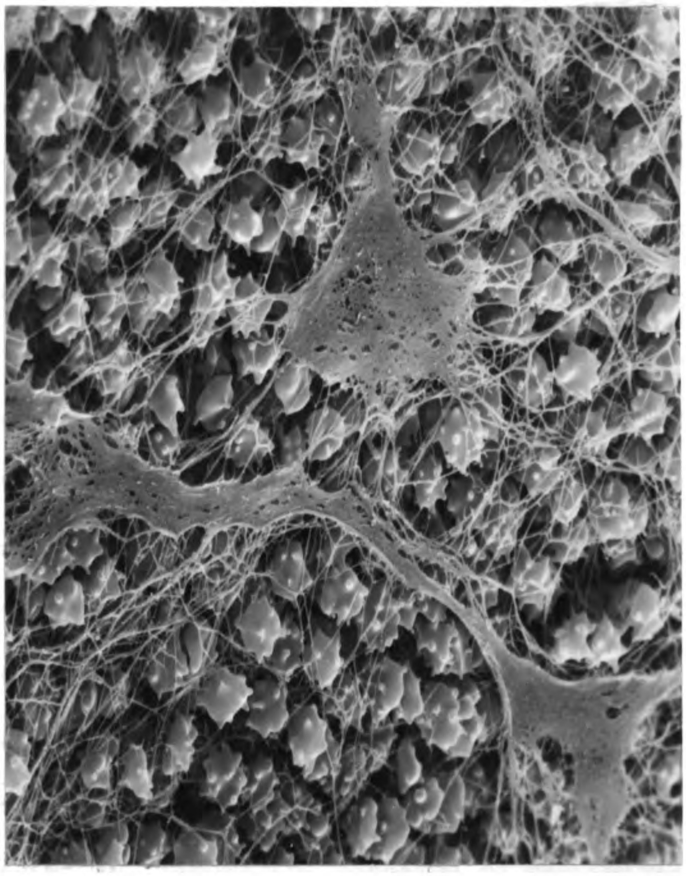

Figure 24. Higher magnification of an area from Figure 23. Notice the densely packed leukocytes and the absence of erythrocytes and alveolar walls. Strands of fibrin, which are more dense in some areas, can be seen throughout the exudate. Gold palladium coated. x 2000.

CONCLUSION

The scanning electron micrographs from the present study illustrate the destruction which occurs in the respiratory epithelium of calves with shipping fever. The ciliary destruction varied from partial loss and degeneration to complete denuding of the individual cells. The microvilli appeared normal, blunted, less abundant or absent as the severity of the disease increased. Extrusion and destruction of the respiratory epithelium left the surface mucosa exposed as the protective cell layer changed from a ciliated epithelium to a layer of nonciliated or undifferentiated epithelium.

With the loss of these vital defense mechanisms apparently due to viral infections, and the loss of the mucous film secreted by the goblet and secretory glands, the invasion by *Pasteurella sp.* or other pathogenic bacteria may have been made easier.

As the disease progressed in severity, the lungs lost their normal architectural structure as the alveolar walls became thickened and the air spaces became smaller. Exudation was apparently related to bacterial invasion and caused the alveoli and bronchioles to be occluded. A severely impaired and badly damaged respiratory organ was the result.

The majority of micrographs illustrated that the lesions were more apparent and more severe and affected greater portions of the respiratory mucosa as the severity of the clinical signs of shipping fever increased.

SUMMARY

Representative animals with characteristic signs of shipping fever were selected from 8 calves which had been purchased from farms or feedlots. The diagnosis at the time of necropsy varied from early bovine respiratory disease (BRD) to acute fibrinous pneumonia caused by *Pasteurella sp.* or other pathogenic organisms. Mucosal epithelium was obtained from 5 calves which had been sick from 3 days to approximately 2 weeks and from 2 normal calves.

The scanning electron microscope was used to study the respiratory mucosa of the nasopharynx, turbinate, trachea, bronchus, bronchioles and alveoli from calves with shipping fever and to compare the tissue with similar micrographs from control calves.

In most areas the normal respiratory mucosa consisted of ciliated cells interspaced with flat or columnar cells. The flat nonciliated cells were densely covered with microvilli. In the airways the tall cells had ciliated cells at the base. In the normal lung tissue the alveoli appeared thin walled with uniform air spaces.

In calves with early BRD associated with *Pasteurella sp.*, the respiratory mucosal cells appeared to be denuded of cilia and appeared swollen, which caused the tissue to appear uneven on the surface. Microvilli were scarce and appeared as short plications. Cell borders were indistinct in some areas and separation of cells was seen in other sections. The bronchioles were filled with exudate and the alveolar walls had thickened.

In animals with bronchopneumonia caused by *Pasteurella sp.*, the mucosal cilia varied from long, slender structures to short stubs. The majority of cells were swollen, giving an uneven appearance to the surface. Microvilli were evident but appeared fewer in number on the cells. Cell borders were visible in most of the sections. Alveolar walls appeared thickened and had smaller air spaces. Bronchioles were filled with exudate.

The calf with an early BRD associated with *Pasteurella sp.*, *Corynebacterium pyogenes* and parainfluenza-3 virus had a nasopharyngeal mucosa which was very swollen, and most of the cells had no cilia or microvilli. The cell borders were not visible.

The calf which died of acute fibrinous pneumonia had most of the mucosal cilia tangled or they were present as broken stubs. Some cells were nonciliated. The microvilli on each cell varied from moderate to none. The surface of many cells was swollen. Pitted and necrotic areas were evident. The lungs had lost their normal architectural structure. Leukocytes and fibrin strands filled the alveoli.

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