sonnel at the laboratory will decide which to use, depending on the history, symptoms, and lesions reported to them. Many times an organ will show microscopic changes but will not be in the system responsible for the clinical signs. The ratio between tissue and 10% formalin should be 1-20. Make it a habit to always carry a few small jars of 10% formalin with you and you will always have it when needed.

### Submission of Specimens for Virus Isolation and Identification[1]

1. Specimens should be fresh and collected, if possible, with aseptic precautions and placed in sterile containers.
2. The source and kind of material depends on the disease. The site of the lesion is usually a good area to sample.
3. No preservatives or fixatives should be added.
4. For isolation purposes, the earlier in the acute stage of the disease the specimen is taken, the better.

   Viruses are usually present in the highest concentration before the onset of clinical signs. Therefore, take the sample as early as possible in the course of the disease.

   Samples from sick animals are preferred rather than samples from animals which have died from the disease.

   Interferon and antibodies may be present in the tissue in later stages of a disease and can interfere with virus isolation procedures.
5. The acutely ill animal will usually have an elevation in temperature. In many viral diseases, a drop in the WBC count will coincide with the rise in temperature. The concentration of virus in the tissue should be highest at this time.
6. Handling of Specimens: Sterile plastic bags for tissue. Swabs in tubes with sterile transport medium.
7. Preservation: Most viruses are heat sensitive. Ship in styrofoam containers filled with crushed ice or dry ice. If dry ice is used, seal the tissue in glass or metal containers so the released $CO_2$ will not inactivate the virus. The best method of shipment is for the owner to take the specimen to the laboratory. Virus isolation and identification is expensive so make sure the specimen is in good condition when it arrives at the laboratory.

   Do not send tissue in 10% formalin and tissue for virus isolation in the same box as freezing will destroy tissue for use in histopathological examination.
8. The standard method of isolation of most bovine viruses is inoculation of bovine kidney cell cultures with the suspected material and incubating the culture until cell damage occurs or until a certain time has elasped. The virus is usually identified by fluorescent antibody tests of cell culture. Fluorescent antibody test can be run directly on some submitted material without culturing.

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# Pulmonary Emphysema of Pastured Cattle

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A significant disease problem of cattle characterized by sudden onset of acute respiratory distress shortly following a change in feed or forage has been known for a number of years. The disease syndrome was described as early as 1830 in Europe and there has been numerous subsequent reports from many areas of the world (17). The disease has many synonyms, but is generally referred to as Acute Bovine Pulmonary Emphysema (ABPE) in the Pacific Northwest and western intermountain regions of the United States. In the Midwest it is often designated as bovine adenomatosis. In other areas of the United States and in several other English-speaking countries it is referred to as a typical interstitial pneumonia of cattle. Local terms that are often used by ranchers and veterinarians include cow asthma, green grass sickness, summer pneumonia, lungers, grunters, panters, and others.

Economic loss to the livestock industry from this disease is extensive. Loss due to death of cattle is considerable, and since the majority of the animals are brood cows, the economic loss substantially exceeds

carcass value. Other economic factors associated with ABPE are possibly even more significant than death losses. Affected cows are often nursing calves and there is subsequently loss of condition and performance of both the cow and the calf. Further, even though the brood cow may survive, she may abort. Also, surviving animals are more susceptible to disease and, consequently, the productive herd life is shortened. Another important monetary factor is the loss of grazing utilization of certain lush summer and fall pastures. Many of these potential feed sources cannot be properly utilized because of the hazards of this disease. Further monetary losses occur because ranchers are forced to alter management procedures by feeding hay and other feeds, moving or altering the cattle into dry lot feeding areas, and/or using alternate pastures. This results in additional feed and labor expenses, poor utilization of available forage and decreased production.

Since many of the economic losses caused by ABPE are rather intangible, reliable estimates of the total annual loss are not available. Some estimates made for individual states vary from one to several million dollars annually (16,22). The total loss to the western United States could be conservatively estimated at several million dollars per year to the livestock industry.

The disease incidence varies from year to year and from one region to another (6,19,22). Causes for this variation are unknown. One factor that is probably involved is the variation in climatic factors which affects either the summer range, the meadows onto which the cattle are transferred, or both. The incidence is increased following exceptionally dry summers which contribute to adverse feed and water conditions of the summer range. On the other hand, if wet climatic conditions or irrigation contribute to exceptionally rapid growth of the forage onto which the cattle are moved, the incidence is increased. Incidence is also apparently influenced by methods of handling and moving of cattle from one feed source to another.

It is the feeling of many people that the disease incidence has increased during the past two decades (2,6,11,14). Reasons for the apparent increase are unknown. It could be due to increased awareness and diagnosis of the condition, changes in the methods in cattle management, or changes in the management of range.

The disease occurs at any time of the year, but is observed most commonly in the summer or early fall (6,14,17). The seasonal incidence is apparently due to the practice of transferring cattle from dry summer range to improved pastures in late summer. With less frequency, it is observed at any time of the year following drastic changes in feed, such as in rotational grazing or when cattle are changed from low to high grain rations. If the disease occurs, it is usually manifested within 10 days after the change.

Onset of the disease usually becomes apparent between two and ten days after a change in feed or forage (3,6,14). The signs are fairly characteristic. The most obvious clinical sign is an increased rate of respiration accompanied by severe dyspnea. An audible expiratory grunt is commonly observed and is a useful diagnostic aid. Another useful finding is that the body temperature is generally normal or only slightly elevated. This helps to differentiate this disease from some of the infectious pneumonias that might have similar clinical signs. Affected cattle stand with the head extended and lowered. As the disease progresses, there is mouth breathing and the tongue is protruded. Affected animals will generally remain standing because it is easier for them to breath in that position. The course may vary from a few hours to a few days after onset of signs. Death comes suddenly and there is usually no struggling once the animal falls. It is often difficult to predict which cattle in an affected group will die, because some that have severe clinical signs will recover, whereas others that have less obvious signs will die unexpectedly. Forced exercise or exertion often results in death.

Morbidity in affected herds is reported to range from 1-100% (18,22). Case fatality usually varies from 0-50% although some outbreaks have noted case fatalities up to 90% (6,18,22).

The causes of the disease remain unknown. A great variety of plant types have been associated with the onset of the disease, but no single plant type, dietary ingredient, or combination of plants or ingredients has been consistently associated as the causative agent (6,11,21). The disease has been associated with both native and improved grass types, various legumes, cruciferous plants such as rape and kale, some weeds, and others. The wide variety of plant types that have been incriminated suggest that a sudden change to any lush forage, regardless of type, might precipitate an outbreak of the disease. In some instances, the ingestion of moldy feed has been incriminated (20).

Since the basic cause and pathogenesis are poorly understood, treatment is generally directed toward relief of clinical signs. This is sometimes rather disappointing. Agents that have been used for treatment include: antihistamine, epinephrine, atropine, corticosteroids, and antibiotics.

Prevention of the disease appears to be more effective than attempting to treat the animals after they become sick. One of the most effective methods of prevention is by controlled management of the cattle at the time a change in forage is made (3,6,14). In this procedure, the cattle are moved to dry lots, fed hay, and placed in the new pasture for gradually increasing periods each day. This adjusts them gradually to the new feed so that a sudden or drastic change does not occur.

There have been numerous hypotheses proposed as to the cause and mechanisms of the disease. Extensive experimental work has been conducted, but to date none have conclusively demonstrated the cause. An allergic phenomenon associated with ingestion or inhalation of sensitizing antigens is often proposed as the basic mechanism of the disease. Another popular proposal is that the lung changes are induced as the result of inhaled gases or other volatile substances which act as irritants to the lung tissues. Another proposal regarding the pathogenesis is that the respiratory disease is a manifestation of indigestion, gastrointestinal intoxication, or enterotoxemia that is precipitated by the change in feed. Each of the above points can be argued with some justification; however, no conclusive evidence to substantiate any of the hypotheses has been demonstrated to date.

Efforts to reproduce the disease under experimental conditions have not been too satisfactory in providing clues to the mysteries of the natural disease. Experimental studies have been conducted by feeding material which incited the disease in natural conditions, (13,22) by inducing allergic lung reactions (1,10), and by forced inhalation of certain gases (7,21). Although these treatments induced respiratory distress and lung changes, they have had limited usefulness in the study of causes and pathogenesis of diet-related pulmonary emphysema because the material fed was so variable, the results inconsistent, the tissue changes were sometimes different than those observed in the natural disease and/or the techniques of induction could not be readily linked to conditions as they occurred in the diet-related disease.

Some recent experimental work has provided promising leads into possible causes and mechanisms of the natural disease. It has been demonstrated that when cattle are given large intraruminal doses of the amino acid tryptophan, a disease is produced which has many similarities to naturally-occurring ABPE (4,8,15).

Following a large dose of tryptophan, there is an increased rate of respiration and progressive dyspnea that begins from one to three days following the administration of the amino acid. Clinical signs are essentially the same as those observed in the natural disease, and onset and course are also similar. At necropsy, the characteristic lesions are severe, diffuse pulmonary edema and interstitial emphysema. Microscopic changes are characterized by thickening of alveolar septa and swelling and proliferation of alveolar lining cells, similar to those observed in the natural disease. Other interesting correlations between the experimental and natural diseases include similarities in age, breed, and species susceptibilities.

Subsequent experimental work has demonstrated that tryptophan does not cause respiratory disease when administered intravenously or intraperitoneally (4). It is only effective when placed in the rumen. This suggests that ruminal fermentation of the amino acid is essential. Further work has demonstrated that administration of indoleacetic acid, a compound closely related to tryptophan, also causes respiratory disease when given intraruminally, but not if injected intravenously (9).

Recent experiments have demonstrated that ruminal microorganisms convert tryptophan and indoleacetic acid to 3-methylindole (3MI) and that both intraruminal and intravenous administration of 3MI cause acute pulmonary edema and emphysema in cattle (5,24). These findings suggest that ruminal fermentation converts tryptophan, indoleacetic acid, and possibly other suitable precursors, to 3MI which is absorbed into the blood and causes pulmonary lesions.

The definitive link between the experimental disease and naturally-occurring ABPE remains to be demonstrated. However, since tryptophan is an amino acid of protein and present in nearly all feeds and since indoleacetic acid is regarded as a plant hormone that is known to be present in rapidly growing plants, the possibility that these components might serve as precursors for ruminal conversion to 3MI cannot be overlooked. At the present time, the hypothesis that indolic compounds act as a possible cause of ABPE seems an appropriate lead in the search for the causation of the natural disease.

The working hypothesis of the current experimental efforts is that tryptophan, indoleacetic acid, or similar indolic compounds, are present in certain feeds or forages; that these are converted to 3MI by the action of rumen bacteria; and that progressive membrane damage occurs in the lungs as a result of vascular transport of 3MI. Further, it is assumed that ruminal conversion of the precursors to 3MI is accelerated under appropriate conditions (increased rumen activity and fermentation) precipitated by sudden changes in feed or forage.

This hypothesis seems reasonable since the most consistent observations of naturally-occurring ABPE are in cattle that have been subjected to conditions which favor heavy consumption of highly digestible forage before the onset of the disease. In most cases, the largest, most hardy animals are affected most severely, especially if they are nursing calves. These cows may be expected to consume larger amounts of the lush feed than other animals in the same pasture. Also, animals that are moved from short, dry ranges or those that are moved considerable distances are obviously hungrier when subjected to the new forage and intake is subsequently increased. These types of conditions are most commonly associated with the onset of the natural disease.

Present research efforts are oriented toward attempts to conclusively demonstrate the link between the natural and experimental diseases, and to define methods to inhibit ruminal conversion of indolic precursors to 3MI. A search is being made for a possible supplement or feed additive that might be

utilized in a practical manner to effectively inhibit rumen fermentation and formation of 3MI. It is anticipated that this approach might provide a practical method for controlling the natural disease.

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# Ova Transfer in the Bovine Animal

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Although ova transfer in the cow was first successfully accomplished in the early 1950's, it took 20 years before it was used for commercial purposes on a practical basis. The early stimulus for the development of ova transfer in cattle was its value for research. Considerable information has been gained concerning reproductive physiology in other species in which ova transfer has been successful for many years; e.g. in the rabbit and swine. This research value may be genetically oriented as well as physiologically oriented. Presently cattle progeny testing is directed mostly at testing the transmitting ability of the male since insufficient offspring numbers limit such testing of the cow. With superovulation and ova transfer, selection based on the female transmitting ability may also be established.

The production of increased numbers of full sibs is obviously a tremendous asset for nutritional studies, for susceptibility to disease studies and for many other types of research.

The introduction of the so-called "exotic" breeds of cattle for cross-breeding purposes provides the greatest immediate stimulus for the commercial application of ova transfer. This is due to the fact that there are severe restrictions against importation of these breeds from foot-and-mouth disease countries. The limited numbers that can be brought in are, therefore, very valuable and the maximum number of offspring from these few is very important for the exotic cattle industry of this country.

In these breeds semen has been much more importable. Thus, the semen is used to produce $F_1$ half-bloods. These are bred back to the same breed giving ¾ bloods. The next generation (⅞ bloods) are considered to be full bloods. Ova transfer is used to increase the number of these percentage-blood animals and "up-blood" these as rapidly as possible. Instead of the traditional pyramid of diminishing numbers being produced as with single births in "breeding-up"