Respiratory Session

Dr. R. A. Curtis, *Presiding*

The Pathogenesis of Shipping Fever in Cattle

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For several years our laboratory worked on the pathogenesis of "Shipping Fever". Many people contributed to the work. I would like to give you some of the results, together with my assessment of what the probable pathogenesis of the disease is at the current state of knowledge. I think the discussion will be of particular interest to you because we combined field observations of the disease under natural conditions with experimental work in the laboratory.

I would like to make four points in this talk which you might keep in mind as we go along:

1) To emphasize that nasal bacterial flora becomes highly significant in the pathogenesis;
2) That normal defense mechanisms are overwhelmed in animals which die of the acute disease but only partially so in those that have or are able to develop resistance;
3) That *Pasteurella hemolytica* is the most significant factor in acute fatal cases;
4) That viruses *may* play a role in the acute disease.

This slide illustrates the basic pathogenesis of the three main morphological types of pneumonia in cattle (Fig. 1). There is an association between the type, the cause and the pathogenesis. The fibrinous lesion of acute Pasteurellosis results from an "explosion" deep in the lung with much vascular damage. As just mentioned, this is characteristic of acute severe "shipping fever". Bronchopneumonia spreads gradually into the lung and is usually caused by viral agents and secondary bacteria causing bronchitis, bronchiolitis and an exudative pneumonia. The disease is called enzootic pneumonia. The third type, interstitial pneumonia, is caused by injury primarily at the alveolar level. The best example is atypical interstitial pneumonia, now assiciated with ingestion of lush pasture containing L tryptophane which is converted to 3M indole in the rumen, is absorbed and is toxic to the lung at the alveolar level.

The textbook fatal case of acute "shipping fever" or Pasteurellosis is a fulminating bacterial fibrinous pneumonia which implies a sudden insult deep in the lung with an agent that causes marked vascular damage which in turn accounts for the edema and fibrin.

The following lesions are typical of the field and natural cases: (Fig. 2, 3, 4). Figures 5 to 8 represent typical lesions in subacute field cases which do not die but are sick and require treatment. These begin as acute cases but become confined

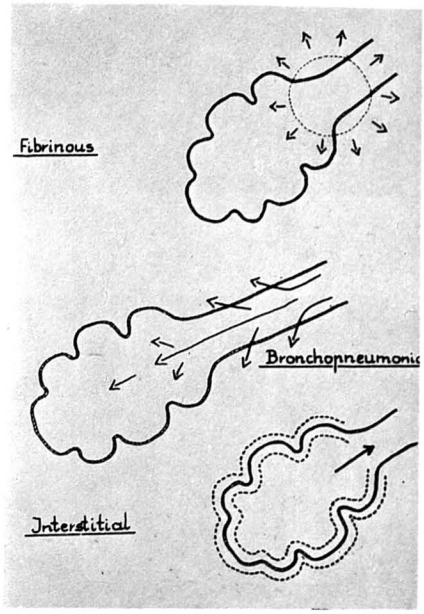


Fig. 1 Types of pneumonia in cattle.

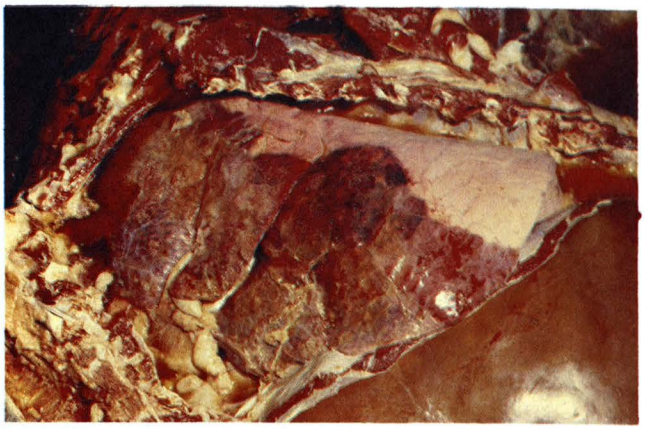
and have typical areas of necrosis (11). These will be discussed again under lung scores. *P. hemolytica* is highly toxic to macrophages in tissue culture and probably accounts for the severely degenerate macrophages which are so characteristic of the lesions in the natural and experimental disease (1) (2). Usually there is no morphological evidence of virus infection in acute fatal cases. However, some IBR occurs with acute fibrinous pneumonia but not usually. PI-3 virus is often implicated in the disease based on serology but viral inclusions are rarely found in acute fatal cases. We will return to the role of "helper" viruses in the pathogenesis later.

My interest in this disease began by seeing the very characteristic and consistent lesions repeatedly in the PM room and also seeing the "pretty" areas of necrosis on the slides of the lesions. Both were a delight for a pathologist.

These observations led to questions. What happens in the animal which allows these classical lesions of acute fatal shipping fever to develop? What is the sequence of events? What are the etiological factors and their role in the disease? These questions in turn led to research programs which attempted to unravel the puzzle.

Initially we tried to very simplistically reproduce the

Fig. 2 Acute fibrinous pneumonia.
Fig. 3 Acute fibrinous pneumonia.
Fig. 4 Acute fibrinous pneumonia - note areas of coagulation necrosis.



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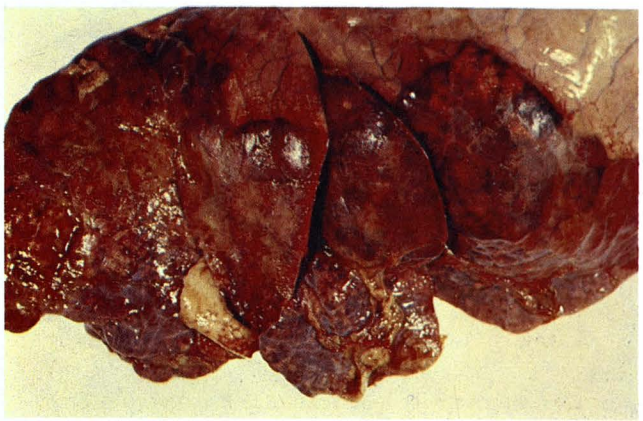

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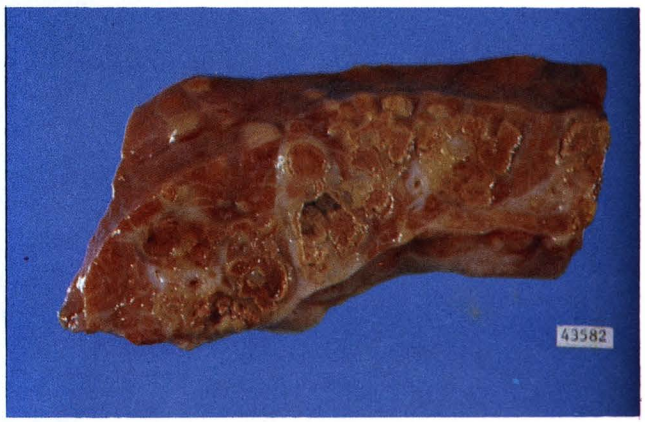

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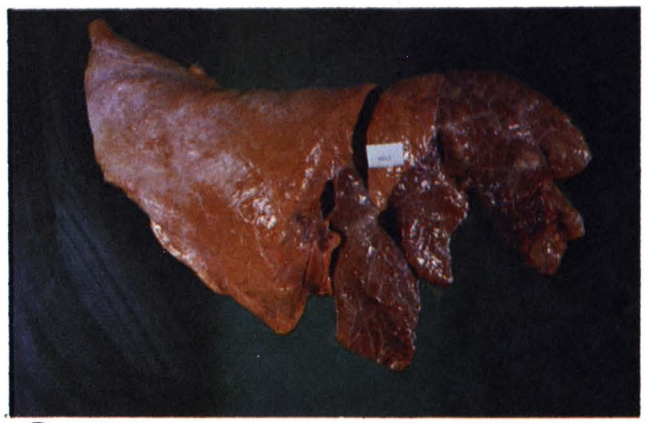

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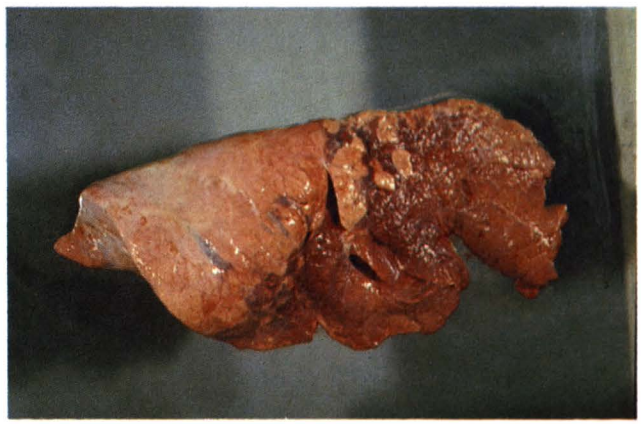

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Fig. 5 Field cases of shipping fever - moderate severity
Fig. 6 Field cases of shipping fever
Fig. 7 Field cases of shipping fever
Fig. 8 Field cases of shipping fever
Fig. 9 Normal nasal mucosa

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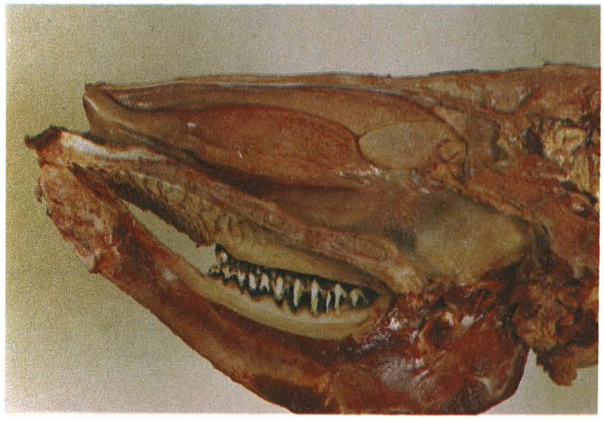

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disease using *P. hemolytica* by aerosol in calves but had negative results. We then tried to increase the virulence of the organism in mice prior to aerosol with no better results. These frustrations led to a new approach. We decided to examine animals shipped from the West and determine why some became sick and died and some had no illness, with others being somewhere in between. As we made observations on these field cases we took problems to the laboratory for detailed study. Thus, the combination of field and labroatory work was very useful. This was something like Drs. Acres and Radostits going on to problem calf scour farms to observe all aspects of the disease in conjunction with laboratory work on the bacterial cause. Dr. Martin will give you the results of extensive field observations on feedlot diseases, particularly shipping fever, which points out the importance of combining research in the laboratory with the real world of the disease (9). Observation of the field situation was probably one of the most important aspects of our work and added stability and credibility to our laboratory work. In order to simplify this presentation, I will deal mainly with field observations first and then move to laboratory work, but keep in mind that these were often going on in parallel.

In our story, the primary event seems to be a rapid buildup of *P. hemolytica* in the nasal flora on the nasal mucosa.

This is the normal nasal mucosa (Fig. 9).

A swab assembly was used to assess the nasal bacterial flora *qualitatively* (which organisms are present) and *quantitatively* (how many of each are present). Dr. Magwood determined the normal nasal flora of calves and found it to be variable over time in normal animals (8) (Table I).

The clearance pattern of the nasal mucosa in cattle is in *two* directions: the posterior regions are cleared toward the pharynx, and the anterior portions to the nostrils. Therefore one might expect to find the nasal flora in the mouth. The organisms live in or on the mucus and can survive there even with the steady ciliary clearance pattern. If heads are split and detailed culturing of several areas of the nasal mucosa conducted, it was often possible to find *P. hemolytica* in animals which had been negative on nasal swabs (10).

Table I Nasal Aerobic Bacterial Flora of Calves in 12 herds on Examination of 790 swabs during a period of seven months.

Bacterial Flora Category and Species	Per Cent Frequency of Isolation
Basal	
Pasteurella multocida	61.3
Pasteurella hemolytica	23.0
Neisseria catarrhalis	46.7
Micrococcus spp.	53.3
Beta-hemolytic **Streptococcus spp.**	10.3
Supplementary	
Moraxella duplex	5.1
Moraxella saccharolytica	0.2
Hemophilus parainfluenzae	0.6
Diplococcus pneumoniae	0.1
Corynebacterium pyogenes	0.2
Saprophytic Corynebacteria	0.4
Streptococcus spp.	15.2
Transient	
Coliform Sub-Group **Escherichia coli, Klebsiella pneumoniae, Paracolobactrum spp., Proteus spp.**	8.2
Dust-type Sub-Group Genera **Flavobacterium, Chromobacterium, Streptomyces, Achromobacter, Bacillus, Pseudomonas, Serratia, Lactobacillus**	23.4

What happens to the nasal bacterial flora under field conditions? Our interest was in finding the difference between those animals which became sick and those which

did not. We decided to separate field animals into two categories and compare them. We designated these two groups as S for sick and W for well. We bought animals off the train on arrival from the West and put them in a shed in a small paddock. We kept them for one month initially and later for only one week. None were treated. They were sold and more purchased at the end of the observation period.

The animals were about five to ten months of age. We handled 14 groups of ten each, one at a time. An animal was designated S for sick if it had a body temperature of 104°F once, followed by three successive days of plasma fibrinogen over 700 mgm%. This was an arbitrary decision based on observation of several groups of animals. If this occurred it did so nearly always in the first week after arrival. This allowed us to do our determinations in one week compared to four weeks in our early groups (12, 14). The lungs were recovered from some after the one week period.

We measured: 1) body temperature,
2) plasma fibrinogen, - a good indicator of illness in cattle,
3) nasal flora a) qualitatively,
b) quantitatively,
4) virus isolation from nasal swabs,
5) serum and nasal antibody to *P. hemolytica,*
6) serum and nasal antibody to PI-3 virus.

There were many differences between S and W animals:

1. body temperature in the first week (Fig. 10),

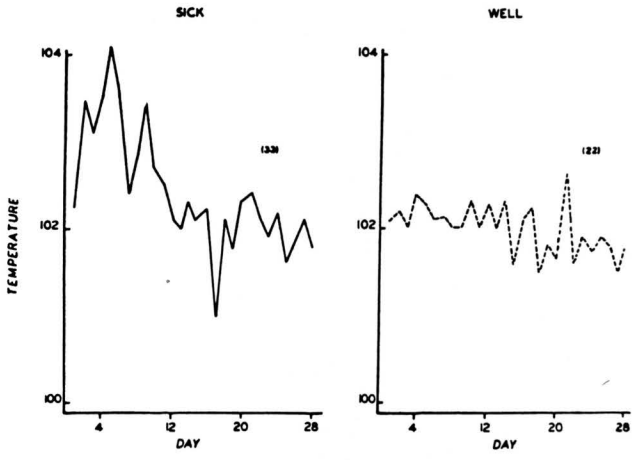

Fig. 10 The mean daily body temperature in degrees Farenheit of 33 sick animals is recorded on the left and 22 well animals on the right. The mean temperature for sick animals is below the required level (104.5°) since the day on which the maximum temperature occurred in individual animals varied.

2. plasma fibrinogen in one week to ten days (Fig. 11),
3. incidence of *P. hemolytica* on the nasal flora (Fig. 12),

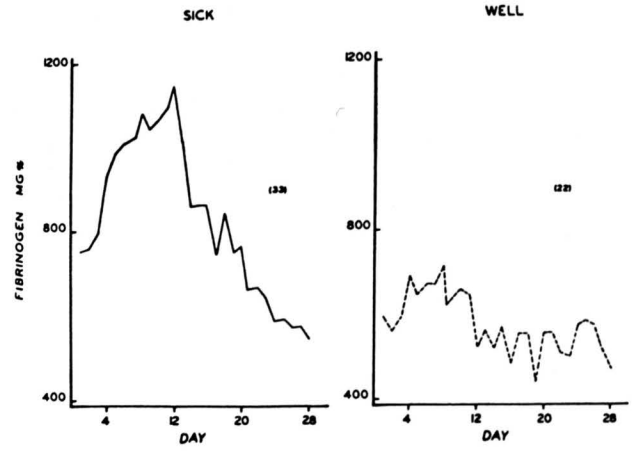

Fig. 11 The mean daily plasma fibrinogen levels of 33 sick animals is shown on the left and 22 well animals on the right.

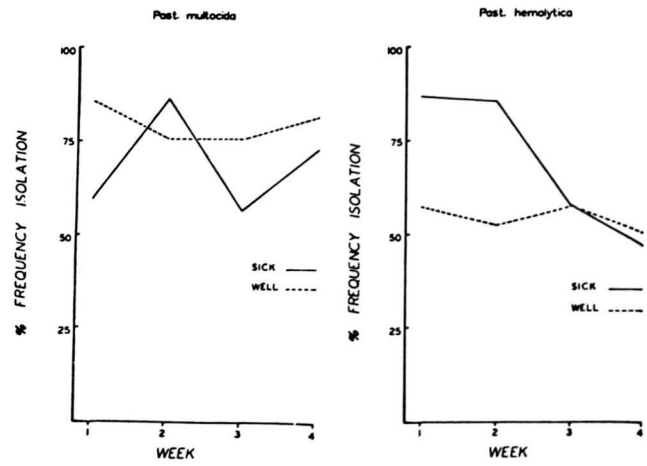

Fig. 12 The frequency of isolation of *P. hemolytica* is recorded on the right and *P. multocida* on the left in sick and well animals. The difference between the two groups in the weeks one and two was statistically significant.

4. numbers of *P. hemolytica* on the nasal mucosa (Fig. 13),
5. *P. hemolytica* serum antibody titres - significant difference between day one and day seven (Fig. 14),

6. PI-3 virus serum antibody titres - *no* significant difference between day one and seven (Fig. 15).

In addition, PI-3 viral isolates were very low. Serum antibody to PI-3 was present in 40% of animals at arrival and increased to 80% over four weeks. Serum antibody to IBR virus was present in 10% on arrival and increased to 25% by one month.

Let's compare S and W further and to do so let us look at two groups, *one mostly S* and *one mostly W* and compare the factors which probably reflect resistance or previous exposure to etiological agents (Table II). These two groups illustrate quite well the main differences between S and W. Note the low titres to *P. hemolytica* in the S group and the rapid rise in titres to *P. hemolytica* in the S animals with relatively similar PI-3 titres in either S or W.

One weakness in our observation was that we did not know the history of these animals in terms of vaccination, source, or time off the range before they arrived in our hands. Therefore we requested Dr. Radostits to assist from Saskatoon. He purchased two groups of calves directly off the range and took samples for all the parameters we were measuring for three successive days and sent the samples to us daily by air. He then put the animals on the train and sent them to us. We could then have an accurate comparison of before and after shipment. One group (F) had five-S and five-W, and the next (G) had ten-W. Group F had no *P. hemolytica* on nasal swabs prior to shipment whereas 20% of group G had the organism. On arrival the S and W animals were each characteristic of the features shown in the previous slides. We think these two groups added credibility to our field observations.

In our field investigations and experimental reproduction of pneumonia we quantitated the amount of pneumonia in

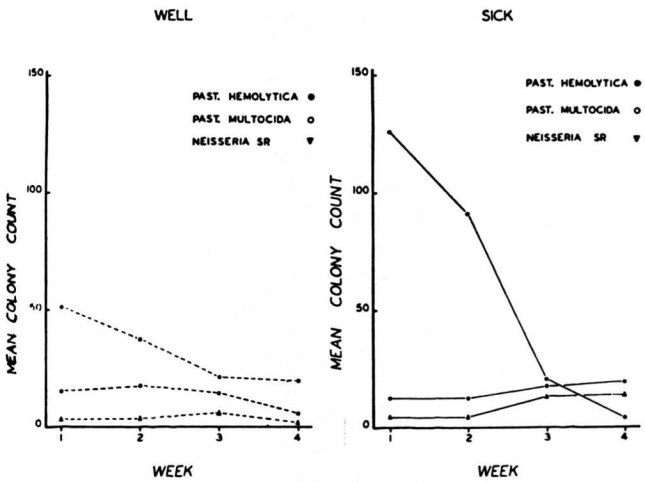

Fig. 13 The relative number of three species of bacteria on the nasal mucosa in sick and well animals is illustrated.

	Day	Group C (9 sick)	D (9 well)
Past. hemolytica	1	8*	16
	7	21	30
	14	130	23
	21	230	24
	28	346	30
Parainfluenza-3	1	14	3
	7	17	5
	14	36	23
	21	50	44
	28	27	12

*Mean of reciprocal of dilutions

Table II Comparison of the Serum Antibody Titres to P. Hemolytica and Parainfluenza-3 Virus in Groups C (9 sick) and D (9 well).

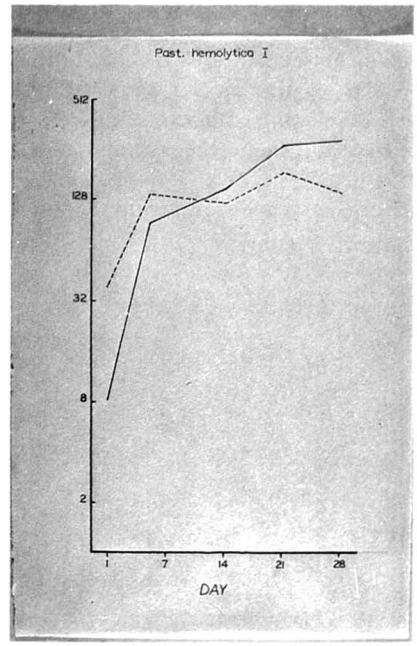

Fig. 14 *P. hemolytica* serum antibody titres.
Dotted line = Sick
Solid line = Well

the lungs by a "Lung Score". The amount of discoloration and consolidation was assessed in each lobe at post mortem examination with four on the right lung and three on the left lung; each lobe was evaluated on a scale of zero to five. Therefore the maximum score was 5 × 7 = 35. Table III illustrates the range of lesions in some of our animals. Most animals are in the high teens and twenties.

Now let's relate clinical illness to lung scores, i.e. compare clinical illness as reflected by temperature and plasma

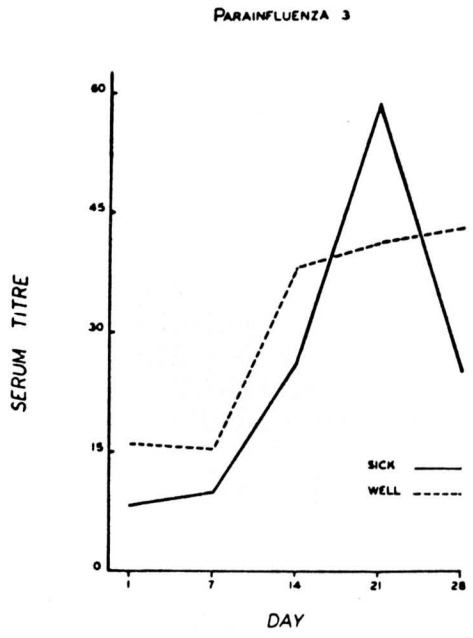

Fig. 15 The mean serum antibody titer to PI-3 virus in sick and well animals is shown. On an overall basis there was no significant difference between the two. In general the well animals had higher titres to PI-3 virus and *P. hemolytica* than the sick at the start of the period of observation.

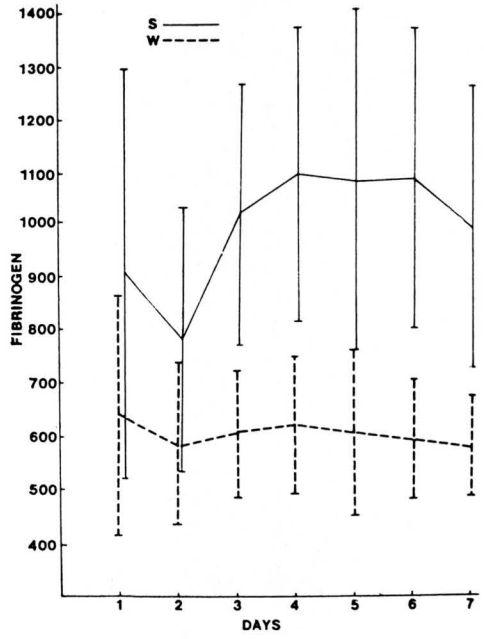

Fig. 16 Daily mean plasma fibrinogen levels with standard deviations for SICK and WELL animals.

Classification			
SICK		WELL	
Animal Number	Score	Animal Number	Score
4646	30	4977	10
4986	30	4978	7
205	27	4976	4
4983	25	206	4
201	26	4979	3
208	22	143	2
4648	22	141	2
4643	21	4984	2
4993	20	204	2
4642	19	138	2
146	19	144	2
139	19	4980	1
4647	18	4991	1
4982	18	145	1
4987	18	137	1
4992	14	142	1
4981	13	202	1
4989	13	140	0
4994	10	203	0
4995	9	207	0
4990	8	209	0
210	3		
4985	2	mean	5.7
mean	17.6		

Table III Individual Lung Scores of 23 SICK and 21 WELL Animals in GROUP 1 and the Comparisons of Mean Scores.

fibrinogen (Fig. 16) to the amount of pneumonia as reflected by lung score (Fig. 17, 18) gradations as follows:

a=lung score 0 - 10
b=lung score of 11 - 20
c=lung score of 21 - 30.

The high levels of plasma fibrinogen and body temperature are clearly related to the amount of pneumonia present in the lungs.

We tried to reproduce the disease in order to have an experimental model which could be used as a positive control in attempts to prevent the disease and did so with reasonable success. We used the experimental model to evaluate antibiotic therapy and intended it for use in immunization trials. The model produced lesions indistinguishable from the natural disease and covered a range from fatal cases to minimal lesions. The average lung score in a control group of six calves was in the low to mid-twenties.

We will now leave the field assessment and consider the avenues of laboratory investigation:

a) how does *P. hemolytica* get into the lung?
b) how do viral agents play a role?

The factors in favour of the *offense* must become dominant over the defense. The normal respiratory *defense* mechanisms must be overcome if pneumonia is to develop

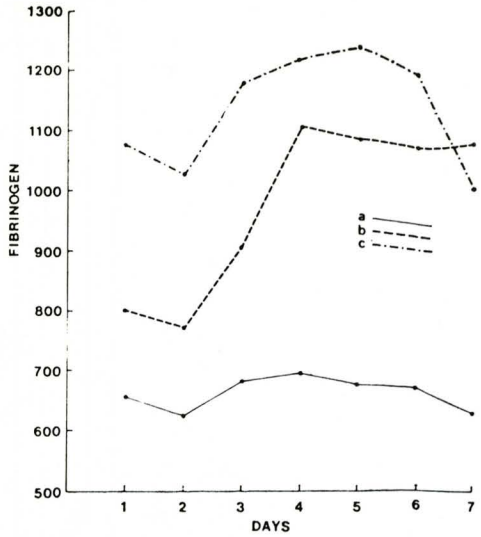

Fig. 17 Daily mean body fibrinogen levels for subgroups a, b and c, divided according to lung scores, (a includes lung scores of 0-10, b, 11-20 and c, 21-30).

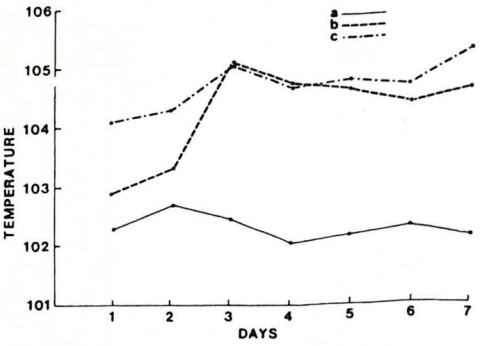

Fig. 18 Daily mean body temperature for subgroups a, b and c, divided according to lung scores, (a includes lung scores of 1-10, b, 11-20, and c, 21-30).

(Table IV). We will concentrate on four and five in this table. Infections must enter the lung attached to small particles (one to two U in size), to get into the deep areas of the lung, otherwise they are eliminated by ciliary clearance. We know *P. hemolytica* is in the nasal flora but how does it get into the lung?

We purchased animals on arrival from the west as discussed earlier. We quantitated the bacterial nasal flora by swabs to determine which animals had high and low levels of *P. hemolytica* in their flora. Using the Anderson air sampler we sampled the air being breathed out by the animals (Fig. 19) and also cannulated the trachea to determine how many organisms were being breathed into the lung from the nasal passage (3) (Fig. 20). The animals were away from all others

Fig. 19 Sampling the expired air.

RESPIRATORY DEFENSE MECHANISMS

1. The cough reflex
2. The physical filtration of the nasopharyngeal region
3. The mucociliary clearance mechanism
4. The alveolar macrophage clearance mechanism
5. The cellular and humoral defenses of the body

Table IV Normal defense mechanisms.

so any *P. hemolytica* could only come from its own nasal mucosa. The Anderson sampler provided a quantitation of numbers and also the particle sizes that were being inhaled. There were very few organisms in exhaled air unless the animal snorted (a surprise). *P. hemolytica* was found in the oral cavity of the animals, if it was present in nasal swabs, even at low levels. This finding suggests that the hairy coats and the water may be a source of spread of infection. Remember that in cattle, nasal clearance occurs partly in an anterior direction and brings organisms to the nasal orifices and that also the tongue is often used to clean off the nose. In addition, if *P. hemolytica* was present in nasal swabs it was found in the tracheal air. Fifty percent of *P. hemolytica* in four to nine, bacteria are retained in the lung in large numbers with peak retention at day seven. Detailed investigation has shown that the retention is not directly

the tracheal air were on particles of the size which get into alveoli.

The question is how does *P. hemolytica* become established in the lung because the normal defense mechanisms of alveolar macrophages should dispose of them completely and rapidly and keep the lung sterile? We now set out to investigate how viral infections interfere with bacterial clearance from the lung in cattle.

There was considerable literature on normal pulmonary clearance of bacteria from the lung of laboratory animals (5). The technique involves providing an aerosol exposure to a group of animals and then determining the number of bacteria which can be cultured from the lung at intervals after the aerosol is completed. Several mice are killed at specific intervals, the lung removed and bacteria cultured and counted (Fig. 21). We performed this in mice with *Staph aureus* according to the literature to standardize our methods and then determined clearance systems in calves for *P. hemolytica.* The clearance curves for *P. hemolytica* in calves were very similar to *Staph aureus* in mice so we now had a standard for comparison to existing literature (Fig. 22) (6).

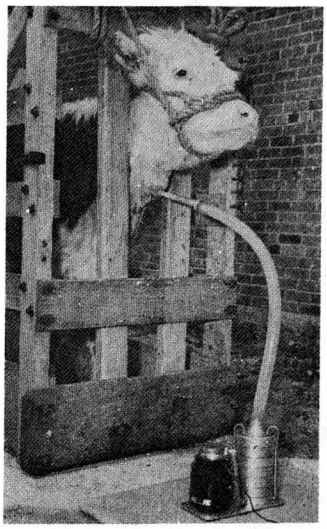

Fig. 20 Sampling the tracheal air. An adapter (close to the external end of the tracheostomy tube) connects the narrow end of the polyethylene tube and sampler to the inserted tracheostomy tube. The picture was taken outside for photographic reasons.

There are many examples of viral agents interfering with pulmonary bacterial clearance in laboratory animals (13). Following virus infection, the peak of viral replication occurs three days later. Lesions caused by the virus begin after the peak of virus production at about the same time as serum antibody to the virus begins to develop. Bacterial clearance from the lung of these virus infected animals is normal until about day four of the virus infection. From day

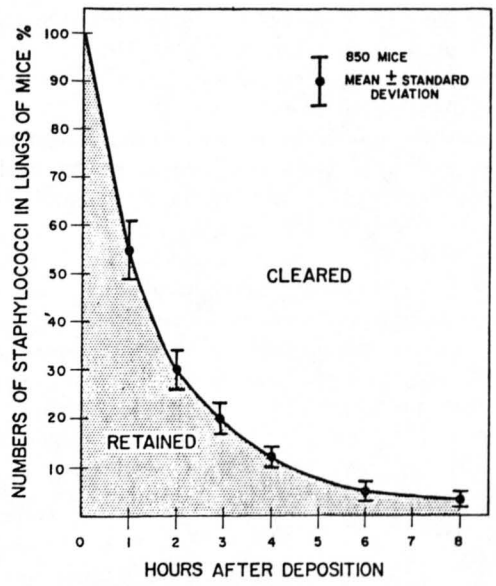

Fig. 21 The clearance of staphylococci from the lungs of mice. Each point on the curve represents the mean percentage of three or more studies with an average of 50 mice in each study. Example: deposition 100,000 ± 13,000; retained at end of one hour, 55,000 ± 7,000; two hours, 30,000 ± 5,000; three hours, 20,000 ± 3,500; four hours, 12,000 ± 2,500; six hours, 5,000 ± 1,000; and eight hours, 4,000 ± 1,000.

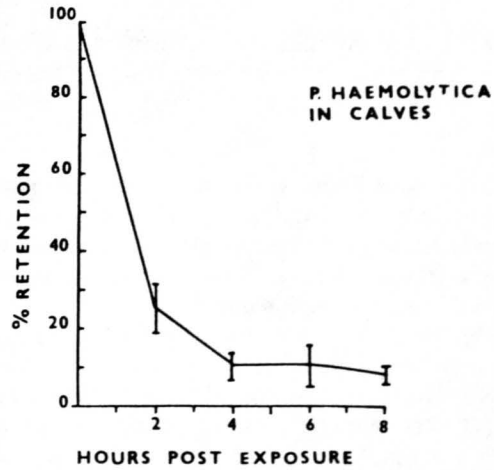

Fig. 22 The clearance of *Pasteurella hemolytica* by calf lungs from 0-8 hours post exposure.

related to the viral lesion but to a defect in the phagocytic process in alveolar macrophages (4, 16). The problem is not with ciliary clearance in bronchioles but with phagocytosis by alveolar macrophages.

The question now arises as to whether a similar type of interference occurs in cattle. An experiment was conducted on calves using PI-3 virus, the viral agent generally assumed to be a factor in shipping fever. Clearance of *P. hemolytica* was examined on day three, seven and 11, after an aerosol of PI-3 virus was administered to groups of calves. Calves were killed and several samples from different areas of the lung were cultured quantitatively. Marked interference with pulmonary bacterial clearance occurred at day seven (7) (Fig. 23). Further work just completed by Dr. Lopez at the Ontario Veterinary College using BVD virus and *Mycoplasma bovis* failed to demonstrate interference in pulmonary clearance of *P. hemolytica* in cattle with these agents. (Personal communication).

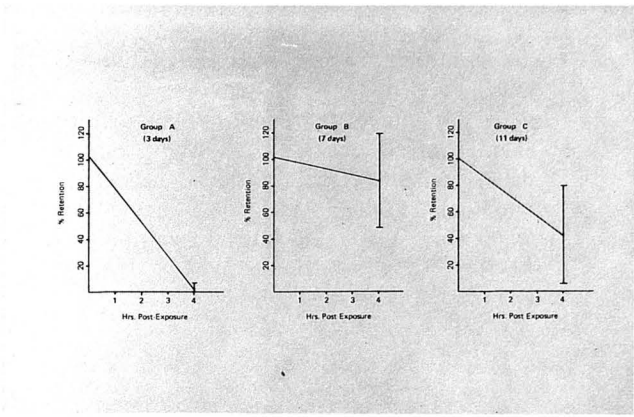

Fig. 23 Four hour clearance curves of *P. hemolytica* in calves at days 3, 7, 11 post exposure to PI-3 virus.

Pasteurella pneumotropica is part of the normal nasal flora in mice but is not cultured from the lungs of normal mice. The next slide illustrates that this organism can be cultured from the lung one week after Sendai virus infection (Fig. 24). Therefore if the low levels of normal nasal bacterial flora are present in the lung during a virus infection, there would logically be many more bacteria entering the lung from an animal that had much larger numbers of bacteria present in its nasal flora. This could be a very significant step in the pathogenesis of shipping fever.

I think that *P. hemolytica* can cause fibrinous pneumonia either with or without the help of a viral agent (15).

IN SUMMARY I have attempted to emphasize to you:—

(1) The role of *P. hemolytica* and nasal flora in this disease;
(2) The possible role of some "helper" viruses; and
(3) The significance of the very susceptible animal in what appears to be a highly contageous and often fatal disease.

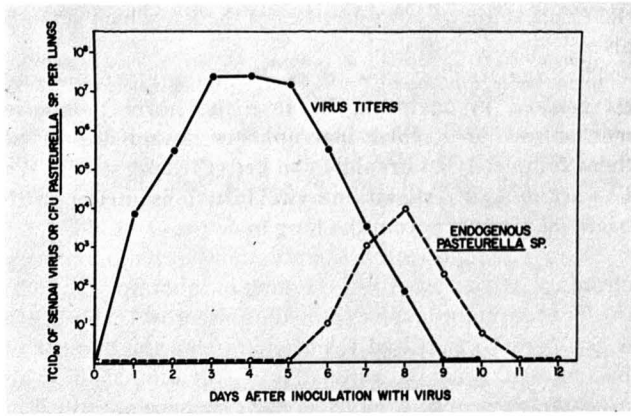

Fig. 24 Virus titers and endogenous *Pasteurella sp.* in the lungs of mice infected with Sendai virus. $TCID_{50}$: Tissue culture infective dose median. CFU: Colony forming units.

The greatest need is an efficient immunizing agent against *P. hemolytica.*

You will be given additional information about assessing the immune status and the importance of immunity to P. hemolytica in this disease from Dr. Wilkie. Dr. Martin will discuss the epidemiology and other factors aside from the etiological agents related to the occurrence of this and perhaps other diseases in shipped feedlot animals. What I have presented will be background to both of these presentations.

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