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DISSERTATION

INFLUENCE OF COLD EXPOSURE ON VENTILATION, RESPIRATORY HEAT LOSS, AND PULMONARY DEPOSITION/CLEARANCE

Submitted by

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In partial fulfillment of the requirements

for the degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 1990

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY DONALD ALAN DIESEL ENTITLED INFLUENCE OF COLD EXPOSURE ON VENTILATION, RESPIRATORY HEAT LOSS, AND PULMONARY DEPOSITION/CLEARANCE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

INFLUENCE OF COLD EXPOSURE ON VENTILATION, RESPIRATORY HEAT LOSS, AND PULMONARY DEPOSITION/CLEARANCE

The possible thermoregulatory benefits of cold-induced changes in breathing pattern (hypoventilation) and the mechanism(s) by which cold stimulates a change in breathing pattern were investigated in male Holstein calves between the ages of 1 and 3 months. The effects of ambient temperatures (T_a) between 4 and 18°C on ventilatory parameters and respiratory heat loss (RHL) were determined in four calves. As T_a decreased, respiratory frequency (f) decreased 29% while tidal volume (V_T) increased 35%. Total ventilation ($\dot{V}_E$) did not change significantly, but the ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}_{O2}$) decreased with decreasing T_a. These changes were opposite to the panting response observed in other studies with animals in the heat. RHL did not increase as T_a decreased and the percentage of metabolic rate attributed to RHL significantly decreased with decreasing T_a. To investigate the relationship of ventilation to expired air temperature (T_E), $\dot{V}_E$ was stimulated by increasing inspired CO_2 in six calves (T_a= 4-6°C). A positive relationship existed between f and T_E. Therefore, calves were capable of conserving respiratory heat during cold exposure by decreasing T_E and by decreasing dead space ventilation while increasing O_2 extraction. Increasing $\dot{V}_E$ during cold exposure had no effect on hypothalamic temperature (T_h) in three calves.

The mechanism by which cold induces a ventilatory pattern change was investigated in six calves. Decreased f and increased V_T were induced by airway cold exposure alone, as well as by exposing the body, but not the airways, to cold air. Blocking airway cold receptors with topical 3% lidocaine during airway cold exposure prevented the ventilatory response, but did not affect T_h. Also, cooling the hypothalamus with a thermode (T_a = 16–18°C) did not produce a ventilatory response. Thus, airway temperature, but not hypothalamic temperature, appears to control ventilation. Hypoventilation is suggested as a mode of heat conservation in cold environments in animals which use panting as a means of increasing evaporative heat loss.

The effect of cold-induced hypoventilation on pulmonary particle deposition was investigated in 10 calves. Deposition of nasally-instilled fluorescent *Pasteurella haemolytica* was significantly higher for cold-exposed calves. It is speculated that the cold-induced respiratory pattern change was responsible. Deposition was greater in apical and mediastinal lung lobes, but the mechanism of this preferential deposition is uncertain.

Nasal mucus velocity (NMV) was measured in four unanesthetized calves at T_a = 2–4°C and at T_a = 16–18°C, using a radiographic tracking technique. NMV was 24% lower during cold exposure. In addition, the effect of mucosal temperature (T_m) on tracheal mucus velocity (TMV) was determined in excised tracheae from seven calves and 10 sheep. A direct relationship exists between T_m and TMV, within the T_m range studied (35.0 – 39.5°C). Tracheal air temperature measurements indicate that conditioning of inspired air is not complete at the tracheal level. Therefore, it is possible that cold-exposure decreases tracheal mucociliary clearance rate.

Since the individual variability of TMV, and NMV, between animals was high, it is speculated that mucociliary clearance rate may be partially genetically determined. Cold-induced decreases in particle clearance, and increases in deposition, may predispose calves to respiratory infection.

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CHAPTER I

INTRODUCTION

Species evolve to acquire adaptations to environmental stresses which enhance the fitness of the species. Conversely, physiological responses to an environmental stress which are detrimental should be selected against. However, there exists, in cattle, a physiological response to cold which seems paradoxical. Unlike humans, cattle exposed to cold change their breathing pattern to one characterized by deeper, slower respirations. While the adaptive benefit of this cold-induced ventilatory pattern is uncertain, there appears to be a significant disadvantage to this ventilatory pattern. Cattle exposed to cold have been shown to hypoventilate, to the extent that pulmonary hypertension occurs as a result of hypoxia (1,5). The possible deleterious effects of pulmonary hypertension, particularly in newborn animals, may include pulmonary edema and cor pulmonale.

One might assume that some benefit is derived from cold-induced hypoventilation to offset the selective disadvantages which seem so apparent. How might hypoventilation in a cold environment significantly benefit an animal? Two possibilities exist. First, since a great deal of body heat is lost through respiration, ventilatory changes can obviously modulate the amount of heat loss (*e.g.* panting). Perhaps cold-induced hypoventilation is *a* thermoregulatory mechanism to reduce heat loss. Second, since cold stress suppresses the immune system, perhaps hypoventilation compensates by altering deposition and/or rate of

clearance of pathogenic particles in the airways and lung. Should hypoventilation result in increased deposition or decreased clearance, it could not be considered advantageous in this regard, but may help to explain the higher incidence of respiratory disease in cattle during the cold seasons. Therefore, this study was undertaken to investigate the possible adaptive benefits or disadvantages of cold-induced hypoventilation in cattle, with regard to respiratory heat loss and particle deposition and clearance.

Respiratory Disease in Cattle

Studies of mechanisms of bovine shipping fever pneumonia span many years. The emphasis, however, was and continues to be placed on the various infectious agents associated with the disease. Early studies demonstrated a predisposition to bacterially-induced pneumonia following viral infection and "stress" (2). A proposed pathogenesis of bovine shipping fever pneumonia has evolved (4), which postulates that there is chilling of the nasopharyngeal mucus membranes which, together with upper airway viral infection, predisposes the nasopharynx to bacterial colonization. These pathogens are then transferred to the lungs by simple drainage or aspiration of infected droplets. Once infected, the progression of bovine respiratory disease is relatively rapid.

The method of penetration of the infectious agent into the alveoli is a topic which requires further investigation. Breathing pattern probably influences pulmonary particle deposition patterns and may also affect airway wall temperature, thereby affecting particle clearance. While the hypoventilatory response might be energetically advantageous under normal circumstances, the same response may increase deposition

and/or decrease clearance of pathogens and subsequently become detrimental with the unnatural density of animals (and pathogens) in a feedlot setting.

Justification

Bovine respiratory disease is a major infectious disease of cattle in the United States, estimated losses being in excess of $500 million per year. In the Western United States it has been estimated that 75% of all feedlot cattle illnesses are associated with diseases of the respiratory tract, and that this is the major cause of economic loss in feedlot animals (3). Major etiological factors include environmental conditions, such as dusty environment, overcrowding, transport stress, and cold exposure. Knowledge relative to responses of the respiratory system to altered environmental conditions would lead to a better understanding of the physiopathological events associated with bovine respiratory disease.

A new approach to our understanding of causative factors in bovine respiratory disease is urgently needed. Progress in recent years toward reducing the incidence of this disease has been largely confined to the identification of the infectious agents and not to the predisposing factors leading to the infection. The purpose of the present study was to determine, in calves, the effects of cold exposure on pathogen deposition in the respiratory tract and on mucociliary function. If a cold-induced change in ventilatory pattern is indeed a contributing factor to the development of bovine respiratory disease, it is important to determine why it occurs and precisely how cold stimulates hypoventilation. This basic information will improve our understanding of the responsiveness of the bovine respiratory system to cold stress and allow

us to assess the interaction between the respiratory and thermoregulatory systems in calves. Defining the pathophysiological changes in the airways of calves that occur during environmental stress will not only improve our understanding of the basic mechanisms of deposition and clearance of pathogens in the lung and lung defense mechanisms, but may also provide the basis for appropriate management and therapy.

Objectives of Study

Three aspects of cold-induced hypoventilation were investigated in this study. Chapter III addresses the effect of hypoventilation upon respiratory heat loss, while Chapters IV and V investigate the effects of cold-exposure upon particle deposition and clearance, respectively.

To discover how cold-induced hypoventilation is stimulated and whether respiratory pattern change acts as a thermoregulatory mechanism, the following objectives were addressed:

1) Determine the adaptive benefit of the respiratory response to cold exposure in calves (a panting animal) by measuring any reduction in respiratory heat loss afforded by hypoventilation in cold environments of varying severity.

2) Determine if the ventilatory response to cold is a reflex in an attempt to preserve brain temperature.

3) Determine if "airway","skin", or hypothalamic cold receptors are primarily responsible for the altered breathing pattern during cold exposure.

To investigate the effects of cold-induced altered breathing pattern upon deposition of particles in the lung, Chapter IV addresses the following objectives:

1) Determine if cold exposure and associated ventilatory changes enhance deposition of *Pasteurella haemolytica* bacteria deep within the lungs of calves.

2) Determine if the amount of particle deposition can be altered by changing the rate and depth of ventilation, using the isolated sheep lung preparation.

The effect of cold and cold-induced hypoventilation upon the clearance rate of particles from the upper airways is addressed in Chapter V. Nasal clearance rate was determined in calves under thermoneutral and cold conditions to assess the effect of acute cold exposure. In addition, isolated tracheae from sheep and calves were used to assess the effect of mucosal temperature upon the transport rate of the tracheal mucosa.

The information provided in this study improves our understanding of the interaction between the respiratory and thermoregulatory systems in calves. Furthermore, the findings of this study provide important information about the susceptibility of bovine respiratory defense mechanisms to cold stress, and the subsequent development of respiratory disease, and assist in elucidating the pathogenesis of bovine respiratory disease.

REFERENCES - CHAPTER I

1. Busch, M.A., A. Tucker and D. Robertshaw. Interaction between cold and altitude exposure on pulmonary circulation of cattle. *J. Appl. Physiol.* 58:948–953, 1985.

2. Hoerlein, A.B. and C.L. Marsh. Studies on the epizootiology of shipping fever in calves. *JAVMA* 131:123–127, 1957.

3. Jensen, R., R.E. Pierson, P.M. Braddy, D.A. Saari, L.H. Lauerman, J.J. England, D.P. Horton and A.E. McChesney. Diseases of yearling feedlot cattle in Colorado. *JAVMA* 169:497–499, 1976.

4. Jensen, R., R.E. Pierson, P.M. Braddy, D.A. Saari, L.H. Lauerman, J.J. England, H. Keyvanfar, J.R. Collier, D.P. Horton, A.E. McChesney, A. Benitez and R.M. Christie. Shipping fever pneumonia in yearling feedlot cattle. *JAVMA* 169:500–506, 1976.

5. Will, D.H., I.F. McMurtry, J.T. Reeves and R.F. Grover. Cold-induced pulmonary hypertension in cattle. *J. Appl. Physiol.* 45:469–473, 1978.

CHAPTER II

REVIEW OF THE LITERATURE

Altered Ventilatory Pattern and Respiratory Heat Loss

In man, ventilation volume is a reasonable predictor of oxygen consumption, since the expired air oxygen content remains relatively constant (30). However, in both sheep and pigs a doubling of oxygen consumption due to cold exposure results in only a 40% increase in minute ventilation, which is possible due to increased oxygen extraction from the inspired air (50,58). Mild cold exposure, with little or no increase in oxygen consumption, causes a decrease in respiratory rate and minute ventilation in calves (23) with a consequent arterial hypoxemia. Bianca and Naf (11) demonstrated a significant positive relationship between ambient temperature and respiratory rate between the temperatures -5 to 25°C in six-month-old calves, with a decrease of 6 breaths/min over a 10°C fall in temperature. McLean *et al.* (69) observed a similar relationship in 3 to 12-year-old cows with a decrease of 10 breaths/min from 18°C to 0°C. Below the lower critical temperature (0°C to -30°C) respiratory rate fell 3 breaths/min for every 10°C decrease in ambient temperature. Although total ventilation was not measured in either of these studies, one might assume that tidal volume increased as ambient temperature decreased. In the guinea pig, cold exposure caused oxygen consumption and total ventilation to double, and the increased ventilation was primarily due to a 70% increase in tidal volume rather than to an increase in

respiratory frequency (12). Taylor (88) also found respiratory rate to be lower at cold temperatures in elands, with a compensatory increase in tidal volume allowing for greater oxygen extraction from the inspired air.

The pulmonary effects of cold-induced hypoventilation, particularly in combination with altitude exposure, have received considerable attention due to implications in the development of high mountain disease (brisket disease) in cattle. Cold exposure has been shown to increase pulmonary artery pressure in calves (23,101), as well as in sheep (25). However, the possible adaptive benefits of a ventilatory pattern change during cold exposure have not been explored.

When heat stressed, cattle change their respiratory pattern by increasing respiratory frequency and decreasing tidal volume (40). This produces an increase in dead space ventilation, but no change in alveolar ventilation, resulting in increased respiratory heat loss (*i.e.* panting) and thereby regulation of body heat content (21). It seems logical that a reverse strategy could occur in a cold environment to reduce respiratory heat loss. In other words, when exposed to cold ambient temperatures, respiratory frequency would decrease and tidal volume would increase, thereby reducing dead space ventilation and respiratory heat loss. Some evidence exists to support this hypothesis in that exhaled air temperatures in humans were found to be lower when exhalation rate was voluntarily slowed from 0.5 to 0.25 liters/sec (85).

Since respiratory heat loss comprises a significant proportion of the total heat production (between 11 and 50% at 15°C in sheep; 19), a change in ventilatory pattern might represent a significant means of energy conservation. Ingram & Legge (50) predicted a savings in heat loss of about 15% in the pig, due to the relatively small (40%) increase in $\dot{V}_E$,

while oxygen consumption doubled with cold exposure. If one could compare cold-exposed animals, which decrease respiratory frequency and increase tidal volume, to the same animals when this response is prevented, a difference in respiratory heat loss between the two would suggest a benefit in energy savings.

Circulatory Adaptations and Preservation of Brain Temperature

Another advantage of hypoventilation under cold conditions may be the preservation of brain temperature. During inspiration, the mucosal lining of the airway is cooled by both convective heat loss from the airway wall to the air, as well as by evaporative heat loss as the air is humidified. Conditioning of inspired air during the resting state is primarily confined to the upper airways (98). During expiration, much of this heat is recovered by the cooled airway wall by convection and condensation of water vapor. However, there is not a complete recovery of heat upon exhalation and some chilling of the blood supplying the nasal mucosa occurs.

In many panting animals the rete mirabile acts as a heat exchanger between venous blood coming from the nasal mucosa and the arterial blood going to the hypothalamus (7,8). Nasal mucosal venous blood may follow one of two paths: blood may flow via the angular oculi vein to the cavernous sinus where heat exchange occurs with arterial blood in the rete mirabile, or blood may be diverted to the facial vein. While the carotid rete mirabile probably plays a minor role in controlling blood flow to the brain (36), selective control of the route of venous return from the nasal mucosa has been demonstrated in reindeer (56). Part of this control may be autonomic, with alpha adrenergic receptors predominant in the angular

oculi vein and beta receptors in the facial vein (56). Part of the control may also be a direct local effect of cold upon the facial vein to decrease intrinsic tone at colder temperatures (102), diverting blood from the angular oculi vein and reducing the cooling of the carotid rete.

Under conditions of heat exposure, increased dead space ventilation (panting) is an effective means of cooling the blood traveling to the brain (89). Selective brain cooling has been demonstrated in the panting ox during exercise, with hypothalamic temperature remaining as much as 1.5°C below core body temperature (26). It seems logical that under cold conditions the "opposite" of panting, or decreased frequency with increased tidal volume, along with a decreased blood flow to the nasal mucosa, would facilitate preservation of brain temperature. One can easily imagine a feedback mechanism by which the temperature of cooler blood received at the hypothalamus under cold conditions elicits hypoventilation, just as panting is part of a feedback loop designed to preserve brain temperature under hot conditions (89). However, goats subjected to central cooling while inspiring air at temperatures from 33 to -17°C showed no local effects on hypothalamic temperature (55), indicating that the function of the carotid rete may not be affected in cold stressed animals.

Mechanism of Stimulation of Hypoventilation

It is possible that specific cold receptors exist in a particular region of the body, which, when stimulated, induce the pulmonary effects, and perhaps also the cardiovascular effects, observed in cattle. Numerous studies have suggested that cold-induced pulmonary and airway events result from stimulation of cold receptors in the airway. Nasal cold

receptors have been suggested by Burgess and Whitelaw (22) as having an inhibitory effect on the ventilatory response to CO_2 in humans, and the frequency of inspiratory efforts during breath holding was decreased by flowing cold air through the upper airway (66). Laryngeal cold receptors have been characterized in dogs (82) and are implicated in the bronchomotor response to cold in cats (51). Tsubone (92) found that nasal thermoreceptors were stimulated by cold exposure and speculated that these receptors might alter the pattern of breathing by regulating inspiratory demand. Peripheral cutaneous cold receptors may be involved in stimulating the hypoventilatory response as well. Cooling of the face induces bronchoconstriction in humans (10,57) and has long been known to elicit the dive response (reflex), which includes reflex apnea (2,90).

Experiments in panting calves indicate that increased respiratory rate during heat exposure is due, in part, to increased peripheral skin temperature (13,32). Skin thermoreceptors may also be involved in ventilatory responses to cold exposure. Bligh (14) postulated that the removal of fleece from sheep results in the exposure of cold receptors, the stimulation of which depresses respiratory frequency. Cooling the skin of calves exposed to 25°C ambient temperature with cold water (lowering skin temperature by almost 6°C) resulted in nearly a 50% reduction in both minute ventilation and respiratory frequency (70).

Cooling of the anterior hypothalamus in the preoptic region has been shown to induce cold defense reactions of shivering, piloerection, cutaneous vasoconstriction, and increased oxygen consumption in a variety of unanesthetized animals, including the cat (1,31,64), dog (34,41,45,46), goat (4), pig (9), monkey (44), and ox (24,32). It is also possible that hypothalamic temperature controls respiratory rate and respiratory heat

loss in animals which thermoregulate by panting. This has been demonstrated through the use of thermodes implanted in the anterior hypothalamus (39,41,54,71), but appears to apply only to warming of the hypothalamus and not cooling. In winter-insulated reindeer, control of respiratory rate by changing hypothalamic temperature could not be demonstrated in animals at -20°C and only occurred in heat stressed animals at 20°C (71). Also, while cooling of the preoptic area reduces respiratory rate in rabbits during spinal cord heating, cooling of both spinal cord and preoptic region had no effect on respiration (39). Therefore, the induction of hypoventilation or reduced respiratory rate by cooling of the hypothalamus has not been confirmed.

Local cooling of the spinal cord failed to affect respiratory rate in sheep (5) or respiratory heat loss in goats (54), while warming of the spinal cord increased respiratory rate and respiratory heat loss in these two studies, respectively. Identical results were obtained in rabbits (60), where it was postulated that responses to thermal stimulation of the spinal cord are mediated by the hypothalamus.

Locating the receptors responsible for the hypoventilatory response to cold would facilitate the study of respiratory heat loss. To directly measure the energy conserved through a mechanism of decreased ventilation, it would be desirable to study an animal under cold conditions that did not exhibit hypoventilation. Manipulation of receptors might allow such a comparison. For example, Burgess and Whitelaw (22) were able to study the effect of cold on nasal receptors by use of a topical anesthetic on the receptor area, and found an increased ventilatory response to CO_2 rebreathing when the receptors were blocked.

Isolation of specific cold receptors responsible for the pulmonary response to cold may be of practical significance in the study of high mountain disease as well. The additive effects of hypoxia and cold on pulmonary hypertension appear to be in part due to hypoventilation (23,101). Should specific cold receptors exist which induce decreased ventilation, it would be interesting to speculate on the effect of blocking (or inhibiting) these receptors on cardiopulmonary parameters, particularly pulmonary vascular resistance.

Bovine Respiratory Disease and Cold Stress

Pathological studies of bovine shipping fever pneumonia strongly implicate *Pasteurella* species as the causative agent, in combination with unspecified environmental stress and viral infection. Subsequent studies have expanded these results and there is little question of the significance of *Pasteurella*, and in some cases *Mycoplasma*, in shipping fever pneumonia (33,53,91). However, *Pasteurella* species normally inhabit the upper airways of cattle, and the lungs are probably routinely exposed to limited amounts of the bacteria. Efforts to induce experimental shipping fever pneumonia have shown that, in the absence of a predisposing viral infection or environmental stress, a regularly reproducible disease state cannot be achieved (33).

Jensen *et al.* (53) proposed that in bovine shipping fever pneumonia chilling of the nasopharyngeal mucous membranes in combination with upper airway viral infection, predisposes the nasopharynx to *Pasteurella* or *Mycoplasma* colonization. Simple drainage or aspiration of infected droplets subsequently transfers these pathogens to the lung. Lung injury is believed to be initiated in the lung parenchyma in pasteurellosis,

rather than from an extension of lesions in conducting airways (83). Once infected, bovine respiratory disease progresses quickly, with extensive atelectasis, exudative pneumonia, and obstructive suppurative bronchiolitis occurring within 12 hours after infection (84).

Pulmonary venoconstriction has been observed in awake instrumented calves exposed to ambient temperatures of 2 to 5°C (38). Simultaneous measurements of pulmonary arterial, pulmonary wedge, and left atrial pressures and cardiac output indicate that cold exposure induces post-capillary vasoconstriction. Such venoconstriction could lead to the development of interstitial pulmonary edema, due to hydrostatic influences, which would be more likely to occur in the dependent portions of the lung. These ventral parts of the lung are predilection sites for many forms of bovine pneumonia (91). The anatomical organization of the bovine lung into discrete lobules, without any air connection, confines the infective process to the lobules, thereby preventing possible dissolution of the pneumonia. The unique anatomical features of the bovine lung have thus been identified as accounting, in part, for the susceptibility of cattle to pneumonic infections. Therefore, both the anatomical arrangement and the circulatory changes that accompany cold exposure represent an ideal medium for bacterial replication.

Yet to be fully addressed is the role of environmental stress (79,80,100) in the development of bovine respiratory disease. Stress has been cited consistently in relation to the epizootiology of shipping fever pneumonia, yet has barely been investigated (33,49,53,91). Respiratory diseases are particularly prevalent when there are sudden falls in temperature in the fall months (52,53,99), when animals, which have been unable to become cold acclimated, are exposed to a sudden severe cold

stress. Cold stress may act in a number of ways to decrease resistance to pneumonia. The effect of stress upon release of adrenal cortical steroids may result in a depressed immune response (42). However, cold exposure may also result in increased deposition and/or decreased clearance of pathogenic particles in the lung and airways as a result of respiratory pattern changes and subsequent cooling of the airways.

Particle Deposition in the Lung

The primary determinant of the location and manner of deposition of an airborne particle in the respiratory tract is the aerodynamic diameter of the particle. In humans, particles of 3 μm and larger are primarily deposited in the nasopharyngeal and tracheobronchial regions (15,61) by impaction. Sedimentation of smaller particles, 0.5 to 3 μm, is a more significant means of deposition in small bronchi, bronchioles and alveolar ducts, while particles <0.5 μm are deposited by diffusion (87). *Pasteurella haemolytica*, the nonmotile, rod-shaped bacteria which are often involved in bovine respiratory disease, measure approximately 1.4 x 0.4 μm. Therefore, sedimentation appears to be the most likely mode for deposition of airborne *Pasteurella* or droplet nuclei containing *Pasteurella*.

The flow rate and residence time of air in the airways and lung is also a factor in determining the location and amount of deposition (48,86), indicating that respiratory pattern influences deposition pattern. In addition, Brain *et al.* (17) found that aerosol inhalation in rats and hamsters resulted in preferential deposition in the apical lobes, indicating that certain pulmonary regions may be predisposed to greater deposition, not explained by pleural pressure gradients. Pityn *et al.*

(76) showed in humans that, while 1 μm particle deposition reflected regional lung ventilation, 3.5 μm particles were preferentially deposited in apical lobes.

It appears unlikely that cold-induced hypoventilation provides any benefit in resistance to bovine pneumonia. In fact, it has been hypothesized that the slow, deep respirations associated with cold exposure may increase the likelihood of infective deposition of bacteria deep within the lung (84). Heyder *et al.* (48) found that deposition of particles of diameter <2.4 μm occurs almost entirely in the alveoli. Since the low flow velocity in the distal parts of the lung does not provide for inertial impaction, deposition of particles <2.4 μm is primarily due to gravitational sedimentation (86). An increase in the deposition of particles in the respiratory tract occurs with decreased respiratory rate (43), since residence time in the lung affects the amount of deposition of particles by gravitational settling. There is also evidence that particle deposition by inertial impact in the tracheo-bronchial tree (where ciliary motion provides clearance) is less at lower respiratory flow rates (87). Thus, at lower air flow rates more particles may avoid being trapped and removed by the tracheo-bronchial ciliated epithelium. Using excised dog lungs, Valberg *et al.* (94) found deposition efficiency to be 42% for slow deep breathing, 21% for an exercising pattern, and 9% for rapid shallow breathing. During slow, deep breathing the parenchyma received a greater than average proportion of the deposition, while the more rapid, shallow pattern deposited more in the large airways.

Particle Clearance from the Airways

In addition to the possible effects of cold-induced ventilatory changes on deposition of bacteria in the lung, clearance may also be altered. Air is conditioned upon inspiration, resulting in warming and humidification, as well as removal by inertial impaction of a large proportion of the airborne particles, including dust, droplet nuclei, and pathogens. Particles deposited in the nasal cavities are moved toward the pharynx by mucociliary action, where the mucus can be swallowed, and pass into the gut. While in the nasal cavities, particles remain subject to possible aspiration and transfer to the lower airways or pulmonary region of the lung (18,95). Nungester and Klepser (74) found that exposure of rats to cold increased the aspiration of mucus material placed in the nose and they attributed this finding to cold-induced interference of closing of the glottis. Particles which escape entrapment in the nasal region may be deposited in the ciliated airways, primarily by sedimentation, and cleared toward the glottis by mucociliary action. As in the nasal cavities, particles along the airway are subject to aspiration and introduction into deeper portions of the lung. Therefore, the slower the clearance of pathogens from the upper airways, the greater the chance for deposition in the alveolar region of the lung where pneumonia is most likely.

Green and Kass (37) concluded that cold stress in mice inhibited clearance of inhaled bacteria from the lung. This was primarily explained by decreased macrophage activity, but the effect of cold on mucociliary clearance was not considered. Proctor *et al.* (77) reported the optimum ambient temperature for human nasal mucosal flow to be 23°C, with a fall in flow rate observed in the anterior and middle parts of the nose above

and below 23°C. Cooling created a greater and more consistent flow rate change than did warming. Studies by McFadden *et al.* (67,68) in humans demonstrate that inspiration of colder air resulted in cooling of the airways and that this cooling was not restricted to the upper airways. Therefore, the cooling of airway walls, which facilitates heat and water recovery upon exhalation, may also result in a decreased mucociliary clearance rate.

A reduction in blood flow to the nasal mucosa may occur in response to the inhalation of cold air (55). There is some evidence suggesting that nasal mucosal blood flow actually is reduced by cold exposure. Preliminary data show that when calves were exposed to 3–6°C, nasal mucosal temperature decreased by 23.2% and expired air temperature decreased by 29.4% with respect to thermoneutral (18–20°C) measurements (93). This may serve the purpose of reducing respiratory heat loss or preserving brain temperature. However, reduced mucosal blood flow is likely to impair the mucociliary function as well as the inflammatory response of the respiratory tract (97).

Inspiration of gases of low temperature and humidity have been reported to alter particle deposition and to reduce mucus clearance (6,81), suggesting an interaction between thermal stimuli and ciliary function. Bohning *et al.* (16) found bronchial transport in donkeys to decrease at least 1.5% per °C decrease in ambient pretest temperature. Yager *et al.* (103) showed a significant change with temperature in the ciliary beat frequency of human ciliated epithelium *in vitro*. Those measured at 25°C beat more slowly than those measured at 37°C, indicating a local effect of temperature. However, Liote *et al.* (62) were unable to show a relationship between ciliary beat frequency and nasal mucosal

transport time in human subjects, but did observe a significant relationship between transport time and the transport rate of collected mucus on the frog depleted-palate model. Similarly, Katz *et al.* (59) were unable to show a relationship of ciliary beat frequency to tracheal mean clearance rate in humans. This indicates that properties of the mucus secretions and/or the presence of cilioactive mediators in the mucus may play an important role in clearance rate. In fact, Phipps *et al.* (75) attributed an age-related increase in tracheal mucus transport velocity in neonatal sheep to elevated secretion of macromolecules, Cl^-, and liquid during the first 2-4 weeks of life, while ciliary beat frequency was unchanged. Hypoxemia has been shown to increase tracheal submucosal gland secretion in dogs (28), a finding which may be of significance for mucociliary clearance during cold-induced hypoventilation. Large variations in mucosal clearance between healthy humans has been reported (3,29,78,104,105) and is perhaps due to the secretory properties of the mucosal layer.

Whether mucosal clearance changes affected by temperature are due to local effects or autonomic reflexes has not been resolved. Cholinergic and beta adrenergic drugs have a ciliostimulatory effect (47), while atropine inhibits ciliary activity and depresses mucociliary transport (27,65). While both vagal and sympathetic nerve stimulation increase mucus secretion rate (35,72,73), autonomic nerve interruption has not produced any changes in ciliary activity, secretory function, or mucociliary transport (20,35,96). However, Lung and Wang (63) recently demonstrated that nasal blood vessels are under predominantly sympathetic control, and speculated that a mechanism for mucosal congestion might involve withdrawal of sympathetic discharge.

Airway clearance mechanisms are normally highly efficient, approaching 100% clearance efficiency. Therefore, the efficiency of clearance mechanisms is extremely important in determining the degree of retention of particles in the lung. If one assumed a normal clearance of 99%, for example, a 1% decrease in clearance to 98% results in a doubling of particle retention (18). Thus, should cold exposure result in even a small reduction in particle clearance rate, the implications in terms of susceptibility to infection would be great.

REFERENCES - CHAPTER II

1. Adams, T. Body-temperature regulation in the normal and cold-acclimatized cat. *J. Appl. Physiol.* 18:772-777, 1963.

2. Angell-James, J.E. and M.D. Daly. Reflex respiratory and cardiovascular effects of stimulation of the receptors in the nose of the dog. *J. Physiol.* 220:673-696, 1972.

3. Andersen, I., G.R. Lundqvist and D.F. Proctor. Human nasal mucosal function in a controlled environment. *Arch. Envir. Health.* 23:408-420, 1971.

4. Andersson, B., R. Grant and S. Larsson. Central control of heat loss mechanisms in the goat. *Acta. Physiol. Scand.* 37:261-280, 1956.

5. Bacon, M. and J. Bligh. Interaction between the effects of spinal heating and cooling and of injections into a lateral cerebral ventricle of noradrenaline, 5-hydroxytryptamine and carbachol on thermoregulation in sheep. *J. Physiol.* 254:213-227, 1976.

6. Baetjer, A.M. Effect of ambient temperature and vapor pressure on cilia-mucus clearance rate. *J. Appl. Physiol.* 23:498-504, 1967.

7. Baker, M.A. A brain-cooling system in mammals. *Sci. Am.* 240:114-122, 1979.

8. Baker, M.A. Brain-cooling in endotherms in heat and exercise. *Ann. Rev. Physiol.* 44:85-96, 1982.

9. Baldwin, B.A. and D.L. Ingram. The influence of hypothalamic temperature and ambient temperature on thermoregulatory mechanisms in the pig. *J. Physiol.* 198:517-529, 1968.

10. Berk, J.L., K.A. Lenner and E.R. McFadden, Jr. Cold induced bronchoconstriction: role of cutaneous reflexes vs. direct airway effects. *J. Appl. Physiol.* 63:659-664, 1987.

11. Bianca, W. and F. Naf. Responses of cattle to the combined exposure, to diurnal temperature rhythm (-5 to 25°C) and to simulated high-altitude (4000m). *Int. J. Biometeor.* 23:299-310, 1979.

12. Blake, C.I. and N. Banchero. Effects of cold and hypoxia on ventilation and oxygen consumption in awake guinea pigs. *Respir. Physiol.* 61:357-368, 1985.

13. Bligh, J. The initiation of thermal polypnoea in the calf. *J. Physiol.* 136:413-419, 1957.

14. Bligh, J. Inhibition of thermal polypnoea in the closely shorn sheep. *J. Physiol.* 168:764-781, 1963.

15. Bohning, D.E., R.E. Alpert, M. Lippman and W.M. Foster. Tracheobronchial particle deposition and clearance. *Arch. Environ. Health* 30:457-462, 1975.

16. Bohning, D.E., R.E. Alpert, M. Lippman and V.R. Cohen. Effect of pretest temperature on aerosol penetration and clearance in donkeys. *J. Appl. Physiol.* 41:920-924, 1976.

17. Brain, J.D., D.E. Knudson, S.P. Sorokin and M.A. Davis. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. *Envir. Res.* 11:13-33, 1976.

18. Brain, J.D. and P.A. Valberg. Deposition of aerosol in the respiratory tract. *Am. Rev. Respir. Dis.* 120:1325-1373, 1979.

19. Brockway, J.M., J.D. McDonald and J.D. Pullar. Evaporative heat loss mechanisms in sheep. *J. Physiol.* 179:554-568, 1965.

20. Brody, J.S., J.A. Waldhausen and D.E. Kuhl. Mucociliary clearance in denervated bronchi. *Clin. Res.* 19:507, 1971. (Abst.)

21. Brody, S. Environmental physiology with special reference to domestic animals. 1. Physiological backgrounds. *Res. Bull. Mo. Agric. Exp. Sta.* 423, 1948.

22. Burgess, K.R. and W.A. Whitelaw. Reducing ventilatory response to carbon dioxide by breathing cold air. *Am. Rev. Respir. Dis.* 129:687-690, 1984.

23. Busch, M.A., A. Tucker and D. Robertshaw. Interaction between cold and altitude exposure on pulmonary circulation of cattle. *J. Appl. Physiol.* 58:948-953, 1985.

24. Calvert, D.T., D.P. Clough, J.D. Findlay and G.E. Thompson. Hypothalamic cooling, heat production and plasma lipids in the ox. *Life Sci.* 11:223-229, 1972.

25. Chauca, D. and J. Bligh. An additive effect of cold exposure and hypoxia on pulmonary artery pressure in sheep. *Res. Vet. Sci.* 21:123-124, 1976.

26. Chesy, G., M. Caputa, W. Kadziela, W. Kozak and A. Lachowski. Selective brain cooling in the ox (*Bos taurus*) during heavy exercise. *J. Therm. Biol.* 10:57-61, 1985.

27. Chopra, S.K. Effect of atropine on mucociliary transport velocity in anesthetized dogs. *Am. Rev. Respir. Dis.* 118:367-371, 1978.

28. Davis, B., R. Chinn, J. Gold, D. [illegible]ovac, J.D. Widdicombe and J.A. Nadel. Hypoxemia reflexly increases secretion from tracheal submucosal glands in dogs. *J. Appl. Physiol.* 52:1416–1419, 1982.

29. Del Donno, M., D. Pavia, J.E. Agnew, M.T. Lopez-Vidriero and S.W. Clarke. Variability and reproducibility in the measurement of tracheobronchial clearance in healthy subjects and patients with different obstructive lung diseases. *Eur. Respir. J.* 1:613–620, 1988.

30. Durnin, J.V.G.A. and R.G. Edwards. Pulmonary ventilation as an index of energy expenditure. *Quart. J. Exp. Physiol.* 40:370–377, 1955.

31. Eliasson, S. and G. Strom. On the localization in the cat of hypothalamic and cortical structures influencing cutaneous blood flow. *Acta Physiol. Scand. Suppl.* 70:113, 1950.

32. Findlay, J.D. and D.L. Ingram. Brain temperature as a factor in the control of thermal polypnoea in the ox (*Bos taurus*). *J. Physiol.* 155:72–85, 1961.

33. Frank, G.H. *Pasteurella haemolytica* and respiratory disease in cattle. *Proc. U.S. Anim. Health Assoc.* 83:153–160, 1979.

34. Fusco, M.M., J.D. Hardy and H.T. Hammel. Interaction of central and peripheral factors in physiological temperature regulation. *Am. J. Physiol.* 200:572–580, 1961.

35. Gallagher, J.T., P.W. Kent, M. Passatore, R.J. Phipps and P.S. Richardson. The composition of tracheal mucus and the nervous control of its secretion in the cat. *Proc. R. Soc. London B* 192:49–76, 1975.

36. Garcia-Villalon, A.L., G. Dieguez, B. Gomez, E. Nava-Hernandez, L. Santamaria and S. Llunch. Mechanics of arteries forming the carotid rete of goat and cattle. *Microvasc. Res.* 37:204–217, 1989.

37. Green, G.M. and E.H. Kass. The influence of bacterial species in pulmonary resistance to infection in mice subjected to hypoxia, cold stress, and ethanolic intoxication. *Brit. J. Exper. Path.* 46:360–366, 1965.

38. Greenlees, K.J. Pulmonary venoconstriction in cold exposed calves. Ph.D. Diss., Colo. State Univ., Ft. Collins, 1983.

39. Guieu, J.D. and J.D. Hardy. Effects of preoptic and spinal cord temperature in control of thermal polypnea. *J. Appl. Physiol.* 28:540–542, 1970.

40. Hales, J.R.S. The partition of respiratory ventilation of the panting ox. *J. Physiol.* 188:P45–P46, 1967.

41. Hammel, H.T., J.D. Hardy and M.M. Fusco. Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. *Am. J. Physiol.* 198:481–486, 1960.

42. Hartmann, H., W. Bruer, A. Herzog, H. Meyer, H. Rhode, F. Schulze and G. Steinbach. The general adaptation syndrome in the calf. IV. Stress conditions – their impact upon antibody levels, following active and passive immunization and upon the topographical distribution of some groups of bacteria in the gastrointestinal system. *Arch. Exp. Vet. Med* 30:553–566, 1976.

43. Hatch, T. Distribution and deposition of inhaled particles in the respiratory tract. *Bact. Rev.* 25:237–240, 1961.

44. Hayward, J.N. and M.A. Baker. Diuretic and thermoregulatory responses to preoptic cooling in the monkey. *Am. J. Physiol.* 214:843–850, 1968.

45. Hellstrom, B. and H.T. Hammel. Some characteristics of temperature regulation in the unanesthetized dog. *Am. J. Physiol.* 213:547–556, 1967.

46. Hemingway, A., T. Rasmussen, H. Wikoff and A.T. Rasmussen. Effects of heating hypothalamus of dogs by diathermy. *J. Neurophysiol.* 3:329–338, 1940.

47. Hermens, W.A.J.J. and F.W.H.M. Merkus. The influence of drugs on nasal ciliary movement. *Pharm. Res.* 4:445–449, 1987.

48. Heyder, J., J. Gebhart and W. Stahlhofen. Inhalation of aerosols. Particle deposition and retention. In: *Generation of Aerosols and Facilities for Exposure Experiments* (K. Willeke, Ed.), Ann Arbor Science, Ann Arbor, MI, 1980, pp. 65–108.

49. Hoerlein, A.B. and C.L. Marsh. Studies on the epizootiology of shipping fever in calves. *JAVMA* 131:123–127, 1957.

50. Ingram, D.L. and K.F. Legge. The effect of environmental temperature on respiratory ventilation in the pig. *Respir. Physiol.* 8:1–12, 1968.

51. Jammes, Y., P. Barthelemy and S. Delpierre. Respiratory effects of cold air breathing in anesthetized cats. *Respir. Physiol.* 54:41–54, 1983.

52. Jensen, R., R.E. Pierson, P.M. Braddy, D.A. Saari, L.H. Lauerman, J.J. England, D.P. Horton and A.E. McChesney. Diseases of yearling feedlot cattle in Colorado. *JAVMA* 169:497–499, 1976.

53. Jensen, R., R.E. Pierson, P.M. Braddy, D.A. Saari, L.H. Lauerman, J.J. England, H. Keyvanfar, J.R. Collier, D.P. Horton, A.E. McChesney, A. Benitez and R.M. Christie. Shipping fever pneumonia in yearling feedlot cattle. *JAVMA* 169:500–506, 1976.

54. Jessen, C. Interaction of air temperature and core temperatures in thermoregulation of the goat. *J. Physiol.* 264:585-606, 1977.

55. Jessen, C. and H. Pongratz. Air humidity and carotid rete function in thermoregulation of the goat. *J. Physiol.* 292:469-479, 1979.

56. Johnsen, H.K. and L.P. Folkow. Vascular control of brain cooling in reindeer. *Am. J. Physiol.* 254:R730-R739, 1988.

57. Josenhans, W.T., G.N. Melville and W.T. Ulmer. The effect of facial cold stimulation on airway conductance in healthy man. *Can. J. Physiol. Pharmacol.* 47:453-457, 1969.

58. Joyce, J.P. and K.L. Blaxter. Respiration in sheep in cold environments. *Res. Vet. Sci.* 5:506-516, 1964.

59. Katz, I., T. Zwas, G.L. Baum, E. Aharenson and B. Belfer. Ciliary beat frequency and mucociliary clearance. *Chest* 92:491-493, 1987.

60. Kosaka, M., E. Simon, R. Thauer and O.E. Walther. Effect of thermal stimulation of spinal cord on respiratory and cortical activity. *Am. J. Physiol.* 217:858-863, 1969.

61. Lee K.P. Lung response to particulates with emphasis on asbestos and other fibrous dusts. *CRC Critical Reviews in Toxicology* 14:33-86, 1985.

62. Liote, H., J.M. Zahm, D. Pierrot and E. Puchelle. Role of mucus and cilia in nasal mucociliary clearance in healthy subjects. *Am. Rev. Respir. Dis.* 140:132-136, 1989.

63. Lung, M.A. and J.C.C. Wang. Autonomic nervous control of nasal vasculature and airflow resistance in the anaesthetized dog.. *J. Physiol.* 419:121-139, 1989.

64. Magoun, H.W., F. Harrison, J.R. Brobeck and S.W. Ranson. Activation of heat loss mechanism by local heating of the brain. *J. Neurophysiol.* 1:101-104, 1938.

65. Marin, M.G., B. Davis and J.A. Nadel. Effect of acetylcholine on Cl^-, and Na^+ fluxes across dog tracheal epithelium *in vitro*. *Am. J. Physiol.* 231:1546-1549, 1976.

66. McBride, B. and W.A. Whitelaw. A physiological stimulus to upper airway receptors in humans. *J. Appl. Physiol.* 51:1189-1197, 1981.

67. McFadden, E.R.,Jr., D.M. Dennison, J.F. Waller, B. Assoufi, A. Peacock and T. Sopwith. Direct recordings of the temperatures in the tracheobronchial tree in normal man. *J. Clin. Invest.* 69:700-705, 1982.

68. McFadden, E.R.,Jr., B.M. Pichurko, H.F. Bowman, E. Ingenito, S. Barns, N. Dowling and J. Solway. Thermal mapping of the airways in humans. *J. Appl. Physiol.* 58:564-570, 1985.

69. McLean, J.A., W.T. Whitmore, B.A. Young and R. Weingardt. Body heat storage, metabolism and respiration of cows abruptly exposed and acclimatized to cold and 18°C environments. *Can. J. Anim. Sci.* 64:641-653, 1984.

70. McMurtry, I.F., J.T. Reeves, D.H. Will and R.F. Grover. Hemodynamic and ventilatory effects of skin-cooling in cattle. *Experientia* 31:1303-1304, 1975.

71. Mercer, J.B., H.K. Johnsen, A.S. Blix and R. Hotvedt. Central control of expired air temperature and other thermoregulatory effectors in reindeer. *Am. J. Physiol.* 248:R679-R685, 1985.

72. Nadel, J.A., B. Davis and R.J. Phipps. Control of mucus secretion and ion transport in airways. *Ann. Rev. Physiol.* 41:369-381, 1979.

73. Nadel, J.A. and B. Davis. Parasympathetic and sympathetic regulation of secretion from submucosal glands in airways. *Federation Proc.* 39:3075-3079, 1980.

74. Nungester, W.J. and R.G. Klepser. A possible mechanism of lowered resistance to pneumonia. *J. Infect. Dis.* 63:94-102, 1938.

75. Phipps, R.J., W.M. Abraham, A.T. Mariassy, P.J. Torrealba, M.W. Sielezak, A. Ahmed, M. McCray, J.S. Stevenson and A. Wanner. Developmental changes in the tracheal mucociliary system in neonatal sheep. *J. Appl. Physiol.* 67:824-832, 1989.

76. Pityn, P., M.J. Chamberlain, T.M. Fraser, M. King and W.K.C. Morgan. The topography of particle deposition in the human lung. *Respir. Physiol.* 78:19-29, 1989.

77. Proctor, D.F., I.B. Andersen and G.R. Lundqvist. Human nasal mucosal function at controlled temperatures. *Respir. Physiol.* 30:109-124, 1977.

78. Puchelle, E., F. Aug, J.M. Zahm and A. Bertrand. Comparison of nasal and bronchial mucociliary clearance in young non-smokers. *Clin. Sci.* 62:13-16, 1982.

79. Robertshaw, D. Cold stress and animal production. In: *Current Veterinary Therapy* (J.L. Howard, Ed.), Saunders, New York, 1981.

80. Robertshaw, D. The environmental physiology of animal production. In: *Environmental Aspects of Housing for Animal Production* (J.A. Clark, Ed.) Butterworths, London, 1981, pp. 3-17.

81. Salah, B., A.T. Dihn Xuan, J.L. Fouilladieu, A. Lockhart and J. Regnard. Nasal mucociliary transport in healthy subjects is slower when breathing dry air. *Eur. Respir. J.* 1:852-855, 1988.

82. Sant'Ambrogio, G., O.P. Mathew, F.B. Sant'Ambrogio and J.T. Fisher. Laryngeal cold receptors. *Respir. Physiol.* 59:35-44, 1985.

83. Slocombe, R.F., F.J. Derksen, N.E. Robinson, A. Trapp, A. Gupta and J.P. Newman. Interactions of cold stress and *Pasteurella haemolytica* in the pathogenesis of pneumonic pasteurellosis in calves: Method of induction and hematologic and pathologic changes. *Am. J. Vet. Res.* 45:1757-1763, 1984.

84. Slocombe, R.F., F.J. Derksen and N.E. Robinson. Interactions of cold stress and *Pasteurella haemolytica* in the pathogenesis of pneumonic pasteurellosis in calves: Changes in pulmonary function. *Am. J. Vet. Res.* 45:1764-1770, 1984.

85. Solway, J., B.M. Pichurko, E.P. Ingenito, E.R. McFadden, Jr., C.H. Fanta, R.H. Ingram, Jr. and J.M. Drazen. Breathing pattern affects airway wall temperature during cold air hyperpnea in humans. *Am. Rev. Respir. Dis.* 132:853-857, 1985.

86. Stahlhofen, W., J. Gebhart and J. Heyder. Experimental determination of the regional deposition of aerosol particles in the human respiratory tract. *Am. Ind. Hyg. Assoc. J.* 41:385-398, 1980.

87. Stuart, B.O. Deposition and clearance of inhaled particles. *Envir. Health Pers.* 55:369-390, 1984.

88. Taylor, C.R. Metabolism, respiratory changes, and water balance of an antelope, the eland. *Am. J. Physiol.* 217:317-320, 1969.

89. Taylor, C.R. and C.P. Lyman. Heat storage in running antelopes: independence of brain and body temperatures. *Am. J. Physiol.* 222:114-117, 1972.

90. Tchobroutsky, C., C. Merlet and P. Rey. The diving reflex in rabbit, sheep, and newborn lamb and its afferent pathways. *Respir. Physiol.* 8:108-117, 1969.

91. Thomson, R.G. A perspective on respiratory diseases in feedlot cattle. *Can. Vet. J.* 21:181, 1980.

92. Tsubone, H. Nasal flow receptors of the rat. *Respir. Physiol.* 75:51-64, 1989.

93. Tucker, A., D. Robertshaw, C.B. Allen and M.L. Wright. Nasal airflow and mucosal blood flow in cold-exposed cranially-sympathectomized calves. *Proc. IUPS* 16:473, 1986. (Abst.)

94. Valberg, P.A., J.D. Brain, S.R. Lemott, S.L. Sneddon and A. Vinegar. Dependence of aerosol deposition site on breathing pattern. *Am. Rev. Respir. Dis. Suppl.* 117:262, 1978. (Abst.)

95. Walsch, T.E. and P.R. Cannon. Immunization of the upper respiratory tract. *Arch. Otolaryngol.* 20:820-836, 1934.

96. Wanner, A. Autonomic control and mucociliary functions. *Chest* 91:49S-51S, 1987.

97. Wanner, A. Circulation of airway mucosa. *J. Appl. Physiol.* 67:917-925, 1989.

98. Webbe, P. Air temperatures in respiratory tracts of resting subjects in cold. *J. Appl. Physiol.* 4:378-382, 1951.

99. Webster, A.J.F. Weather and infectious disease in cattle. *Vet. Rec.* 108:183-187, 1981.

100. Webster, A.J.F. Environmental stress and the physiology, performance and health of ruminants. *J. Anim. Sci.* 57:1584-1593, 1983.

101. Will, D.H., I.F. McMurtry, J.T. Reeves and R.F. Grover. Cold-induced pulmonary hypertension in cattle. *J. Appl. Physiol.* 45:469-473, 1978.

102. Winquist, R.J. Temperature sensitive tone in the rabbit facial vein: myogenic mechanism for cranial thermoregulation? *Science* 207:1001-1002, 1980.

103. Yager, J.A., H. Ellman and M.J. Dulfano. Human ciliary beat frequency at three levels of the tracheobronchial tree. *Am. Rev. Respir. Dis.* 121:661-665, 1980.

104. Yeates, D.B., N. Aspin, H. Levison, M.T. Jones and A.C. Bryan. Mucociliary tracheal transport rate in man. *J. Appl. Physiol.* 39:487-495, 1975.

105. Yergin, B.M., K. Saketkhoo, E.D. Michealson, S.M. Serafini, K. Kovitz and M.A. Sackner. A roentgenographic method for measuring nasal mucus velocity. *J. Appl. Physiol.* 44:964-968, 1978.

CHAPTER III

THERMOREGULATORY BENEFIT AND MECHANISM OF STIMULATION OF COLD-INDUCED HYPOVENTILATION

Introduction

This study investigated the mechanism(s) and the thermoregulatory significance of cold-induced hypoventilation in cattle, and tested the hypothesis that **hypoventilation is an adaptive strategy to maintain brain temperature and decrease respiratory heat loss, thereby representing an indispensable mechanism for energy conservation**. To test this hypothesis, three specific objectives were addressed. First, this study determined the thermoregulatory benefit of the ventilatory response to cold exposure in calves by measuring any reduction in respiratory heat loss afforded by hypoventilation. Second, the effect of cold-induced hypoventilation upon hypothalamic temperature was investigated to determine if the ventilatory response to cold is a reflex to preserve brain temperature or if the hypoventilatory response is mediated by a decrease in hypothalamic temperature. Third, the relative role of peripheral and airway cold stimulation in the ventilatory response of calves was evaluated by partitioning the thermal environments of body and airway.

Materials and Methods

ANIMALS: Experiments were conducted using male Holstein calves between the ages of 1 and 3 months and weighing between 45 and 55 kg. Calves were purchased locally at 1 week of age and maintained in an indoor holding facility (20-25°C, 35-55% relative humidity). They were maintained on a diet of milk replacer (2 liters, 2 times per day) and Startena medicated calf chow (Purina, Inc.) *ad libitum*. Calves were not fed the morning of an experiment. Data were collected between the hours of 1000 and 1500. During exposures, calves were held in a stanchion either inside a temperature-controlled insulated box (1 x 2 x 1.5 m) or cold room (7 x 4 m, 4-6°C).

METHODS: Temperatures were measured using copper-constantan thermocouples (36 gauge wire) connected to a digital thermometer (Doric Trendicator 400 A). The copper and constantan were electrically welded so that the temperature sensitive area was confined to the tip. Skin temperature (T_{sk}) was measured on the trunk, 10 cm below mid-dorsal along the 8th rib, with thermocouple tips being fixed by cyanoacrylate adhesive. A thermocouple was placed 20 cm into the rectum to determine core body temperature (T_b).

Hypothalamic temperature (T_h) was measured in three calves by inserting a thermocouple into a surgically placed guide tube, following the technique of Dmi'el and Robertshaw (4), such that the temperature sensitive tip was at the sealed end of the guide tube (12 gauge). This surgery was performed following sedation with xylazine (0.2 mg/kg) and maintenance of anesthesia with halothane (1%). The correct position of the guide tube was predetermined from skull measurements. A 2 cm incision

was made through the skin along the midline of the skull, followed by penetration of the skull bone with a sterile 1/8" drill bit. The dura was penetrated with a sterile needle. The guide tube, prefabricated from a 12 gauge spinal needle, was inserted into the brain through the hole at such an angle as to place the tip in the preoptic area of the hypothalamus. To secure the guide tube, three stainless steel screws were placed in the skull approximately 1 cm from the tube and equidistant from each other. A length of 80 lb test steel cable was wrapped around the probe and out to each screw, in a clover leaf fashion. To provide additional support for the guide tube, the tube, screws, and cable were imbedded in dental acrylic approximately 0.5 cm thick. The skin was closed around the tube and over the dental acrylic. The exposed end of the guide tube was protected from bumping by a plastic cup (with bottom removed) sutured to the skin around the tube. The end of the tube was covered with a removable rubber cap to prevent blockage of the lumen of the tube. Three days were allowed for recovery from surgery before data were collected.

The guide tube was also used as a thermode in an experiment to lower hypothalamic temperature by circulating cold ethanol through the tube. This was accomplished by circulating ethanol through a reservoir surrounded by dry ice. The ethanol was circulated through polyethylene tubing, using a perfusion pump, to a spinal needle (16 gauge) which was placed inside the guide tube. The ethanol returned from the guide tube, by way of polyethylene tubing, to the reservoir. Heating the probe was accomplished by circulating hot water through the system. The location of the tip of the guide tube, in the pre-optic area of the hypothalamus, was verified by dissection of each calf following the experiments.

Oxygen and CO_2 concentrations of expired air were determined using a mass spectrometer (Perkin-Elmer, Model 1100 Medical Gas Analyzer) by sampling from a mixing chamber. End-tidal CO_2 was also determined to enable estimation of dead space volume (V_D) using the Bohr equation as described in Appendix A. The mass spectrometer was calibrated daily using gas mixtures of known concentration. Arterial blood samples were obtained from three of the calves (from a cannulated saphenous artery) and analyzed for pH, Pa_{O2}, and Pa_{CO2}, using a blood gas analyzer (Radiometer ABL30). Hematocrit was also determined for each blood sample. Three samples (2.5 ml) were obtained from each calf during each treatment or temperature range. Sampling was begun 10 min following the initiation of data collection and individual samples were then taken at 10 min intervals. Pa_{CO2} measurements were used to correct the estimates derived by mass spectrometry for end-tidal CO_2 concentrations (F_{ETCO2}), since there was some delay in the response of the recorder used to record F_{ETCO2}. The magnitude of the correction was linearly correlated ($P<0.05$) with respiratory frequency (f), therefore the following equation was used to adjust F_{ETCO2}:

$$F_{ETCO2} = -13.84 * f + 1.59 \quad (r = 0.72)$$

No correction was made for those trials in which blood samples were taken; instead F_{ETCO2} was determined from Pa_{CO2} measurements and used in the dead space equation in Appendix A.

Nasal airflow was measured using a fabricated nasal mask (Figure 3.1), with dead space = 87 ml (ATPS), two one-way respiratory valves, and a gas flowmeter (American Meter Company) which integrated expired air flow. A harness was fabricated from nylon web material to

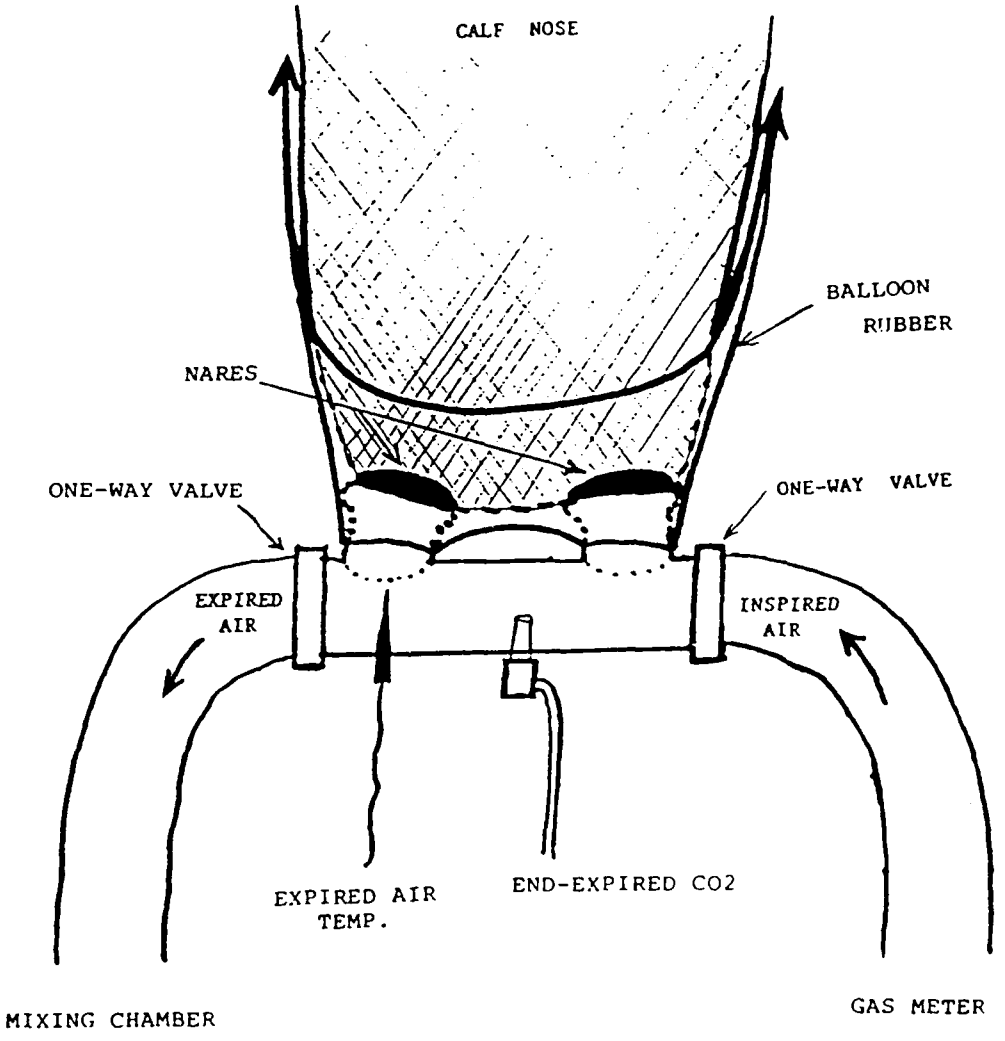


Figure 3.1 Nasal respiratory mask.

hold the mask. The calves were trained to accept the harness and mask. Total ventilation ($\dot{V}_E$), respiratory frequency (f), and expired air temperature (T_E) measurements were made simultaneously, over a continuous period. Inspired relative humidity (RH), ambient temperature (T_a), inspired air temperature (T_I), T_{sk}, T_b, inspired O_2 concentration ($F_{I\ O2}$), and inspired CO_2 concentration ($F_{I\ CO2}$) were recorded during experimental trials at 10 min intervals. Oxygen and CO_2 concentrations of expired air ($F_{E\ O2}$ and $F_{E\ CO2}$, respectively) were determined by sampling from the mixing chamber at 2-min intervals. Total ventilation, oxygen consumption ($\dot{V}_{O2}$), and CO_2 production ($\dot{V}_{CO2}$) were calculated using the equations in Appendix B. Tidal volume (V_T) was derived from $\dot{V}_E$ divided by f. Alveolar volume (V_A) was determined from V_T minus V_D. Dead space ventilation ($\dot{V}_D$) and alveolar ventilation ($\dot{V}_A$) were determined from V_D and V_A times f, respectively. A thermocouple (40 gauge) was placed into the mask in order to continuously monitor T_E. End-tidal CO_2 was determined by continuous sampling of expired air at the nares (mass spectrometer response time = 250 msec). T_E and F_{ETCO2} tracings were obtained during continuous sampling of expired air using a 2-channel recorder (Kipp & Zonen).

Relative humidity (RH) of inspired air was determined, at 10 min intervals, using a RH indicator (Abbeon Cal, Inc). The barometric pressure was determined daily prior to an experimental period using a mercurial barometer (Princo Instruments, Inc.). Body weight was also measured daily, before each experimental period, using a Wadler livestock scale. Ambient water vapor pressure during the experiments was between 4 and 10 Torr for all experiments. RH of expired air was assumed to be 100%. Convective respiratory heat loss (CHL), evaporative respiratory heat loss (EHL), and total respiratory heat loss (RHL) were determined

from the measurements using the equations described by Varene and Kays (21), Appendix C.

The use of the nasal mask also allowed exposure of the airway to thermal conditions differing from that to which the body was exposed. To expose the calf to a cold environment while breathing warm air, the calf was placed in a stanchion in the cold room and supplied warm air to the nasal probe from the insulated box. RH of inspired air was measured inside the box and T_I was determined at the intake valve of the nasal mask. To expose the calves to a warm environment while breathing cold air, the calves were placed inside the warm insulated box and supplied air to the mask from the surrounding cold room. Inspired air RH was determined as the RH of the cold room and T_I was determine at the intake valve of the mask.

Calves experienced a one week training period to become accustomed to the temperature controlled box and nasal mask. During this training period, the animals wore the mask while inside the box for 2-4 hr each day. Following the training period, calves readily accepted wearing the mask and standing in the insulated box with no apparent stress.

PROTOCOL:

Experiment 1: The effect of ambient temperature (T_a) on ventilation was determined in four calves for the following temperature ranges: 4-6, 8-10, 12-14, and 16-18°C. Temperature was regulated within the insulated box by use of a heater at the rear of the box and a variable speed fan at the front which pulled air out of the box (Figure 3.2). This system provided control of temperature within a 2°C range inside the box. Measurements were made over a continuous period of at least 50 min for

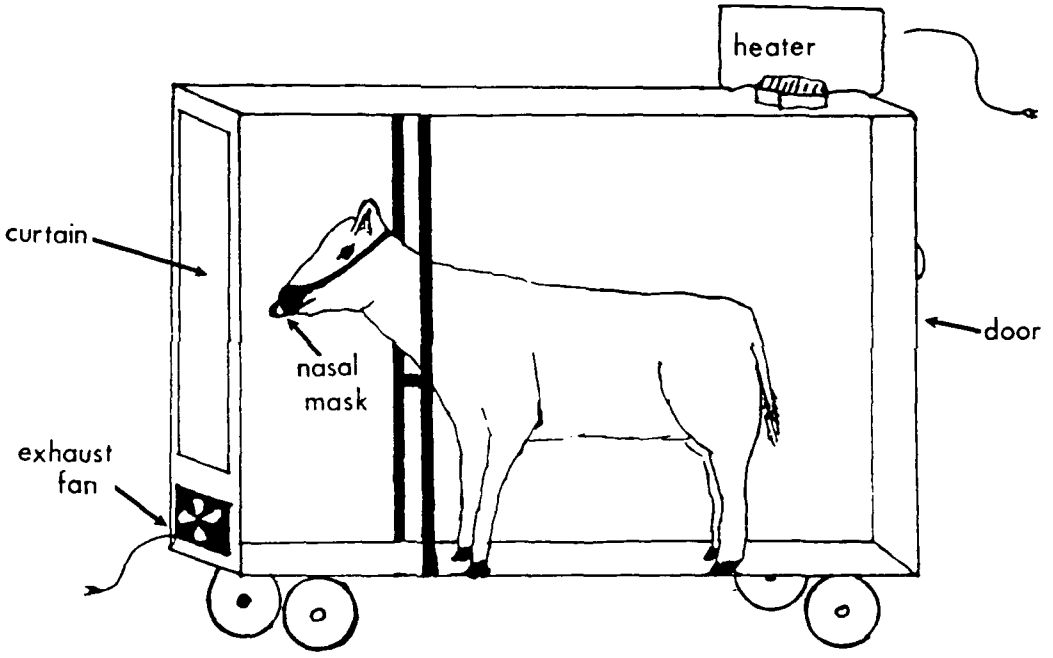


Figure 3.2 Temperature controlled box.

each calf at each temperature range. The measurement periods were performed consecutively, allowing approximately 20 min exposure to a temperature before beginning to collect data. Trials on two different days were conducted on each calf at each temperature range, for an overall balanced sample of 8 trials per temperature. The order of the temperature periods was such that each calf was evaluated once during a sequence of increasing temperatures and once during decreasing temperatures. The effect of T_a on various parameters was determined using two-way ANOVA with provisions for repeated measures and the Student-Newman-Kuels (SNK) test. Also, the linear relationship of T_a to various respiratory parameters was determined using least-squares regression and an F-test. Unless otherwise stated, significance was determined as $P<0.05$.

Experiment 2: To determine how cold stimulates the ventilatory response, it was necessary to separate the thermal environment of the airway from that of the body. Use of the cold room, in combination with the nasal mask and insulated box, allowed the following combinations of thermal exposure:

Treatment	Body	Airway	
1	TN	TN	
2	TN	COLD	
3	COLD	TN	
4	COLD	COLD	
5	TN	COLD	(+ lidocaine)

TN = thermoneutral (16-18°C)

COLD = 4-6°C

Measurements were made over a continuous period of at least 50 min for each calf at each treatment. Trials on two different days were conducted on each calf at each treatment, for an overall balanced sample of 12 trials per treatment. The effect of treatment on various parameters was determined using two-way ANOVA with provisions for repeated measures and the SNK test. Unless otherwise stated, significance was determined as $P<0.05$.

Treatment #5 was conducted to differentiate an airway receptor response from a hypothalamic cooling response. Topical 3% lidocaine (3 ml through each nostril) was applied to the nasal mucosa of calves subjected to the same thermal conditions as Treatment #2. This should have negated the effect of local airway receptors, but not affect a hypothalamic cooling response. Respiratory and temperature measurements were begun 5 min following lidocaine application and limited to a period of 30 min, to avoid taking measurements after the effect of lidocaine had worn off.

Data collected from these five different exposures were used to determine how the hypoventilatory response was stimulated. In other words, hypoventilation may be the result of either stimulation of receptors in the airway and/or skin, cooling of blood to the hypothalamus, or some combination of these. Treatment #4 above has already been shown to result in hypoventilation in calves (3,24). If a change in ventilation were observed with Treatment #3, this would indicate that cutaneous cold receptors elicit the respiratory change. Should Treatment #2 alter ventilation, the respiratory change might be mediated by airway cold receptors or by cooling of the blood perfusing the hypothalamus.

Three additional calves were prepared with hypothalamic probes for measurement of hypothalamic temperature and exposed to Treatments 1, 2,

4, and 5. Trials lasting 20 min were conducted for each treatment. Duplicate trials were performed on 2 of the 3 calves for a total sample of 5 trials per treatment. The same measurements as stated above were made, with the addition of recording T_h at 2 min intervals.

Experiment 3: A comparison of respiratory heat loss in cold-exposed calves (T_a = 4.1 ± 0.2°C, RH = 82 ± 1%) at different levels of ventilation was achieved by increasing the inspired CO_2 concentration, thereby providing a means for comparison of the relationship between f, $\dot{V}_E$, and RHL. A mixture of CO_2 and air was supplied to calves via the nasal mask. After the appropriate time period to allow stabilization of ventilation and metabolic rate, the f, $\dot{V}_E$, temperature and humidity measurements listed earlier were obtained for a continuous period of at least 10 min. At least 5, and no more than 9, trials were obtained for each calf, including those obtained for normal inspired CO_2 concentration, for a total sample of 46 trials. From the above data, the linear relationship of T_E and RHL to f and $\dot{V}_E$ was determined using forward selection multiple linear regression and F-tests.

The three calves which were prepared with hypothalamic probes were also exposed to increased inspired CO_2 concentrations as described above. In addition to making the measurements stated above, T_h was also recorded at 2 min intervals. Three trials were conducted on calf #229 and 7 trials were conducted on each of calves #111 and #222, for a total sample of 17 trials.

Experiment #4: The hypothalamic probe was used as a thermode to cool the preoptic region of the hypothalamus while the calves were exposed to an ambient temperature of 16-18°C. After collecting baseline data for at least 10 min, the hypothalamus was cooled for a period of 20 min. This

period was followed by an additional 10 min of recovery measurements. During this experiment, f, $\dot{V}_E$, and T_E were continuously monitored, while T_b and T_{sk} were recorded at 2 min intervals. One trial was conducted on the first calf (#229), and two trials were conducted on each of the other two calves (#111 and #222) for a total sample of 5 trials. In addition, to assess the function and placement of the thermode, hot water was circulated through the probe for 4 to 8 min while ventilatory measurements were taken (in calves #111 and #222).

Control data consisted of the average value obtained during both baseline measurement periods (including before and after cooling the probe) for a total of at least 20 min of measurements per trial. Data from the 20 min period of hypothalamic cooling were compared to control values, along with data from hypothalamic heating periods, using two-way ANOVA and the SNK test, with significance determined as $P<0.05$.

Results

VENTILATORY AND METABOLIC RESPONSES TO AMBIENT TEMPERATURE: The data collected on individual calves in experiment #1 are presented in Appendices D and E. As T_a decreased over the range studied, f decreased by 29% (comparing the 4-6°C value with the 16-18°C value) while V_T increased by 35%, resulting in no change in $\dot{V}_E$ (Table 3.1). This change in respiratory pattern resulted in a decrease in dead space ventilation ($\dot{V}_D$) of 21.6 ml/min•kg and an increase in alveolar volume (V_A) of 3.36 ml/kg, but no significant change in alveolar ventilation ($\dot{V}_A$).

Convective heat loss was increased by 0.015 W/kg (88% increase) with decreasing T_a over the temperature range studied, even though T_E decreased with T_a by 5.1°C (Table 3.2). However, EHL did not change with T_a. As a

result, RHL (the sum of CHL and EHL) did not increase with decreasing T_a. In addition, since $\dot{V}_E$ did not change, the RHL per liter (in joules/liter) did not increase with decreasing T_a (Table 3.2).

Although $\dot{V}_{O2}$ and MR showed significant increases ($P<0.01$) over the temperature range studied (Tables 3.1 and 3.2), these were primarily due to increases at the lowest temperature range (4-6°C). Regression of MR or $\dot{V}_{O2}$ versus T_a over the upper three temperature ranges did not show significant negative relationships. Therefore, the lower critical temperature for these calves can be assumed to be between 6 and 8°C. The ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}_{O2}$) and the alveolar ventilatory equivalent ($\dot{V}_A/\dot{V}_{O2}$) were both positively correlated ($P<0.01$) with T_a. The linear relationships of respiratory parameters to ambient air temperature are graphically represented in Figures 3.3 and 3.4.

The change in ventilatory pattern at colder temperatures resulted in increased O_2 extraction from the inspired air (Figure 3.4). V_T, f, $\dot{V}_D$, and V_A are all linearly related to O_2 extraction ($P<0.01$). In spite of increased O_2 extraction, analysis of arterial blood (Table 3.3) revealed hypoxemia ($P<0.05$) in conjunction with slight hypercapnia ($P=0.059$) during cold conditions. The linear relationship of Pa_{O2} and Pa_{CO2} to T_a are graphically represented in Figure 3.5.

MECHANISM OF STIMULATION OF HYPOVENTILATION: The data collected on individual calves in experiment #2 are presented in Appendices F and G. Treatment #2 demonstrated that inspiration of cold air, while the animal was exposed to a 16-18°C environment, caused decreases in f, $\dot{V}_D$, and T_E, as well as increases in V_T and O_2 extraction when compared to Treatment #1 (Table 3.4). Therefore, cooling of the airways alone was sufficient to

elicit a change in ventilatory pattern. However, Treatment #3 (exposure to a cold environment while inhaling warm air) also caused identical changes in ventilatory pattern.

The effect of treatment on blood gas tensions is presented in Table 3.5. Compared to no cold exposure at all (Treatment #1), Pa_{O_2} was lower as a result of cold stimulation of the skin, but was not affected by breathing cold air. There was no significant difference in Pa_{CO_2} or pHa between treatments, although Pa_{CO_2} appeared to mirror Pa_{O_2}, with cold stimulation of the skin resulting in a higher Pa_{CO_2}, but no effect due to cold exposure of airways alone.

To determine if airway cold stimulation (Treatment #2) produced a ventilatory change by direct airway cold stimulation, lidocaine was applied to the nasal mucosa in Treatment #5. The values for f and V_T for Treatment #5 were similar to those for Treatment #1, in spite of the cold inspired air. T_E for Treatment #5 was significantly lower than T_E for Treatment #1.

To further investigate the possibility of hypothalamic control of ventilation in a cold environment, T_h was measured in three additional calves. The data collected on these calves are presented in Appendix H. No difference was observed in T_h between Treatments #1, 2, 4, or 5, nor did the T_b - T_h difference change (Table 3.6).

Data collected on individual calves in experiment #4 are presented in Appendix I. When the hypothalamic probe was used as a cold thermode to reduce hypothalamic temperature (T_a = 16 - 18°C), there was no change in f, $\dot{V}_E$, V_T, T_b, T_{sk}, or T_E (Table 3.7). Heating the probe resulted in an increase in f as expected, with no significant change in $\dot{V}_E$. In addition, T_E increased with thermode heating. One thermode trial on calf #111 is

represented in Figure 3.6 to demonstrate the effectiveness of the thermode during heating and the lack of response to thermode cooling.

EFFECT OF VENTILATORY CHANGES ON T_E AND RHL: The data collected on individual calves in experiment #3 are presented in Appendix J. When ventilation was stimulated with an increased inspired CO_2 concentration, T_E was significantly increased ($P<0.01$) by the resulting increased f. The inclusion of $\dot{V}_E$ influence on T_E in a multiple linear regression was significant ($P<0.05$) and allows prediction of T_E by the following equation ($r = 0.64$):

$$T_E = 12.1 + (0.592)f - (0.0105)\dot{V}_E \qquad \text{Equation 1}$$

To ascertain the accuracy of this equation in estimating T_E, the T_E predicted by Equation 1 for Treatment #2 of experiment #2 (mean $T_I = 4.2°C$) can be compared to the measured value. Based upon Equation 1, the T_E for Treatment #2 would be 19.5°C, which closely approximates the actual measured T_E value of 19.7°C. RHL was also significantly increased ($P<0.01$) by increases in f and $\dot{V}_E$ in experiment #3, resulting in the following multiple linear regression equation ($r = 0.94$):

$$RHL = -0.0868 + (0.000559)\dot{V}_E + (0.00670)f \qquad \text{Equation 2}$$

This equation appears to be accurate in that it predicts a RHL of 0.105 W/kg for Treatment #2, compared to the measured value of 0.103 W/kg.

When ventilation was driven by increased inspired CO_2 concentration in calves prepared with the hypothalamic probe (data in Appendix K), the $T_b - T_h$ difference was not increased. There was no correlation between either T_h or the T_b-T_h difference versus f, $\dot{V}_I$, or $\dot{V}_T$ ($n = 17$).

Table 3.1. Effects of ambient temperature on respiratory parameters.

	4-6°C	8-10°C	12-14°C	16-18°C
T_a (°C)	4.7 ± 0.4	9.0 ± 0.1	13.1 ± 0.1	17.1 ± 0.2
f (b/min)*	12.48± 0.77‡	13.62± 0.86‡	14.36± 1.31‡	17.66± 1.22
$\dot{V}_E$ (ml/min•kg)	157.0 ± 9.2	142.3 ± 6.9	140.3 ± 7.6	165.9 ± 11.2
V_T (ml/kg)*	12.76± 0.77‡	10.70± 0.78	10.20± 0.81	9.46± 0.39
V_D (ml/kg)	3.80± 0.29	3.57± 0.17	3.42± 0.21	3.86± 0.25
V_A (ml/kg)*	8.96± 0.63‡	7.13± 0.66	6.78± 0.66	5.60± 0.31
$\dot{V}_D$ (ml/min•kg)*	47.6 ± 4.6‡	48.4 ± 3.5‡	48.3 ± 4.6‡	69.2 ± 7.8
$\dot{V}_A$ (ml/min•kg)	109.4 ± 5.8	93.9 ± 4.8	92.0 ± 4.1	96.7 ± 4.4
$\dot{V}_{O_2}$ (ml/min•kg)*	6.96± 0.27‡	5.74± 0.36	5.49± 0.36	5.44± 0.22
$\dot{V}_{CO_2}$ (ml/min•kg)*	5.86± 0.30‡	4.76± 0.31	4.60± 0.29	4.65± 0.20
RER	0.84± 0.02	0.84± 0.01	0.84± 0.01	0.85± 0.02
O_2 extr (Torr)*	41.2 ± 1.8‡	37.2 ± 1.5‡	36.1 ± 1.9‡	30.8 ± 1.5
$\dot{V}_E/\dot{V}_{O_2}$*	22.6 ± 0.9‡	25.0 ± 0.9‡	26.0 ± 1.5‡	30.4 ± 1.4
$\dot{V}_A/\dot{V}_{O_2}$*	15.7 ± 0.6‡	16.5 ± 0.4	17.0 ± 0.6	17.8 ± 0.5

Values are means ± SE; n = 8. T_a, ambient air temperature; f, respiratory frequency; $\dot{V}_E$, minute ventilation; V_T, tidal volume; V_D, dead space volume; $\dot{V}_A$, alveolar volume; $\dot{V}_D$, dead space ventilation; $\dot{V}_A$, alveolar ventilation; $\dot{V}_{O_2}$, oxygen consumption; $\dot{V}_{CO_2}$, expired CO_2 volume; RER, respiratory exchange ratio; O_2 extr, O_2 extraction; $\dot{V}_E/\dot{V}_{O_2}$, ventilatory equivalent for O_2; $\dot{V}_A/\dot{V}_{O_2}$, alveolar ventilatory equivalent.

* $P<0.01$ for linear correlation with ambient temperature.

‡ $P<0.05$ compared to 16-18°C range.

Table 3.2. Effects of ambient temperature on body, skin and expired temperatures and respiratory heat loss.

	4-6°C	8-10°C	12-14°C	16-18°C
Vapor pressure (Torr)	5.4 ± 0.2	5.8 ± 0.3	7.0 ± 0.3	8.6 ± 0.4
T_b (°C)	39.5 ± 0.1	39.8 ± 0.1	39.8 ± 0.1	39.5 ± 0.1
T_{sk} (°C)*	31.8 ± 0.3‡	32.8 ± 0.4‡	33.4 ± 0.3	34.3 ± 0.3
T_E (°C)*	18.8 ± 1.0‡	20.2 ± 0.8	22.2 ± 0.9	23.9 ± 1.2
CHL (W/kg)*	0.032± 0.003‡	0.023 ± 0.002	0.019± 0.002	0.017± 0.004
EHL (W/kg)	0.060± 0.008	0.060 ± 0.007	0.066± 0.008	0.083± 0.012
RHL (W/kg)	0.092± 0.011	0.084 ± 0.009	0.084± 0.011	0.100± 0.015
MR (W/kg)*	2.36 ± 0.10‡	1.95 ± 0.12	1.86 ± 0.12	1.85 ± 0.08
RHL/MR (%)†	3.9 ± 0.4	4.1 ± 0.3	4.6 ± 0.5	5.3 ± 0.7
RHL/liter (J/l)	35.0 ± 3.2	34.7 ± 2.5	35.5 ± 3.4	35.2 ± 4.0

Values are means ± SE; n = 8. Vapor pressure, ambient water vapor pressure; T_b, body temperature; T_{sk}, skin temperature; T_E, expired air temperature; CHL, convective respiratory heat loss; EHL, evaporative respiratory heat loss; RHL, total respiratory heat loss; MR, metabolic rate; %RHL/MR, percentage of metabolic rate attributed to respiratory heat loss; RHL/liter, heat loss per liter of expired air.

* $P<0.01$ for linear correlation with ambient temperature.

‡ $P<0.05$ compared to 16-18°C range.

† $P=0.054$

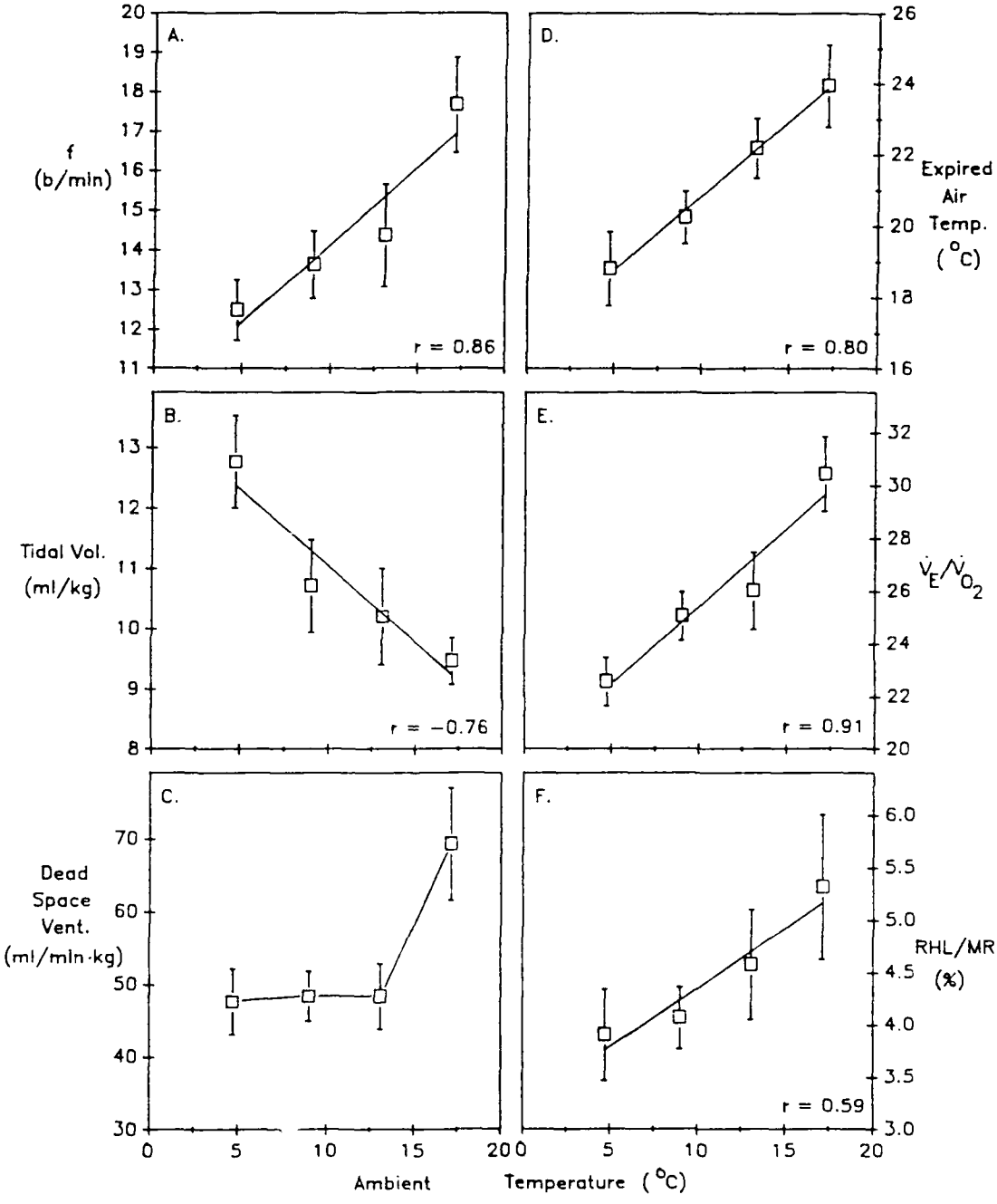


Figure 3.3. Effect of ambient temperature on respiratory pattern, expired air temperature, ventilatory equivalent for O_2, and respiratory heat loss/metabolic rate. Values are means ± SE; n=8. Regression equations for lines are as follows: y = 0.39x + 10.2 (A); y = -0.26x + 13.7 (B); y = 0.43x + 16.6 (D); y = 0.59x + 19.6 (E); y = 0.12x + 3.19 (F).

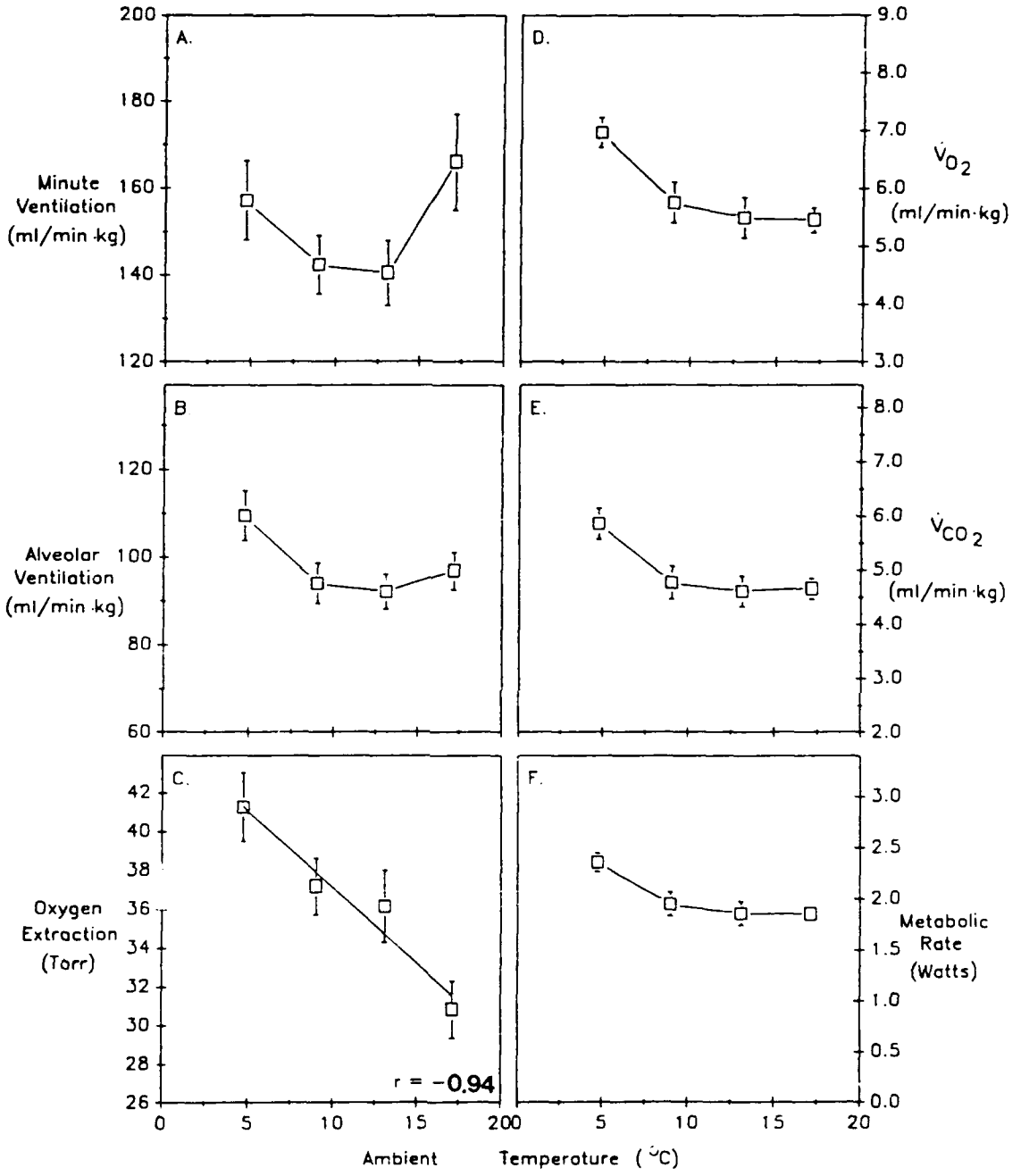


Figure 3.4. Effect of ambient temperature on total ventilation, alveolar ventilation, metabolic rate, and oxygen extraction. Values are means ± SE; n=8. Regression equation for panel C is: y = -0.76x + 45.0.

Table 3.3. Effects of ambient temperature on blood gas parameters.

	4-6°C	8-10°C	12-14°C	16-18°C
Pa_{O2} (Torr)*	66.2 ± 2.7	65.1 ± 2.0	67.0 ± 1.4	76.3 ± 2.2
Pa_{CO2} (Torr)‡	52.0 ± 0.8	50.9 ± 0.8	50.5 ± 0.7	47.2 ± 0.9
pH_a	7.37 ± 0.01	7.36 ± 0.01	7.37 ± 0.01	7.37 ± 0.01

Values are means ± SE; n = 9. Pa_{O2}, arterial oxygen tension; Pa_{CO2}, arterial carbon dioxide tension; pH_a, arterial pH.

* $P<0.05$ for linear correlation with ambient temperature.

‡ $P=0.059$ for linear correlation with ambient temperature.

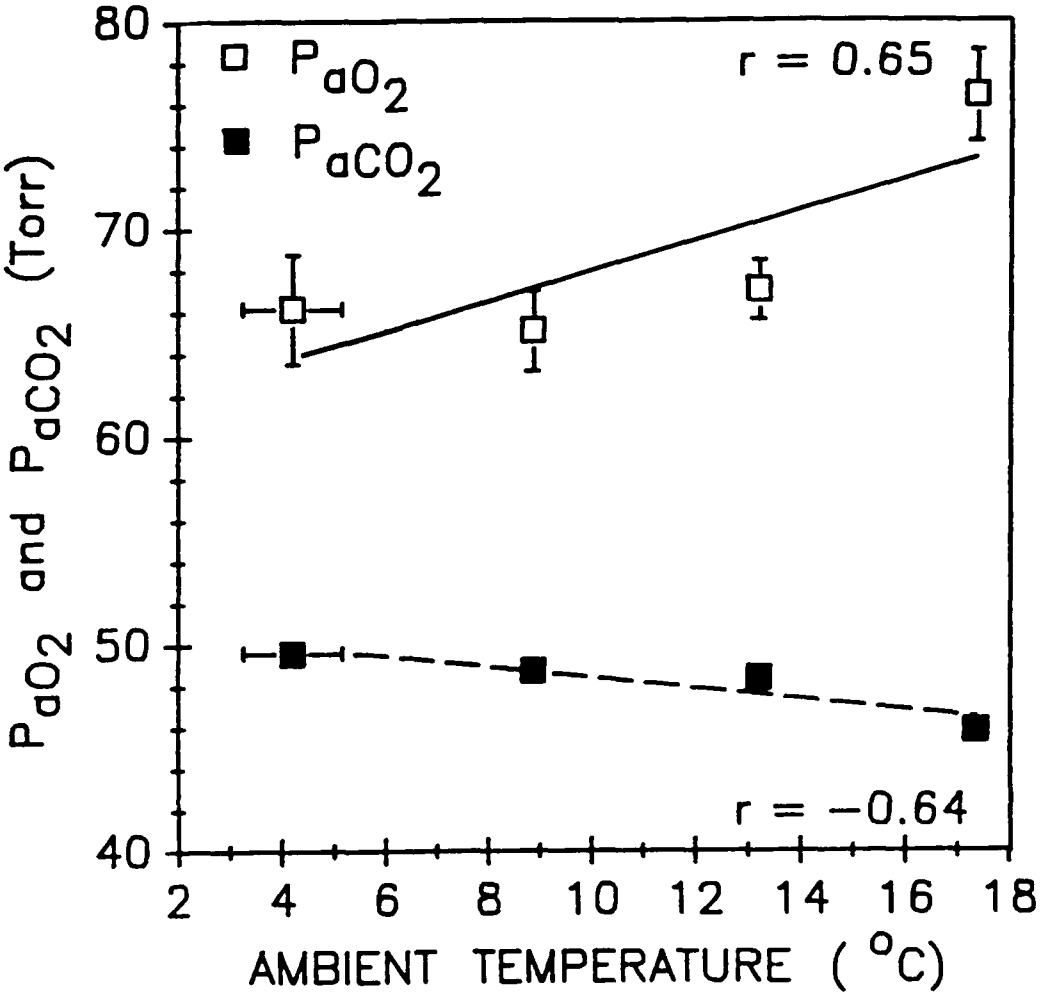


Figure 3.5. Effect of ambient temperature (T_a) on arterial P_{aO2} (solid line) and P_{aCC2} (dashed line). Values are means ± SE; n=9. Regression equations for lines are: $y = 0.74x + 60.6$ (T_a vs P_{aO2}; $P<0.05$) and $y = -0.33x + 51.8$ (T_a vs P_{aCO2}; $P=0.059$). Measurements were made at Ft. Collins, Colorado, altitude 1525 m.

Table 3.4. Effect of partitioning of body/airway temperature exposure on ventilatory parameters.

	Treatment				
	1	2	3	4	5
T_a(°C)	17.0 ± 0.2	17.4 ± 0.2	4.3 ± 0.2	4.6 ± 0.3	17.2 ± 0.2
T_I (°C)	17.0 ± 0.2	4.2 ± 0.3	16.0 ± 0.6	4.6 ± 0.3	4.4 ± 0.2
T_E (°C)	23.9 ± 1.0‡	19.7 ± 1.0*	21.5 ± 0.6‡	17.9 ± 0.8*	20.6 ± 1.1*
T_b (°C)	39.4 ± 0.1	39.6 ± 0.1	39.3 ± 0.2	39.3 ± 0.2	39.6 ± 0.1
T_{sk} (°C)	34.2 ± 0.3‡	34.3 ± 0.5‡	31.1 ± 0.4*	30.5 ± 0.7*	33.8 ± 0.5‡
f (b/min)	18.46 ± 0.92‡	15.26 ± 0.73‡*	13.87 ± 0.64*	12.38 ± 0.52*	18.22 ± 1.04‡
$\dot{V}_E$ (ml/min·kg)	173.2 ± 8.3	159.8 ± 5.7	167.7 ± 5.7	156.3 ± 6.3	157.9 ± 6.0
V_T (ml/kg)	9.43 ± 0.26‡	10.64 ± 0.44‡*	12.30 ± 0.57*	12.75 ± 0.51*	8.92 ± 0.50‡
V_D (ml/kg)	3.89 ± 0.17	3.88 ± 0.17	4.03 ± 0.19	3.63 ± 0.23	3.36 ± 0.25*
V_A (ml/kg)	5.54 ± 0.21‡	6.76 ± 0.34‡*	8.27 ± 0.41‡*	9.12 ± 0.44*	5.56 ± 0.32‡
$\dot{V}_D$ (ml/min·kg)	72.5 ± 5.5‡	58.8 ± 3.2*	55.0 ± 2.1*	45.2 ± 3.5*	59.2 ± 3.1*
$\dot{V}_A$ (ml/min·kg)	100.7 ± 3.5	101.0 ± 3.6	112.7 ± 4.1	111.1 ± 4.0	98.7 ± 4.9
$\dot{V}_{O2}$ (ml/min·kg)	5.68 ± 0.20‡	5.77 ± 0.16‡	7.29 ± 0.24*	7.06 ± 0.19*	5.62 ± 0.21‡
O_2 extr (torr)	30.6 ± 1.0‡	33.3 ± 0.86‡*	39.8 ± 0.83‡*	41.7 ± 1.23*	32.7 ± 0.78‡*

Values are means ± SE; n = 12. T_a, ambient air temperature; T_I, inspired air temperature; T_E, expired air temperature; T_b, body temperature; T_{sk}, skin temperature; f, respiratory frequency; $\dot{V}_E$, minute ventilation; V_T, tidal volume; V_D, dead space volume; V_A, alveolar volume; $\dot{V}_D$, dead space ventilation; $\dot{V}_A$, alveolar ventilation; $\dot{V}_{O2}$, oxygen consumption; O_2 extr, oxygen extraction.

* $P<0.05$ compared with Treatment #1.
‡ $P<0.05$ compared with Treatment #4.

Table 3.5. Effects of partitioning of body/airway temperature exposure on blood gas parameters.

Trt #	1	2	3	4	5
Pa_{O2} (Torr)	76.3±2.2‡	77.8±3.3‡	65.0±3.4*	66.2±2.6*	66.2±2.0*
Pa_{CO2} (Torr)	47.3±0.9	46.4±0.9	51.9±1.5	52.0±0.8	50.0±1.1
pH_a	7.37±0.004	7.38±0.004	7.37±0.008	7.37±0.006	7.38±0.002

Values are means ± SE; n = 9 for treatments #1-4, n = 8 for treatment #5. Pa_{O2}, arterial oxygen tension; Pa_{CO2}, arterial carbon dioxide tension; pH_a, arterial pH.

* $P<0.05$ compared with treatment #1.

‡ $P<0.05$ compared with treatment #4.

Table 3.6. Effect of partitioning of body/airway temperature exposure on hypothalamic temperature.

	Treatment			
	1	2	4	5
f (b/min)	19.90± 1.21‡	16.11± 0.84*	15.18± 0.75*	18.02± 0.59‡
$\dot{V}_E$ (ml/min•kg)	182.9 ± 13.9	173.3 ± 10.1	171.4 ± 9.1	179.1 ± 8.8
V_T (ml/kg)	9.20± 0.45	10 77± 0.33	11.38± 0.63	9.96± 0.47
T_b (°C)	39.1 ± 0.2	39.1 ± 0.2	39.0 ± 0.2	39.1 ± 0.2
T_h (°C)	38.8 ± 0.1	38.8 ± 0.1	38.8 ± 0.2	38.8 ± 0.2
T_{sk} (°C)	33.2 ± 0.2‡	33.1 ± 0.4‡	30.1 ± 0.6*	33.6 ± 0.6‡
T_E (°C)	19.3 ± 1.5	16.8 ± 1.6	15.0 ± 1.3	17.4 ± 1.9

Values are means ± SE; n = 5. f, respiratory frequency; $\dot{V}_E$, minute ventilation; V_T, tidal volume; T_b, body temperature; T_h, hypothalamic temperature; T_{sk}, skin temperature; T_E, expired air temperature.

* $P<0.05$ compared to Treatment #1.

‡ $P<0.05$ compared to Treatment #4.

Table 3.7. Effect of hypothalamic temperature on respiratory pattern.

	Control	Cold	Warm
f (b/min)	15.20 ± 0.94	15.41 ± 0.88	21.59 ± 2.99*
$\dot{V}_E$ (ml/min•kg)	163.3 ± 8.1	171.6 ± 12.0	204.2 ± 21.9
V_T (ml/kg)	10.80 ± 0.45	11.10 ± 0.26	9.61 ± 0.50*
T_b (°C)	38.8 ± 0.2	38.7 ± 0.2	39.0 ± 0.1
T_{sk} (°C)	34.2 ± 0.3	34.1 ± 0.3	34.5 ± 0.3
T_E (°C)	24.0 ± 1.0	24.1 ± 0.7	26.4 ± 0.7*

Values are means ± SE; n = 5 for control and cold thermode, n = 4 for warm thermode. f, respiratory frequency; $\dot{V}_E$, minute ventilation; V_T, tidal volume; T_b, body temperature; T_{sk}, skin temperature; T_E, expired air temperature. All measurements were made at T_a = 16-18°C.

* $P<0.05$ compared to control.

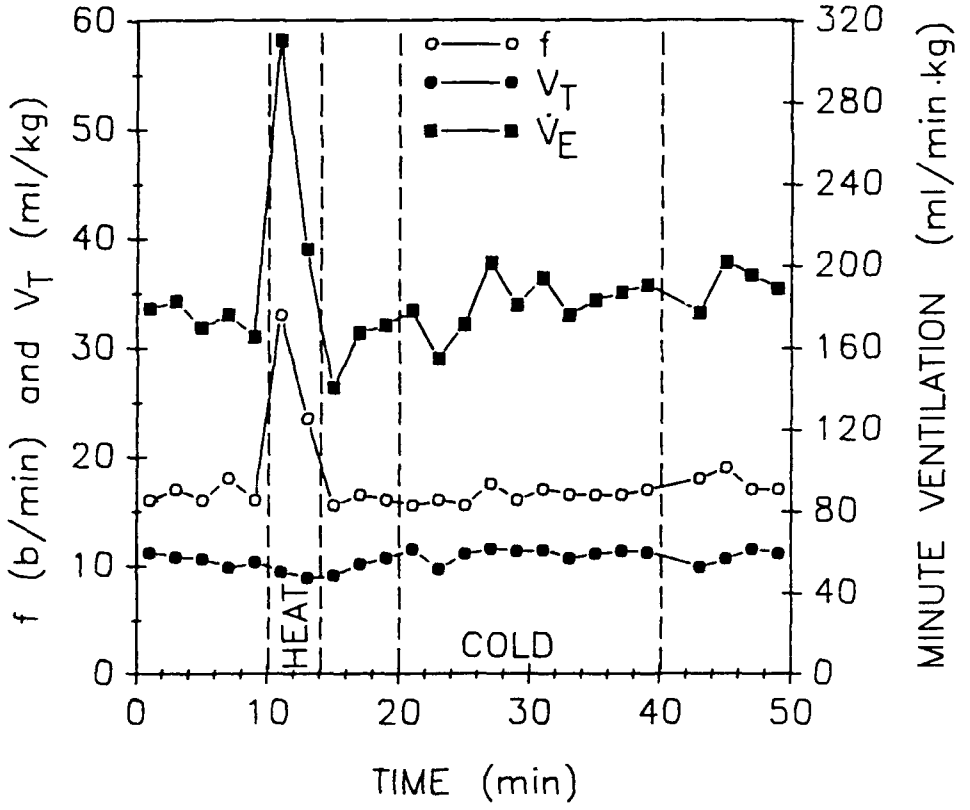


Figure 3.6. Effect of hypothalamic thermode heating and cooling on breathing pattern in calf #111.

Discussion

EVALUATION AND COMPARISON OF RESULTS: The cold-induced changes in ventilatory parameters observed in the present study can be compared with results reported by Busch *et al.* (3). Using cold temperatures of -2 to 1°C, they obtained a similar $\dot{V}_{O2}$ (6.82 ml/min-kg) to that of this study's coldest temperature (T_a = 4-6°C), but $\dot{V}_A$ was 9.7% greater, $\dot{V}_E$ was 12.7% greater, f was 3.9 breaths/min higher and V_T was 13% less. Also, at temperatures of 14-16°C, Busch *et al.* (3) observed $\dot{V}_{O2}$, $\dot{V}_A$, $\dot{V}_E$, and f values greater than comparable measurements in this study. These differences may be due to the way in which ventilation was measured. The apparatus used in this study was designed for minimal dead space (87 ml ATPS) and fit only over the nose of the calves, not the entire muzzle. This allowed the calf to move its mouth and tongue freely. Since calves are obligatory nose breathers, this design allowed estimation of total ventilation while providing the animal with more freedom and comfort. Busch (2) used a mask that fit over the entire muzzle, which is somewhat more confining. In addition, this mask had considerably more dead space (200 to 250 ml), which would vary depending upon the dimensions of the calf's muzzle. A greater amount of dead space would result in the need for increased ventilation and explain the differences observed between the two studies. Will *et al.* (24) obtained ventilatory measurements on calves at 15°C and found $\dot{V}_{O2}$ and $\dot{V}_E$ values nearly identical to the values observed in this study, with f values of 21 breaths/min and V_T of 7.8 ml/kg. After 48 hours of cold exposure (-5 to 0°C) they found no change in $\dot{V}_E$, in spite of a 57% increase in $\dot{V}_{O2}$. The ventilatory equivalent for O_2 data of the present study shows a similar relative hypoventilation with the cold-induced increase in metabolic demand.

Pa_{O2} decreased from 76.3 Torr at 16-18°C to 66.2 Torr at 4-6°C, while Pa_{CO2} increased from 47.2 to 52.0 Torr in this study, (Table 3.3). These can be compared to similar changes (Pa_{O2}, -8.8 Torr and Pa_{CO2}, +4.5 Torr) measured by Busch *et al.* (3), although the Pa_{O2} and Pa_{CO2} at the "control" temperature (14-16°C) were 66.8 and 40.0 Torr, respectively. The blood gas data from the present study are comparable to those of Will *et al.* (24). They measured a Pa_{O2} of 70 Torr and Pa_{CO2} of 42 Torr at 15°C, with Pa_{O2} decreasing by 7-10 Torr and Pa_{CO2} increasing by 4-6 Torr at T_a of -5 to 0°C. All of these changes in blood gases suggest a possible combination of alveolar hypoventilation and ventilation-perfusion imbalance in causing the slight arterial hypoxemia and hypercapnia, since Pa_{CO2} changes would be expected to mirror Pa_{O2} more closely in the case of hypoventilation alone (Figure 3.5). It should be noted that the slight hypoxemia induced by cold exposure in the previous studies (3,24) was sufficient to increase pulmonary artery pressure.

In this study, RHL per liter of expired air, measured at a T_I of 16 to 18°C, was 35.2 J/l, estimated with a T_E of 23.9°C. RHL per liter has been estimated for cattle by Varene and Kays (21) using data from Langman *et al.* (15). They predicted a RHL of 63 J/l at T_I = 21.1°C and 68% inspired relative humidity. Although this estimate is somewhat higher than that measured in this study, it was a mathematical prediction made for larger cattle (200 kg) at 21.1°C and was based upon a considerably higher T_E (30.5°C). Expired air temperatures measured in this study are comparable to T_Es determined at similar inspired air temperatures for reindeer (1,16) and the elephant seal (8).

Equation 1 predicts a T_E of 21.2°C for the measured f and $\dot{V}_E$ values of Treatment #1 of experiment #2 (T_I and T_a both = 17.0°C). This prediction

of T_E in a cold environment, if hypoventilation did not occur, is 3.3°C greater than that actually measured during cold-exposure (Treatment #4). Thus, it appears that hypoventilation at T_a = 4.6°C (T_E = 17.9°C) enables a calf to expire air at cooler temperatures. The difference in T_E between Treatment #1 and Treatment #4 was 6.0°C, 3.3°C of which was due to the respiratory pattern change and the remainder (2.7°C) due to the lower T_I of Treatment #4.

THERMOREGULATORY IMPLICATIONS OF HYPOVENTILATION: The term 'thermoneutral' is sometimes used to describe ventilatory parameters of animals at basal metabolic rate. While this may be appropriate for humans and some animals, it is probably not an appropriate description (with the exception of $\dot{V}_{O2}$) of an animal which uses panting to regulate body temperature. Obviously, the ventilatory pattern during panting is different from the non-panting state, yet both exist within the zone of thermoneutrality. However, 'non-panting' should not imply constancy of f and V_T within the thermoneutral zone. The data presented in this study indicate that no portion of the thermoneutral zone of calves is characterized by stable f or V_T. In other words, the change in respiratory pattern observed with heat-stress (panting) is part of a continuum throughout the thermoneutral zone. With heat stress, f increases and V_T decreases resulting in an increase in $\dot{V}_D$ but no change in $\dot{V}_A$ (6). In the present study, cold-exposure resulted in decreased f and increased V_T, subsequently $\dot{V}_D$ decreased while $\dot{V}_A$ remained constant (Figure 3.3 and 3.4). Since panting is an effective means of maintaining thermoneutrality by increasing RHL, it seems logical that the changes in respiratory pattern observed with decreasing T_a might serve the purpose of decreasing RHL.

Kaminski *et al.* (14) had previously speculated that cold-induced changes in breathing which affect respiratory heat loss in ponies contribute to maintenance of thermal balance.

Although one might expect RHL to increase with decreasing T_a, a significant decrease in RHL was not observed. The major determinants of RHL are $\dot{V}_E$ and the T_E - T_I gradient. $\dot{V}_E$ did not increase with decreasing T_a, even in the face of increased MR. As a result the ventilatory equivalent for O_2 is positively correlated with T_a. This could occur if O_2 extraction increased as T_a decreased, resulting from the ventilatory pattern change (*i.e.* increased V_T with decreased f and $\dot{V}_D$). Similar results have been observed in other panting animals, such as pigs (9) and sheep (13). Taylor (19) also observed a similar relationship between ventilatory pattern and O_2 extraction in the eland, however f was positively related to rectal temperature. A relationship between T_b and either f, V_T or O_2 extraction was not observed in the present study. Therefore, it is not essential for T_b to decrease in order for a ventilatory change to occur at lower T_a. Although calves in this study increased O_2 extraction at colder temperatures, some degree of hypoxemia was tolerated as T_a decreased (Figure 3.5).

The demonstrated advantage of the ventilatory pattern change observed in the eland was a decrease in respiratory water loss during cool nights (19); however, this indicates a decrease in evaporative respiratory heat loss, which is also a potential benefit of decreased f with increased V_T. While the hot, arid environment of the eland probably makes water conservation an essential selective factor, energy conservation is also a potential benefit of decreased f with increased V_T.

Even though CHL increased with decreasing T_a, the increase in CHL would have been even greater had changes in ventilatory pattern not occurred. The derived Equation 1 demonstrates the positive relationship between f and T_E, presumably by allowing better heat exchange upon exhalation. Had ventilation not changed from the 16-18°C values (f = 17.7, $\dot{V}_E$ = 165.9), the T_E predicted by Equation 1 at 4-6°C would be 20.8°C, or 2.0°C higher than measured. As a result, the heat loss per liter of expired air (J/l) did not significantly increase as T_a decreased. The relative contribution of RHL to total MR is expressed as a percentage in Table 3.2 and shows a positive relationship with T_a. During expiration the recovery of heat added to the inspired air was 74% at the coldest temperature. This is comparable to 75% recovery determined by Langman (16) in cold-exposed reindeer.

Treatments #2 and #4 of experiment #2 also allow for comparison of RHL at similar T_I, but different respiratory patterns. The f when only the airways were cold exposed (Treatment #2) is almost 3 breaths/minute greater than Treatment #4, and V_T is 2.11 ml/kg less for Treatment #2 compared to Treatment #4. The calculated RHL for Treatments #2 and #4 are 0.103 and 0.085 W/kg, respectively, representing a 21% increase in RHL as a result of the higher f and lower V_T. In a similar comparison of airway cold exposure plus topical lidocaine application (Treatment #5) with Treatment #4, a 28% increase in RHL results from an increase in f of 5.84 breaths/minute and V_T decrease of 3.83 ml/kg. It should be noted that $\dot{V}_E$ was not significantly different between Treatments 2, 4, and 5.

Equation 2 predicts a RHL of 0.134 W/kg for the f and $\dot{V}_E$ measured in Treatment #1 (T_a and T_I = 17.0°C). Therefore, hypoventilation at 4-6°C (Treatment #4) provides a 36% reduction in RHL from that predicted for

ventilatory values at 17.0°C. This reduction in RHL represents an adaptive benefit provided by cold-induced changes in breathing pattern.

CONTROL OF BREATHING PATTERN: To further understand the adaptive significance of cold-induced hypoventilation, it would be enlightening to know how cold stimulates the ventilatory response. Should cold stimulation of the airway alone induce a change in ventilation, one might assume that the benefit lies either in enhanced conditioning of inspired air, decreased RHL (thermoregulation), or maintenance of hypothalamic temperature. A response elicited following deactivation of airway cold receptors would support the latter, as would a response elicited by cooling of the hypothalamus. Should chilling the skin alone induce a ventilatory response, this would most likely indicate a thermoregulatory mechanism.

The differences between Treatments #2 and #1 demonstrate the effect of cooling the airway, while the body is kept at a warmer temperature. The pattern of ventilation (f and V_T) changed significantly, while total ventilation did not change. However, the change was not of the same magnitude as was achieved by cooling both the skin and the upper airways, since treatment #4 elicited a lower f and higher V_T than Treatment #2.

Experiments involving localized cooling of the skin and airways would suggest that cold receptors at both sites contribute to the overall change in breathing pattern associated with cold exposure. Cooling of the skin, but not the airways (Treatment #3), resulted in a greater change in ventilatory pattern than did cooling of the airways alone (Treatment #2). This treatment resulted in a f and V_T which were not significantly different from complete cold exposure (Treatment #4). Therefore, while

cooling of the airways can contribute to the ventilatory pattern change observed with cold exposure, it appears that cutaneous cold stimulation is the more important input resulting in altered respiratory frequency and tidal volume. This is reflected in the blood gas data for Treatments 1-4, (Table 3.5). With cold exposure of the skin (Treatment #3), Pa_{O2} was 11.3 Torr lower than 'thermoneutral' conditions (Treatment #1). However, Pa_{O2} was not affected by breathing cold air. This appears to be due to the combined effects of increased oxygen consumption without a significant increase in total ventilation. Hypoventilation during Treatments #3 and #4 is also evident by comparison of the ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}_{O2}$). The ventilatory equivalent for Treatments #1 and #2 is 30.5 and 27.7, respectively, while Treatments #3 and #4 have ventilatory equivalents of 23.0 and 22.1, respectively.

The relatively low Pa_{O2} value obtained for Treatment #5, where the topical application of lidocaine resulted in a 'thermoneutral' respiratory pattern in spite of breathing cold air, cannot be explained by the ventilatory equivalent for O_2, 28.1. A possible explanation of this result for Treatment #5 may be ventilation-perfusion imbalance as a result of uneven bronchoconstriction induced by aspirated lidocaine.

A ventilatory response controlled by both airway and skin cold stimulation would allow for fine tuning to the environment. For example, a cold, wet climate would be expected to stimulate the skin receptors to a greater degree due to decreased insulating ability of the wet fur. However, the airways would be stimulated to a lesser degree in wet weather, since less evaporative heat loss from the mucosal lining would occur when inspired air is of higher humidity. Conversely, should the

climate be cold, but arid, there would be less stimulation to the skin and an increased heat loss from the upper airways.

Although cold exposure of the airways alone resulted in an altered breathing pattern, this does not resolve the question of whether the response is elicited by thermoreceptors in the airway or by cooling of blood perfusing the hypothalamus. In an attempt to resolve this question, Treatment #2 was repeated, but with 3% lidocaine applied topically to the nasal mucosa (Treatment #5). This resulted in a return of the ventilatory pattern to that of Treatment #1. Although not shown in Table 3.3, a two-way ANOVA comparison of Treatment #2 and #5 revealed a significant difference in f and V_T, but no difference in $\dot{V}_E$. These results indicate a role for airway thermoreceptors in regulating ventilatory pattern. In addition, one might predict that such a change should not occur as a result of lidocaine application if T_h is the controlling factor in governing ventilatory pattern during cold exposure. Tucker *et al.* (20) did not observe any difference in T_h between 'thermoneutral' (18-20°C) and cold exposed (3-6°C) calves, even though nasal mucosal temperature decreased by 23.2%. Similarly, measurement of T_h in this study (Table 3.6) showed no difference in T_h or the T_b - T_h difference between Treatments #1 and #4. T_h was also unaltered by Treatment #5, which one might expect to decrease T_h, since the animal is inspiring cold air but at a respiratory pattern typical of a warm environment. Therefore, the manipulations of this study were unable to demonstrate an effect of environmental temperature or respiratory pattern on T_h. This is further supported by T_h data obtained during increased inspired CO_2 concentration, which showed no correlation between T_h or T_b - T_h difference and respiratory pattern (f, $\dot{V}_E$, V_T).

To further investigate this question, the hypothalamic probe was used as a thermode in experiment #4 to cool the hypothalamus (Table 3.7; Figure 3.6). The accurate placement of the probe in the preoptic region of the hypothalamus was verified in all calves by dissection following the experiments. Heating of the probe produced a marked increased in f and decreased V_T (Table 3.7). Since the thermode could be effectively heated at the tip using this apparatus, and during cooling, fragments of frozen ethanol could be observed entering the probe circulation, it seems certain that cooling of the hypothalamus occurred. $\dot{V}_E$, f, and V_T were not influenced by cooling of the thermode, therefore it is concluded that hypoventilation does not serve a protective function to maintain brain temperature as panting does during heat stress.

Protection of hypothalamic temperature would most likely be more crucial at high ambient temperatures, where protein denaturation is a potential problem. Thus, panting provides an important adaptive benefit in allowing T_b to rise above T_h. However, temperatures below that of normal T_b do not pose the same risk to the hypothalamus. While there is an optimal operating temperature for the brain, slightly cooler temperatures do not present the same danger of tissue damage as do slightly higher than normal temperatures. It seems more likely that hypoventilation provides an adaptive benefit in energy conservation by decreasing respiratory heat loss.

Whatever the benefit of cold induced hypoventilation (altered breathing pattern), it presumably outweighs the disadvantage of the slight hypoxia that results. Just as hypoxic pulmonary vasoconstriction is an adaptive local mechanism which becomes deleterious in cattle in an unusual environment (high altitude), cold-induced hypoventilation may be an

adaptive mechanism which becomes deleterious in unusual environments. For example, cold exposure in combination with altitude can increase susceptibility to high mountain disease in cattle (12,23). Also, cold-induced change in breathing pattern may increase susceptibility to pneumonia in a feed-lot setting by increasing pathogenic particle deposition in the lung (17). An increase in the deposition of particles in the respiratory tract occurs with decreased respiratory rate (7,18) since residence time in the lung affects the amount of deposition of particles by gravitational settling. The normal adaptive strategy of hypoventilation during cold exposure may therefore become detrimental under unnatural conditions, such as the feedlot. Franzen (5) found the incidence of pneumonia in feedlot cattle to be correlated with the concentration of airborne particles. Knowledge of the effects of cold exposure upon particle deposition and clearance may further clarify the significance of the higher incidence of respiratory disease incurred following sudden falls in temperature during the fall and winter months (10,11,12,22).

Conclusions

Cold-induced hypoventilation in calves is the result of a mechanism by which respiratory heat loss is minimized. While cold exposure (4-6°C) did not result in a significant change in total ventilation, respiratory frequency was reduced, tidal volume increased, and dead space ventilation decreased. These cold-induced respiratory pattern changes are opposite to those observed during heat-induced panting. The respiratory pattern change prevents an increase in respiratory heat loss with decreasing environmental temperature and results in a significant decrease in the

proportion of metabolic rate devoted to respiratory heat loss. When ventilation was increased by increasing inspired CO_2 at T_a = 4-6°C, a positive relationship existed between respiratory frequency and expired air temperature. Therefore, calves are capable of conserving respiratory heat during cold exposure by decreasing expired air temperature and dead space ventilation, while increasing oxygen extraction and tolerating slight hypoxemia.

Cutaneous thermoreceptors are primarily responsible for the ventilatory changes resulting from cold exposure, although airway thermoreceptors probably also have a regulatory role. Decreased respiratory frequency and increased tidal volume stimulated by inspiration of cold air was prevented by topical application of lidocaine to the nasal mucosa, indicating the ventilatory response is due to airway thermoreceptors rather than hypothalamic cooling. Hypothalamic temperature did not decrease at the coldest temperatures utilized for this study, and increasing ventilation by increasing inspired CO_2 at T_a = 4-6°C did not affect hypothalamic temperature. Cooling of the pre-optic area of the hypothalamus did not alter total ventilation or ventilatory pattern, therefore, unlike panting, hypoventilation does not appear to be a mechanism by which the brain is protected from over-cooling.

REFERENCES - CHAPTER III

1. Blix, A.S. and K.J. Helge. Aspects of nasal heat exchange in resting reindeer. *J. Physiol.* 340:445-454, 1983.

2. Busch, M.A. Mechanisms of cold-induced pulmonary hypertension in cattle. Ph.D. Diss., Colo. State Univ., Ft. Collins, 1981.

3. Busch, M.A., A. Tucker and D. Robertshaw. Interaction between cold and altitude exposure on pulmonary circulation of cattle. *J. Appl. Physiol.* 58:948-953, 1985.

4. Dmi'el, R. and D. Robertshaw. The control of panting and sweating in the black Bedouin goat: a comparison of two modes of imposing a heat load. *Physiol. Zool.* 56:404-411, 1983.

5. Franzen, D.K. Airborne particle concentration associated with pneumonia in feedlot cattle. Master's Thesis, Colo. State Univ., Ft. Collins, 1984.

6. Hales, J.R.S. The partition of respiratory ventilation of the panting ox. *J. Physiol.* 188:P45-P46, 1967.

7. Hatch, T. Distribution and deposition of inhaled particles in the respiratory tract. *Bact. Rev.* 25:237-240, 1961.

8. Huntley, A.C., D.P. Costa and R.D. Rubin. The contribution of nasal countercurrent heat exchange to water balance in the northern elephant seal, *Mirounga angustirostris*. *J. Exper. Biol.* 113:447-454, 1984.

9. Ingram, D.L. and K.F. Legge. The effect of environmental temperature on respiratory ventilation in the pig. *Respir. Physiol.* 8:1-12, 1968.

10. Jensen, R., R.E. Pierson, P.M. Braddy, D.A. Saari, L.H. Lauerman, J.J. England, D.P. Horton, and A.E. McChesney. Diseases of yearling feedlot cattle in Colorado. *JAVMA* 169:497-499, 1976.

11. Jensen, R., R.E. Pierson, P.M. Braddy, D.A. Saari, L.H. Lauerman, J.J. England, H. Keyvanfar, J.R. Collier, D.P. Horton, A.E. McChesney, A. Benitez and R.M. Christie. Shipping fever pneumonia in yearling feedlot cattle. *JAVMA* 169:500-506, 1976.

12. Jensen, R. and D.R. Mackey. Brisket disease. In: *Diseases of Feedlot Cattle* (3rd ed.), Lea and Febiger, Philadelphia, PA, 1979, p.257-261.

13. Joyce, J.P. and K.L. Blanter. Respiration in sheep in cold environments. *Res. Vet. Sci.* 5:506-516, 1964.

14. Kaminski, R.P., H.V. Forster, G.E. Bisgard, L.G. Pan and S.M. Dorsey. Effect of altered air temperature on breathing in ponies. *J. Appl. Physiol.* 58:1585-1591, 1985.

15. Langman, V.A., G.M.O. Maloiy, K. Schmidt-Nielsen and R.C. Schroter. Nasal heat exchange in the giraffe and other large mammals. *Respir. Physiol.* 37:325-333, 1979.

16. Langman, V.A. Nasal heat exchange in a northern ungulate, the reindeer (*Rangifer tarandus*). *Respir. Physiol.* 59:279-287, 1985.

17. Slocombe, R.F., F.J. Derksen and N.E. Robinson. Interactions of cold stress and *Pasteurella haemolytica* in the pathogenesis of pneumonic pasteurellosis in calves: Changes in pulmonary function. *Am. J. Vet. Res.* 45:1764-1770, 1984.

18. Stuart, B.O. Deposition and clearance of inhaled particles. *Envir. Health Pers.* 55:369-390, 1984.

19. Taylor, C.R. Metabolism, respiratory changes, and water balance of an antelope, the eland. *Am. J. Physiol.* 217:317-320, 1969.

20. Tucker, A., D. Robertshaw, C.B. Allen and M.L. Wright. Nasal airflow and mucosal blood flow in cold-exposed cranially-sympathectomized calves. *Proc. IUPS* 16:473, 1986. (Abst.)

21. Varene, P. and C. Kays. A graphic analysis of respiratory heat exchange. *J. Appl. Physiol.* 63:1374-1380, 1987.

22. Webster, A.J.F. Weather and infectious disease in cattle. *Vet. Rec.* 108:183-187, 1981.

23. Will, D.H. and A.F. Alexander. High mountain (brisket) disease. In: *Bovine Medicine and Surgery*, Am. Vet. Publ., Santa Barbara, CA, 1970, p. 412-430.

24. Will, D.H., I.F. McMurtry, J.T. Reeves and R.F. Grover. Cold-induced pulmonary hypertension in cattle. *J. Appl. Physiol.* 45:469-473, 1978.

CHAPTER IV

EFFECTS OF COLD AND BREATHING PATTERN ON PARTICLE DEPOSITION

Introduction

Although a cold-induced change in breathing pattern appears to provide an adaptive benefit by reducing respiratory heat loss, there may be a disadvantage to slow, deep breathing. Since decreased respiratory rate increases the residence time of air in the lung, particle deposition in the respiratory tract by gravitational sedimentation is greater (9,19). This possibility of increased particle deposition may be detrimental in environments where airborne dust and pathogens are concentrated, such as the feedlot. To explain the higher incidence of bovine respiratory disease following sudden falls in temperature, the following hypothesis is proposed. **During acute cold stress in calves, cold-induced changes in breathing pattern will increase deposition of airborne particles within the lung**. To test this hypothesis, two objectives were addressed. First, this study determined if cold exposure and associated ventilatory changes enhance deposition of *Pasteurella haemolytica* deep within the lung. Second, isolated, ventilated sheep lungs were used to determine if the amount of particle deposition is altered by changing respiratory rate and volume of each breath.

Materials and Methods

BACTERIAL DEPOSITION DURING COLD EXPOSURE: Ten male Holstein calves between the ages of 1 and 3 months and weighing 45-90 kg were used to investigate the effects of cold exposure upon deposition of pathogenic organisms in the lung (experiment #5). Calves were purchased, housed, and fed as described in Chapter III. Five calves were exposed to 16-18°C ambient temperature and 5 were exposed to 4-6°C. Calves exposed to the colder T_a were moved to the cold room 24 hr prior to the experiment. Calves exposed to 16-18°C were placed in the insulated box (Figure 3.2) immediately prior to the experimental period. The measurements mentioned in Chapter III for experiments #1 and #2 were obtained over a 50-60 min period for each calf during this experiment.

Calves were administered a solution containing 3.45×10^9 colony forming units (CFU) of *Pasteurella haemolytica* per ml normal saline. The bacteria were treated with bis-benzamide (Hoechst #33258) to induce fluorescence. Two 12 ml syringes were used simultaneously to deliver bacteria via cannulae (PE-190, inner diameter 1.19 mm) placed in each nostril. An infusion pump (Harvard Apparatus, Model 975) was utilized to deliver the bacterial solution to the nasal cavity of the calf at a rate of 0.049 ml/min/syringe. The tips of the nasal cannulae were located approximately 15 cm beyond the nares to instill the bacterial solution in the nasal cavities. Instillation occurred over a period of 102 min, to deliver a total of 10.0 ml of the bacterial solution, or 3.45×10^{10} CFU. Preliminary experiments showed that this rate of application was slow enough to prevent the solution from running out of the nostrils.

Following the nasal instillation period, the calves were weighed and then killed with a captive bolt gun. The lung was immediately excised

and weighed. Two lung tissue samples, approximately 1 cm^3 each, were cut from the lung at specific sites and frozen. One sample was placed in a vial and frozen, while the other sample was frozen in O.C.T. Compound cryofixative (Tissue-Tek, Miles, Inc.) for cryosectioning. The period of time from completion of instillation until freezing of lung samples was approximately 40 min. The sites of sampling were right apical lobe dorsal (RAD) and ventral (RAV), right cardiac lobe dorsal (RCD) and ventral (RCV), right diaphragmatic lobe dorsal (RDD) and ventral (RDV), in addition to similar sites from the left lobes (i.e. LAD, LAV, LCD, LCV, LDD, and LDV). A site was also sampled from the mediastinal lobe (MED). For the first three calves studied, tissue samples were obtained aseptically from each of the seven lobes specified (at mid-lobe), homogenized with normal saline, and placed on both blood agar and chocolate agar (except for the first calf, #728) media. The culture plates were then placed in an incubator (39.5°C). Bacteria were collected from colonies on these culture dishes two days after inoculation. The positive identification of *P. haemolytica* was made microscopically.

One of the lung samples from each site was evaluated for bis-benzamide using fluorescent spectrophotometry. A technique was adapted from Brunk *et al.* (3) and Labarca and Paigen (14) in which bis-benzamide was used to complex with DNA and assay for nanogram quantities of DNA in cellular homogenates. Tissue samples were weighed (wet weight) and then buffer was added (30ml/g of lung tissue). The buffer consisted of 2M NaCl, 0.05M Na_2HPO_4, and 0.002M EDTA at 7.4 pH (14). The tissue was then homogenized using a polytron blender (Brinkman Instruments), then centrifuged (2045 relative centrifugal force) for 2 min to separate debris. The fluid fraction was removed from the centrifuge tube and

placed in a cuvette for spectrophotometric analysis. A fluorescent spectrophotometer (MFP-2A, Hitachi Perkin-Elmer) was used with the excitation wavelength set at 356 nm and emission read at 475 nm wavelength. The filter was set at 430 nm and a 10 nm slit was used. The emission wavelength was selected based on preliminary scanning emission readings on samples from normal lung to which bis-benzamide-labeled *P. haemolytica* were added. The peak emission from these samples was at 475 nm. Samples were run at room temperature (21°C).

Standards were made from normal lung homogenate to which known concentrations of *P. haemolytica* labeled with bis-benzamide were added. The standards were run on the spectrophotometer either immediately before or after running all 13 samples from a particular calf lung. A linear regression equation was calculated using the standard concentrations, and the bacterial concentration per gm of tissue (wet weight) for the lung samples was determined using the standard regression.

Precautions were taken to rinse glassware and cuvette with deionized water and no soap. The samples were kept in complete darkness until ready to be analyzed. The percent dry weight of each lung was also determined from lung samples to evaluate any bias which could result from using wet lung weights when preparing samples.

Lung samples frozen in O.C.T. were sectioned 15 μm thick using a cryostat (Model CTI, International Equipment Company) and placed upon separate microscope slides treated with Histo-stik. Slides were stored in a freezer and kept in complete darkness until they were used for microscopic data collection. Slides were labeled on one end with the calf number and the lung sample site. These labels were then covered with a piece of tape. A complete set of 13 slides for each of two calves, one

4-6°C exposure and one 16-18°C exposure, were combined and randomized. One or two tissue slides from an untreated calf lung were also included in the slide tray. The slides, totaling 27 or 28, were then numbered sequentially. This coding technique was utilized to eliminate observer bias while evaluating the slides for fluorescent bacteria. Following evaluation of the entire set of slides, the tape was removed and calf number and sample site were recorded.

Evaluation of samples for fluorescent bacteria was made using a Laborlux 12 microscope (Leitz), with an H2 filter block, which excites in the range of 420-490 nm wavelength light and suppresses above 515 nm. A 100 X oil immersion lens (Leitz Wetzlar, Germany, 100/1.25 OEL) provided a field of view 140 μm in diameter. Therefore, a count was made for a cylinder with a total volume of 2.309×10^{-7} cm^3 (radius = 70 μm; thickness = 15 μm). Non-drying immersion oil was used (Cargille, Type B). A drop of FA mounting media (9 parts glycerol with 1 part FAWB) was placed on a tissue section before covering with a glass cover slip.

A field of view of the tissue section was randomly chosen. The total number of fluorescent bacteria within the field of view was recorded. Focusing up and down through the tissue section was necessary to make an accurate count. Twenty such field counts were made for each lung sample by moving the slide in a step-wise pattern. To avoid loss of fluorescence over time, only 10 field counts were made consecutively, then the slide was replaced in total darkness for 20-30 min after which another 10 field counts were made. The average bacterial count from the 20 field counts was used as an estimation of the bacterial concentration at that lung sample site. Since there was some background fluorescence in the lung tissue, the data from untreated calf lung samples were averaged and

used as a baseline. The baseline count was subtracted from each sample mean. Data were therefore in the form of bacteria/cm^3 of lung tissue. The weight of 1 cm^3 of collapsed wet lung was determined to be 0.727 $\pm$ 0.026 gm (n = 6). All data were therefore converted to bacteria/gm of lung to allow comparison with data derived by spectrophotometric analysis. Data were analyzed using a split-plot ANOVA and an SNK test to compare apical, cardiac, and diaphragmatic lobes. The mediastinal lobe was compared with other sample sites and with the three lobe groupings using a contrast analysis.

AIRBORNE PARTICLE DEPOSITION IN ISOLATED LUNG: To determine if the pattern of breathing influences the deposition of airborne particles in the lung (experiment #6), freshly excised sheep lungs were placed inside a breathing apparatus (artificial lung) similar to that used by Esch *et al.* (5) (Figure 4.1). Fourteen western range ewes, 4 to 5 years old and weighing 45 to 65 kg, were used for this experiment. Sheep were brought from an outdoor holding pen on the morning on which the lungs were to be excised. Sheep were anesthetized with pentobarbital and exsanguinated. Lung and heart were excised *en bloc* and the trachea cut just below the larynx. Lungs were inflated to examine for leaks and discarded if a leak was detected or if there were any gross pathological lesions.

Following gross inspection and weighing of the lung, the trachea was cannulated and the cannula attached to the bulkhead connector on one end of the artificial thorax. The lung was suspended from a horizontal rod running through the aorta. Thus the lung was in a horizontal position, with the heart intact, to duplicate the normal *in vivo* lung position as closely as possible.

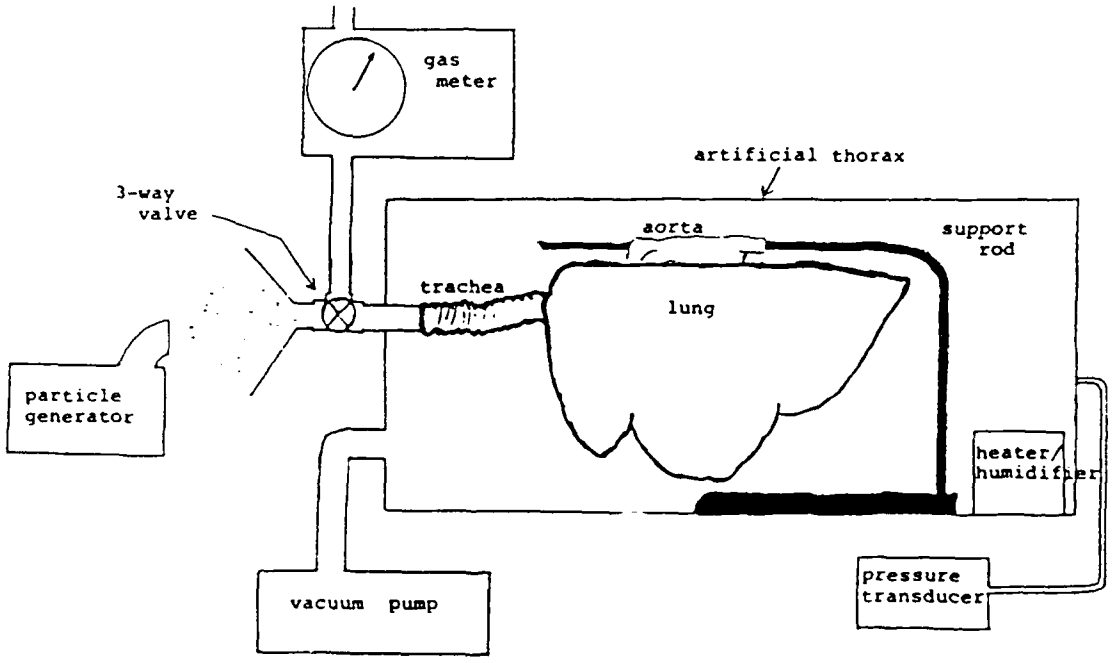


Figure 4.1. Isolated sheep lung ventilation apparatus.

The lung was ventilated by applying rhythmic vacuum oscillations to the artificial thorax, using a cyclic ventilator (Model 115-6, Monaghan, Denver). Exhalation was achieved by the elastic recoil of the lung as vacuum was released. Total ventilation was adjusted to a predetermined volume, prior to any experimental manipulations, using a dry gas meter (American Meter Company) and a one-way valve connected to the tracheal cannula. Humidity inside the artificial thorax was maintained at 100% and ambient temperature maintained between 36 and 38°C. Air drawn into the tracheal cannula was at room temperature (21°C). Once the vacuum was adjusted to produce the desired respiratory rate and tidal volume, the gas meter was removed from the tracheal cannula. The tracheal cannula was then exposed continuously for 40 min to a $BaSO_4$ suspension aerosol produced by a DeVilbis humidifier. The geometric mean diameter of the airborne particles was 0.545 μm (as measured by a laser aerosol spectrometer, Appendix L), which corresponds to a mass median aerodynamic diameter of 2.33 μm.

Following the 40 min exposure to the aerosol, the lungs were removed from the artificial thorax, inflated to 7 cm H_2O pressure, and radiographed (1.6 mA, 42 kVp). Seven of the lungs were ventilated at 12 b/min and tidal volume between 9.92 and 13.18 ml/kg. Two of these 7 lungs were exposed to H_2O aerosol containing no $BaSO_4$ and served as controls for evaluating radiographs. The remaining 7 lungs were ventilated at 20 b/min and tidal volume between 6.86 and 8.22 ml/kg. Again, 2 of these 7 lungs were exposed to H_2O aerosol containing no $BaSO_4$ and also served as the controls for evaluating radiographs. Thus, there were five lungs representing each respiratory pattern exposed to the $BaSO_4$ aerosol and four lungs which served as controls which were not exposed to the $BaSO_4$ (two for

each respiratory pattern). Vacuum applied to the artificial thorax at the 12 b/min rate was approximately 20 cm H_2O pressure and at 20 b/min, the vacuum was approximately 15 cm H_2O.

Total ventilation (BTPS) was calculated from the gas meter reading, assuming a body temperature of 39.5°C. Body weight was not determined, but for the purpose of calculating a $\dot{V}_E$ estimate, body weight was assumed to be the combined lung and heart weight multiplied by a factor of 50 (1). Tidal volume was calculated as $\dot{V}_E$ divided by f.

Radiographs were evaluated by means of a fabricated densitometer (Figure 4.2). Specific sites of each lung lobe on the radiograph were used for comparison. The sample sites were right apical lobe (2 sites), right cardiac lobe (2 sites), right diaphragmatic lobe (4 sites), left cardiac lobe (2 sites), and left diaphragmatic lobe (4 sites). A separate radiograph was made of the left apical lobe, since the heart obscured this lobe on the whole lung radiograph. This radiograph was obtained at a magnification of 3 x (2.5 mA and 46 kVp). Three sample sites were used from each left apical lobe radiograph. Small sections of film were cut from the radiograph at these predetermined sites and then inserted into 35 mm slide frames (window dimension = 23 x 35 mm). These slides were then each placed in the densitometer to obtain a reading which indicated the relative opacity of the film (arbitrary units). A low reading therefore corresponded to a lighter film section which resulted from the presence of more $BaSO_4$ in the lung. The readings for the four control lungs were averaged for each lobe and used as a baseline. Readings for the $BaSO_4$-exposed lungs were then subtracted from baseline readings to obtain a number which was directly related to the amount of $BaSO_4$ present. Data for each lobe were analyzed using one-way ANOVA with sub-sampling. Total lung data were analyzed using repeated measures ANOVA.

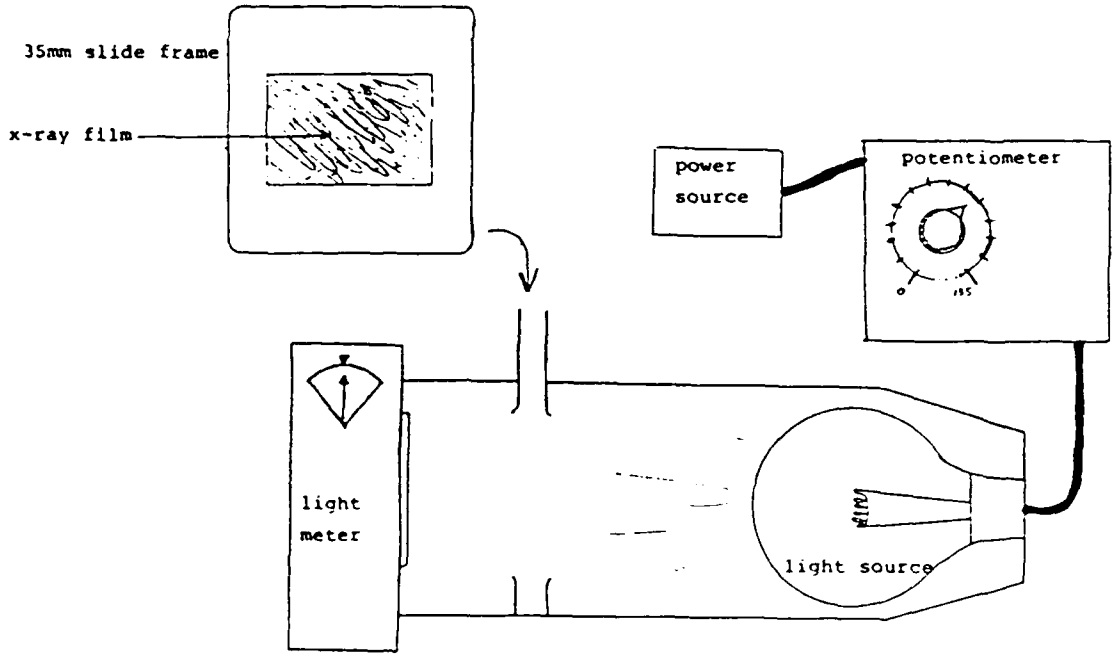


Figure 4.2. Fabricated densitometer for evaluating relative opacity of x-ray film.

Lung tissue samples were taken from the left apical lobe of each lung and assayed for barium content using atomic absorbance flame spectrophotometry (Varian AA1275 series, Techtron). To accomplish this assay, samples were freeze dried and then homogenized. A 0.5 g sample was weighed into a ceramic crucible and then ashed. The ash was dissolved in 1 ml 0.6N HNO_3 and 1 ml 0.4% KCl was added to suppress the partial ionization of barium in the flame. An acetylene flame with nitrous oxide support was used. The wavelength used for making measurements was 553.6 nm, the spectral band pass (slit) was 0.5 nm, and the lamp current was 10 mA. The results from these measurements were compared to the radiograph measurements of the left apical lobe, using least squares linear regression, to evaluate the densitometer technique for evaluating the radiographs.

Results

PULMONARY DEPOSITION OF INTRANASALLY INSTILLED BACTERIA: Data collected on individual calves in experiment #5 are presented in Appendix M. Background information concerning the body and lung weights, T_b, T_a, and respiratory parameters for each treatment group are presented in Table 4.1. The groups differed only in respiratory frequency, tidal volume, and oxygen consumption ($P<0.05$). Cold exposure (3.9 $\pm$ 0.3°C) resulted in a 25% increase in oxygen consumption over that measured under thermoneutral conditions (17.2 $\pm$ 0.3°C). Although total ventilation was not significantly different between treatments, V_T was 32% higher during cold exposure and f was 5.5 b/min lower. Therefore, ventilatory pattern, but not total ventilation, was affected by the cold stress used for this

experiment. To focus on the effects of this pattern change and adjust for variations in $\dot{V}_E$ between calves, the results for deposition were standardized for $\dot{V}_E$.

The ANOVA table for spectrophotometric analysis of particle deposition standardized for $\dot{V}_E$ is shown in Appendix N. The means $\pm$ SE for spectrophotometric analysis of deposition, adjusted for $\dot{V}_E$, are shown in Table 4.2. Cold exposure resulted in a 66% increase ($P<0.05$) in bacterial deposition. When not adjusting for $\dot{V}_E$, a 53% greater bacterial deposition during cold exposure was not statistically significant ($P=0.11$). There was no effect of cold exposure upon the deposition pattern within the lung. Deposition (standardized for $\dot{V}_E$) did not differ between dorsal and ventral sites. However, apical lobes exhibited higher deposition ($P<0.05$) than cardiac and diaphragmatic lobes (combined cold and thermoneutral treatment data). During the thermoneutral exposure, the apical lobe deposition was 46% higher ($P<0.05$) than that of the cardiac and diaphragmatic lobes, while during cold exposure the apical lobe deposition was 28% greater ($P=0.052$) than that of the cardiac and diaphragmatic lobes. Contrast comparison of mediastinal lobe deposition versus all other sample sites showed no significant difference. However, a contrast comparison of mediastinal lobe versus either apical, cardiac, or diaphragmatic lobes showed that the mediastinal lobe is similar in deposition to the apical lobes, but statistically different ($P<0.05$) from the cardiac and diaphragmatic lobes (Appendix N).

The analysis of variance tables for fluorescent microscopic data analysis of particle deposition adjusted for total ventilation are presented in Appendix O. The microscopic counts of fluorescent *P. haemolytica* did not reveal any difference between thermoneutral and cold

treatments (Table 4.2), whether adjusted for $\dot{V}_E$ or unadjusted. In addition, the fluorescent microscopy data did not reveal any difference in deposition pattern within the lung between treatments. There was no difference between dorsal and ventral sites, and the three lobes (apical, cardiac, diaphragmatic) were not different in the degree of deposition. Contrast comparison of the mediastinal lobe with all other sample sites and also with other lobe groupings (apical, cardiac, diaphragmatic) showed no significant differences (Appendix O). Although the fluorescent microscopy data were not normally distributed, a square root transformation of the data to achieve normality did not change the outcome of the statistical analyses.

Deposition, as determined by fluorescent spectrophotometric analysis, was similar for the thermoneutral treatment and two times greater for the cold treatment ($P<0.05$) when compared to respective treatments for which deposition was determined by fluorescent microscopy. Comparison of fluorescent microscopy data with data from spectrophotometric analysis did not show a significant correlation ($r = 0.16$).

DEPOSITION OF AIRBORNE PARTICLES IN ISOLATED SHEEP LUNGS: Data collected on individual sheep lungs in experiment #6 are presented in Appendix P. Background information on ventilatory parameters used during the experiment for the two treatment groups is presented in Table 4.3. Body weight (estimated from lung weight) was not different between treatments. Respiratory frequency was controlled manually and set to 20 b/min for Treatment #1 and 12 b/min for Treatment #2. Total ventilation was controlled manually to achieve similar values for $\dot{V}_E$ for all lungs. Tidal

volume was significantly higher for Treatment #2 ($P<0.05$). Although an attempt was made to manipulate ventilatory pattern and keep $\dot{V}_E$ constant for all lungs in both treatment groups, some slight variation in $\dot{V}_E$ did occur. Therefore, all estimates of deposition were adjusted by dividing by total ventilation.

The analysis of variance tables for each lobe and the total lung are presented in Appendix Q. Means $\pm$ SE for each lobe and the total lung are presented in Table 4.4. There was no statistical difference ($P=0.18$) between the total deposition for lungs in Treatment #1 versus Treatment #2. Radiographic analysis by lobe revealed a treatment difference only for the left apical lobe ($P<0.05$), with a 134% greater deposition for Treatment #2. Atomic absorbance spectrophotometric analysis for barium in the left apical lobe did not reveal a statistical difference in deposition between treatments ($P=0.11$). However, comparison of radiographic results for left apical lobe with atomic absorbance spectrophotometric analysis for samples from the left apical lobe revealed a significant correlation ($r = 0.85$; $P<0.01$). Data not adjusted for total ventilation revealed no differences between treatments for all lobes, including atomic absorbance spectrophotometric analysis of samples from the left apical lobe for barium content.

Table 4.1. Respiratory parameters and body weight in calves exposed to thermoneutral and cold conditions during experiment #5.

	THERMONEUTRAL	COLD
Body weight (kg)	61 ± 8	49 ± 2
Lung weight (kg)	0.81 ± 0.08	0.65 ± 0.01
% dry weight (lung)	19.6 ± 0.5	20.9 ± 0.3
T_b (°C)	39.1 ± 0.2	39.6 ± 0.1
T_{sk} (°C)	33.4 ± 0.4	31.6 ± 0.8
f (b/min)	18.13 ± 1.49	12.64 ± 0.74*
$\dot{V}_E$ (ml/min-kg)	155.2 ± 8.5	143.4 ± 7.5
V_T (ml/kg)	8.71 ± 0.59	11.49 ± 0.87*
$\dot{V}_{O2}$ (ml/min-kg)	5.25 ± 0.20	6.56 ± 0.33*
RER	0.88 ± 0.02	0.84 ± 0.01

Values are means ± SE; n = 5. T_b, body temperature; T_{sk}, skin temperature; f, respiratory frequency; $\dot{V}_E$, minute ventilation; V_T, tidal volume; $\dot{V}_{O2}$, oxygen consumption; RER, respiratory exchange ratio.
THERMONEUTRAL = 16-18°C and COLD = 4-6°C.

* $P<0.05$ compared to the thermoneutral treatment.

Table 4.2. Effect of cold exposure on pulmonary deposition of nasally-instilled *Pasteurella haemolytica*.

	n	THERMONEUTRAL	COLD
Total lung	65	0.98 ± 0.07	1.63 ± 0.10*†
Dorsal	30	1.06 ± 0.11	1.60 ± 0.13
Ventral	30	0.91 ± 0.08	1.56 ± 0.16
Apical lobe	20	1.24 ± 0.13	1.85 ± 0.17
Cardiac lobe	20	0.86 ± 0.11‡	1.39 ± 0.15
Diaphragmatic lobe	20	0.84 ± 0.10‡	1.50 ± 0.19
Mediastinal lobe	5	0.97 ± 0.27	2.19 ± 0.22
Apical-dorsal	10	1.26 ± 0.21	1.88 ± 0.25
Cardiac-dorsal	10	1.02 ± 0.19	1.59 ± 0.17
Diaphr.-dorsal	10	0.89 ± 0.17	1.33 ± 0.22
Apical-ventral	10	1.22 ± 0.18	1.82 ± 0.25
Cardiac-ventral	10	0.70 ± 0.08	1.20 ± 0.23
Diaphr.-ventral	10	0.80 ± 0.12	1.61 ± 0.31
Total lung (microscopic anal.)	65	1.41 ± 0.14	0.82 ± 0.06

Values are means ± SE x 10^5, determined for 5 calves for each treatment. Unless otherwise specified, values were determined by spectrophotometric analysis. All values were adjusted for total ventilation, therefore units are CFU·kg·min/ml (CFU = Colony Forming Units).

* $P<0.05$ compared to thermoneutral treatment.

‡ $P<0.05$ for lobes compared to apical lobe for that treatment.

† $P<0.05$ compared to microscopic analysis for that treatment.

Table 4.3. Body weight and respiratory parameters for treatments during experiment #6.

TREATMENT #	1	2
Body weight (kg)	52.2 ± 3.7	56.0 ± 2.8
f (b/min)	20	12
$\dot{V}_E$ (ml/min·kg)	153.2 ± 3.9	137.4 ± 8.1
V_T (ml/kg)	7.66 ± 0.20	11.45 ± 0.67*

Except for f, values are means ± SE; n = 5. f, respiratory frequency; $\dot{V}_E$, minute ventilation; V_T, tidal volume.

* $P<0.05$ compared to the Treatment #1.

Table 4.4. Effects of respiratory pattern on particle deposition in isolated, ventilated sheep lungs.

LOBE	n	TREATMENT #1	TREATMENT #2
Left-apical	15	1.21 ± 0.25	2.83 ± 0.27*
Left-cardiac	10	2.11 ± 0.28	1.82 ± 0.34
Left-diaphr.	20	2.05 ± 0.32	2.91 ± 0.28
Right-apical	10	1.84 ± 0.41	1.80 ± 0.42
Right-cardiac	10	1.86 ± 0.32	2.89 ± 0.37
Right-diaphr.	20	2.14 ± 0.32	3.19 ± 0.26
Total lung	85	1.88 ± 0.14	2.70 ± 0.14
Left-apical(ppm Ba/$\dot{V}_E$)	5	0.164 ± 0.025	0.247 ± 0.038

Values are means ± SE, determined for 5 calves for each treatment. Unless specified, units are arbitrary units determined by densitometer readings from radiographs, divided by total minute ventilation ($\dot{V}_E$).

* $P<0.05$ compared to Treatment #1.

Discussion

EFFECTS OF COLD EXPOSURE ON PULMONARY DEPOSITION OF BACTERIA: The results indicate that the intranasal instillation technique is an effective means of introducing bacteria into the inspired air. Viable, colony forming *P. haemolytica* were obtained from lung sample cultures from treated calves. Grey and Thomson (7) had previously found tracheal air to contain airborne *P. haemolytica* only in calves with positive nasal swabs for *P. haemolytica*. However, they did not observe a direct correlation between the numbers in the nasal flora and the numbers found in the tracheal air. Perhaps this is due to other factors important to the aspiration of nasal fluids, such as respiratory pattern, nasal airway resistance, or viscosity of nasal secretions.

A total quantity of 3.45×10^{10} CFU of *P. haemolytica* were administered to each calf during experiment #5. To compare this to the amount of deposition, one must assume that the pattern of deposition is relatively uniform throughout the lung. Using the mean lung weight and mean $\dot{V}_E$ from Table 4.1 and the mean deposition from Table 4.2 for the thermoneutral treatment, the estimated total deposition would be 1.23×10^{10}, or 36% of the amount instilled. For the cold treatment, the total deposition predicted by spectrophotometric analysis would be 1.52×10^{10} or 44% of the amount instilled. These percentages are comparable to previously reported fractional pulmonary deposition of inhaled particles <5 μm (19). Grey and Thomson (7) determined that approximately half of the airborne *Pasteurella*-containing droplets found in tracheal air were of this aerodynamic diameter (<5 μm). However, one cannot assume that 100% of the bacteria delivered by intranasal instillation is aspirated, and the actual number of bacteria which are inhaled is probably somewhat

less than the total amount delivered to the nasal cavity. On the other hand, one must consider the unequal distribution within the lung. For example, the calculations made here for total deposition use total lung weight. However, deposition was only determined for parenchymal sites and not for the airways. A large proportion of the lung weight is composed of airways, yet compared to the parenchyma the airway surface area is relatively small. In addition, Valberg *et al.* (20) determined that deposition in airways was 20% of that for parenchyma during slow deep breathing. Therefore, rather than multiplying deposition/gm by total lung weight, it would be more appropriate to use some fraction of total lung weight which represented the parenchyma.

The effect of cold upon respiratory pattern resulted in greater deposition of bis-benzamide-labeled bacteria, as determined by spectrophotometric analysis (Figure 4.3). This 66% increase in pathogen deposition would subsequently increase the workload of alveolar macrophages. In addition, the effect of the stress of cold exposure upon release of adrenocortical steroids may simultaneously attenuate immune responses (8). Thus, the possibility of respiratory infection as a result of increased deposition due to cold-induced respiratory pattern changes may be compounded by the effect of cold on pulmonary clearance.

Although there was not a significant increase in deposition unadjusted for $\dot{V}_E$, exposure to colder temperatures than used in experiment #5 would most likely result in significantly greater deposition as $\dot{V}_E$ eventually increased with metabolic demand. Ventilatory parameters were not determined for temperatures below 2°C, however, previous research by Joyce and Blaxter (13) with sheep and Ingram and Legge (12) with pigs supports the prediction that as $\dot{V}_{O2}$ increases with colder temperatures,

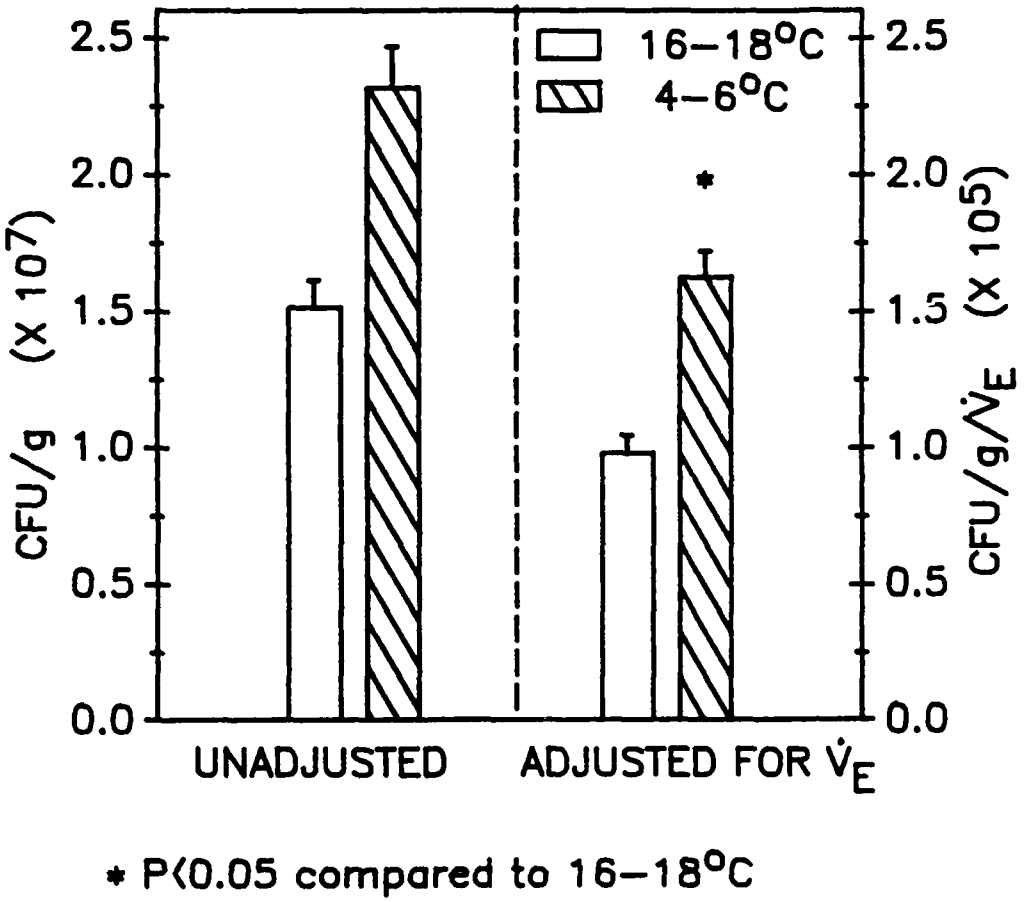


Figure 4.3. Effect of cold exposure on pulmonary deposition of nasally instilled *Pasteurella haemolytica*. Values are means ± SE; n=65 (* $P<0.05$ compared to 16-18°C).

calves would increase $\dot{V}_E$ by increasing f and maintaining a relatively large V_T. It would be interesting to determine the effect of colder temperatures on pulmonary deposition.

Deposition, as determined by fluorescent microscopy, did not show a difference in deposition due to cold exposure. In addition, fluorescent microscopy data did not correlate with data derived by fluorescent spectrophotometry. One explanation for this may be non-uniform clearance of fluorescent *P. haemolytica* from the lung. While counting fluorescent *P. haemolytica* within the microscopic field, background fluorescence was ignored, while sharp images resembling *P. haemolytica* were counted. However, the lag time between initiation of instillation of bacteria and freezing of lung samples was as much as 2.5 hr. During this period it is likely that some fluorescent bacteria were phagocytized by macrophages and lysed, so that the sharp image was destroyed. In such instances, these bacteria were not counted in the fluorescent microscopic analysis of deposition, but would be detected by the spectrophotometric analysis for bis-benzamide. If this is an accurate evaluation of the discrepancy between the two forms of data, then it appears that pulmonary macrophage activity may have been greater during the cold treatment. A statistical comparison of the thermoneutral treatment data showed no difference between fluorescent microscopy and spectrophotometry data, while comparison of the cold treatment data (Table 4.2) indicated that the fluorescent microscopy data are less than that of the spectrophotometry data ($P<0.05$). Therefore, rather than resulting in depression of macrophage activity at the level of cold exposure used in experiment #5, there may be enhanced macrophage activity, perhaps due to the greater

presence of pathogens in the lung. These conclusions, however, are derived from a number of assumptions and the spectrophotometric data appear to be a more accurate assessment of deposition, since:

1) spectrophotometry is an objective measurement compared to the subjective identification and counting of bacteria by fluorescent microscopy;

2) fluorescent microscopy was made difficult by background fluorescence, and some subjective decisions were made;

3) fluorescent microscopy counts were made over a unit volume of lung and then data were converted to grams, which assumes a uniform weight-to-volume ratio throughout all lung samples.

Therefore, the balance of the discussion will refer only to results determined from spectrophotometric analysis.

Increased deposition resulting from a slower, deeper respiratory pattern has been previously shown in excised dog lungs (20) and is supported by the physical properties of particle movement and deposition (9,10,19). Whether the increase in deposition observed in this study also increases the risk of respiratory infection is still open to debate. However, Slocombe *et al.* (17) have previously postulated that the relationship between stress and respiratory disease in cattle may be related to changes in ventilatory pattern.

Although the pattern of deposition did not vary with treatment in this study, it is interesting to note that the mediastinal and apical lobes exhibited the highest particle deposition (Figure 4.4). The phenomenon of preferential apical lobe deposition has been observed in other quadrupeds. For example, Brain *et al.* (2) observed in rats and

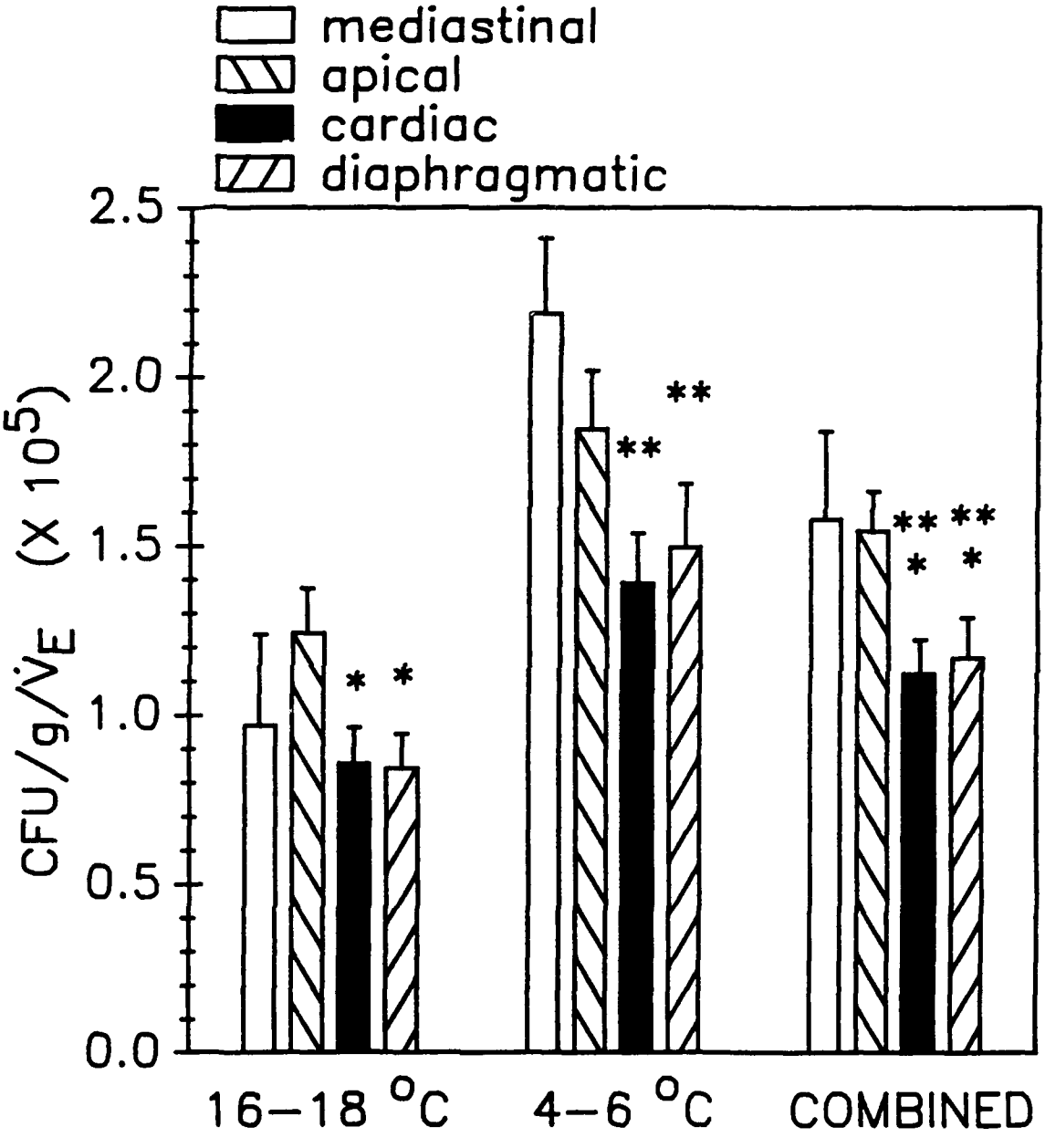


Figure 4.4. Regional lung deposition of *Pasteurella haemolytica*. Values are means ± SE; n=20 for apical, cardiac, and diaphragmatic lobes, n=5 for mediastinal lobe (* $P<0.05$ compared to apical lobes; ** $P<0.05$ compared to mediastinal lobe).

hamsters that there was preferential deposition of aerosol in the apicallobes. Interpretation of these results is difficult, since these animals often position themselves vertically and thereby alter the gravitational vector upon the lung. Valberg *et al.* (20) also noticed increased deposition in the base-to-apex, as well as the dorsal-to-ventral, direction in isolated, ventilated dog lungs. Chamberlain *et al.* (4) demonstrated in humans that regional deposition of particles 1 μm aerodynamic diameter corresponds to the regional ventilation. However, Pityn *et al.* (15) showed that larger particles (3.5 μm) were preferentially deposited in upper rather than lower regions of the lung. It is interesting to note that silicosis and pneumoconiosis in coal workers often involves the upper zones of the lungs first (16).

While an explanation of a dorsal-ventral difference could be based upon gravitationally-dependent differential ventilation of the lung, a difference in deposition between lobes of the horizontally positioned lung of quadrupeds is more elusive. Sneddon and Brain (18) observed a greater deposition in the apex of the lung in rats, regardless of body position. A possible explanation of this phenomenon may exist in differences in regional compliance of the lung, with the apical, and perhaps mediastinal, lobe being more compliant. If this were true, then these lobes would tend to receive greater ventilation, and perhaps increased particle deposition by sedimentation. Frank (6) observed that, at all distending pressures tested, the apical lobes of excised dog lungs contained a greater volume of gas/gm of tissue than did lower lobes. However, Hubmayr *et al.* (11) found less expansion of apical lobes compared to lower lobes between functional residual capacity and total lung capacity in supine dogs.

Another possible explanation for preferential deposition in apical lobes may be differential filtration of particles by the airways, similar to the theory proposed by Pityn *et al.* (15) for humans. This would require that flow to the basilar lobes exceed flow to the apical lobes, causing less impaction and deposition of particles along airways leading to apical lobes, thereby allowing more particles to penetrate to the pulmonary region of the lung. This might occur as the result of the intrapleural pressure gradient in the vertically-positioned lung, but must be due to some other factor in the horizontally-positioned lung. The cephalocaudal gradient of regional expansion differences observed by Hubmayr *et al.* (11) supports this hypothesis. Preferential deposition in apical regions could also be the result of differences in airway resistance or other aerodynamic differences between lobes. In support of this theory, Sneddon and Brain (18) compared breathing mixtures of different densities and observed, in rats, an enhancement of apex-to-base differential deposition with an SF_6-O_2 breathing mixture compared to He-O_2.

EFFECT OF VENTILATION PATTERN ON AIRBORNE PARTICLE DEPOSITION: Radiographic analysis of isolated, ventilated sheep lungs exposed to a $BaSO_4$ aerosol did not reveal any differences in deposition except for the left apical lobe ($P<0.05$). This lobe was treated differently from the other lobes in that analysis was made from a separate 3 x magnification radiograph of this lobe.

The correlation of radiographic analysis with atomic absorbance spectrophotometry analysis for barium in samples from the left apical lobe indicate that this radiographic technique may be effective in detecting the level of deposition within the lung. However, magnification of the

lung may be essential. Also, the left apical lobe is not as thick as other lobes, which may allow for better radiographic analysis. Therefore, for larger lobes, this technique may require sectioning of the lobe to approximately 5 cm or less in thickness.

Due to differences in thickness of different lobes, radiographic analysis does not permit comparison of lobes within a lung. Therefore, radiographic analysis has several disadvantages and provides no advantage over atomic absorbance spectrophotometry, unless it is desired to make measurements while keeping the lung intact. It is doubtful that this radiographic technique would be effective in whole animals, unless deposition is great.

Atomic absorbance spectrophotometry for barium did not show a significant difference between treatments (P=0.11), although the means show a trend which supports the hypothesis of greater deposition with a slower, deeper respiratory pattern (Treatment #2). In retrospect, atomic absorbance spectrophotometric analysis of samples from all lobes may have provided a more accurate assessment of differences in deposition. For future isolated, ventilated lung experiments I would recommend either a longer exposure period or more concentrated aerosol, and would discourage radiographic analysis for determining deposition within the lung.

Conclusions

Cold exposure results in a change in the ventilatory pattern of calves, which enhances the deposition of bacteria deep within the lung. It is possible that, associated with this increase in bacteria deposition, there is a compensatory increase in alveolar macrophage activity. Although apical and mediastinal lobes received the largest burden of

particle deposition, this regional difference is probably not due to gravitationally-dependent differential ventilation of the lung.

REFERENCES - CHAPTER IV

1. Altman, P.L. and D.S. Dittmer. *Biological Handbook: Growth, including Reproduction and Morphological Development*, Federation of American Societies for Experimental Biology, Washington D.C., 1962, pp. 416-417.

2. Brain, J.D., D.E. Knudson, S.P. Sorokin and M.A. Davis. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. *Envir. Res.* 11:13-33, 1976.

3. Brunk, C.F., K.C. Jones and T.W. James. Assay for nanogram quantities of DNA in cellular homogenates. *Anal. Biochem.* 92:497-500, 1979.

4. Chamberlain, M.J., W.K.C. Morgan and S. Vinitski. Factors influencing the regional distribution of inhaled particles in man. *Clin. Sci.* 64:69-78, 1983.

5. Esch, J.L., D.M. Spektor and M. Lippmann. Effect of lung airway branching pattern and gas composition on particle deposition. II. Experimental studies in human and canine lungs. *Exp. Lung Res.* 14:321-348, 1988.

6. Frank, N.R. A comparison of static volume-pressure relations of excised pulmonary lobes of dogs. *J. Appl. Physiol.* 18:274-278, 1963.

7. Grey, C.L. and R.G. Thomson. *Pasteurella haemolytica* in the tracheal air of calves. *Can. J. Comp. Med.* 35:121-128, 1971.

8. Hartmann, H., W. Bruer, A. Herzog, H. Meyer, H. Rhode, F. Schulze and G. Steinbach. The general adaptation syndrome in the calf. IV. Stress conditions - their impact upon antibody levels, following active and passive immunization and upon the topographical distribution of some groups of bacteria in the gastrointestinal system. *Arch. Exp. Vet. Med.* 30:553-566, 1976.

9. Hatch, T. Distribution and deposition of inhaled particles in the respiratory tract. *Bact. Rev.* 25:237-240, 1961.

10. Heyder, J., J. Gebhart and W. Stahlhofen. Inhalation of aerosols. Particle deposition and retention. In: *Generation of Aerosols and Facilities for Exposure Experiments* (K. Willeke, Ed.), Ann Arbor Science, Ann Arbor, MI, 1980, pp. 65-108.

11. Hubmayr, R.D., B.J. Walters, P.A. Chevalier, J.R. Rodarte and L.E. Olsen. Topographic distribution of regional lung volume in anesthetized dogs. *J. Appl. Physiol.* 54:1048-1056, 1983.

12. Ingram, D.L. and K.F. Legge. The effect of environmental temperature on respiratory ventilation in the pig. *Respir. Physiol.* 8:1-12, 1968.

13. Joyce, J.P. and K.L. Blanter. Respiration in sheep in cold environments. *Res. Vet. Sci.* 5:506-516, 1964.

14. Labarca, C. and K. Paigen. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* 102:344-352, 1980.

15. Pityn, P., M.J. Chamberlain, T.M. Fraser, M. King and W.K.C. Morgan. The topography of particle deposition in the human lung. *Respir. Physiol.* 78:19-29, 1989.

16. Sargent, E.N. and W.K.C. Morgan. In: *Induced Disease* (L. Preger, Ed.), Grune and Stratton, New York, 1980, pp. 257-316.

17. Slocombe, R.F., F.J. Derksen and N.E. Robinson. Interactions of cold stress and *Pasteurella haemolytica* in the pathogenesis of pneumonic pasteurellosis in calves: Changes in pulmonary function. *Am. J. Vet. Res.* 45:1764-1770, 1984.

18. Sneddon, S.L. and J.D. Brain. Persistent apex to base gradients of aerosol deposition in rats. *Respir. Physiol.* 46:113-124, 1981.

19. Stuart, B.O. Deposition and clearance of inhaled particles. *Envir. Health Pers.* 55:369-390, 1984.

20. Valberg, P.A., J.D. Brain, S.R. Lemott, S.L. Sneddon and A. Vinegar. Dependence of aerosol deposition site on breathing pattern. *Am. Rev. Respir. Dis. Suppl.* 117:262, 1978. (Abst.)

CHAPTER V

EFFECTS OF COLD EXPOSURE ON NASAL AND TRACHEAL MUCOCILIARY CLEARANCE

Introduction

Bovine respiratory disease is usually initiated by a viral infection of the upper airways, often in combination with some stress, such as cold exposure (12). Viral infection may act in several ways to potentiate the development of pneumonia, including decreasing mucociliary clearance rate. Mucociliary clearance in the upper airways may also be influenced by environmental temperature (3,20). While a cold-induced change in respiratory pattern resulted in increased particle deposition in previous experiments (Chapter IV), a simultaneous decrease in clearance would act to further increase the overall burden of pathogens in the lung. Should clearance of the upper airways become significantly impaired, this may permit bacteria to invade the lungs, either through drainage or aspiration. The same mucosal circulatory adjustments and ventilatory pattern changes which provide for conservation of respiratory heat may also result in decreased mucociliary clearance, predisposing the animal to pneumonia, particularly in conjunction with viral infection of the upper airways.

To test the hypothesis that cold exposure reduces mucociliary clearance of upper airways, two objectives were addressed. First, nasal mucociliary clearance was determined for calves in a warm environment and compared to that measured in a cold environment. Second, isolated calf and sheep tracheae were used to assess the effect of mucosal temperature upon the transport rate of the tracheal mucosa.

Materials and Methods

NASAL MUCOSAL CLEARANCE DURING COLD EXPOSURE: Four male Holstein calves between the ages of 1 and 3 months and weighing 45-55 kg were used to investigate the effects of cold exposure on mucociliary clearance rate of the nasal mucosa (experiment #7). Calves were purchased, housed, and fed as described in Chapter III. All measurements were made in an environmental chamber, previously described as the cold room in Chapter III. During mucociliary measurements, calves were held in a stanchion which immobilized the head, allowing x-ray of the nasal region of the skull. While not being utilized for measurements, the calves were maintained in a pen (0.6 x 1.3 m) which allowed complete freedom of movement.

Two temperature ranges were utilized for making measurements, 16-18°C and 2-4°C. At the 16-18°C exposure, ambient water vapor pressure was approximately 11 Torr, while at the 2-4°C exposure, ambient water vapor pressure was approximately 6 Torr. Five measurements of nasal mucus velocity (NMV) were made for each calf at each temperature. Calves were placed in the environmental chamber 24 hr prior to beginning measurements. Measurements were made during the morning (0800-1000) and during the afternoon (1500-1700), such that individual measurements were made several hours apart. Thus, each calf was maintained at a particular

temperature for 3 days to obtain 5 individual measurements of NMV at that temperature. Two of the calves experienced the colder temperature prior to the thermoneutral temperature, while the remaining two calves experienced the thermoneutral temperature first. To assist in making measurements from radiographs and verify accurate distance measurements, radio-opaque metal disks were sutured to one side of the calf's muzzle.

Measurements consisted of radiographic analysis of the velocity of movement of a droplet of a tantalum powder/paraffin oil mixture placed upon the nasal mucosa. The tantalum/oil mixture was applied by syringe through PE tubing which was inserted into the nostril approximately 10-15 cm. An initial radiograph (100 mA, 70 kVp) of the droplet within the nasal cavity was obtained immediately following placement of the tantalum/oil droplet. Another radiograph was obtained 5 min following the initial radiograph. For instances when the calf snorted, disrupting the droplet, this procedure was immediately repeated. The distance which the droplet traveled was measured (in mm) from comparison of the two radiographs and velocity (mm/min) was calculated. The effect of temperature on NMV was evaluated using two-way ANOVA with provisions for repeated measures.

EFFECT OF TEMPERATURE ON TRACHEAL MUCOCILIARY ACTION: Seven male Holstein calves, 2 to 8 weeks old and weighing 35-55 kg, and 10 western range ewes, 4 to 5 years old and weighing 45-65 kg, were used in experiment #8 to investigate the effects of mucosal temperature upon mucociliary clearance rate of the tracheal mucosa. Calves were purchased from a local dairy (Dickinson Dairy) at 1 to 2 days of age, maintained indoors at approximately 21°C, and fed as described in Chapter III. Sheep were kept in an outdoor facility subject to local environmental conditions, and brought indoors 1 hr prior to the experiment.

The animals were anesthetized with pentobarbital and then exsanguinated. A section of the trachea 10 cm long, just beneath the larynx, was carefully removed, then slit open along the membranous wall and pinned open on a board at a 25 degree upward slant following the technique of Ahmed *et al.* (1). The board was placed inside a temperature controlled chamber which consisted of a water bath filled with water to just below the bottom edge of the tracheal board. A plexiglass cover allowed viewing of the trachea while maintaining constant temperature and 100% humidity within the chamber. An antifog solution was used on the plexiglass to prevent the "window" from fogging up The temperature within the chamber was adjusted by changing the thermostat of the water bath.

To measure tracheal mucous velocity (TMV), a grid was placed just above (5 mm) the surface of the trachea. The grid consisted of parallel, horizontal bars spaced at 1 cm intervals. Aluminum and/or plastic particles, 1 mm square and 0.1 mm thick, were placed upon the mucosal surface. TMV was determined by timing the particles as they travelled the 1 cm distance from one grid bar to the next. The tip of a thermocouple was placed in contact with the tracheal mucosa to measure mucosal temperature (T_m). TMV measurements were made within the T_m range of 35.0 to 39.5°C. The temperature of the chamber was either slowly increased or decreased (alternating for each successive trachea preparation) to acquire several TMV estimates across the T_m range of interest. Data were evaluated using analysis of co-variance to test the data for significant correlation of TMV versus T_m and to test the equality of slopes for separate tracheae.

TRACHEAL AIR TEMPERATURE MEASUREMENTS: Tracheal air temperature was measured in five unanesthetized calves at 16.5 to 21.0°C and also at approximately 2°C. In addition, tracheal air temperature was measured in two calves at -10°C. A 16 gauge spinal needle was inserted through the skin and into the lumen of the trachea, under local lidocaine anesthesia. A 40 gauge thermocouple was then placed into the trachea through the needle and the needle was withdrawn. The thermocouple was slowly withdrawn until the maximum difference between inspired and expired tracheal air temperature was achieved, indicating that the tip of the thermocouple was not in contact with the airway wall. Inspired and expired tracheal air temperature, as well as body temperature and respiratory frequency were determined over a continuous 4 to 10 min period.

Results

EFFECT OF COLD EXPOSURE ON NMV: Exposure of calves to cold temperature (2-4°C) resulted in a 24% decrease (P=0.0033) in NMV (8.0 $\pm$ 0.4 mm/min) compared to thermoneutral exposure at 16-18°C (10.5 $\pm$ 0.4 mm/min). There may be some difference in NMV between the calves utilized for this experiment, as demonstrated by the "between calf" variability (P=0.0511). However, there was no significant treatment*calf interaction (P=0.8342), indicating that the relative change in NMV between treatments was similar for all calves. The data collected on individual calves in experiment #7 are presented in Appendix R and the ANOVA table is presented in Appendix S.

EFFECT OF MUCOSAL TEMPERATURE ON TMV: Tracheal air temperature did not decrease significantly (P>0.05) during cold exposure (Table 5.1) except

during inhalation at the coldest temperature tested (-10°C). Although tracheal mucosal temperature was not determined in this experiment, intuitively it should cycle between inspired and expired tracheal air temperatures, but perhaps closest to expired tracheal air temperature. Tracheal air temperature measurements collected on individual calves are presented in Appendix T. There was a significant correlation ($P<0.01$) between T_m and TMV ($r = 0.48$). The calf*T_m interaction ($P = 0.3281$) demonstrates that there is no statistical difference between the slopes of the linear regressions of T_m and TMV for the 7 calves. Therefore, the overall linear regression equation of the pooled data for TMV (mm/min) as determined by T_m (within the range of 35.0 to 39.5°C) is:

$$TMV = -9.91(\pm 5.50SE) + 0.80(\pm 0.15SE)*T_m$$

While this equation provides an accurate prediction of the slope of the TMV versus T_m relationship, the predicted intercept is of little value, since the TMV estimate at a specific T_m is significantly different ($P<0.0001$) between calves. The linear regressions of TMV versus T_m for individual calf tracheae are presented in Figure 5.1. Three of the calves (#1, 6, and 7) did not show a significant correlation, while calves #2, 3, 4, and 5 demonstrated a correlation ($P<0.05$) between TMV and T_m. Data collected on individual calf tracheae in experiment #8 are presented in Appendix U and the ANOVA table is presented in Appendix V.

Analysis of co-variance revealed that the slopes of the regressions of TMV versus T_m for the 10 sheep tracheae were not equal. The linear regressions of TMV versus T_m for individual sheep tracheae are presented in Figure 5.2. Seven of the sheep tracheae (#2, 3, 4, 7, 8, 9, and 10)

showed a significant ($P<0.05$) relationship of TMV to T_m. No correlation could be made between the slope of the regression or average TMV for a trachea versus the pre-test environmental temperature to which each individual sheep was exposed. Data collected on sheep tracheae are presented in Appendix W and the ANOVA table is presented in Appendix X.

Table 5.1. Effect of cold exposure on tracheal air temperature.

	THERMONEUTRAL	COLD	EXTREME COLD
	(n = 5)	(n = 5)	(n = 4)
T_a (°C)	18.5 ± 0.8	2.0 ± 0.2	-10.4 ± 1.4
f (b/min)	23.1 ± 1.6	15.8 ± 1.8*	18.4 ± 1.8
T_b (°C)	38.7 ± 0.3	38.7 ± 0.2	38.4 ± 0.4
T_{ti} (°C)	37.7 ± 0.1	37.2 ± 0.4	36.0 ± 0.3*
T_{te} (°C)	38.3 ± 0.3	38.3 ± 0.3	36.8 ± 0.2*

Values are means ± SE. T_a, ambient temperature; n, sample size; f, respiratory frequency; T_b, body temperature; T_{ti}, tracheal air temperature during inspiration; T_{te}, tracheal air temperature during expiration.

* $P<0.05$ compared to thermoneutral treatment (T_a = 18.5°C).

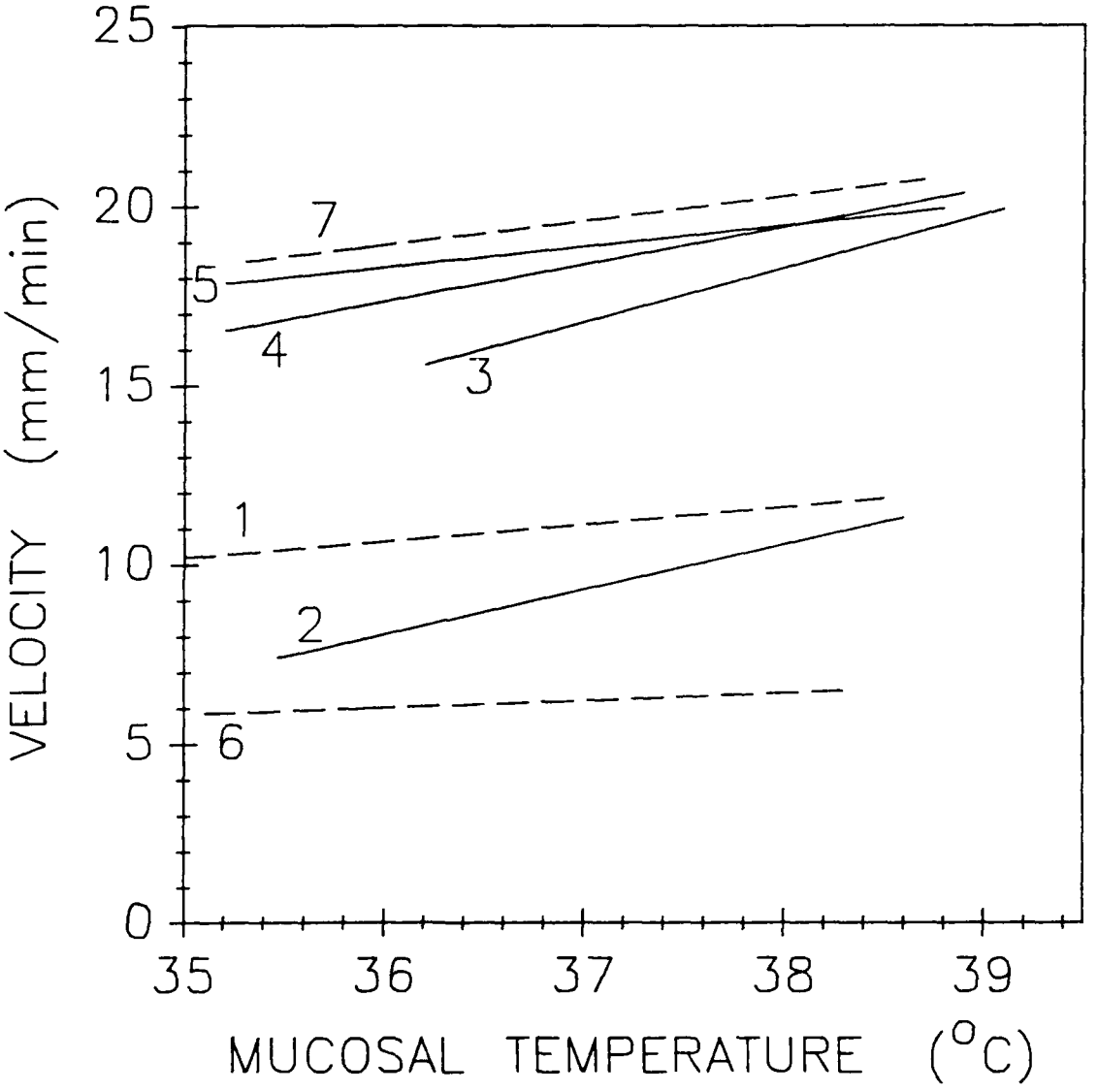


Figure 5.1 Effect of ambient temperature on tracheal mucus velocity for seven calves, *in vitro* (solid lines, $P<0.05$ for linear correlation; dashed line, no significant correlation).

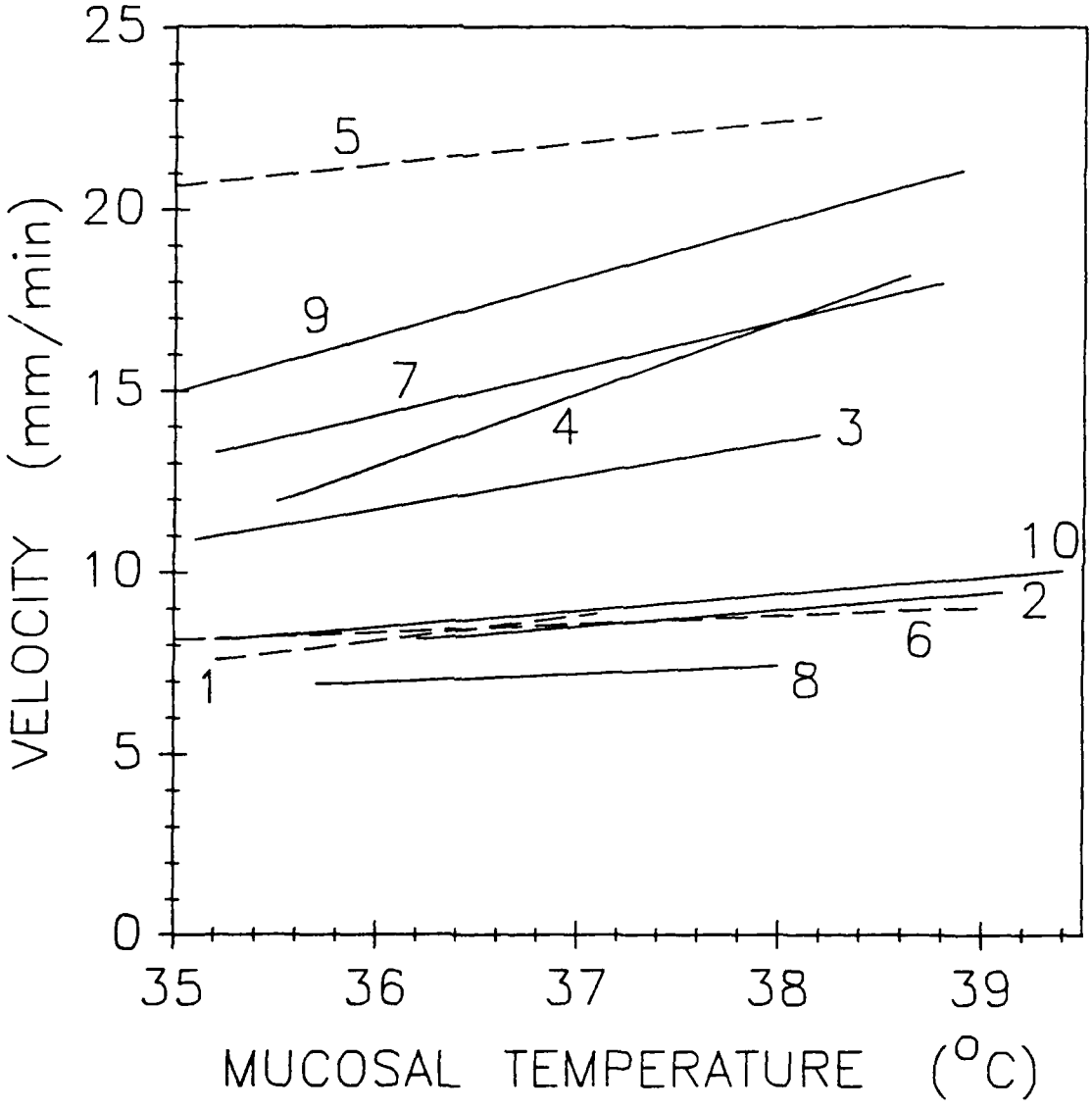


Figure 5.2 Effect of ambient temperature on tracheal mucus velocity for ten sheep, *in vitro* (solid lines, $P<0.05$ for linear correlation; dashed line, no significant correlation).

Discussion

Comparison of NMV estimates for individual calves showed potential differences between calves (p=0.0511; Appendix S). Differences in TMV (P<0.0001) between calf, as well as sheep, tracheae were also great (Figures 5.1 and 5.2). Large variations in mucosal clearance have been reported in previous studies (2,8,21,25,26) and evidently are due to some intrinsic characteristic of the individual animal which was controlled neither in these previous studies, nor in the present study.

If one assumes the nasal mucosal temperature to be approximately equal to expired air temperature for a particular treatment, then a mucosal temperature of 23.9 and 17.9 would be assumed for Treatments #1 and #2, respectively (see Chapter III, Table 3.2). Assuming the change in NMV with temperature to be linear between Treatment #1 and #2, this would be a 0.4 mm/min/°C decrease in NMV as mucosal temperature decreases. Decreased NMV during cold exposure (Figure 5.3) may be the result of either an autonomic reflex and/or a direct temperature effect on the nasal mucosa. Since a direct effect of temperature was demonstrated for tracheal mucus velocity, and experiments in Chapter III show a decrease in T_E with cold exposure at 4-6°C, a direct effect of cold on NMV seems certain. However, one cannot rule out a contributing autonomic influence on the nasal mucosa during cold exposure in this experiment. Bohning *et al.* (4) observed a direct linear correlation between pretest outdoor temperature and bronchial mucociliary clearance rate in donkeys, indicating some influence upon mucosal activity other than direct cooling of the mucosa. Although several investigators have described the effects of autonomic nerve interruption and stimulation (6,9,17,18), as well as cholinergic and adrenergic drug administration on mucus production and

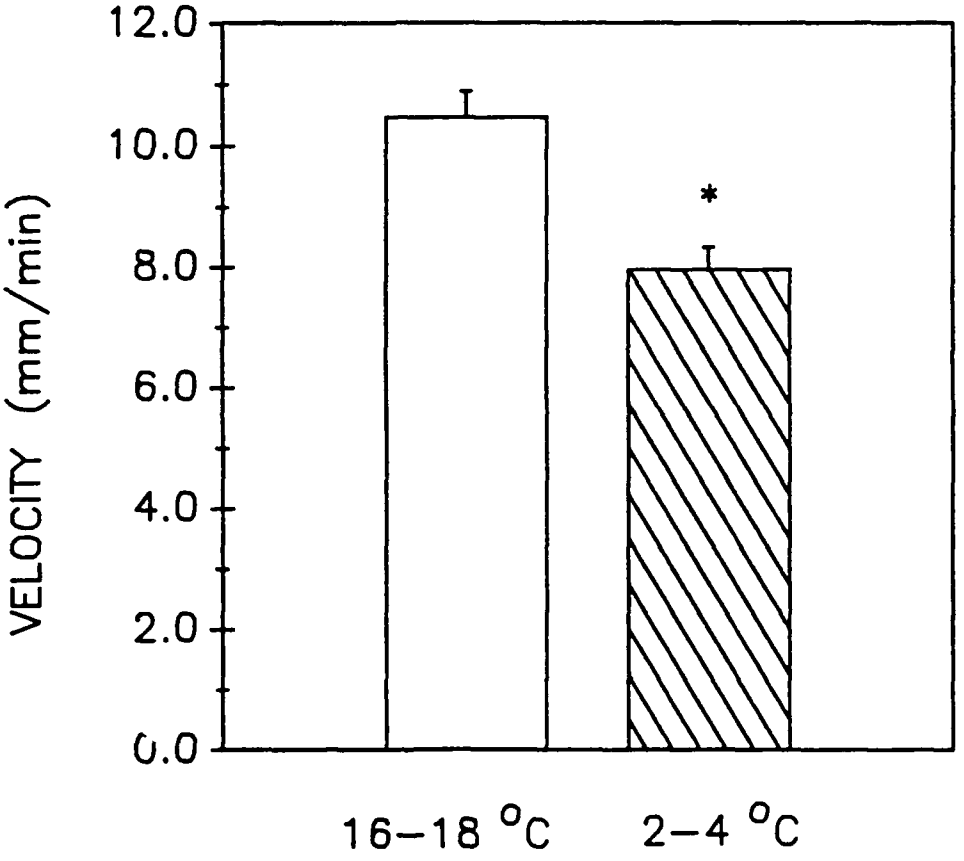


Figure 5.3 Effect of cold exposure on nasal mucus velocity in four calves. Values are means ± SE; n=20 (* $P<0.01$ compared to 16-18°C).

mucociliary clearance (7,10,14), the results often appear contradictory. There is a paucity of information concerning autonomic influences on mucociliary response to perturbations, such as cold. Such investigations may resolve some of the seemingly contradictory findings concerning autonomic control of mucociliary clearance.

Tracheal mucociliary clearance, measured *in vitro*, was twice as responsive to temperature (0.8 mm/min/°C) as NMV. Several possible explanations exist for this difference. First, the mucosal temperature ranges differ between NMV measurements (approximately 17.9 to 23.9°C) and TMV measurements (35 to 39.5°C). Second, there may be intrinsic differences between the tracheal and nasal mucosa. Third, TMV measurements involved only the direct effect of cold, while NMV may have been influenced by other factors, such as the autonomic nervous system. In addition, Wong and Yeates (24) found that ciliary beat frequency is elevated by local tissue incision, although the mechanism of activation is not clear. Thus, the tracheal excision procedure may also be responsible for comparatively high TMV measurements.

To predict tracheal mucus velocity for a young calf, based upon the data at 18.5°C, one might assume tracheal mucosal temperature as being the average of the inspired and expired air temperatures (Table 5.1). Therefore, at 38.0°C tracheal mucosal temperature, the predicted TMV would be 20.5 mm/min. If one uses expired tracheal air temperature to estimate mucosal temperature, TMV would be 20.7 mm/min. This is comparable to tracheal mucus velocities ranging from 20 to 45 mm/min measured at body temperature by other investigators in excised tracheal tissue from a variety of animals (11,13), but higher than velocities measured by

Whaley *et al.* (22) for young adult anesthetized dogs (9.7 mm/min), and by Phipps *et al.* (19) for unsedated 8-week-old sheep (9.6 mm/min).

The estimated TMV at a tracheal mucosal temperature of 36.2°C (average of inspired and expired tracheal air temperatures at T_a = -10.4°C) would be 19.0 mm/min. If one chooses to use the expired tracheal air temperature of 36.8°C at T_a = -8.0°C, TMV would be 19.5 mm/min. As T_a drops even lower, one would assume that tracheal air temperature would also decrease. Conditioning of cold inspired air in humans has been shown to extend into the distal parts of the tracheo-bronchial tree (15,16). Although this clearance rate is only 6-7% lower (depending on the method of estimating tracheal mucosal temperature from tracheal air temperature) than that predicted for an ambient temperature of 18.5°C, it may represent a much greater retention of particles in the lung. For example, normally airway clearance mechanisms are almost 100% efficient. Therefore, if one assumes a normal clearance of 99%, a 1% decrease in clearance efficiency results in nearly a 2-fold increase in retention (5). As clearance rate decreases, the amount of time during which pathogenic particles are subject to aspiration deep into the lung is lengthened. There is *in vitro* evidence that bacteria produce factors which cause ciliary slowing, dyskinesis, and stasis (23). Therefore, the rapidity of movement of these pathogens may be critical in preventing concentrated release of such factors, and subsequent penetration of the mucociliary barrier and bacterial colonization.

Other factors, besides the direct effect of mucosal temperature, may compound the effect of cold exposure on tracheal mucociliary clearance. The wide variety of responses obtained from the sheep tracheae is evidence of this. It appears that the sheep tracheae responses may be

divided into two groups (Figure 5.2). Tracheal clearance rates for tracheae 1, 2, 6, 8, and 10 seem to be depressed compared to tracheae 3, 4, 5, 7, and 9. The sheep used for this experiment were not controlled for age, pretest temperature, or possible airway and respiratory infection. Age has been shown to be a critical factor in determining mucociliary clearance in dogs (22) and sheep (19). Tracheae were obtained from these animals immediately after they were brought indoors from a variable and sometimes harsh outdoor environment. Calves, however, were maintained in a controlled indoor environment and all were young (2-8 week old) animals. Calf tracheal clearance rates can be divided into high and low clearance groups, with lower rates measured for tracheae 1, 2, and 6, compared to 3, 4, 5, and 7. Figure 5.4 demonstrates this difference between calf tracheae by comparison of the mean of TMV measurements between 37 and 39°C. This dichotomy cannot be explained and may be due to some intrinsic characteristic of the individual calf. Wong and Yeates (24) obtained results suggesting that the temporal relationship in the response of ciliary beat frequency to surgical trauma may be genetically determined. Perhaps, the individual variability in mucociliary clearance rate observed in this study is genetically determined. Should this be true, resistance to respiratory and airway infections may be increased by selective breeding of animals with high mucociliary clearance rates.

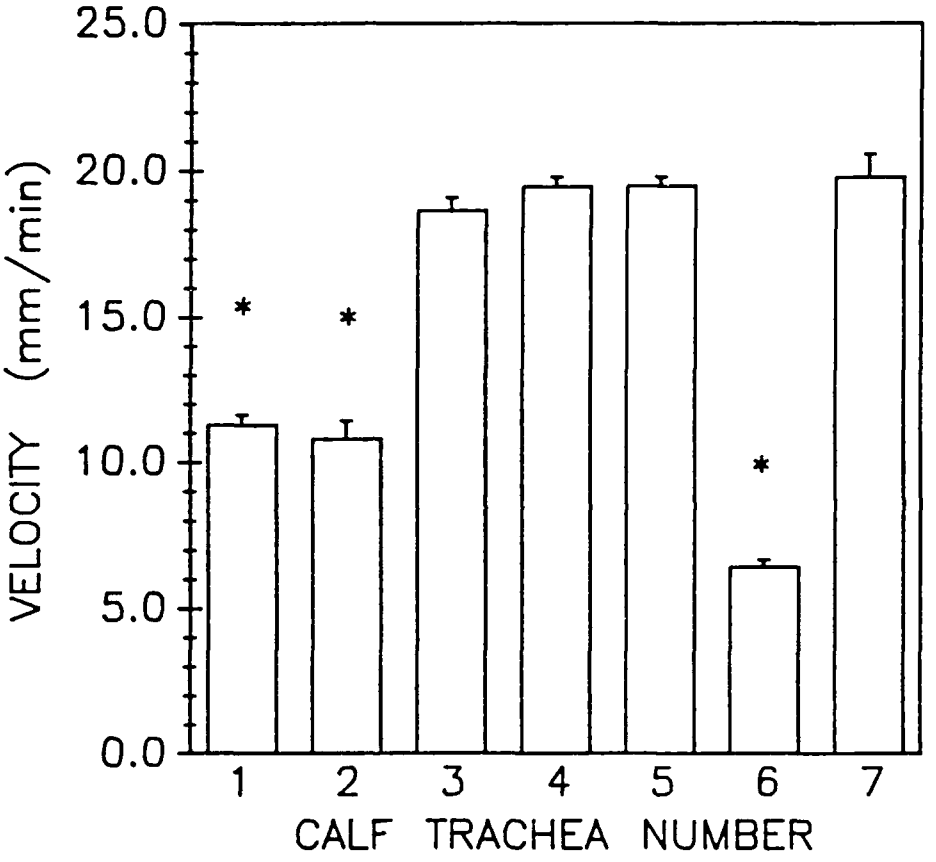


Figure 5.4 Comparison of tracheal mucus velocity for seven calves at mucosal temperature of 37-39°C. Values are means ± SE (* $P<0.05$ compared to calves 3, 4, 5, and 7).

Conclusions

Nasal and tracheal mucociliary clearance is quite variable between individual animals, which may, in part, explain why some animals in an otherwise homogeneous group are more susceptible to respiratory infections than others. In addition, nasal mucociliary clearance is significantly depressed by cold exposure (2-4°C). This decrease in mucociliary clearance *in vivo* is probably due to a combination of direct temperature effects and autonomic reflexes. Tracheal mucociliary clearance *in vitro* is also depressed by decreasing mucosal temperature. During extreme cold exposure, conditioning of inspired air is not complete at the tracheal level, therefore, tracheal mucociliary clearance rate may be decreased during cold exposure in the intact animal. It is postulated that slowing of tracheal and nasal clearance mechanisms increases the opportunity for pathogen colonization of these upper airways, as well as increasing the opportunity of aspiration of these same pathogens deep into the lung.

REFERENCES - CHAPTER V

1. Ahmed, T., A.J. Januszkiewicz, J.F. Landa, A. Brown, G.A. Chapman, P.J. Kenny, R.D. Finn, J. Bondick and M.A. Sackner. Effect of local radioactivity on tracheal mucous velocity of sheep. *Am. Rev. Respir. Dis.* 120:567-575, 1979.

2. Andersen, I., G.R. Lundqvist and D.F. Proctor. Human nasal mucosal function in a controlled environment. *Arch. Envir. Health* 23:408-420, 1971.

3. Baetjer, A.M. Effect of ambient temperature and vapor pressure on cilia-mucus clearance rate. *J. Appl. Physiol.* 23:498-504, 1967.

4. Bohning, D.E., R.E. Alpert, M. Lippman and V.R. Cohen. Effect of pretest temperature on aerosol penetration and clearance in donkeys. *J. Appl. Physiol.* 41:920-924, 1976.

5. Brain, J.D. and P.A. Valberg. Deposition of aerosol in the respiratory tract. *Am. Rev. Respir. Dis.* 120:1325-1373, 1979.

6. Brody, J.S., J.A. Waldhausen and D.E. Kuhl. Mucociliary clearance in denervated bronchi. *Clin. Res.* 19:507, 1971. (Abst.)

7. Chopra, S.K. Effect of atropine on mucociliary transport velocity in anesthetized dogs. *Am. Rev. Respir. Dis.* 118:367-371, 1978.

8. Del Donno, M., D. Pavia, J.E. Agnew, M.T. Lopez-Vidriero and S.W. Clarke. Variability and reproducibility in the measurement of tracheobronchial clearance in healthy subjects and patients with different obstructive lung diseases. *Eur. Respir. J.* 1:613-620, 1988.

9. Gallagher, J.T., P.W. Kent, M. Passatore, R.J. Phipps and P.S. Richardson. The composition of tracheal mucus and the nervous control of its secretion in the cat. *Proc. Roy. Soc. London B.* 192:49-76, 1975.

10. Hermens, W.A.J.J. and F.W.H.M. Merkus. The influence of drugs on nasal ciliary movement. *Pharm. Res.* 4:445-449, 1987.

11. Hill, L. The ciliary movement of the trachea studied *in vitro*. *Lancet* 215:802-805, 1928.

12. Jensen, R., R.E. Pierson, P.M. Braddy, D.A. Saari, L.H. Lauerman, J.J. England, H. Keyvanfar, J.R. Collier, D.P. Horton, A.E. McChesney, A. Benitez and R.M. Christie. Shipping fever pneumonia in yearling feedlot cattle. *JAVMA* 169:500-506, 1976.

13. Kensler, C.J. and S.P. Battista. Chemical and physical factors affecting mammalian ciliary activity. *Am. Rev. Respir. Dis.* 93:93-102, 1965.

14. Marin, M.G., B. Davis and J.A. Nadel. Effect of acetylcholine on Cl^-, and Na^+ fluxes across dog tracheal epithelium *in vitro*. *Am. J. Physiol.* 231:1546-1549, 1976.

15. McFadden, E.R., Jr., D.M. Dennison, J.F. Waller, B. Assoufi, A. Peacock and T. Sopwith. Direct recordings of the temperatures in the tracheobronchial tree in normal man. *J. Clin. Invest.* 69:700-705, 1982.

16. McFadden, E.R., Jr., B.M. Pichurko, H.F. Bowman, E. Ingenito, S. Barns, N. Dowling and J. Solway. Thermal mapping of the airways in humans. *J. Appl. Physiol.* 58:564-570, 1985.

17. Nadel, J.A., B. Davis and R.J. Phipps. Control of mucus secretion and ion transport in airways. *Ann. Rev. Physiol.* 41:369-381, 1979.

18. Nadel, J.A. and B. Davis. Parasympathetic and sympathetic regulation of secretion from submucosal glands in airways. *Fed. Proc.* 39:3075-3079, 1980.

19. Phipps, R.J., W.M. Abraham, A.T. Mariassy, P.J. Torrealba, M.W. Sielezak, A. Ahmed, M. McCray, J.S. Stevenson and A. Wanner. Developmental changes in the tracheal mucociliary system in neonatal sheep. *J. Appl. Physiol.* 67:824-832, 1989.

20. Proctor, D.F., I.B. Andersen and G.R. Lundqvist. Human nasal mucosal function at controlled temperatures. *Respir. Physiol.* 30:109-124, 1977.

21. Puchelle, E., F. Aug, J.M. Zahm and A. Bertrand. Comparison of nasal and bronchial mucociliary clearance in young non-smokers. *Clin. Sci.* 62:13-16, 1982.

22. Whaley, S.L., B.A. Muggenburg, F.A. Seiler and R.K. Wolff. Effect of aging on tracheal mucociliary clearance in beagle dogs. *J. Appl. Physiol.* 62:1331-1334, 1987.

23. Wilson, R. and P.J. Cole. The effect of bacterial products on ciliary function. *Am. Rev. Respir. Dis.* 138:S49-S53, 1988.

24. Wong, L.B. and D.B. Yeates. Stimulation of tracheal ciliary beat frequency by local tissue incision. *J. Appl. Physiol.* 68:411-416, 1990.

25. Yeates, D.B., N. Aspin, H. Levison, M.T. Jones and A.C. Bryan. Mucociliary tracheal transport rate in man. *J. Appl. Physiol.* 39:487-495, 1975.

26. Yergin, B.M., K. Saketkhoo, E.D. Michealson, S.M. Serafini, K. Kovitz and M.A. Sackner. A roentgenographic method for measuring nasal mucus velocity. *J. Appl. Physiol.* 44:964-968, 1978.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Cold exposure in calves results in changes in respiratory pattern which appear to provide an adaptive benefit in reducing respiratory heat loss. Over the range of temperatures studied (4-18°C), respiratory frequency decreased 29% and tidal volume increased 35%, while total ventilation did not change. Oxygen extraction increased by 34% and the respiratory ratio ($\dot{V}_E/\dot{V}_{O2}$) decreased by 26% with decreasing temperature. This cold-induced change in respiratory pattern prevents an increase in respiratory heat loss despite a 28% increase in oxygen consumption. Therefore, the proportion of metabolic rate devoted to respiratory heat loss is reduced significantly at cold temperatures. Just as panting is a mechanism by which heat loss may be increased during heat exposure, a reduced respiratory frequency is a mechanism to reduce heat loss during cold exposures, a mechanism by which body heat is conserved.

The respiratory pattern changes caused by cold exposure may be detrimental under certain circumstances. This study showed an increase in pulmonary particle deposition during cold exposure of calves. When airborne dust and pathogen concentrations are high, such as in the feedlot (4), this increase in particle deposition may overwhelm the lung defense mechanisms. This may be the case particularly for apical and mediastinal lobes which typically receive the largest burden of deposited particles.

The phenomenon of preferential deposition in apical lobes has been observed previously in other animals. The mechanism by which deposition is increased in this region is not understood, but is probably not the result of gravity dependent factors.

The slight hypoxemia, which results from cold-induced respiratory changes, may also become detrimental under certain circumstances. For example, the combination of cold and altitude may increase pulmonary vascular resistance and result in high mountain disease (2,9). Pulmonary edema fluid, possibly associated with hypoxic pulmonary vasoconstriction, would provide an excellent medium for bacterial growth and colonization once deposited in the lung. Hypoxemia has also been shown to increase tracheal sub-mucosal gland secretion (3) and therefore probably affects airway defense mechanisms.

The hypoventilatory response to cold exposure is due to stimulation of primarily cutaneous, but also upper airway, thermoreceptors. Exposure of the body, but not the airway, to cold air resulted in respiratory frequency and tidal volume values similar to cold exposure of the entire animal. Inspiration of cold air in a warm environment also decreased respiratory frequency and increased tidal volume, but not to the same extent as whole body cold exposure. Topical lidocaine application during cold air inspiration prevented the ventilatory pattern change, indicating that hypoventilation resulted from airway thermoreceptor stimulation, rather than stimulation of the hypothalamus. Cooling of the pre-optic area of the hypothalamus did not influence total ventilation, respiratory frequency, or tidal volume. It is speculated that results of this study may find application in the treatment of fever, as a symptom of respiratory infection. Rather than simply a symptom, fever has been proposed as

a mechanism by which body temperature is elevated to combat infection (6). Perhaps, in the context of this study, the ventilatory pattern is returned to thermoneutral parameters by elevated body temperature, thereby eliminating the detrimental effects of hypoventilation.

While increasing deposition, cold exposure may simultaneously depress mucociliary clearance of the nasal and tracheal airways, as shown in this study, and perhaps decrease clearance from lower conducting airways (1) as well as the pulmonary region of the lung (5). Such a decreased clearance may be more detrimental to certain animals, in light of the inherent individual variability in mucociliary clearance rates between animals. Young animals, which typically have lower mucociliary clearance rates than adults (7,8), may be particularly vulnerable to bacterial colonization of airways following cold-induced depression of mucociliary clearance. Mucociliary clearance rates varied significantly between individual animals, for unknown reasons. If this variance has a genetic component, selective breeding may be a means of enhancing resistance to airway and respiratory infection.

In conclusion, although cold-induced respiratory pattern changes may be beneficial in reducing respiratory heat loss, the resulting hypoxemia, increased pulmonary particle deposition, and depressed mucociliary clearance may be detrimental, particularly for young animals in crowded settings where pathogen exposure is high.

REFERENCES - CHAPTER VI

1. Bohning, D.E., R.E. Alpert, M. Lippman and V.R. Cohen. Effect of pretest temperature on aerosol penetration and clearance in donkeys. *J. Appl. Physiol.* 41:920-924, 1976.

2. Busch, M.A., A. Tucker and D. Robertshaw. Interaction between cold and altitude exposure on pulmonary circulation of cattle. *J. Appl. Physiol.* 58:948-953, 1985.

3. Davis, B., R. Chinn, J. Gold, D. Popovac, J.D. Widdicombe and J.A. Nadel. Hypoxemia reflexly increases secretion from tracheal submucosal glands in dogs. *J. Appl. Physiol.* 52:1416-1419, 1982.

4. Franzen, D.K. Airborne particle concentration associated with pneumonia incidence in feedlot cattle. Master's Thesis, Colo. State Univ., Ft. Collins, 1984.

5. Green, G.M. and E.H. Kass. The influence of bacterial species in pulmonary resistance to infection in mice subjected to hypoxia, cold stress, and ethanolic intoxication. *Brit. J. Exper. Pathol.* 46:360-366, 1965.

6. Kluger, M.J., D.H. Ringler and M.R. Anver. Fever and survival. *Science* 188:166-168, 1975.

7. Phipps, R.J., W.M. Abraham, A.T. Marriassy, P.J. Torrealba, M.W. Sielezak, A. Ahmed, M. McCray, J.S. Stevenson and A. Wanner. Developmental changes in the tracheal mucociliary system in neonatal sheep. *J. Appl. Physiol.* 67:824-832, 1989.

8. Whaley, S.L., B.A. Muggenburg, F.A. Seiler and R.K. Wolff. Effect of aging on tracheal mucociliary clearance in beagle dogs. *J. Appl. Physiol.* 62:1331-1334, 1987.

9. Will, D.H., I.F. McMurtry, J.T. Reeves and R.F. Grover. Cold-induced pulmonary hypertension in cattle. *J. Appl. Physiol.* 45:469-473, 1978.

APPENDICES

APPENDIX A

CALCULATION OF DEAD SPACE VOLUME (ml/kg)

$$V_D = V_T * (F_{ETCO2} - F_{E\ CO2})/(F_{ETCO2} - F_{I\ CO2}) - \text{correction}$$

where,

V_T	=	tidal volume (BTPS)
F_{ETCO2}	=	end-tidal CO_2 fraction
$F_{E\ CO2}$	=	mean expired CO_2 fraction
$F_{I\ CO2}$	=	ambient inspired CO_2 fraction
correction	=	mask dead space (BTPS) = 87ml (ATPS)

APPENDIX B

CALCULATION OF TOTAL VENTILATION, O_2 CONSUMPTION, AND CO_2 PRODUCTION (ml/min-kg)

I. $\dot{V}_E(BTPS) = \dot{V}_I * ratio * [(T_b + 273)/(T_a + 273)] * [(P_B - P_{H2O})/(P_B - P_{Tb})]$

where, $\dot{V}_I$ = total inspiratory ventilation (ml/min-kg) ATPS

T_b = body temperature (°C)

T_a = ambient air temperature (°C)

P_B = barometric pressure (Torr)

P_{H2O} = ambient water vapor pressure (Torr)

P_{Tb} = water vapor pressure at body temperature (Torr)

$ratio = [1 - (F_{IO2} + F_{ICO2})]/[1 - (F_{EO2} + F_{ECO2})]$

where, F_{IO2} = ambient O_2 fraction

F_{ICO2} = ambient CO_2 fraction

F_{EO2} = mean expired O_2 fraction

F_{ECO2} = mean expired CO_2 fraction

II. $\dot{V}_{O2}(STPD) = (\dot{V}_I(STPD) * F_{IO2}) - (\dot{V}_E(STPD) * F_{EO2})$

where, $\dot{V}_I(STPD) = \dot{V}_I(ATPS) * [273/(273 + T_a)] * [(\ - P_{H2O})/760]$

$\dot{V}_E(STPD) = \dot{V}_I(STPD) * ratio$

III. $\dot{V}_{CO2}(STPD) = (\dot{V}_E(STPD) * F_{ECO2}) - (\dot{V}_I(STPD) * F_{ICO2})$

APPENDIX C

CALCULATION OF RESPIRATORY HEAT LOSS (watts/kg)

$$RHL = CHL + EHL$$

$$CHL = \dot{V}_E(STPD) * [cp * p(T_E - T_I)]/60$$

$$EHL = \dot{V}_E(ATPS) * [(T_E+273)/(T_I+273) * (P_B-P_{H2O})/(P_B-P_E)] * [l * (w_E-w_I)]/60$$

$$w_I = P_{H2O}/[r * (T_I + 273)] * mw$$

$$w_E = P_E/[r * (T_E + 273)] * mw$$

where,

- $\dot{V}_E$ = total expiratory ventilation
- cp = specific heat (J/g/°C)
- p = density (g/ml)
- l = latent heat of vaporization (J/g)
- w_I = inspired water (g)
- w_E = expired water (g)
- P_B = barometric pressure (Torr)
- P_{H2O} = ambient water vapor pressure (Torr)
- P_E = expired air water vapor pressure (Torr)
- T_E = expired air temperature
- T_I = inspired air temperature
- r = ideal gas constant (ml-Torr/mol-°K)
- mw = molecular weight of H_2O (g/mol)

APPENDIX D

EXPERIMENT #1 -INDIVIDUAL CALF DATA

Calf #119

T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$	V_T	V_D
17.8	60	9.17	39.6	34.3	17.55	20.9	143.0	8.15	3.40
16.6	71	10.06	39.4	32.6	18.37	24.1	150.8	8.21	3.18
12.9	63	7.03	39.6	32.7	15.22	20.3	128.[illegible]	8.43	3.08
13.0	76	8.54	39.7	33.2	10.91	19.1	110.7	10.15	2.97
9.1	59	5.11	39.6	32.3	13.95	17.8	117.4	8.42	2.87
9.1	82	7.10	39.7	32.5	12.38	18.9	122.9	9.93	3.03
5.0	78	5.10	39.3	31.7	15.08	23.2	178.5	11.84	4.33
6.0	90	6.31	39.6	32.5	15.19	23.7	162.7	10.71	3.75

$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
4.59	3.85	.838	.006	.047	.053	1.55	3.4	29.5
4.60	4.17	.905	.016	.065	.081	1.58	5.1	28.4
4.42	3.85	.870	.014	.048	.062	1.51	4.1	31.9
4.65	3.79	.814	.010	.030	.040	1.56	2.6	38.4
4.32	3.68	.852	.015	.041	.056	1.47	3.8	33.9
4.87	4.20	.862	.017	.039	.056	1.66	3.4	36.6
7.62	6.34	.833	.047	.101	.148	2.57	5.7	39.0
6.26	5.64	.901	.042	.088	.130	2.15	6.0	35.8

T_a, T_b, T_{sk}, T_E = °C; RH, RHL/MR = %; P_{H2O}, O_2extr = Torr; f = b/min; $\dot{V}_E$, $\dot{V}_{O2}$, $\dot{V}_{CO2}$ = ml/min-kg; V_T, V_D = ml/kg; CHL, EHL, RHL, MR = W/kg.

Appendix D Cont'd

Calf #658

T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$	V_T	V_D
16.4	67	9.98	39.6	34.6	22.93	26.4	201.4	8.78	4.41
17.4	54	8.05	39.6	35.6	20.50	27.0	203.6	9.93	4.01
13.2	58	6.60	39.7	33.2	18.97	23.8	132.0	6.96	2.74
13.0	64	7.19	39.8	34.1	18.23	19.6	146.4	8.03	2.89
9.3	63	5.54	39.7	32.4	16.11	20.4	148.5	9.22	3.60
8.9	66	5.64	39.8	33.7	16.35	17.9	133.3	8.15	3.21
5.0	78	5.10	39.3	31.7	15.08	23.2	178.5	11.84	4.33
6.0	90	6.31	39.6	32.5	15.19	23.7	162.7	10.71	3.75

$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
5.62	4.75	.846	.029	.111	.140	1.90	7.4	25.7
6.07	5.62	.926	.028	.134	.162	2.09	7.8	27.9
3.92	3.36	.858	.020	.071	.092	1.33	6.9	27.4
5.72	5.06	.884	.014	.050	.064	1.96	3.3	36.3
5.35	4.56	.853	.024	.064	.088	1.81	3.4	33.2
5.16	4.39	.850	.017	.045	.062	1.75	3.5	35.7
7.62	6.34	.833	.047	.101	.148	2.57	5.7	39.0
6.26	5.64	.901	.042	.088	.130	2.15	6.0	35.8

Appendix D Cont'd

Calf #466

T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$	V_T	V_D
17.3	56	8.29	39.5	33.9	19.98	25.9	199.3	9.97	4.82
17.3	55	8.14	39.6	34.5	14.44	21.1	166.4	11.52	4.64
13.0	53	5.95	39.5	32.6	17.91	23.8	184.3	10.29	4.26
13.4	67	7.72	39.7	32.9	12.83	23.1	149.3	11.64	3.33
9.0	60	5.16	39.3	31.7	16.21	22.3	169.4	10.45	4.03
9.0	78	6.72	39.6	31.7	12.93	20.3	166.6	12.88	3.93
2.7	84	4.67	39.3	31.0	10.77	16.7	168.8	15.67	4.75
4.5	84	5.31	39.5	30.8	12.58	16.1	198.8	15.80	4.52

$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
6.22	5.07	.814	.025	.117	.142	2.09	6.8	28.6
5.84	4.89	.837	.009	.062	.071	1.98	3.6	32.3
6.31	5.17	.819	.029	.104	.133	2.12	6.3	31.4
6.63	5.67	.856	.021	.070	.090	2.25	4.0	41.0
6.10	4.95	.811	.033	.089	.121	2.05	5.9	32.8
7.02	6.17	.880	.027	.064	.091	2.40	3.8	39.0
7.56	6.34	.838	.034	.055	.090	2.56	3.5	41.1
7.98	7.36	.922	.033	.057	.090	2.75	3.3	37.5

Appendix D Cont'd

Calf #220

T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$	V_T	V_D
16.9	48	6.93	39.2	34.0	14.22	18.6	139.6	9.82	3.44
17.4	56	8.34	39.8	35.2	13.31	27.4	123.5	9.28	3.02
13.2	52	5.92	40.0	34.7	10.02	21.7	131.9	13.16	3.94
13.1	67	6.93	40.2	33.9	10.76	26.1	139.7	12.98	4.12
8.7	58	4.89	40.1	34.8	10.42	20.3	130.5	12.53	3.76
9.0	74	6.37	40.2	33.1	10.63	24.0	149.4	14.06	4.08
5.0	81	5.30	39.1	32.0	9.24	18.1	115.2	12.46	2.63
3.9	79	4.78	39.9	31.0	11.15	17.3	144.6	12.96	4.42

$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
5.55	4.39	.791	.003	.045	.048	1.86	2.6	36.0
5.05	4.42	.875	.018	.083	.100	1.72	5.8	37.9
5.96	4.80	.806	.016	.062	.078	2.00	3.9	41.2
6.29	5.13	.815	.026	.090	.116	2.11	5.5	41.4
5.92	4.19	.814	.022	.058	.080	1.99	4.0	41.5
7.22	5.97	.827	.032	.083	.115	2.44	4.7	44.5
6.54	5.43	.831	.022	.041	.062	2.21	2.8	51.8
7.17	5.54	.778	.028	.049	.078	2.37	3.3	44.7

APPENDIX E

EXPERIMENT #1 - BLOOD GAS ANALYSIS

CALF#	T_a(°C)	Pa_{O_2}(Torr)	Pa_{CO_2}(Torr)	pH_a	Hct(%)
658	17.4	84.4	44.0	7.386	29
		84.9	45.9	7.370	28
		85.7	44.2	7.386	27
	13.0	70.8	53.2	7.346	22
		70.7	52.3	7.343	21
		73.0	51.7	7.358	22
	8.9	62.1	53.5	7.369	23
		63.0	53.1	7.375	22
		64.7	53.2	7.378	22
	6.0	74.6	49.8	7.353	24
		76.0	51.7	7.334	23
		75.0	51.1	7.348	24
466	17.3	73.9	45.6	7.365	39
		74.5	46.5	7.365	40
		71.6	48.1	7.362	40
	13.4	60.6	52.4	7.375	41
		68.4	47.8	7.409	40
		63.5	51.4	7.391	40
	9.0	78.6	47.6	7.380	40
		59.8	53.0	7.353	40
		66.2	49.8	7.382	41
	2.7	60.4	53.3	7.357	40
		68.8	49.1	7.391	40
		67.4	48.8	7.390	41
220	17.4	70.0	51.0	7.344	32
		69.4	51.0	7.358	32
		72.6	49.0	7.376	32
	13.2	69.3	47.7	7.366	37
		64.2	48.8	7.357	37
		62.8	49.1	7.359	37
	8.7	68.1	47.8	7.342	37
		58.8	51.0	7.332	37
		64.5	49.3	7.344	37
	3.9	59.7	55.4	7.371	36
		56.1	54.8	7.372	36
		57.4	53.8	7.380	36

APPENDIX F

EXPERIMENT #2 - INDIVIDUAL CALF DATA

Calf #685

Trt	T_I	T_a	RH	P_{H2O}	T_b	T_{ac}	f	T_E	$\dot{V}_E$
1	17.7	17.7	68	10.33	38.9	34.5	21.43	28.0	191.5
1	17.5	17.5	64	9.06	39.0	34.4	22.25	26.4	208.1
2	3.8	16.6	85	5.11	39.4	36.2	18.34	23.1	151.5
2	4.8	17.7	75	4.84	39.3	30.1	18.48	22.0	175.7
3	17.5	4.3	35	5.25	38.7	31.2	14.25	21.6	163.4
3	15.6	4.6	43	5.71	38.2	31.6	11.75	19.6	146.4
4	3.9	3.9	85	5.15	38.0	29.6	12.58	16.5	151.6
4	4.8	4.8	86	5.55	38.7	24.5	11.96	18.2	145.0
5	4.4	15.8	85	5.33	39.3	31.2	21.88	13.8	182.8
5	4.2	16.8	85	5.26	39.4	36.5	21.61	24.8	176.1

V_T	V_D	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
8.94	3.72	6.20	5.75	.929	.029	.122	.150	2.14	7.0	30.1
9.35	4.15	6.80	6.25	.919	.027	.119	.145	2.35	6.2	30.4
8.26	2.90	5.34	4.45	.832	.043	.084	.127	1.80	7.0	32.3
9.51	3.57	5.98	5.47	.915	.044	.091	.135	2.06	5.1	31.7
11.47	3.30	6.62	5.65	.853	.010	.081	.091	2.25	4.0	37.1
12.46	3.74	7.54	5.91	.784	.009	.059	.068	2.51	2.7	46.2
12.05	3.44	6.80	5.90	.867	.028	.047	.075	2.32	3.2	40.9
12.13	2.96	7.15	6.00	.839	.028	.050	.079	2.42	3.3	44.9
8.36	2.85	6.69	4.87	.728	.025	.040	.065	2.20	3.0	32.6
8.15	2.30	5.93	4.82	.814	.053	.112	.165	1.99	8.3	30.7

See Appendix D for units. T_I = °C.

Appendix F Cont'd

Calf #155

Trt	T_I	T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$
1	16.8	16.8	66	9.47	39.4	34.0	18.30	20.7	171.5
1	15.0	15.0	57	7.29	39.3	32.4	18.18	20.0	179.7
2	4.1	17.4	80	4.92	40.0	34.4	15.96	16.8	182.3
2	3.2	17.8	89	5.13	40.0	36.2	15.70	14.2	174.8
3	15.8	4.3	40	5.38	39.4	29.4	13.05	20.6	151.7
3	13.3	4.3	44	5.04	39.2	29.0	11.56	18.5	158.8
4	5.2	5.2	81	5.37	39.1	29.8	12.92	13.0	173.2
4	3.8	3.8	86	5.17	39.4	28.2	11.23	16.9	150.2
5	2.7	17.6	76	4.22	40.0	34.0	15.50	15.8	170.6
5	4.8	18.1	91	5.87	40.4	35.1	19.80	25.9	168.9

V_T	V_D	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
9.37	3.76	5.43	4.50	.830	.010	.053	.062	1.83	3.4	28.9
9.88	4.14	6.15	4.90	.797	.013	.064	.077	2.06	3.7	31.0
11.42	5.09	6.09	5.02	.824	.033	.058	.092	2.05	4.5	30.6
11.13	3.86	6.46	5.41	.838	.028	.041	.069	2.19	3.2	34.1
11.62	4.35	6.79	5.16	.761	.011	.068	.079	2.25	3.5	40.4
13.74	4.29	7.06	5.89	.834	.012	.061	.073	2.38	3.1	40.6
13.40	4.11	7.91	6.21	.785	.020	.035	.054	2.64	2.0	41.3
13.38	2.64	7.16	6.00	.837	.029	.048	.076	2.42	3.1	43.8
11.01	4.86	6.10	4.77	.782	.032	.054	.086	2.03	4.2	32.5
8.53	3.78	5.34	5.28	.987	.051	.112	.163	1.88	8.7	30.2

Appendix F Cont'd

Calf #119

Trt	T_I	T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$
1	17.8	17.8	60	9.17	39.6	34.3	17.55	20.9	142.9
1	16.6	16.6	71	10.06	39.4	32.6	18.37	24.1	150.8
2	3.1	17.4	88	5.04	40.0	35.1	13.85	16.3	148.6
2	3.1	18.5	87	4.98	39.7	32.2	18.85	16.7	147.3
3	14.2	3.2	46	5.59	39.8	32.4	15.33	22.2	150.8
3	13.5	4.2	50	5.80	39.8	32.7	13.99	21.4	169.2
4	4.8	4.8	92	5.94	40.0	33.2	14.32	18.6	136.8
4	5.9	5.9	89	6.20	39.5	31.9	11.51	16.7	150.5
5	3.9	17.3	84	5.12	39.5	30.4	22.13	22.0	132.8
5	3.8	17.5	88	5.29	39.7	31.6	20.95	18.9	132.3

V_T	V_D	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
8.15	3.40	4.59	3.85	.838	.006	.047	.053	1.55	3.4	29.5
8.21	3.18	4.60	4.17	.905	.016	.065	.081	1.58	5.1	28.4
10.73	3.47	5.48	4.52	.826	.028	.045	.073	1.84	4.0	33.8
7.81	3.25	4.96	4.15	.837	.029	.046	.075	1.68	4.5	30.9
9.84	3.45	6.04	4.95	.820	.017	.076	.093	2.03	4.6	36.7
12.10	4.45	7.46	5.98	.802	.019	.079	.098	2.50	3.9	40.1
9.56	2.81	5.75	4.52	.785	.027	.047	.074	1.92	3.8	38.3
13.08	3.22	6.84	5.68	.830	.024	.041	.065	2.31	2.8	41.7
6.00	2.10	4.16	3.53	.847	.035	.067	.102	1.41	7.2	28.9
6.32	2.40	4.53	3.82	.843	.029	.050	.079	1.53	5.2	31.5

Appendix F Cont'd

Calf #658

Trt	T_I	T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$
1	16.4	16.4	67	9.98	39.6	34.6	22.93	26.4	201.4
1	17.4	17.4	54	8.05	39.6	35.6	20.50	27.0	203.6
2	6.4	18.2	71	5.12	39.4	34.8	15.20	24.1	148.0
2	5.0	17.4	87	5.69	39.8	35.8	14.83	23.7	170.4
3	13.8	5.6	40	4.73	39.3	31.6	17.56	24.7	203.0
3	18.6	5.0	39	6.27	39.8	32.2	17.54	26.4	151.8
4	5.0	5.0	78	5.10	39.3	31.7	15.08	23.2	178.5
4	6.0	6.0	90	6.31	39.6	32.5	15.19	23.7	162.7
5	6.4	17.6	75	5.41	39.5	34.9	15.64	19.5	150.2
5	5.1	17.8	86	5.67	39.8	35.1	22.30	19.5	172.6

V_T	V_D	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
8.78	4.41	5.62	4.75	.846	.029	.111	.140	1.90	7.4	25.7
9.93	4.01	6.07	5.62	.926	.028	.134	.162	2.09	7.8	27.9
9.74	4.15	5.01	4.12	.823	.038	.090	.128	1.69	7.6	31.0
11.49	3.91	5.65	5.15	.912	.046	.096	.142	1.95	7.3	30.9
11.56	3.75	8.27	7.18	.867	.032	.133	.165	2.82	5.8	37.6
8.66	2.76	6.12	5.30	.866	.017	.105	.122	2.07	5.9	37.3
11.84	4.33	7.62	6.34	.833	.047	.101	.148	2.57	5.7	39.0
10.71	3.75	6.26	5.64	.901	.042	.088	.130	2.15	6.0	35.8
9.61	4.01	5.41	4.33	.801	.029	.060	.089	1.81	4.9	32.8
7.74	3.29	5.82	5.19	.892	.036	.067	.103	2.00	5.2	31.4

Appendix F Cont'd

Calf #466

Trt	T_I	T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$
1	17.3	17.3	56	8.29	39.5	33.9	19.98	25.9	199.3
1	17.3	17.3	55	8.14	39.6	34.5	14.44	21.1	166.4
2	4.2	16.9	75	6.54	38.9	34.6	16.28	20.9	196.7
2	4.2	16.8	81	5.05	39.5	33.8	12.01	17.4	154.2
3	17.2	4.2	30	4.41	38.9	28.6	13.25	20.2	205.2
3	18.3	3.8	30	4.73	39.2	31.2	11.07	20.2	167.3
4	2.7	2.7	84	4.67	39.3	31.0	10.77	16.7	168.8
4	4.5	4.5	84	5.31	39.5	30.8	12.58	16.1	198.8
5	4.1	17.2	78	4.79	39.1	34.2	17.81	20.3	184.8
5	4.4	17.2	87	5.38	39.6	33.1	13.96	19.8	157.7

V_T	V_D	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
9.97	4.82	6.22	5.07	.814	.025	.117	.142	2.09	6.8	28.6
11.52	4.64	5.84	4.89	.837	.009	.062	.071	1.98	3.6	32.3
12.08	4.42	6.80	5.46	.802	.048	.081	.129	2.28	5.7	31.3
12.84	4.44	6.06	4.84	.799	.030	.052	.082	2.03	4.0	35.7
15.48	5.03	8.44	7.08	.839	.009	.096	.105	2.85	3.7	37.6
15.12	4.85	7.19	5.94	.826	.005	.077	.081	2.42	3.3	39.2
15.67	4.75	7.56	6.34	.838	.034	.055	.090	2.56	3.5	41.1
15.80	4.52	7.98	7.36	.922	.033	.057	.090	2.75	3.3	37.5
10.38	3.25	6.58	5.10	.776	.043	.083	.127	2.19	5.8	32.1
11.30	4.16	5.92	4.70	.795	.036	.065	.101	1.98	5.1	34.1

Appendix F Cont'd

Calf #220

Trt	T_I	T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$
1	16.9	16.9	48	6.93	39.2	34.0	14.22	18.6	139.6
1	17.4	17.4	56	8.34	39.8	35.2	13.31	27.4	123.5
2	4.7	17.7	73	4.68	39.3	33.2	12.10	17.1	139.3
2	4.0	16.9	76	4.64	39.6	35.7	11.52	23.8	129.0
3	17.3	4.5	36	5.33	39.8	30.4	11.90	22.0	161.6
3	17.2	4.0	30	4.41	39.5	33.2	15.23	20.9	183.2
4	5.0	5.0	81	5.30	39.1	32.0	9.24	18.1	115.2
4	3.9	3.9	79	4.78	39.9	31.0	11.15	17.3	144.6
5	4.3	16.8	73	4.55	39.5	33.7	13.05	22.3	135.5
5	4.4	16.9	76	4.77	39.6	35.6	13.98	25.2	130.3

V_T	V_D	$\dot{V}_{O2}$	$\dot{V}_{CO2}$	RER	CHL	EHL	RHL	MR	RHL/MR	O_2extr
9.82	3.44	5.55	4.39	.791	.003	.045	.048	1.86	2.6	36.0
9.28	3.02	5.05	4.42	.875	.018	.083	.100	1.72	5.8	37.9
11.51	4.04	5.82	4.57	.785	.025	.048	.073	1.94	3.8	37.8
11.20	3.48	5.55	4.69	.846	.037	.078	.115	1.88	6.1	39.5
13.58	4.54	7.56	5.98	.791	.011	.082	.093	2.53	3.7	42.6
12.03	3.87	8.39	7.68	.916	.010	.091	.101	2.89	3.5	42.7
12.46	2.63	6.54	5.43	.831	.022	.041	.062	2.21	2.8	51.8
12.96	4.42	7.12	5.54	.778	.028	.049	.078	2.37	3.3	44.7
10.38	4.06	5.69	4.49	.789	.036	.062	.098	1.90	5.2	38.1
9.32	3.33	5.28	4.47	.846	.039	.088	.127	1.79	7.1	37.3

APPENDIX G

EXPERIMENT #2 - BLOOD GAS ANALYSIS

CALF#	Trt #	Pa_{O_2}(Torr)	Pa_{CO_2}(Torr)	pH_a	Hct(%)
658	1	84.4	44.0	7.386	29
		84.9	45.9	7.370	28
		85.7	44.2	7.386	27
	2	85.7	42.4	7.357	28
		93.2	43.9	7.374	28
		87.4	45.9	7.367	28
	3	65.5	50.1	7.360	23
		60.5	51.8	7.359	24
		86.3	47.5	7.391	24
	4	74.6	49.8	7.353	24
		76.0	51.7	7.334	23
		75.0	51.1	7.348	24
	5	64.5	52.9	7.372	23
		70.6	49.8	7.382	22
		64.8	53.0	7.384	22
466	1	73.9	45.6	7.365	39
		74.5	46.5	7.365	40
		71.6	48.1	7.362	40
	2	67.6	46.9	7.374	40
		79.6	45.2	7.385	40
		79.7	44.2	7.395	41
	3	67.1	49.1	7.382	39
		71.6	47.9	7.401	40
		63.7	48.9	7.396	39
	4	60.4	53.3	7.357	40
		68.8	49.1	7.391	40
		67.4	48.8	7.390	41
	5	69.9	46.5	7.371	41
		75.9	44.4	7.384	41
220	1	70.0	51.0	7.344	32
		69.4	51.0	7.358	32
		72.6	49.0	7.376	32
	2	66.5	50.6	7.376	32
		66.6	49.5	7.386	33
		74.0	48.6	7.398	33
	3	61.6	56.9	7.335	36
		48.9	59.8	7.340	35
		59.8	55.4	7.353	36
	4	59.7	55.4	7.371	36
		56.1	54.8	7.372	36
		57.4	53.8	7.380	36
	5	57.9	50.6	7.380	34
		64.7	50.7	7.386	34
		61.0	52.1	7.382	35

APPENDIX H

EXPERIMENT #2 - HYPOTHALAMIC TEMPERATURE DATA

TRT#	CALF#	f	$\dot{V}_E$	V_T	T_b	T_h	T_{sk}	T_E
1	229	18.27	185.5	10.16	38.8	38.7	33.7	19.7
1	111(a)	19.39	198.5	10.27	39.6	39.0	33.0	24.8
1	222(a)	19.05	162.7	8.54	39.4	38.9	32.8	16.7
1	111(b)	24.65	224.0	9.09	38.8	38.6	33.4	19.3
1	222(b)	18.13	143.8	7.93	38.8	38.6	33.1	16.2
2	229	17.31	191.1	11.08	38.7	38.6	33.6	21.6
2	111(a)	15.35	180.4	11.77	39.7	39.2	32.4	19.0
2	222(a)	16.08	157.1	9.77	39.4	39.0	31.8	17.0
2	111(b)	18.35	195.0	10.63	39.0	38.8	33.7	12.3
2	222(b)	13.45	142.9	10.62	38.6	38.4	34.1	14.3
4	229	15.30	177.8	11.62	38.6	38.5	31.4	19.2
4	111(a)	14.89	138.2	9.40	39.4	39.0	27.8	15.6
4	222(a)	15.40	177.7	11.54	39.5	39.1	30.7	11.4
4	111(b)	17.50	192.9	11.03	39.2	39.1	30.7	14.7
4	222(b)	12.80	170.6	13.33	38.5	38.4	30.0	14.2
5	229	18.60	188.5	10.17	38.6	38.6	33.9	23.9
5	111(a)	16.60	192.0	11.58	39.7	39.0	31.8	18.7
5	222(a)	18.96	188.0	9.91	39.6	39.0	32.9	15.1
5	111(b)	19.33	182.4	9.46	39.3	39.2	35.0	12.9
5	222(b)	16.60	144.5	8.70	38.5	38.3	34.4	16.5

f = respiratory frequency, b/min; $\dot{V}_E$ = minute ventilation, ml/min-kg; V_T = tidal volume, ml/kg; T_b = body temperature, °C; T_h = hypothalamic temperature, °C; T_{sk} = skin temperature, °C; T_E = expired air temperature, °C.

APPENDIX I

EXPERIMENT #4 - INDIVIDUAL CALF DATA

THERMODE TEMP	CALF#	f	$\dot{V}_E$	V_T	T_b	T_{sk}	T_E
CONTROL	229	18.00	176.4	9.80	38.3	33.8	20.6
CONTROL	111(a)	16.83	176.2	10.47	38.6	33.8	23.2
CONTROL	222(a)	14.23	176.9	12.43	39.2	34.3	25.4
CONTROL	111(b)	13.65	141.3	10.35	39.0	33.8	25.8
CONTROL	222(b)	13.29	145.8	10.97	39.0	35.2	25.1
COLD	229	17.85	197.7	11.08	38.3	34.1	21.9
COLD	111(a)	16.40	181.8	11.08	38.5	33.6	24.4
COLD	222(a)	16.00	192.8	12.05	39.2	34.2	25.7
COLD	111(b)	13.60	143.8	10.57	38.8	33.6	25.2
COLD	222(b)	13.20	141.8	10.74	38.9	35.2	23.5
WARM	111(a)	28.25	259.2	9.18	38.6	34.3	24.4
WARM	222(a)	17.12	186.8	10.91	39.2	34.2	26.0
WARM	111(b)	25.00	214.7	8.59	39.0	34.0	27.4
WARM	222(b)	16.00	156.3	9.77	39.0	35.4	27.6

See Appendix H for units.

APPENDIX J

EXPERIMENT #3 - INDIVIDUAL CALF DATA

CALF	T_a	RH	P_{H2O}	T_b	T_{sk}	f	T_E	$\dot{V}_E$	V_T	CHL	EHL	RHL
685	3.9	85	5.15	38.0	29.6	12.58	16.5	151.6	12.05	.028	.047	.075
685	4.8	86	5.55	38.7	24.5	11.96	18.2	145.0	12.13	.028	.050	.079
685	3.5	85	5.01	39.1	31.6	20.50	24.0	238.8	11.65	.071	.144	.216
685	4.2	86	5.32	38.5	24.5	18.00	21.0	271.1	15.06	.067	.125	.192
685	4.2	86	5.32	38.2	24.0	23.75	20.6	439.9	18.52	.106	.196	.302
155	5.2	81	5.37	39.1	29.8	12.92	13.0	173.2	13.40	.020	.035	.054
155	3.8	86	5.17	39.4	28.2	11.23	16.9	150.2	13.38	.029	.048	.076
155	3.6	87	5.16	39.3	28.8	28.30	20.3	632.3	22.34	.153	.276	.429
155	3.6	87	5.16	39.3	29.0	22.30	18.3	499.7	22.41	.106	.181	.288
155	3.6	87	5.16	39.4	29.0	18.70	16.2	377.2	20.17	.069	.111	.180
155	3.3	85	4.94	39.1	33.4	14.80	20.7	319.7	21.60	.081	.147	.228
155	5.8	87	6.02	39.3	33.7	17.20	22.0	360.4	20.96	.085	.172	.256
155	3.7	84	5.02	39.4	33.5	25.50	22.3	515.6	20.22	.139	.270	.409
119	4.1	89	5.47	40.0	32.5	15.22	21.8	148.2	9.74	.038	.072	.109
119	4.8	92	5.94	40.0	33.2	14.32	18.6	136.8	9.56	.027	.047	.074
119	5.9	89	6.20	39.5	31.9	11.51	16.7	150.5	13.08	.024	.041	.065
119	3.0	94	5.34	40.2	33.2	18.70	19.0	249.2	13.32	.058	.094	.152
119	5.2	94	6.24	40.3	33.7	22.00	20.0	250.6	11.39	.054	.096	.150
119	5.2	94	6.24	40.3	33.7	24.30	21.5	283.5	11.66	.067	.125	.192
119	1.9	89	4.68	38.8	30.8	11.20	14.9	156.0	13.93	.030	.043	.072
119	2.0	85	4.50	39.0	31.0	12.50	14.6	171.9	13.75	.032	.047	.078
119	4.7	90	6.41	39.2	31.7	13.50	15.2	198.0	14.66	.030	.044	.074
658	5.0	78	5.10	39.3	31.7	15.08	23.2	178.5	11.84	.047	.101	.148
658	6.0	90	6.31	39.6	32.5	15.19	23.7	162.7	10.71	.042	.088	.130
658	4.5	75	4.74	39.5	31.9	18.70	21.5	299.1	16.00	.074	.150	.224
658	4.4	75	4.70	39.5	31.7	22.00	23.5	286.9	13.04	.080	.170	.249
658	3.9	75	4.54	39.4	31.6	24.50	23.3	384.1	15.68	.108	.226	.334
658	5.5	89	6.03	39.9	31.8	19.71	24.7	229.6	11.65	.064	.137	.201
658	5.7	89	6.11	40.0	32.3	21.73	23.3	264.1	12.15	.067	.139	.206
466	4.0	76	4.64	39.6	29.3	16.70	18.0	310.4	18.59	.063	.115	.179
466	1.9	76	3.99	39.6	29.6	19.70	21.3	352.6	17.90	.099	.183	.282
466	2.3	72	3.89	39.4	28.9	18.10	19.4	334.3	18.10	.083	.150	.233
466	4.5	72	4.55	39.2	31.7	15.90	18.7	341.0	21.45	.070	.137	.207
466	4.6	72	4.45	39.4	31.2	12.50	17.1	237.6	19.01	.043	.079	.122
466	3.0	70	3.98	39.2	30.1	16.40	18.8	436.4	26.61	.100	.185	.285
466	3.5	76	4.48	39.4	29.2	14.04	15.2	199.6	14.22	.034	.058	.092
466	2.7	84	4.67	39.3	31.0	10.77	16.7	168.8	15.67	.034	.055	.090
466	4.5	84	5.31	39.5	30.8	12.58	16.1	198.8	15.80	.033	.057	.090
220	4.6	74	4.74	39.9	30.6	17.20	22.0	291.1	16.92	.073	.152	.225
220	4.5	71	4.49	39.9	30.6	23.30	24.9	378.4	16.24	.112	.252	.364
220	4.2	72	4.45	39.8	30.6	19.00	24.6	222.9	11.73	.066	.145	.211
220	2.2	71	3.81	39.3	33.1	17.40	16.9	255.0	14.66	.055	.093	.148
220	3.8	73	4.39	39.6	34.4	22.10	17.7	259.6	11.75	.053	.096	.149
220	3.1	75	4.29	39.3	33.3	23.10	19.6	338.1	14.64	.081	.149	.231
220	5.0	81	5.30	39.1	32.0	9.24	18.1	115.2	12.46	.022	.041	.062
220	3.9	79	4.78	39.9	31.0	11.15	17.3	144.6	12.96	.028	.049	.078

See Appendix D for units.

APPENDIX K

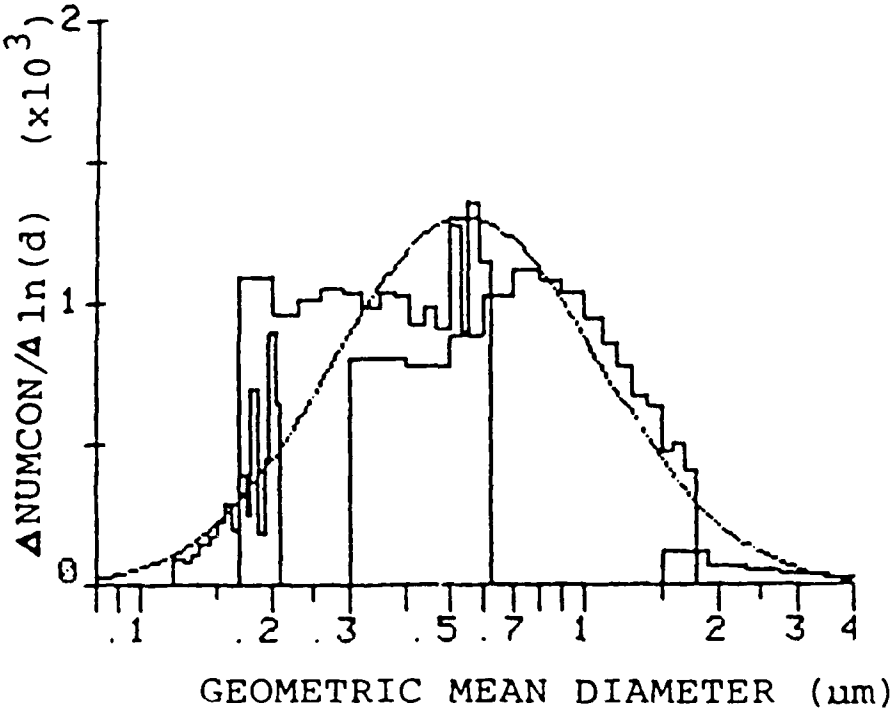
EXPERIMENT #3 - HYPOTHALAMIC TEMPERATURE DATA

Calf#	f	$\dot{V}_E$	V_T	T_b	T_h	T_{ac}	T_E
229	15.30	177.8	11.62	38.60	38.54	31.4	19.2
	22.40	358.3	16.00	38.54	38.32	31.1	20.0
	19.31	265.5	13.75	38.50	38.38	31.1	18.8
111(6 Nov)	14.89	138.2	9.40	39.37	39.03	27.8	15.6
	22.10	362.4	16.40	39.40	39.04	27.9	18.9
	19.30	272.3	14.11	39.34	38.98	27.5	19.7
	29.10	446.5	15.34	39.24	38.80	28.2	20.1
111(10 Nov)	17.50	192.9	11.03	39.23	39.11	30.7	14.7
	23.60	365.6	15.50	38.89	38.83	31.0	17.4
	20.20	227.6	11.27	39.05	38.93	31.1	14.8
222(7 Nov)	15.40	177.7	11.54	39.46	39.14	30.7	11.4
	18.30	254.6	13.92	39.52	39.08	31.2	12.5
	23.00	304.7	13.25	39.37	38.90	31.4	15.7
	23.60	266.7	11.30	39.05	38.73	31.7	17.4
222(9 Nov)	12.80	170.6	13.33	38.52	38.42	30.0	14.2
	19.00	430.8	22.67	38.40	38.33	29.6	17.0
	22.70	548.9	24.18	38.30	38.20	29.8	18.0

See Appendix H for units.

APPENDIX L

$BaSO_4$ PARTICLE GENERATOR ANALYSIS



Geometric Mean Diameter (GMD) = 0.5451 μm
Geometric Standard Deviation (σ_g) = 1.993
Mass Median Volumetric Diameter (MMVD)
MMVD = antilog[log GMD + 6.9(log σ_g)^2]
ρ = density of $BaSO_4$ solution = 1.06 g/ml
Mass Median Aerodynamic Diameter (MMAD) = MMVD$\sqrt{\rho}$

MMVD = 2.267 μm
MMAD = 2.334 μm

APPENDIX M

EXPERIMENT #5 - INDIVIDUAL CALF DATA

Calf #	728	119	658	100
Weight (kg)	90.7	52.2	49.0	46.3
Lung wt. (kg)	1.14	0.72	0.67	0.74
%dry wt.-lung	18.0	20.6	19.0	19.4
T_b (°C)	38.5	39.2	39.5	39.8
T_{sk} (°C)	32.0	32.6	33.9	34.0
T_E (°C)	30.3	24.8	30.3	20.3
f (b/min)	19.21	14.11	21.80	15.18
$\dot{V}_E$ (ml/min-kg)	142.1	137.5	186.0	157.2
V_T (ml/kg)	7.40	9.74	8.53	10.35
V_D (ml/kg)	2.40	2.10	4.19	2.86
$\dot{V}_{O2}$ (ml/min-kg)	4.96	5.15	4.93	6.03
$\dot{V}_{CO2}$ (ml/min-kg)	4.45	4.48	4.69	5.17
RER	0.897	0.870	0.951	0.857

Lobe	FM	SPEC	FM	SPEC	FM	SPEC	FM	SPEC
RAD	4.34	3.62	2.46	2.29	1.12	1.73	0.92	0.73
RCD	4.19	0.91	2.49	2.88	1.24	2.26	0.98	0.44
RDD	4.01	0.34	3.39	2.29	0.71	0.22	0.89	2.22
LAD	4.82	2.62	3.30	1.27	1.51	2.19	0.71	0.58
LCD	8.27	1.05	2.61	1.42	0.95	2.19	0.56	0.58
LDD	5.03	0.62	2.44	2.15	1.30	2.19	0.86	1.03
RAV	4.64	2.48	2.67	1.71	1.63	2.34	0.68	1.18
RCV	5.71	1.19	3.00	1.12	1.27	1.28	0.89	0.28
RDV	6.28	0.77	2.82	0.69	0.98	1.73	0.56	1.48
LAV	3.89	2.77	2.40	1.56	1.42	1.28	1.57	2.07
LCV	3.95	0.91	2.20	0.54	0.95	1.88	0.77	1.33
LDV	4.76	1.19	3.12	0.54	1.27	3.10	0.83	1.33
MED	4.37	2.05	2.91	1.93	1.30	2.49	0.65	0.88

FM = fluorescent microscopy, CFU/g X 10^7; SPEC = fluorescent spectrophotometry, CFU/g X 10^7; Calf #'s 728, 119, 658, 100, and 229 were exposed to 16-18°C and Calf #'s 685, 155, 466, 220, and 241 were exposed to 4-6°C.

Appendix M Cont'd

Calf #	229	685	155	466
Weight (kg)	68.0	56.7	45.4	46.3
Lung wt. (kg)	0.77	0.63	0.63	0.66
%dry wt.-lung	21.0	21.2	21.4	20.9
T_b (°C)	38.7	39.2	39.5	39.9
T_{sk} (°C)	34.3	31.8	33.6	29.5
T_E (°C)	28.6	24.8	12.5	19.8
f (b/min)	20.34	13.32	10.02	12.13
$\dot{V}_E$ (ml/min-kg)	153.1	118.5	140.6	151.2
V_T (ml/kg)	7.53	8.90	14.04	12.46
V_D (ml/kg)	2.72	1.86	3.05	3.92
$\dot{V}_{O2}$ (ml/min-kg)	5.18	5.54	6.33	6.74
$\dot{V}_{CO2}$ (ml/min-kg)	4.40	4.58	5.44	5.61
RER	0.849	0.826	0.860	0.833

Lobe	FM	SPEC	FM	SPEC	FM	SPEC	FM	SPEC
RAD	1.57	2.03	1.42	2.73	1.60	2.07	0.80	1.70
RCD	1.42	1.26	1.45	1.86	2.76	1.63	1.27	1.84
RDD	0.95	1.10	1.24	2.15	2.32	1.33	0.86	1.70
LAD	1.60	2.03	1.07	2.88	2.02	2.52	1.04	2.14
LCD	1.21	2.81	0.38	2.15	3.51	2.37	1.27	2.29
LDD	2.38	1.41	1.75	1.12	1.36	1.33	0.80	0.67
RAV	1.30	0.32	1.18	2.59	1.12	2.15	1.16	2.21
RCV	0.95	0.79	0.89	0.69	2.79	1.03	0.68	1.70
RDV	1.60	0.63	1.04	3.90	2.41	1.63	0.77	2.14
LAV	1.84	2.97	0.77	2.15	2.61	1.48	0.71	1.84
LCV	0.95	1.57	1.81	2.00	2.49	1.92	0.65	2.14
LDV	0.44	1.41	1.63	2.73	2.85	2.07	0.77	1.70
MED	1.01	0.16	1.16	2.73	2.02	1.78	0.50	2.72

Appendix M Cont'd

Calf #	220	241
Weight (kg)	46.7	52.2
Lung wt. (kg)	0.65	0.70
%dry wt.-lung	19.8	21.1
T_b (°C)	39.7	39.6
T_{sk} (°C)	33.3	29.9
T_E (°C)	17.3	25.4
f (b/min)	13.54	14.20
$\dot{V}_E$ (ml/min-kg)	142.2	164.2
V_T (ml/kg)	10.50	11.57
V_D (ml/kg)	3.39	4.00
$\dot{V}_{O2}$ (ml/min-kg)	6.58	7.61
$\dot{V}_{CO2}$ (ml/min-kg)	5.65	6.27
RER	0.859	0.824

Lobe	FM	SPEC	FM	SPEC
RAD	0.86	1.65	1.24	3.79
RCD	0.77	1.72	0.95	3.62
RDD	0.59	1.20	1.24	3.46
LAD	0.32	1.50	0.74	5.92
LCD	0.92	1.65	0.98	4.61
LDD	1.12	1.95	0.00	4.44
RAV	1.42	1.80	0.95	5.76
RCV	0.59	3.44	1.07	0.82
RDV	0.74	0.90	0.86	1.65
LAV	0.50	1.50	0.83	4.61
LCV	1.07	0.60	1.07	3.62
LDV	0.98	0.30	0.59	4.44
MED	0.41	3.29	1.51	4.77

APPENDIX N

EXPERIMENT #5 - ANOVA TABLES FOR FLUORESCENT SPECTROPHOTOMETRIC ANALYSIS

SOURCE	df	MS	F	p
Trt	1	13.56083853	6.66	0.0326
Calf(Trt)	8	2.03750109	-	-
Site ‡	12	0.58321732	1.84	0.0523
Trt*Site	12	0.24938450	0.79	0.6632
Residual	96	0.31714692	-	-
Total	129			
Trt	1	10.72740241	5.57	0.0459
Calf(Trt)	8	1.92581525	-	-
Lobe	2	2.09501913	6.77	0.0018
DV	1	0.27093243	0.88	0.3518
Trt*Lobe	2	0.03546559	0.11	0.8919
Trt*DV	1	0.09190535	0.30	0.5871
Lobe*DV	2	0.59289428	1.92	0.1527
Trt*Lobe*DV	2	0.18792760	0.61	0.5469
Residual	100	0.30955837	-	-
Total	119			

‡ Contrasts with Mediastinal Lobe:

Med. vs All Sites	1	0.83799874	2.64	0.1073
Med. vs Apical Lobe	1	0.01179648	0.04	0.8475
Med. vs Cardiac Lobe	1	1.66298461	5.24	0.0242
Med. vs Diaphr. Lobe	1	1.34202327	4.23	0.0424

APPENDIX O

EXPERIMENT #5 - ANOVA TABLES FOR FLUORESCENT MICROSCOPIC ANALYSIS

SOURCE	df	MS	F	p
Trt	1	11.57328297	1.13	0.3184
Calf(Trt)	8	10.22409887	-	-
Site ‡	12	0.06736327	0.50	0.9089
Trt*Site	12	0.11228287	0.84	0.6130
Residual	96	0.13424010	-	-
Total	129			

Trt	1	10.77414949	1.12	0.3200
Calf(Trt)	8	9.58504201	-	-
Lobe	2	0.08702325	0.62	0.5377
DV	1	0.02415621	0.17	0.6781
Trt*Lobe	2	0.05547117	0.40	0.6727
Trt*DV	1	0.04053562	0.29	0.5909
Lobe*DV	2	0.10926405	0.78	0.4594
Trt*Lobe*DV	2	0.01183290	0.08	0.9187
Residual	100	0.13938608	-	-
Total	119			

‡ Contrasts with Mediastinal Lobe:

Med. vs All Sites	1	0.08872635	0.66	0.4182
Med. vs Apical Lobe	1	0.02181689	0.16	0.6877
Med. vs Cardiac Lobe	1	0.16928402	1.26	0.2643
Med. vs Diaphr. Lobe	1	0.07439690	0.55	0.4584

APPENDIX P

EXPERIMENT #6 - INDIVIDUAL SHEEP LUNG DATA

Sheep	Wt.	f	$\dot{V}_E$	V_T	Ba^{++}	LA	LC	LD	RA	RC	RD
02	63.5	12	120.7	10.06	24.0	19	38	44	20	32	57
						19	13	44	34	52	49
						18		61			56
								41			52
03	59.5	12	119.0	9.92	46.4	44	38	45	0	35	62
						45	24	46	17	50	52
						42		61			50
								58			48
04	55.5	12	136.0	11.33	26.2	24	29	26	11	27	45
						24	3	32	17	51	30
						26		35			35
								41			36
05	47.0	12	158.1	13.18	41.4	61	42	37	54	50	44
						66	37	30	69	58	49
						66		39			45
								45			36
06	54.5	12	153.4	12.78	29.4	49	16	19	13	18	33
						44	5	14	29	17	19
						44		26			24
								26			24
11	56.5	20	148.0	7.40	19.2	2	40	48	24	23	57
						7	12	41	20	36	32
						0		33			44
								54			43
12	64.0	20	141.5	7.08	28.2	20	57	54	45	54	71
						14	28	57	58	20	66
						10		48			54
								63			39
13	44.5	20	164.4	8.22	23.7	23	39	34	51	43	39
						21	26	30	32	30	39
						19		32			26
								39			30

Sheep - sheep #;Wt. - estimated body wt, kg; f - ventilation frequency, b/min; $\dot{V}_E$ - minute ventilation, ml/min-kg; V_T - tidal volume, ml/kg; Ba^{++} - barium concentration, ppm; LA, LC, LD, RA, RC, RD - deposition determined by radiograph densitometer, AU.

Appendix P Cont'd

Sheep	Wt.	f	$\dot{V}_E$	V_T	Ba^{++}	LA	LC	LD	RA	RC	RD
15	51.0	20	157.1	7.86	38.0	44	28	21	11	20	26
						45	21	26	3	1	13
						48		14			16
								2			17
16	45.0	20	154.8	7.74	15.9	12	43	18	30	34	13
						3	27	0	5	22	16
						14		0			0
								1			1

APPENDIX Q

EXPERIMENT #6 - ANOVA TABLES FOR RADIOGRAPHIC ANALYSIS

LOBE	SOURCE	df	MS	F	p
Left-apical	Trt	1	0.19602083	5.60	0.0455
	Lung(Trt)	8	0.03502475	80.12	0.0001
	Sample{Lung(Trt)}	20	0.00043717	-	-
	Total	29			
Left-cardiac	Trt	1	0.00435125	0.42	0.5349
	Lung(Trt)	8	0.01035023	1.06	0.4579
	Sample{Lung(Trt)}	10	0.00604280	-	-
	Total	19			
Left-diaphr.	Trt	1	0.07430440	1.02	0.3421
	Lung(Trt)	8	0.07285631	24.65	0.0001
	Sample{Lung(Trt)}	30	0.00295538	-	-
	Total	39			
Right-apical	Trt	1	0.00010580	0.00	0.9553
	Lung(Trt)	8	0.03161118	5.23	0.0089
	Sample{Lung(Trt)}	10	0.00604280	-	-
	Total	19			
Right-cardiac	Trt	1	0.05345780	3.40	0.1026
	Lung(Trt)	8	0.01574055	1.85	0.1791
	Sample{Lung(Trt)}	10	0.00851740	-	-
	Total	19			
Right-diaphr.	Trt	1	0.11025000	1.56	0.2475
	Lung(Trt)	8	0.07085480	24.48	0.0001
	Sample{Lung(Trt)}	30	0.00289478	-	-
	Total	39			
Total	Trt	1	0.19149820	2.13	0.1826
	Lung(Trt)	8	0.08993191	-	-
	Lobe	5	0.03202260	1.33	0.2709
	Trt*Lobe	5	0.03114673	1.29	0.2855
	Lobe*Lung(Trt)	40	0.02405678	6.18	0.0001
	Sample	110	0.00389000	-	-
	Total	169			

APPENDIX R

EXPERIMENT #7 - INDIVIDUAL CALF DATA

CALF #	VELOCITY (mm/min)	
	T_a = 2-4°C	T_a = 16-18°C
550	5.6	9.3
	6.6	10.5
	5.6	10.0
	7.8	10.5
	6.8	8.5
1142	6.5	7.3
	7.2	11.3
	9.2	10.0
	4.6	13.2
	10.8	8.8
653	7.8	11.0
	7.8	13.5
	10.0	10.9
	8.9	8.8
	10.1	9.7
659	9.2	10.1
	8.0	15.4
	7.9	9.2
	9.9	9.9
	9.0	11.5

APPENDIX S

EXPERIMENT #7 -

ANOVA TABLE FOR NASAL MUCUS VELOCITY ANALYSIS

SOURCE	df	MS	F	p
Trt	1	0.62750250	73.40	0.0033
Calf	3	0.07799583	9.12	0.0511
Trt*Calf	3	0.00854917	0.29	0.8342
Residual	32	0.02975125	-	-
Total	39			

APPENDIX T

TRACHEAL AIR TEMPERATURE - INDIVIDUAL CALF DATA

Calf#	T_a(°C)	f(b/min)	T_b(°C)	TrachealAir Temp.(°C) Inspired	Expired
653	2.5	12.0	38.3	36.0	37.9
659	2.5	15.0	39.0	37.3	38.9
142	1.9	17.5	38.9	37.4	38.5
111	1.6	21.8	39.3	38.3	38.8
222	1.5	12.6	37.9	37.1	37.5
653	19.2	22.0	37.9	37.6	37.9
659	19.0	25.0	38.6	37.6	38.1
142	21.0	19.5	38.8	37.6	38.2
111	16.5	20.7	38.8	37.6	37.8
222	16.6	28.5	39.5	38.2	39.5
111	-8.0	21.1	39.0	35.4	36.8
222	-8.1	13.8	37.7	35.6	36.8
333	-13.2	16.5	37.6	36.1	36.3
444	-12.2	21.0	39.1	36.9	37.3

APPENDIX U

EXPERIMENT #8 - INDIVIDUAL CALF DATA

Calf	T_m	TMV	Calf	T_m	TMV	Calf	T_m	TMV
1	37.2	11.5	3	36.8	17.1	5	37.0	18.8
1	38.4	12.0	3	36.6	17.1	5	37.2	20.7
1	38.5	11.8	3	36.2	15.0	5	37.5	19.4
1	37.4	11.1	4	35.2	17.6	5	37.8	22.2
1	37.5	10.0	4	35.5	18.2	5	37.9	19.4
1	34.6	9.7	4	35.7	20.0	5	38.1	20.0
1	35.2	9.7	4	35.8	17.6	5	38.4	19.4
1	36.0	10.5	4	36.0	18.8	5	38.6	18.2
1	36.4	12.0	4	36.2	17.6	5	38.9	20.0
1	36.4	12.0	4	36.2	18.8	6	38.3	6.3
2	38.6	11.5	4	36.4	20.0	6	37.9	5.8
2	38.1	11.8	4	36.6	18.2	6	37.6	6.7
2	38.0	10.9	4	36.7	19.4	6	37.0	6.9
2	37.6	9.0	4	36.8	18.2	6	36.8	7.0
2	36.9	8.7	4	36.8	17.1	6	36.6	6.2
2	36.7	8.8	4	37.2	18.2	6	36.3	5.7
2	36.4	7.7	4	37.3	17.1	6	36.1	5.4
2	36.1	8.1	4	37.8	18.2	6	35.8	5.9
2	35.8	7.9	4	37.9	20.0	6	35.5	6.1
2	35.5	8.2	4	38.0	18.8	6	35.1	5.8
2	35.2	7.4	4	38.0	18.2	7	38.7	20.0
3	37.4	17.1	4	37.8	20.7	7	38.2	23.1
3	37.7	17.1	4	38.0	20.0	7	38.0	20.7
3	37.9	19.4	4	38.0	20.7	7	37.9	20.0
3	38.2	20.0	4	38.3	21.4	7	37.8	18.2
3	38.5	20.7	4	38.3	20.0	7	37.6	14.6
3	38.8	20.7	4	38.5	19.4	7	37.4	18.2
3	39.0	20.7	4	38.6	20.0	7	37.2	20.7
3	39.0	18.8	4	38.8	20.0	7	37.0	23.1
3	39.1	19.4	5	35.2	16.2	7	37.0	19.4
3	38.4	18.2	5	35.8	17.6	7	36.8	26.1
3	38.4	17.1	5	36.1	19.4	7	36.8	19.4
3	38.2	19.4	5	36.4	15.4	7	36.4	20.0
3	38.0	17.1	5	36.6	15.8	7	36.0	15.4
3	37.8	16.2	5	36.8	17.1	7	35.3	17.6

Calf = calf #; T_m = mucosal temperature, °C; TMV = tracheal mucus velocity, mm/min.

APPENDIX V

EXPERIMENT #8 -

ANOVA TABLE FOR CALF TRACHEAL MUCUS VELOCITY ANALYSIS

SOURCE	df	MS	F	p
T_m	1	67.105606	29.57	0.0001
Calf	6	412.513080	181.75	0.0001
T_m*Calf	6	2.660637	1.17	0.3281
Residual	91	2.269622	-	-
Total	104			

APPENDIX W

EXPERIMENT #8 - INDIVIDUAL SHEEP DATA

Sheep	T_m	TMV	Sheep	T_m	TMV	Sheep	T_m	TMV
1	36.0	7.7	3	35.2	13.3	6	35.5	8.0
1	36.0	7.5	3	35.2	11.1	6	35.2	8.1
1	35.2	7.9	4	35.5	12.2	6	35.0	8.6
1	36.0	8.4	4	36.8	15.8	7	37.6	14.3
1	36.4	9.0	4	37.2	16.7	7	38.1	15.8
1	36.7	8.3	4	37.5	17.6	7	38.4	16.2
1	37.1	9.1	4	37.7	18 2	7	38.6	15.8
2	36.2	9.2	4	38.2	18.2	7	38.8	15.8
2	36.3	8.0	4	38.4	16.7	7	38.3	23.1
2	36.7	7.7	4	38.4	17.1	7	37.6	16.7
2	36.8	8.8	4	38.2	15.8	7	37.7	20.7
2	37.2	9.7	4	38.2	15.8	7	37.7	16.2
2	37.3	8.8	4	38.5	16.7	7	37.4	20.0
2	37.6	7.8	4	38.7	18.2	7	37.4	16.2
2	37.2	8.6	4	37.5	17.1	7	36.8	14.6
2	37.5	8.3	4	38.0	18.2	7	36.8	14.3
2	37.9	9.2	4	36.5	14.0	7	36.7	13.6
2	37.9	9.7	4	36.6	12.8	7	36.5	14.0
2	38.2	9.7	4	36.4	13.6	7	36.3	13.3
2	37.5	7.8	4	36.2	12.2	7	36.3	14.0
2	38.2	8.4	4	35.5	10.7	7	36.1	11.5
2	37.8	7.8	5	35.4	16.2	7	36.1	15.4
2	38.4	8.7	5	36.4	20.0	7	35.9	15.0
2	38.7	9.4	5	36.7	19.4	7	35.9	16.2
2	39.1	9.7	5	36.9	21.4	7	35.8	11.5
2	38.7	10.3	5	36.1	20.0	7	35.5	13.3
3	35.8	9.2	5	37.3	26.1	7	35.6	16.7
3	36.3	8.8	5	37.5	28.6	7	35.4	14.6
3	37.4	12.2	5	37.8	23.1	7	35.4	12.0
3	38.2	14.0	5	38.2	21.4	7	35.2	14.6
3	38.2	14.3	5	38.2	22.2	8	35.7	6.9
3	38.2	13.0	6	36.8	8.3	8	36.0	6.6
3	36.8	12.2	6	37.4	7.5	8	36.0	7.2
3	37.7	15.0	6	38.8	9.4	8	36.0	7.1
3	37.8	13.6	6	39.0	9.2	8	36.4	7.0
3	36.5	13.6	6	38.3	8.8	8	36.9	7.4
3	37.1	13.0	6	38.8	9.1	8	37.4	7.5
3	37.2	12.2	6	37.2	8.6	8	37.7	7.1
3	36.7	10.9	6	37.5	8.8	8	37.8	7.5
3	36.1	12.0	6	36.4	8.1	8	38.0	7.4
3	36.4	13.6	6	36.5	8.4	9	35.0	15.0
3	36.6	14.0	6	36.5	9.8	9	35.2	13.6
3	35.1	10.7	6	36.2	8.2	9	35.4	14.0

Sheep - sheep number; T_m - mucosal temperature, °C; TMV- tracheal mucus velocity, mm/min.

Appendix W Cont'd

Sheep	T_m	TMV
9	35.5	12.5
9	36.0	18.2
9	36.1	20.0
9	36.3	17.1
9	36.4	18.2
9	36.6	19.4
9	36.7	16.2
9	36.8	17.6
9	37.1	18.2
9	37.2	19.4
9	37.4	22.2
9	37.5	17.6
9	37.6	20.7
9	38.0	19.4
9	38.2	17.6
9	38.4	20.7
9	38.6	20.7
9	38.7	18.8
9	38.9	20.7
10	39.2	9.7
10	39.4	10.2
10	39.4	11.1
10	38.9	11.8
10	38.0	9.5
10	38.2	10.9
10	37.9	8.1
10	37.6	7.6
10	37.6	8.7
10	37.3	8.4
10	37.1	7.8
10	37.0	7.5
10	36.9	8.4
10	36.7	8.3
10	36.6	8.6
10	36.5	9.0
10	36.4	8.7
10	36.0	7.6
10	35.6	9.7
10	35.6	10.0
10	35.7	9.2
10	35.2	8.4
10	35.2	8.2

APPENDIX X

EXPERIMENT #8 -

ANOVA TABLE FOR SHEEP TRACHEAL MUCUS VELOCITY ANALYSIS

SOURCE	df	MS	F	p
T_m	1	194.268329	82.80	0.0001
Sheep	9	347.176928	147.97	0.0001
T_m*Sheep	9	8.698792	3.71	0.0003
Residual	151	2.346312	-	-
Total	170			