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EFFECTS OF HYPOXEMIA ON THE FEBRILE RESPONSE OF YOUNG LAMBS TO BACTERIAL PYROGEN

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Effects of Hypoxemia on the Febrile Response of Young Lambs to Bacterial Pyrogen" submitted by Flora Ricciuti in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Experiments were done on eight young lambs to investigate the effects of hypoxemia on the temperature, metabolic and cardiovascular responses to intravenous administration of a small dose of bacterial pyrogen (lipopolysaccharide extracted from Salmonella Abortus Equi; SAE). Each lamb was anesthetized with halothane and prepared for measurements of cardiac output, arterial and mixed-venous hemoglobin oxygen saturations, body-core and ear-skin temperatures. No sooner than three days after surgery, measurements were made during a one-minute control period and during one-minute experimental periods at 10 minute intervals for 120 minutes following administration of 0.3 ug of bacterial pyrogen in sterile saline. In the control (normoxemic) condition, the lambs were given air to breathe (F_{IO2}=0.21) such that their arterial hemoglobin oxygen saturation was maintained at approximately 90%. In the experimental (hypoxemic) condition, alveolar hypoxia was developed by reducing the animals' F_{IO2} such that the arterial hemoglobin oxygen saturation was maintained at approximately 50%. Administration of SAE produced a short-lived fever of about 0.8^{o}C in the normoxemic lambs, whereas no change in body-core temperature was observed in the hypoxemic lambs following administration of SAE. In the normoxemic

condition, the increase in body-core temperature was preceded by the onset of shivering and a surge in total body oxygen consumption. The increase in total body oxygen consumption was primarily met by an increase in total body oxygen extraction during the rising phase of the febrile response. Cardiac index, heart rate, and systemic oxygen transport increased during the peak body-core temperature response. Systemic arterial blood pressure did not change significantly during the febrile response; however, pulmonic arterial blood pressure increased following the administration of SAE. In the hypoxemic condition, shivering occurred, but there was no surge in total body oxygen consumption, and no change in the body-core temperature following administration of SAE. Thus, we conclude that hypoxemia attenuates the febrile response of young lambs to bacterial pyrogen.

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INTRODUCTION

I. Thermoregulation in the Newborn:

The physiology of temperature regulation in the developing organism is complex. Various afferent, central, and effector mechanisms necessary to maintain homeostasis and ensure survival may not be mature. Although newborn mammals of several species are homeothermic, the range of environmental temperatures over which they can regulate body temperature successfully is restricted when compared to that of the adult. For example, the newborn has disadvantages such as a relatively large surface area to body mass ratio and poor thermal insulation (Brück, 1961). Understanding the responses of young mammals to thermal stimuli, and the factors which limit these responses will be the main focus of this background discussion.

The literature reveals considerable variation in the ability of young mammalian species to maintain thermal stability. In the immature newborn such as rabbits, cats, dogs, and man, thermoregulatory mechanisms are not completely functional at birth (Hull, 1973). That is, within narrow climatic limits they can maintain body temperature, but beyond the narrow limits, they rapidly become hypothermic or hyperthermic. The more mature newborn (ie, lambs and guinea pigs) can maintain a stable deep body temperature over a wide range of ambient temperatures even within an hour or two of birth (Hull,

1973). Young lambs, for example, are able to vasoconstrict in the cold, vasodilate in the heat, and change their respiratory heat loss (ie, panting) in relation to the environmental temperature. They are also capable of shivering and nonshivering thermogenesis in order to maintain a stable body temperature to cold exposure (Alexander, 1961; Hull, 1973). For the purpose of the following discussion, references will be made primarily to the mature newborn, namely the lamb and guinea pig.

(a) Responses of the Normoxemic Newborn to Cold Exposure:

In response to cold environmental temperatures, the lamb and guinea pig can increase heat production several times the basal rate (Alexander, 1961; 1962; Alexander & Williams, 1970; Brück & Wünnenburg, 1966; Blatteis, 1975). For example, in lambs, as heat loss increases on severe cold exposure, the maximum (or summit) metabolism is reached at the remarkable level of some 3-5 times the basal rate (Alexander, 1975). The early studies of Alexander & Williams (1970) have demonstrated that young lambs (under 3 days of age) can increase cardiac output and heart rate by 50-100% during summit metabolism. In fact, the cardiovascular and metabolic adjustments occurring during severe cold exposure have also been reported with relatively small decreases in ambient temperature (Sidi et al, 1983b).

The methods by which the newborn can achieve the metabolic response to cold include shivering as well as nonshivering thermogenesis. In general, newborn mammals with advanced motor development (i.e. lamb and guinea pig) appear to shiver the most when exposed to severe cold temperatures. Although the contribution of shivering thermogenesis to the cold-induced metabolic response in lambs has yet to be clearly determined, Alexander & Williams (1968) have suggested that shivering thermogenesis supplies about 60% of summit metabolism. That is, under conditions where thermogenesis is maximally stimulated and body temperature starts to fall, the potential for shivering thermogenesis just exceeds that for nonshivering thermogenesis. In fact, an increase in blood flow to the non-respiratory muscles has been reported in young lambs when exposed to severe cold (Alexander et al, 1973). In the guinea pig, the relative contribution of shivering to heat production in response to cold increases over the first 2-3 weeks of life (Bruck & Wunnenburg, 1966).

Many newborn mammals do not shiver and yet they can increase their metabolic rate on cold exposure. Studies investigating the nonshivering component of the newborn's response to cold have confirmed the thermogenic function of the brown adipose tissue (Heim & Hull, 1966a), and have shown that the cold response is sympathetically-mediated (Hull & Segall, 1965). For example, stimulation of the

sympathetic nerves causes a rise in the temperature of the tissue. In addition, there is evidence that norepinephrine is probably the mediator at the sympathetic nerve endings. Infusion of norepinephrine, like cold exposure, increased thermogenesis in the brown adipose tissue, as well as its blood flow and oxygen consumption (Heim & Hull, 1966a). It was also demonstrated that the thermogenic response to norepinephrine and to cold exposure is blocked by the catecholamine antagonist, propranolol (Heim & Hull, 1966b). It is important to note that the thermogenic capacity of young mammals will depend not only on the presence of this specialized tissue, but also on the total mass of the heat-producing tissue, the perfusion of this tissue, and the oxygen content of the blood.

Although the utilization of one or both of these effector mechanisms differs depending on the species and the degree of maturity, there is evidence to suggest that shivering and nonshivering thermogenesis form a "meshed" control system (Brück et al, 1971). For example, when newborn (as well as cold-adapted) guinea pigs are cooled externally, nonshivering thermogenesis is initiated first and shivering occurs in addition only after more severe cooling. According to Brück et al (1971), the cervical spinal cord is the region that preferentially receives the heat that is generated in the interscapular brown adipose tissue via vascular connections. This explains the

observations that with external cooling, nonshivering thermogenesis is initiated by the cooling of temperature sensors in the skin, leading to an increase in the temperature of the interscapular brown adipose tissue and in the cervical vertebral canal. The direction of the temperature gradient between the interscapular adipose tissue and the cervical vertebral canal indicates that heat flows from the adipose tissue to the spinal cord. As a result of the spinal cord temperature being maintained at a high level, shivering remains suppressed. However, shivering starts as soon as the cervical spinal cord temperature begins to fall following the blocking of nonshivering thermogenesis by an adrenergic B-receptor blocking agent. Thus in this manner, the two effector systems, shivering and nonshivering thermogenesis in the guinea pig, are interlocked.

The following section will deal with how these two effector mechanisms, responsible for the metabolic response to cold in young mammals, may be limited to some extent by the supply of oxygen to the thermogenic tissues.

(b) <u>Responses of the Hypoxemic Newborn to Cold Exposure:</u>

Hypoxemia is a complex stress. Before an attempt can be made at summarizing the major findings on the effects of hypoxemia on the metabolic response to cold, it is important to review some of the cardiovascular adjustments

which occur during hypoxemia. The cardiovascular response of the hypoxemic newborn is largely characterized by a dramatic and early rise in heart rate and cardiac output, with an increase in pulmonary pressure, and a decrease in the systemic vascular resistance (Sidi et al, 1983a; Sidi et al, 1983a). Also accompanying these changes is a redistribution of blood flow to the vital organs with the heart and brain flow increasing, and the skin, G.I. tract, and kidney flow decreasing. Hypoxemia has also been reported to attenuate the increases in oxygen consumption, cardiac output, and heart rate seen during cold exposure in normoxemic lambs (Sidi et al, 1983b).

The metabolic response of newborn mammals to cold exposure is particularly susceptible to hypoxemia. Several reports have shown that alveolar hypoxia (F_{IO2} = 0.10 - 0.12) attenuates the metabolic response to cold in neonates of several species (Alexander & Williams, 1970; Blatteis, 1964; Hill, 1959; Moore, 1959; Varnai et al, 1971). The early studies of Hill (1959) showed that kittens responded to 10% oxygen by a reduction in metabolic rate. More importantly, she demonstrated that the fall in oxygen consumption occurred only when the animals were at an ambient temperature below their thermal neutral environment. The thermal neutral environment is generally defined as the temperature range at which metabolism is minimal while normal body temperature is maintained (Bruck,

1961; Dawes, 1968). Hypoxemia has also been shown to have an inhibitory effect on body temperature in newborn mammals (Bonora & Gautier, 1987; Mortola & Rezzonico, 1988). According to Hill (1959), a fall in body temperature observed in these newborns is related to a parallel decrease in oxygen consumption which occurs when the ambient temperature is below the thermal neutral environment. It seems then that the reduction in oxygen consumption and body temperature occurring during hypoxemia may be closely correlated to the thermoregulatory heat production and heat conservation.

The precise mechanism for the hypoxemic suppression of the metabolic response to cold has not yet been clarified. Both nonshivering (NST) and shivering thermogenesis (ST) have been investigated. It has been suggested that the hypoxemic effect on cold-induced thermogenesis may result from the depression of the nonshivering thermogenic component. For example, as stated previously, cold-induced chemical thermogenesis in the brown adipose tissue is sympathetically mediated, thus the hypoxemic depression of this thermogenesis may be due in part to the inhibition of the calorigenic action of norepinephrine. In fact, in newborn rabbits (Blatteis, 1964), it has been shown that the increase in oxygen consumption produced by norepinephrine is smaller during hypoxemia than during normoxemia in both a thermoneutral and a cold environment.

One must also consider the possibility that hypoxemia may reduce the blood supply to the brown adipose tissue. Heim & Hull (1966a) eliminated this possibility by demonstrating that hypoxia (10% oxygen) did not reduce the increase in blood flow to the brown adipose tissue occurring during noradrenalin infusion in newborn rabbits. However, local oxygen consumption and heat production in this tissue was reduced. Thus, newborn mammals which depend largely on NST for heat production in the cold (ie, newborn rabbits) have been shown to be especially sensitive to hypoxemia.

The observations on the effects of hypoxemia on shivering thermogenesis are more complex. Several studies have shown that shivering is remarkably resistant to hypoxemia (Blatteis, 1964; Hemmingway & Birzis, 1956; Hemmingway & Nahas, 1952). Others have reported that hypoxemia reduces shivering in newborn (Cross et al, 1959) as well as adult mammals (von Euler & Soderberg, 1958; Gautier et al, 1987). The magnitude of reduction in shivering and body temperature was also found to vary with the severity of the hypoxic exposure (Gautier et al, 1987). There also seems to be further disagreement with respect to the suggested mechanism by which hypoxemia suppresses shivering. Although there have been no systematic studies investigating the mechanism for shivering thermogenesis in young mammals, early studies (von Euler & Soderberg, 1958; Mott, 1963) with adult anesthetized mammals have

demonstrated that this suppression might be due to the excitation of the systemic arterial chemoreceptors since carotid denervation prevented the depression of shivering. In a more recent study with unanesthetized cats (Gautier et al, 1987) it was demonstrated that carotid denervation did not prevent the depression of shivering by ambient hypoxia. Undoubtedly, a number of methodological differences do exist and may in part account for the differences in results. From these observations, it is evident that the hypoxic depression of the total metabolic response to cold requires further investigation to clearly elucidate all the mechanisms involved.

It appears then that in several newborn species hypoxemia limits the extra thermogenic metabolism at low ambient temperatures, resulting in a decrease in oxygen consumption and body-core temperature. Considerably more is known about hypoxemia in the newborn subjected to acute increases in oxygen demand brought about by stress such as cold, than about its effects during a perturbation such as fever. Both of these disturbances result in considerable metabolic and thermogenic effort. However, the effect of hypoxemia on the febrile response of young animals remains largely unknown.

II. Fever in the Newborn:

(a) Responses of the Normoxemic Newborn to Bacterial Pyrogen:

There are numerous reports in the literature concerning the human newborn experiencing severe naturally-occurring infections without developing fever (Epstein, 1951; Bergstrom et al, 1972). Although no systematic studies have been conducted to determine why human neonates do not readily develop fever, a number of laboratory animal studies have established that the febrile response to exogenous pyrogens (i.e. endotoxin), in doses that can cause fever in adult animals, does not develop in their newborn counterparts until some days after birth (Pittman et al, 1973, 1974; Blatteis, 1975, 1977; Kasting et al, 1979b). The precise number of days is species specific and related to their rate of postnatal maturation. Two species, namely the lamb and guinea pig, because of their relatively mature thermoregulatory systems have been studied extensively with respect to the development of fever in the newborn.

The initial experiments investigating the effects of endotoxin in young lambs, demonstrated that if 4-hour old lambs were given intravenous (i.v.) injections of bacterial endotoxin (derived from Salmonella abortus equi), they did not develop a fever (Pittman et al, 1973, 1974; Kasting et

al, 1979b). These authors also found that 60-hour old lambs remained afebrile with a dose (0.3ug) that produced a 1°C fever in adult sheep. Furthermore, they demonstrated that the lambs receiving an initial dose at 4hr, then a second dose at 60hr, became febrile with similar characteristics to those observed in the adult animal. Thus, they concluded that immunological sensitization to endotoxin may be necessary before fever can occur for the first time in young lambs. Further studies, however, showed that sensitization to endotoxin is not a prerequisite for fever production because an increasing number of animals become more responsive to endotoxin with increasing age (Kasting et al, 1979b; Pittman et al, 1974), and with increasing dosages of endotoxin or endogenous pyrogen. (Kasting et al, 1979b). In addition to these observations in young lambs, it was also found that several ewes, especially those near parturition, did not develop fever to bacterial endotoxin. Pregnant ewes seem to lose their ability to respond to bacterial pyrogens with fever some 4 days before term and at least 5 hours after parturition (Kasting et al, 1978).

Kasting et al (1980) argue that a similar mechanism inhibits the development of fever in the ewe and the lamb, and have presented data that increased neural secretion of the hormone arginine vasopressin (Cooper et al, 1979; Kasting et al, 1983) might be this endogenous antipyretic.

For example, they showed that AVP perfusions into the region of the septum (approximately 2-3mm anterior to the anterior commissure) of normal ewes prevented endotoxin fever, whereas perfusions into other brain areas (i.e. posterior hypothalamus, anterior hypothalamus, and preoptic hypothalamus) as well as peripheral perfusions had no effect on fever production (Cooper et al, 1979). AVP perfusions also failed to decrease the body temperature of nonfebrile animals (Kasting et al, 1981). These results seem to suggest that AVP can specifically antagonize only febrile changes in body temperature. Furthermore, the central nervous system (CNS)-mediated antipyretic effects of AVP can be antagonized by an AVP analog. Kasting & Wilkinson (1987) showed that 3-day rat pups failed to respond to endotoxin with a fever (similar to neonates of other species), but when the central AVP antipyretic receptors were blocked, the pups were able to raise their body temperature behaviorally to febrile levels.

Thus research in several laboratories has now demonstrated that AVP has antipyretic effects when administered into the CNS of many species (Cooper et al, 1979; Kasting et al, 1979a; Naylor et al, 1985; Zeisberger et al, 1981). This evidence is further strengthened by the observations that hemorrhage, which is a potent stimulus for AVP release into both the blood and the cerebrospinal fluid, specifically reduced fevers in sheep

without affecting thermoregulatory ability in the cold (Kasting et al, 1981). It might be added here as well that another stimulus for the release of AVP is hypoxemia. When hypoxemia occurs in fetal and newborn lambs, the plasma AVP concentrations have been found to increase (Daniel et al, 1984; Rurak, 1978). However, the effect of hypoxemia on the modulation of the febrile response in the newborn remains largely unknown.

Although fever production in the newborn depends largely on the maturity of the subject, a number of studies have established that the autonomic and behavioral thermoregulatory ability of the newborn are present and functional from birth (Blatteis, 1975). For example, Blatteis (1975) demonstrated that although newborn guinea pigs did not elicit the expected heat production responses to endotoxin at both cool and neutral temperatures, they showed typical responses to cold, which evokes the same pattern of thermoeffector activity as do pyrogens (Brück, 1978). Similar findings of neonates demonstrating competent thermoregulatory activities after birth have also been reported in the newborn lamb (Alexander, 1961; Brück & Wünnenburg, 1966).

Further studies have also shown that the thermoeffector mechanisms that underlie febrigenesis are different in different thermal environments (Székely & Komaroni, 1978; Nisho & Kanoh, 1980; Szelényi & Székely, 1979). For

example, in thermoneutrality, fever is generated by a combination of thermoeffectors in order to increase heat production and decrease heat loss. In a warm environment, inhibition of heat loss appears to be responsible for the rise in body temperature with little or no increase in heat production (Szelényi & Székely, 1979). In the cold, however, fever is produced by a further increase in thermogenesis, since heat conservation is already at its maximum.

Based on the observations that both the febrile response and the thermogenic response to cold involve heat production and heat conservation, it was suggested that similar mechanisms are probably involved in raising the animals body temperature in each situation (Blatteis, 1977). Therefore, in the young animal in which brown adipose tissue has a major role in the thermogenic response to cold (Dawkins & Hull, 1964; Smith & Horwitz, 1969), it is possible that brown adipose tissue may also contribute to heat production during fever. Data obtained from studies in newborn guinea pigs challenged with endotoxin, have shown that increased oxygen consumption is accompanied by vigorous shivering in the older animals (16 to 32 days old), whereas increased oxygen consumption was accompanied by a rise in brown adipose tissue temperature in the younger animals (8 days old) (Blatteis, 1975). Thus, endotoxin-induced thermogenesis gradually shifted from

nonshivering to shivering means during the first month of life (Blatteis, 1975; Nisho & Kanoh, 1980). Furthermore, it has also been shown that endotoxin-induced nonshivering thermogenesis is sympathetically-controlled since it can be blocked by propranolol in newborn, as well as in cold-acclimatized guinea pigs (Blatteis, 1976b).

(b) Responses of the Hypoxemic Newborn to Bacterial Pyrogen:

It is now generally accepted that fever production in young animals results from an increase in heat production (including both nonshivering and shivering thermogenesis), as well as an increase in heat conservation. These effector mechanisms are also utilized by young animals in an attempt to maintain body temperature in response to cold exposure. Previous experiments have shown that hypoxemia impairs the thermoregulatory responses of young animals to cold. At the present time, however, it is not known whether hypoxemia affects the development of fever in young animals.

PURPOSE

The purpose of our present experiments was to investigate whether or not hypoxemia alters the body temperature, metabolic, or cardiovascular responses of young lambs to bacterial pyrogen.

HYPOTHESIS

Hypoxemia will attenuate the febrile response of young lambs to bacterial pyrogen.

METHODS

Eight lambs ranging in age from 21 to 27 days were studied. Each lamb was separated from its ewe 24 hours after birth, and was housed in our laboratory at an ambient temperature of 25°C in a Plexiglass cage with continuous access to milk (Lamb Milk Replacer). The lambs were among other lambs, fed and slept ad libitum and soon became accustomed to the surroundings and laboratory personnel.

Surgical Preparation

Each lamb underwent one operation prior to study. Anesthesia was induced by having the lamb breathe 5% halothane in oxygen through a mask. The trachea was then intubated with a cuffed endotracheal tube and the anesthesia was maintained by ventilating the lamb's lungs with 1% halothane in oxygen. An electrocardiogram, end-tidal CO_2 levels, and rectal body temperature were

monitored during surgery. The body temperature was kept near 39°C with a heating pad, and the end-tidal CO_2 levels were kept near 5% with a volume-cycled ventilator.

The operation was done between 16 and 19 days of age. A left thoracotomy was performed in the fourth intercostal space and the pericardium was incised in order to expose the heart and great vessels. A precalibrated ultrasonic flow transducer (Model T101, 12mm S-Series; Transonic System Inc., Ithaca, N.Y.) was placed around the pulmonary artery for continuous measurements of pulmonary blood flow (ie, cardiac output; Qp). A double-lumen fiberoptic catheter oximeter (model 1270A; Shaw Catheter Oximeter System, Mountain View, CA; 90% response to a step change in SaO2 within 5 seconds) treated with TDMAC heparin complex (Polysciences Inc., Warrington, PA) was placed via a stab wound and secured with a purse-string suture into the pulmonary artery to measure pulmonary arterial blood pressure and mixed-venous hemoglobin oxygen saturation (SvO2). A double-lumen fiberoptic catheter oximeter treated with TDMAC heparin complex was also inserted to the thoracic aorta via a femoral artery for continuous measurements of systemic arterial blood pressure and arterial hemoglobin oxygen saturation (SaO2). In addition, a copper/constantan thermocouple (Ret-1, Sensortek Inc., Clifton, NJ) with a flexible vinyl covered soft tip was inserted into the inferior vena cava (IVC) via a femoral

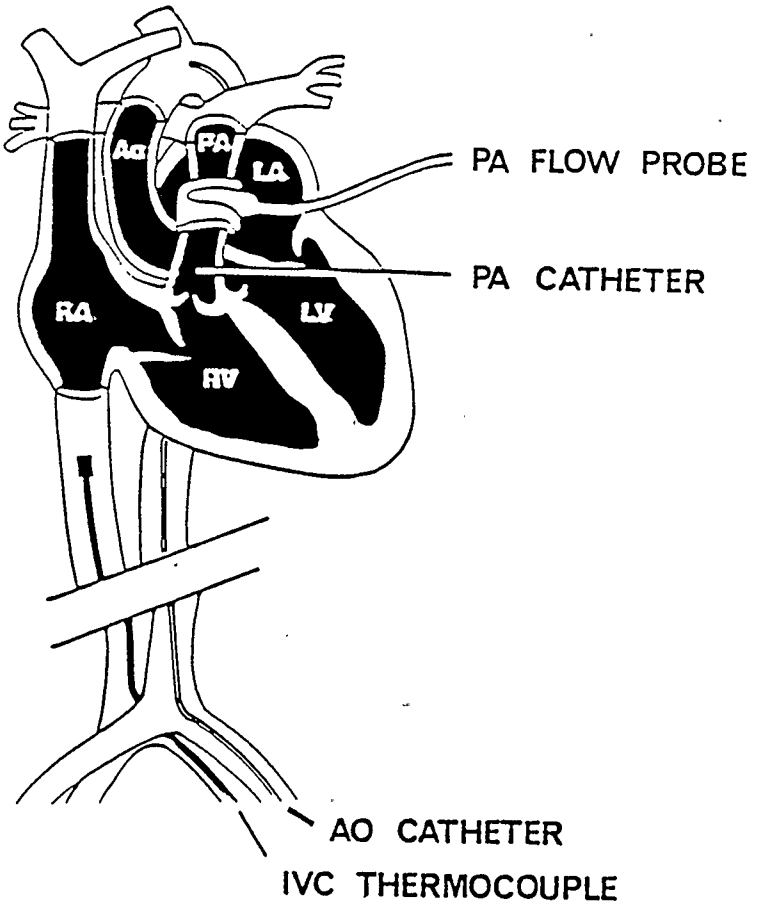


Figure 1. Surgical instrumentation for cardiovascular and metabolic measurements in the young lamb.

vein, and served as a measure of body-core temperature (Figure 1). A surface probe (SST-1, Sensortek Inc.) was attached (Vetbond; Tissue Adhesive, St. Paul, MN) onto the mid-dorsal surface of one ear pinna and served as a measure of skin-surface temperature.

Electrodes for the following recordings were also implanted: electrocorticogram (recorded from electrodes placed through burr holes to lie over the parietal cortex), electro-oculogram (recorded from electrodes placed at the inner and outer canthus of the right eye), nuchal electromyogram (recorded from electrodes placed in the dorsal cervical musculature) and a diaphragm electromyogram (recorded from electrodes placed transabdominally into the muscle fiber adjacent to the lateral margin of the central tendon). A reference wire was also sutured into the subcutaneous tissue of the scalp. The electrodes were made in our laboratory and were paired, teflon-coated multistranded stainless steel wires (AS 633; Cooner Wire Co., Chatsworth, CA); approximately three millimeters of the tip of each was bared for implantation. The proximal end of each wire was bared and soldered to the appropriate pin of an 18-pin electrical plug. This in turn was connected to 4 differential high-impedance probes (7HIP5G, Grass Medical Instruments, Quincey, MA) during a study.

The lambs were allowed to recover from surgery in a Shor-line intensive care unit for small animals (Schroer

Manufacturing Company, Kanas City, MO). They were then placed back in their plexiglass study cage in our laboratory, however, they were not studied until at least three days following the surgery. The lambs also received antibiotics daily (Penicillin G & Dihydrostreptomycin) beginning on the day of surgery.

Conditions of Observations

The experiments took place in a sound attenuating chamber no sooner than 3 days after surgery. The chamber is equipped with a viewing window as well as a closed circuit video system to observe the lambs. Temperature, sound, and lighting can be precisely controlled in this chamber. The animal's catheters, electrodes, and cables were connected to the recording equipment located outside the chamber at least 24 hours prior to the commencement of any experiment. A partition was also placed in the cage to prevent the lamb from turning around. The lamb, however, was still able to lie down, stand up, and feed ad libitum.

For each experiment, the optical connectors were connected to the optical modules of the oximeter processors and the vascular catheters were connected to strain gauge manometers (Gould P23ID, Gould Inc., Oxnatd, CA) using rigid pressure monitoring tubing. The strain gauge manometers were placed at the approximate level of the heart when the animal was lying down. Daily in vivo

calibrations of the fiberoptic catheter oximeters were done after the hemoglobin oxygen saturations of the arterial and venous blood were determined using an IL282 Co-oximeter. The flow transducer cable was connected to a Transonic T101D Ultrasonic Flowmeter (Transonic Systems Inc., Ithaca, NY) and the thermocouples were connected to an Iso-Thermix Unit (Columbus Instruments International Corp., Columbus, Ohio) which was interfaced with a Zenith Lap-Top Computer (Model ZA-180-21). The 18-pin electrical plug was connected to the differential high impedance probes. A heavy duty cable connects the differential high impedance probes to A.C. preamplifiers (Model 7P5 Wide Band A.C. EEG Preamplifier, Grass Medical Instruments, Quincy, MA) in the adjacent room.

The behavioral state of each animal was defined by the following electrophysiological criteria: During wakefulness, the electrocorticogram (ECOG) shows a fast-wave, low-voltage pattern with occasional eye movements and tonic activity on the nuchal electromyogram (EMG). During quiet sleep, the ECOG shows a slow-wave, high-voltage pattern with no eye movements and tonic activity on the nuchal EMG. During active sleep, the ECOG shows a fast-wave, low voltage pattern with rapid eye movements on the electro-oculogram (EOG) and no activity on the nuchal EMG.

Experimental Protocol

During an experiment, systemic and pulmonary arterial blood pressures and hemoglobin oxygen saturations, pulmonary blood flow and the electrophysiological signals were recorded on a Grass model 7 polygraph (Grass Medical Instruments, Quincy, Mass) and were digitized at 167 Hz (Zenith AT, Data Translation 2801A A/D, DATAQ WFS-200 Hardware Scroller, and Codas Data Acquisition Software) and stored on an optical laser disk. The following calculations were made from the measured variables: arterial oxygen content (CaO_2; Hb concentration [g/dL] X Hb O_2 binding capacity [1.36 mL O_2/g Hb] X SaO_2), mixed-venous oxygen content (CvO_2; Hb concentration [g/dL] X Hb O_2 binding capacity [1.36 mL O_2/g Hb] x SvO_2), systemic oxygen transport (SOT; Qp X CaO_2), total body oxygen consumption (VO_2; Qp X [CaO_2-CvO_2]), and total body oxygen extraction ([VO_2/SOT] X 100). Hemoglobin concentration was measured using an IL282 Co-oximeter, which was calibrated with whole blood using the cyanmethemoglobin technique.

I. <u>Establishment of Thermal Neutral Environment:</u>

Measurements were made continuously while the lambs breathed air at seven different environmental temperatures (ie, 10, 15, 20, 25, 30, 35, 40°C). Each animal was maintained at any one given environmental temperature for approximately 90 minutes. The sequence of environmental

temperatures was alternated between animals to avoid any sequencing effects due to the time of day and natural circadian temperature variation (ie, animal 1: 10-15-20-25-30-35-40^{o}C; animal 2: 40-35-30-25-20-15-10^{o}C). Once the variables were acquired, measurements of total body oxygen consumption were made consistently during the last 30 minutes of each 90 minute period when the lamb was in a quiet state (ie, quiet wakefulness or quiet sleep) in order to determine minimal oxygen consumption and its corresponding environmental temperature (ie, thermoneutrality). This environmental temperature was then used in the following experiments.

II. Hypoxemia and the Febrile Response to Bacterial Pyrogen:

In these experiments, the lambs were subjected to two different conditions: (i) control (normoxemia) condition and (ii) experimental (hypocapnic hypoxemia) condition. During the normoxemic condition, the lambs were given air to breathe such that their arterial hemoglobin oxygen saturation was maintained at approximately 90%. The medical air was introduced to these animals at a constant flow rate via a pediatric nasal cannula secured to their muzzle. During the hypoxemic condition, the animal's F_{IO2} was reduced such that their arterial hemoglobin oxygen saturation was maintained at approximately 50%. Thus

alveolar hypoxemia was developed by introducing pure nitrogen to the animal via the nasal pediatric cannula. Carbon dioxide levels were not controlled, thus the animals became hypocapnic. Once the arterial hemoglobin oxygen saturations were stabilized in each condition, the animals were challenged with a bacterial pyrogen. The bacterial pyrogen used was a purified lipopolysaccaride (LPS) extracted from Salmonella Abortus Equi (SAE). The intravenous (iv) administration of 0.3 ug of bacterial pyrogen in sterile saline was done when the lamb was in quiet wakefulness or quiet sleep. Measurements for analysis were made during a one-minute control period when the lamb was in quiet wakefulness or quiet sleep and during one-minute experimental periods at 10 minute intervals for 120 minutes following iv administration of SAE. The experimental and control conditions were conducted on each animal on separate days, and were alternated between animals to avoid any sequencing effects.

Measurements of pH and blood gases (ie, pCO_2, pO_2) were made from arterial blood samples taken prior to each experiment (control values), and every 30 minutes for 120 minutes following administration of SAE. Base deficit was calculated using the pH and pCO_2 measured at the animal's body-core temperature, and the hemoglobin concentration. Arterial blood samples for the analysis of lactate/pyruvate levels were also taken prior to each experiment (control

values) as well as 60 and 120 minutes following administration of SAE. Lactate and pyruvate concentrations were determined by an enzymatic spectrophotometric method (Sigma Chemical Company) (Fleischer, 1970).

Statistical Analysis

I. Establishment of Thermal Neutral Environment:

For each animal an average value for every variable at each environmental temperature was determined. To analyze the data statistically, a one-way ANOVA for repeated measures of the same variable followed by a Student Newman Keuls' multiple comparison test was performed in order to determine if environmental temperature (ie, 10, 15, 20, 25, 30, 35, 40^{o}C) affected the body-core temperature, skin-surface temperature, total body oxygen consumption, total body oxygen extraction, systemic oxygen transport, cardiac output, heart rate, systemic arterial blood pressure, systemic vascular resistance, pulmonary arterial blood pressure, arterial hemoglobin oxygen saturations and mixed-venous hemoglobin oxygen saturations.

II. Hypoxemia and the Febrile Response to Bacterial Pyrogen:

For each animal an average value for each variable during the control period and during the experimental periods was determined. To analyze the data statistically,

a 2-factor ANOVA for repeated measures of the same variable followed by a Student Newman Keuls' multiple comparison test was performed in order to determine if the condition (NOR vs HYP) and/or time (control vs 10m, 20m, 30m, etc.) affected the body-core temperature, total body oxygen consumption, total body oxygen extraction, systemic oxygen transport, cardiac output, heart rate, systemic arterial pressure, systemic vascular resistance, pulmonary arterial blood pressure, arterial hemoglobin saturations and mixed venous hemoglobin oxygen saturations (Winer, 1971; Zar, 1979).

RESULTS

I. Establishment of Thermal Neutral Environment:

Exposure of these young lambs (21±1 days of age) to a range of environmental temperatures produced dramatic changes in the total body oxygen consumption. At the lowest environmental temperature of 10°C, there was a sharp rise in total body oxygen consumption (13.1 ± 2.9 mLO_2/min/Kg; presented as mean ± 1SD) accompanied by the occurrence of vigorous shivering [Figure 2(a)]. Total body oxygen consumption gradually decreased to a reach a minimum at the environmental temperature of 25°C (8.5 ± 1.2 mLO_2/min/Kg) while a constant body-core temperature was maintained at 40.1 ± 0.5°C. As the environmental

temperatures were increased, total body oxygen consumption again increased to reach a value of 11.0 ± 2.4 mLO_2/min/kg at 40°C. All lambs at this end of the temperature range demonstrated panting [Figure 2(a)].

The body-core temperature at the environmental temperature of 25°C (minimal oxygen consumption) did not differ significantly from the body-core temperatures at the lower environmental temperatures (10 - 20°C) [Figure 2(b)]. There was, however, a significant rise in the body-core temperature of these lambs at the highest environmental temperatures of 35 & 40°C [Figure 2(b)].

In addition to the changes in heat production (assessed indirectly by measuring changes in total body oxygen consumption), there were also vasomotor changes to effect heat conservation and heat dissipation. Peripheral vasomotor changes were assessed by measuring changes in the ear skin-surface temperature. A decrease in the skin-surface temperature was observed at the lower environmental temperatures of 10°C to 20°C [Figure 2(c)], reflecting peripheral vasoconstriction. At the environmental temperature of 25°C, the skin-surface temperature increased significantly (reflecting peripheral vasodilatation) and continued to increase as the environmental temperatures were increased.

As far as we are aware, these experiments are the first to provide information about oxygen extraction and delivery

in young mammals attempting to thermoregulate across a range of environmental temperatures. For example, these data provide evidence that the increased metabolic demand occurring during exposure to low environmental temperatures (10 & 15^{o}C) was met primarily by an increase in total body oxygen extraction [Figure 2(d)], as well as an increase in systemic oxygen transport [Figure 2(e)]. In contrast, at the highest environmental temperatures of 35 & 40^{o}C, the increase in metabolic demand was met primarily by an increase in systemic oxygen transport, with no significant increase in the total body oxygen extraction or decrease in the mixed-venous oxygen saturations.

Cardiac index and heart rate also increased significantly relative to the values obtained at 25^{o}C [Figure 3(b&c)]. There were no significant changes in the pulmonary arterial pressures across this range of environmental temperatures; however, the systemic arterial pressures and the systemic vascular resistances decreased significantly as the environmental temperatures were increased to 35 & 40^{o}C [Figure 3(d&e)].

Figure 2. (a) Total body oxygen consumption; (b) body-core temperature; (c) ear-skin temperature; (d) total body oxygen extraction; (e) systemic oxygen transport in young lambs (n=7) exposed to a range of environmental temperatures (10, 15, 20, 25, 30, 35, 40^{o}C). Data are presented as means ± 1 SD. * = represents a significant difference at the 0.05 level from the value at the environmental temperature of 25^{o}C for each variable. 's' = represents the occurrence of shivering; 'p' = represents the occurrence of panting.

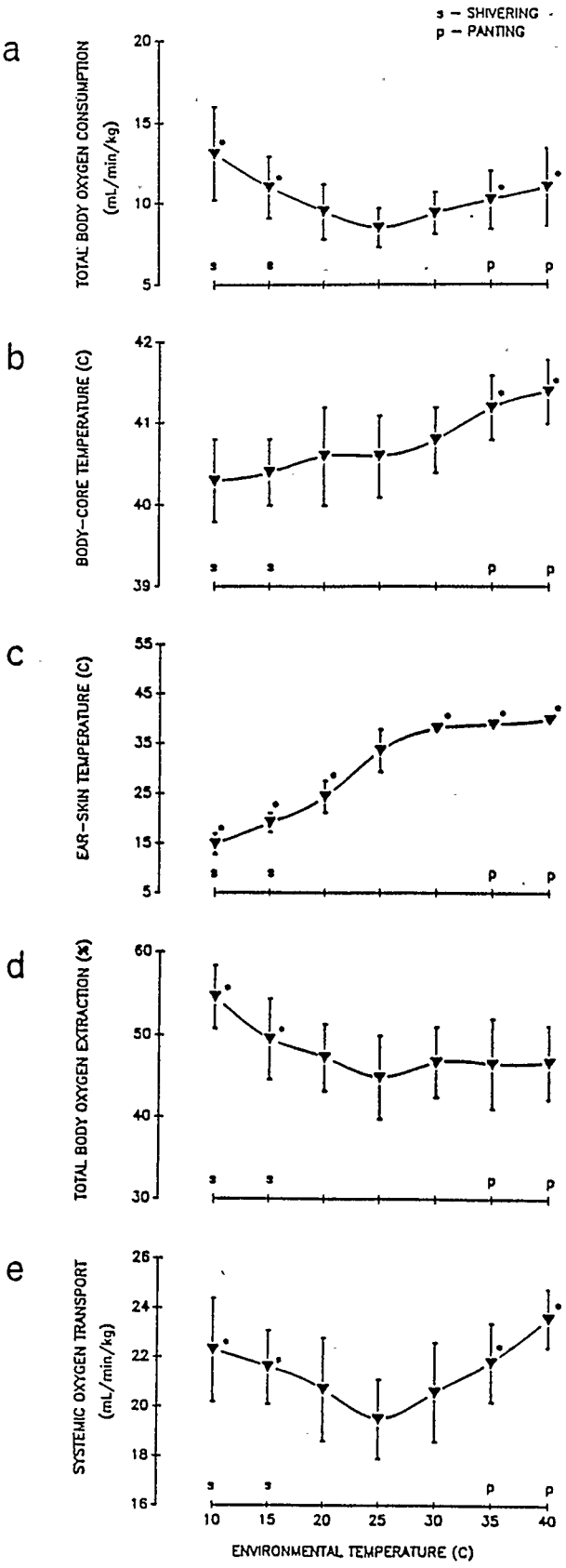
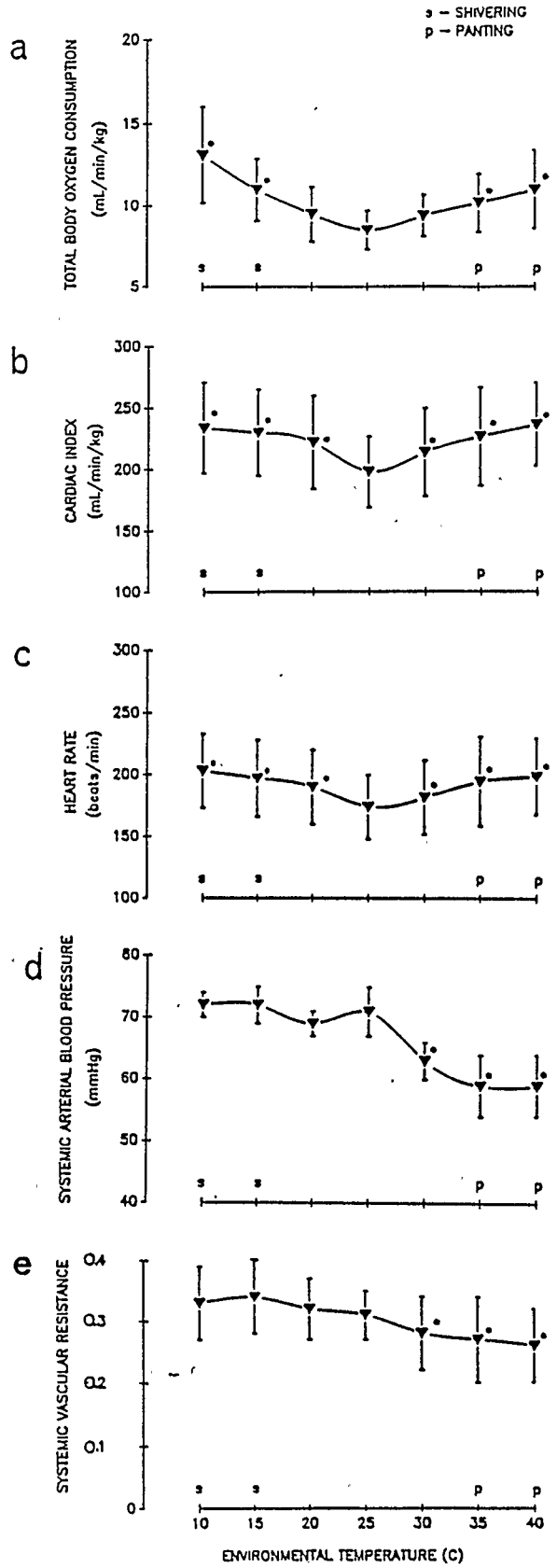

s – SHIVERING
p – PANTING
a
TOTAL BODY OXYGEN CONSUMPTION (mL/min/kg)
20
15
10
5
b
BODY–CORE TEMPERATURE (C)
42
41
40
39
c
EAR–SKIN TEMPERATURE (C)
55
45
35
25
15
5
d
TOTAL BODY OXYGEN EXTRACTION (%)
60
50
40
30
e
SYSTEMIC OXYGEN TRANSPORT (mL/min/kg)
26
24
22
20
18
16
s
s
p
p
10
15
20
25
30
35
40
ENVIRONMENTAL TEMPERATURE (C)

Figure 3. (a) Total body oxygen consumption; (b) cardiac index; (c) heart rate; (d) systemic arterial blood pressure; (e) systemic vascular resistance in young lambs exposed to a range of environmental temperatures (10, 15, 20, 25, 30, 35, 40^{o}C). Data are presented as means ± 1 SD. * = represents a significant difference at the 0.05 level from the value at the environmental temperature of 25^{o}C for each variable. 's' = represents the occurrence of shivering; 'p' = represents the occurrence of panting.


s – SHIVERING
p – PANTING
a
TOTAL BODY OXYGEN CONSUMPTION (mL/min/kg)
20
15
10
5
b
CARDIAC INDEX (mL/min/kg)
300
250
200
150
100
c
HEART RATE (beats/min)
300
250
200
150
100
d
SYSTEMIC ARTERIAL BLOOD PRESSURE (mmHg)
80
70
60
50
40
e
SYSTEMIC VASCULAR RESISTANCE
0.4
0.3
0.2
0.1
0
s s p p
10 15 20 25 30 35 40
ENVIRONMENTAL TEMPERATURE (C)

II. Effects of Hypoxemia on the Febrile Response of Young Lambs to Bacterial Pyrogen:

Administration of 0.3 ug of SAE produced a short-lived fever of about 0.8°C in normoxemic lambs studied at an environmental temperature of 25°C. Body-core temperature was significantly increased above control level at 50 minutes following intravenous administration of SAE and continued to be elevated for approximately 40 minutes (Figure 4). In contrast, there was no increase in the body-core temperature following administration of SAE in the hypoxemic lambs.

In the normoxemic condition, the increase in body-core temperature following SAE was preceded by the onset of shivering and a surge in total body oxygen consumption (Table 1). Shivering was identified by the phasic activity of the nuchal electromyogram, and occurred consistently in all lambs at 20, 30, and 40 minutes following SAE administration (Figure 4). The increase in total body oxygen consumption was initially met by an increase in the total body oxygen extraction (Table 1). Cardiac index, heart rate, and systemic oxygen transport increased during the peak body-core temperature response (Tables 1 & 2). Systemic arterial blood pressure did not change significantly during the febrile response; however, pulmonary arterial pressure increased following administration of SAE (Table 2).

In contrast, total body oxygen consumption did not increase in the hypoxemic lambs following administration of SAE; however, these animals continued to shiver (Figure 4). Similarly, total body oxygen extraction and systemic oxygen transport did not increase following the bacterial pyrogen (Table 1). Total body oxygen extraction, however, was significantly elevated in the hypoxemic condition, whereas the systemic oxygen transport was reduced. Baseline values of cardiac index and heart rate were significantly elevated, but did not change following administration of SAE (Table 2). Pulmonary arterial pressure was also elevated in these lambs with no further significant increases following SAE. Similarly, the systemic arterial pressure did not change following administration of SAE, however, it was significantly reduced in the hypoxemic lambs (Table 2).

In addition to an increase in heat production during the development of fever in the normoxemic condition, there was also evidence for vasomotor changes to effect heat conservation in these lambs that initially demonstrated cutaneous vasodilatation at an environmental temperature of 25^{o}C. In the normoxemic lambs, administration of SAE resulted in a decrease in skin-surface temperature, reflecting peripheral vasoconstriction [Figure 5(b)], which was followed by an increase in the body-core temperature [Figure 5(a)]. Evidence for peripheral vasoconstriction

(ie, a decrease in skin-surface temperature) was also demonstrated by the hypoxemic lambs; however, in these animals, it was not followed by an increase in body-core temperature (Figure 5(c&d)]. Cutaneous vasodilatation (ie, an increase in skin-surface temperature) reappeared in both the normoxemic and hypoxemic lambs approximately 80 minutes following SAE administration.

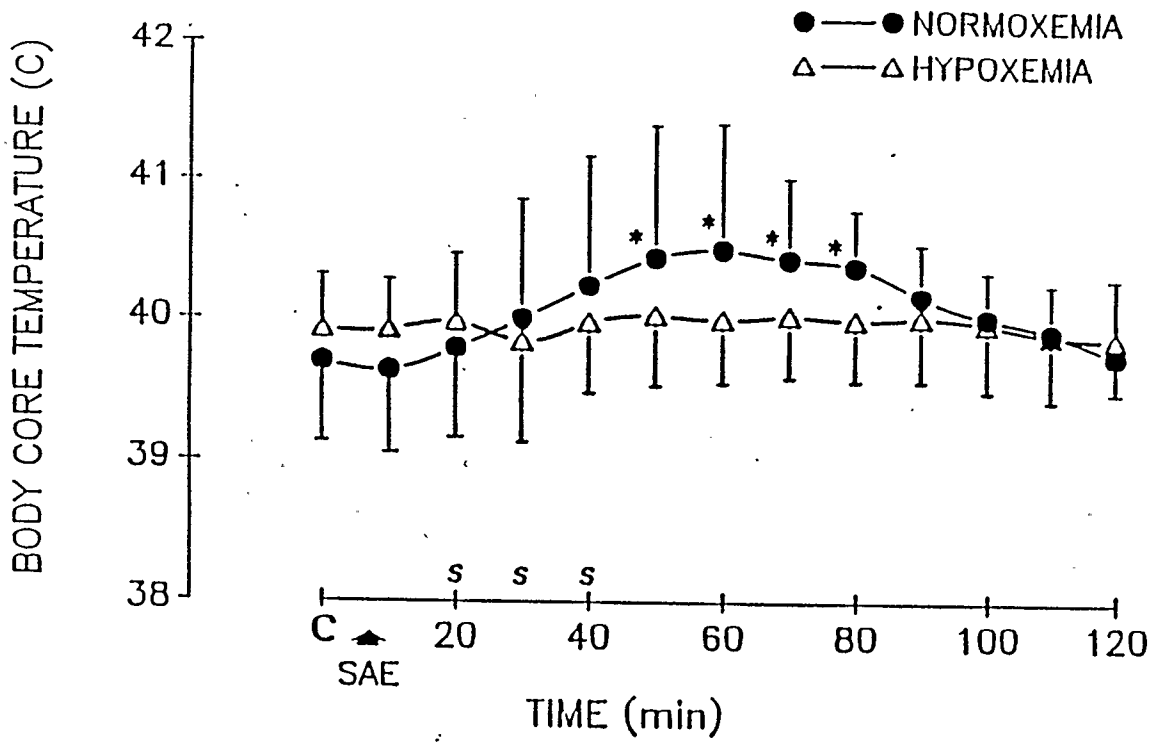


Figure 4. Body-core temperature in young lambs (n=8) before and after i.v. administration of 0.3 ug of SAE during the normoxemic and hypoxemic conditions. Body-core temperature measurements are shown for an initial control period and 10 minute intervals following the SAE. Data are presented as means ± 1 SD. * = represents a significant difference at the 0.05 level from the control value within each condition. 's'= represents the occurrence of shivering.

TABLE 1. Effect of intravenous administration of 0.3 ug of SAE on systemic oxygen transport and systemic oxygen utilization in young lambs.

Variable	Control	10m	20m	30m	40m	50m	60m	70m	80m	90m	100m	110m	120m
Total Body Oxygen Consumption (mL/Kg/min)													
NOR	9.4 ±3.3	9.4 ±3.0	10.6 ±3.0	12.2* ±4.5	12.7* ±3.6	11.3 ±2.6	11.8 ±3.5	11.3 ±2.8	10.0 ±2.1	9.6 ±2.3	9.2 ±2.8	9.1 ±2.4	9.2 ±2.8
HYP	10.3 ±2.5	9.9 ±1.8	9.6 ±1.8	9.6 ±2.2	10.3 ±2.4	10.2 ±2.5	10.3 ±2.5	9.7 ±1.5	10.0 ±1.6	10.2 ±1.8	10.2 ±2.2	10.4 ±2.3	10.6 ±2.5
Systemic Oxygen Transport (mL/Kg/min)													
NOR	19.3 ±4.0 #	19.2 ±4.1	20.1 ±4.3	20.5 ±6.7	21.0 ±5.3	20.8 ±5.0	23.0* ±4.4	21.1 ±4.4	20.3 ±4.4	19.5 ±4.6	18.9 ±4.8	18.2 ±4.8	18.5 ±4.4
HYP	13.9 ±3.8	14.0 ±3.1	13.2 ±2.6	13.4 ±2.9	14.2 ±4.2	14.8 ±3.9	14.9 ±4.4	14.5 ±4.2	14.4 ±4.2	15.1 ±4.0	15.2 ±4.3	15.3 ±4.3	15.4 ±4.6
Total Body Oxygen Extraction (%)													
NOR	44.4 ±11.2 #	46.5 ±9.6	49.7 ±9.2	57.0* ±6.5	57.1* ±6.5	50.7 ±4.0	49.6 ±9.1	50.6 ±9.7	49.0 ±10.5	49.2 ±10.5	47.1 ±9.1	47.1 ±9.3	47.3 ±9.9
HYP	71.8 ±15.2	66.7 ±15.4	69.0 ±15.8	69.7 ±16.4	71.4 ±16.7	67.5 +16.8	68.8 ±15.4	65.6 ±13.3	68.3 ±10.9	65.9 ±11.4	65.9 ±13.4	67.4 ±12.8	62.6 ±13.5

TABLE 1 (CONTINUED)

Arterial Hemoglobin Oxygen Saturation (%)

NOR	90±3	90±3	90±2	92±3	90±3	91±3	91±3	89±5	90±4	91±3	91±4	91±4	92±4
HYP	52±3	52±3	51±7	49±4	50±4	51±5	51±3	51±6	51±2	53±4	51±3	52±5	52±2

Mixed Venous Hemoglobin Oxygen Saturation (%)

NOR	50±9	48±8	46±8	40±5*	40±5*	46±6	46±6	44±8	46±10	46±10	47±11	48±9	48±10
HYP	15±10	18±8	16±8	16±10	15±9	17+9	16±8	19±6	16±6	19±6	18±7	18±5	21±3

Values are means ± 1 SD. $p < 0.05$ by MANOVA and Student Newman Keuls' is indicated as follows: * = control vs. time after administration of SAE; # = control (NOR) vs. control (HYP).

TABLE 2. Effect of intravenous administration of 0.3 ug of SAE on cardiovascular variables in young lambs.

Variable	Control	10m	20m	30m	40m	50m	60m	70m	80m	90m	100m	110m	120m
Cardiac Index (mL/Kg/min)													
NOR	190±37	194±42	199±34	198±40	201±33	201±38	214±56*	205±42*	201±38	193±45	186±41	181±35	182±38
HYP	257±46#	251±44	252±45	251±46	252±53	264±54	266±57	262±52	258±50	263±47	268±60	268±51	265±57
Heart Rate (Beats Per Minute)													
NOR	153±26	151±35	161±31	163±33	160±28	169±23*	189±28*	177±30*	157±24	152±28	152±29	148±33	147±27
HYP	219±42#	211±37	207±40	198±44	215±38	219±42	222±46	213±37	205±39	205±37	212±34	213±25	207±26
Systemic Arterial Blood Pressure (mmHg)													
NOR	74±7	75±6	75±6	76±9	77±8	78±7	80±7	77±9	76±9	77±10	73±8	70±5	72±6
HYP	65±9#	67±9	64±8	68±10	67±11	67±11	68±11	65±4	64±5	65±7	66±6	68±7	67±6

TABLE 2 (CONTINUED)

Systemic Vascular Resistance (mmHg/mL/min/Kg)

NOR	0.36 ±0.08 #	0.39 ±0.10	0.37 ±0.05	0.38 ±0.09	0.39 ±0.07	0.38 ±0.08	0.38 ±0.06	0.37 ±0.07	0.38 ±0.07	0.40 ±0.09	0.39 ±0.09	0.41 ±0.10	0.40 ±0.10
HYP	0.27 ±0.08	0.29 ±0.06	0.26 ±0.06	0.28 ±0.08	0.28 ±0.10	0.26 ±0.07	0.26 ±0.07	0.25 ±0.06	0.26 ±0.08	0.25 ±0.06	0.26 ±0.06	0.27 ±0.09	0.26 ±0.08

Pulmonic Arterial Blood Pressure (mmHg)

NOR	17±6 #	18±4	21±6	26±7*	31±7*	27±8*	28±8*	24±8	23±8	23±9	21±5	18±4	19±4
HYP	28±6	28±7	30±10	30±8	29±7	30±7	29±6	29±5	30±7	29±7	29±7	31±6	31±5

Values are means ± 1 SD. $p < 0.05$ by MANOVA and Student Newman Keuls' is indicated as follows: * = control vs. time after administration of SAE; # = control (NOR) vs. control (HYP).

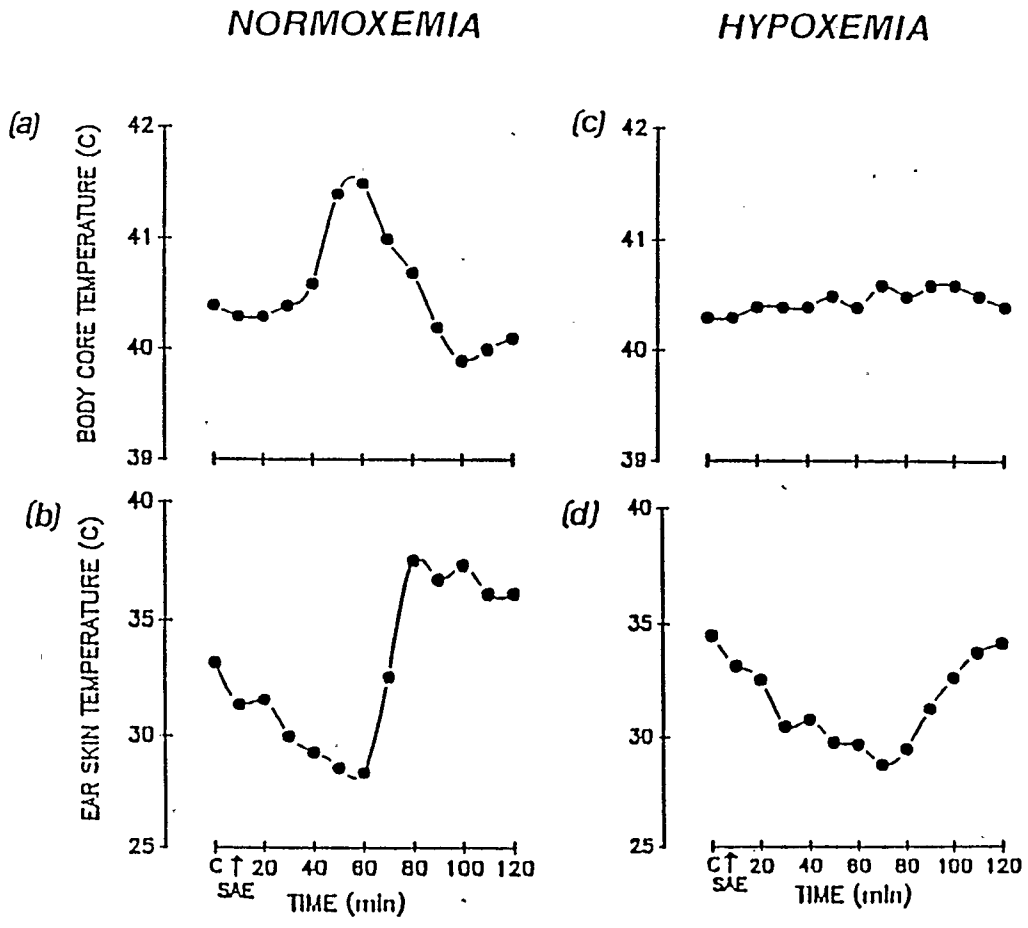


Figure 5. Plots showing body-core temperature and ear-skin temperature in a single lamb following the administration of SAE in the normoxemic and hypoxemic condition.

Blood gas data and hemoglobin oxygen saturations from arterial and mixed venous blood are shown in Tables 1 & 3. In the hypoxemic condition, arterial blood CO_2 tension (P_{aCO2}) decreased to about 25 Torr at an arterial hemoglobin oxygen saturation of 50%. Arterial blood PO_2 (P_{aO2}) decreased to approximately 35 Torr, with no further significant changes following administration of SAE. Thus, with a reduced F_{IO2}, the P_{aO2} fell and the lambs developed a respiratory alkalosis as evidenced by an increased pHa and a decreased P_{aCO2} (Table 3). Furthermore, there were no signs that these lambs developed a metabolic acidosis since the blood lactate concentrations were not significantly elevated (Table 4), nor was there a significant change in the base deficit (Table 3).

TABLE 3. Blood Gas Data in Young Lambs (n=8) before and after SAE administration for Normoxemic and Hypocapnic Hypoxemic Conditions.

	NORMOXEMIA					HYPOCAPNIC HYPOXEMIA				
	C	30	60	90	120	C	30	60	90	120
pH	7.43* ±.03	7.43 ±.03	7.43 ±.03	7.43 ±.03	7.42 ±.04	7.52* ±.05	7.50 ±.06	7.53 ±.07	7.51 ±.06	7.51 ±.05
pCO_2 (torr)	34.9* ±6.3	37.2 ±8.7	36.0 ±8.2	36.7 ±7.3	37.7 ±8.1	24.9* ±4.6	23.6 ±5.2	22.3 ±6.3	23.0 ±6.9	23.1 ±6.4
pO_2 (torr)	75.7* ±6.7	74.1 ±8.3	73.4 ±8.9	75.2 ±8.4	75.7 ±7.6	33.8* ±5.7	32.6 ±7.1	31.1 ±6.5	31.1 ±6.5	32.5 ±7.5
BE/BD (mEq/L)	0.11 ±2.8	0.31 ±1.7	0.41 ±2.6	0.24 ±2.4	0.44 ±2.4	-0.56 ±2.1	-2.2 ±3.2	-1.7 ±3.5	-1.9 ±4.5	-2.1 ±4.7

Values are means ± 1SD. $p < 0.05$ by MANOVA and Student Newman Keuls' is indicated as follows: * = control (NOR) vs. control (HYP).

TABLE 4. Blood Lactate Concentrations (mmol/L) in Young Lambs (n=8) before and after SAE Administration for Normoxemic and Hypocapnic Hypoxemic Conditions; Values are means ± 1 SD.

	Control	60	120
NORMOXEMIA	1.12 ±.55	1.17 ±.22	1.04 ±.20
HYPOCAPNIC HYPOXEMIA	1.25 ±.41	1.21 ±.77	1.31 ±.90

DISCUSSION

Our experiments provide new information about the factors which may limit the temperature, metabolic and cardiovascular responses of young lambs to a small dose of bacterial pyrogen. Studied within their thermoneutral zone, we found that administration of 0.3 ug of SAE produced a short-lived fever of about 0.8°C in normoxemic lambs which was not observed following the administration of SAE in hypoxemic lambs.

I. Defining the Thermal Neutral Environment:

Few studies have actually documented the thermal neutral temperature range of newborn mammals. Our experiments not only define thermoneutrality, but also provide new information about the oxygen utilization and transport in young lambs attempting to thermoregulate across a range of environmental temperatures. Thermoneutrality -- usually defined as the environmental temperature at which metabolism is minimal while a normal body temperature is maintained (Brück, 1961; Alexander, 1974; Stainer, Mount & Bligh, 1984) -- was found to be 25°C for these lambs (21±1 days old). According to Mount (1979), within the thermoneutral zone, not only is the metabolic rate at a minimum, but it is "independent" of the environmental temperature. Thus, if the environmental temperature rises or falls within the thermal neutral

environment, temperature regulation is maintained by peripheral vasodilatation or vasoconstriction, or increased heat dissipation (ie, sweating or panting). Our data suggest that the peripheral vasomotor changes occurring at thermoneutrality were minimal since the skin-surface temperature in these lambs was found to be in a mid-range between a vasoconstricted and vasodilated state observed at the other environmental temperatures [Figure 1(c)].

At the low environmental temperatures (ie, 10 & 15^{o}C), the lambs were able to maintain a normal body-core temperature -- a response very characteristic of homeotherms (Mount, 1979). This was accomplished by the significant rise in total body oxygen consumption accompanied by vigorous shivering, and most likely nonshivering thermogenesis (Alexander, 1961; Alexander & Williams, 1968), demonstrated in several other species (Blatteis, 1975; Dawkins & Hull, 1964). Although changes in the plasma concentration of various metabolites to confirm thermogenic activity during exposure to the low environmental temperatures were not measured in these lambs, previous studies (Alexander et al, 1972; Alexander & Mills, 1968a) have reported an increase in the plasma concentration of glucose, lactate acid, free fatty acids, and glycerol during cold exposure in young lambs. According to Alexander et al (1968b), this is probably due to stimulation of the sympathetic nervous system by cold

exposure, since they are mimicked when cathecholamines are infused into lambs under thermoneutral conditions.

(A) Oxygen Utilization and Transport Below the Thermal Neutral Environment:

The increased metabolic oxygen demand occurring at the lower environmental temperatures (ie, 10 & 15^{o}C) was met by an increase in total body oxygen extraction and an increase in systemic oxygen transport. In addition, there was a significant decrease in skin-surface temperature, reflecting peripheral vasoconstriction. These findings seem to suggest that there is a redistribution of blood flow towards the thermogenic tissues (Alexander et al, 1973), possibly aiding the effector mechanisms of shivering and nonshivering thermogenesis discussed above known to improve insulation on cold exposure. The increase in cardiac index seen in these lambs is similar to previous experiments (Alexander & Williams, 1970) and suggests that cardiac output is finely adjusted to the metabolic requirements of the thermogenic tissues.

In addition to an increase in heat production occurring at the lower environmental temperatures, there was also evidence for a reduction of heat loss, as seen by a decrease in the skin-surface temperature. Studies on tissue blood flow in lambs (Alexander et al, 1973) have shown that blood flow through the skin of the extremities

and the trunk as well as through the nasal turbinals almost ceases under cold conditions.

(B) Oxygen Utilization and Transport Above the Thermal Neutral Environment:

In contrast to the above findings, the increased metabolic oxygen demand occurring at the higher environmental temperatures (ie, 35 & 40^{o}C) was met primarily by an increase in systemic oxygen transport as a result of an increase in cardiac output. The effective increase in cardiac output and oxygen delivery -- most likely away from the thermogenic tissues and towards the periphery (as seen by the increased skin-surface temperature and decreased systemic vascular resistance; Figures 1c & 2e) -- no doubt aided the effector mechanisms of heat dissipation. In fact, previous experiments investigating regional blood flows (Hales, 1973) in sheep have reported that a mild heat stress resulted in a significant increases in blood flow to the tissues important in heat exchange including the nasal mucosa, tongue, external pinnae, and skin of the legs and body. Hales (1973) also reported that the proportion of cardiac output passing through the arterio-venous anastomoses (AVA's) (abundant in the heat exchange tissues mentioned above) increased significantly during a heat stress,

indicating that the AVA's may be temperature sensitive and may play a major role in regulating convective heat loss.

Also associated with the rise in total body oxygen consumption was a slow but significant rise in body-core temperature, suggesting that the capability of the heat-dissipating systems was exceeded. For example, there is a possibility that the temperature gradient between the skin surface and environmental air no longer existed in these lambs since their skin-surface temperatures were similar to the environmental temperatures in the upper range; therefore, no convective heat loss would have been expected to occur (Hill, 1962). Thus, as a result of overloading the heat-dissipating systems, body-core temperature significantly increased at the higher environmental temperatures. Previous studies have shown that normal lambs pant and sweat (Alexander, 1974), but panting (ie, respiratory heat loss) is the major route of heat loss in the hot zone. In fact, although water loss from the skin (ie, sweating) also increases proportionately with increasing environmental temperatures in young lambs, the rate of increase is about half that of respiratory heat loss (Alexander, 1974). Although the lethal limits for lambs have not been well defined, at high environmental temperatures, if the heat-dissipating mechanisms are exceeded, body-core temperature and metabolic rate (Q_{10} Effect) will increase with ensuing death in hyperthermia.

II. Effects of Hypoxemia on the Febrile Response in Young Lambs to Bacterial Pyrogen:

(A) Responses in the Normoxemic Lambs:

The increase in body-core temperature following administration of SAE was preceded by the onset of shivering thermogenesis in these lambs and according to previous studies (Harris et al, 1985; Székely et al, 1973) nonshivering thermogenesis as well. These effector responses were followed by a surge in total body oxygen consumption (Table 1). The increased metabolic oxygen demand occurring during the febrile response was met initially by an increase in the total body oxygen extraction, followed by an increase in the systemic oxygen transport. For example, during the rising phase of the febrile response, there was an increase in total body oxygen extraction and most likely a redistribution of blood flow to the tissues involved in the thermogenic response (Riedel & Hales, 1983; Harris et al, 1985; Blatteis et al, 1988). During the peak of the febrile response, the increased metabolic oxygen demand was met by an increase in systemic oxygen transport as a result of an increased cardiac output. The increased cardiac output, although primarily the result of increased oxygen demand, may also be the result of a marked renal vasodilatation occurring during fevers induced by bacterial pyrogens (Cranston,

1959; Cooper et al, 1960). The cardiac output response we observed (ie, no change during the rising phase of fever and an increase during the peak phase of fever) is similar to what has recently been reported in young rabbits (Harris et al, 1985), but different from that obtained by Blatteis et al (1988) in whethers. Blatteis et al (1988) found that cardiac output was near baseline levels at the peak of the febrile response. The reason for the apparent discrepancy between our data and those of Harris et al (1985), and the data of Blatteis et al (1988) is not clear and requires further investigation.

All of the normoxemic lambs demonstrated a shivering thermogenic response to administration of bacterial pyrogen. This response preceded the surge in total body oxygen consumption (Figure 4). Similar findings have been reported in young rabbits (Harris et al, 1985) where it was shown that blood flow to the skeletal muscles of the forelimbs and hindlimbs doubled during the rising phase of fever with visible signs of shivering, indicating increased metabolic activity of the skeletal muscles. Furthermore, previous reports have suggested that the occurrence of shivering thermogenesis as a mechanism of increased heat production depends not only on the temperature at which the animal is reared, but also on the environmental temperature in which the animal is kept after administration of a bacterial pyrogen. For example, Blatteis (1976a) found

that in cold-acclimated adult guinea pigs nonshivering thermogenesis replaced shivering thermogenesis in a thermoneutral environmental in supplying the necessary heat for fever production. In an environmental temperature below the thermal neutral temperature (ie, 7^{o}C), shivering occurred in both the non-cold and cold-acclimated endotoxin-treated guinea pigs. Although the animals in our experiments were reared and studied at their thermoneutral temperature, and demonstrated both shivering and nonshivering thermogenesis, the relative contribution of shivering versus nonshivering thermogenesis to the overall metabolic response is not clear and will be speculated upon in the following section.

(B) Responses in the Hypoxemic Lambs:

Administration of 0.3 ug of SAE produced no significant rise in the body-core temperature of hypoxemic lambs (Figure 4) and no surge in the total body oxygen consumption as seen in the normoxemic condition (Table 1). Previous studies have shown that alveolar hypoxia alters the nonshivering thermogenic functioning of the brown adipose tissue of newborn mammals whether it is induced by cold exposure (Dawkins & Hull, 1964; Hill, 1959) or sympathetic stimulation (Blatteis, 1964; 1966). It has also been reported that brown adipose tissue nonshivering thermogenesis is the prevailing mechanism of heat

production in response to endotoxin (Blatteis, 1976b; 1977; Harris et al, 1985). Thus based on these findings, our results would suggest that hypoxemia alters the endotoxin-induced nonshivering thermogenic functioning of the brown adipose tissue, possibly responsible for the attenuated febrile response seen in these lambs. Because metabolism of the brown adipose tissue is an oxidative process (Dawkins & Hull, 1964; Smith & Horwitz, 1969; Cannon et al, 1978), the mechanism by which hypoxemia alters the nonshivering thermogenic functioning of the brown adipose tissue might be related to an oxygen insufficiency at the mitochondrial level. Although no systematic in vitro studies on the effects of hypoxia on the biochemical properties of neonatal brown adipose tissue have been conducted, it has been suggested that the mechanism of thermogenesis in the brown adipose tissue is an uncoupling of mitochondrial respiration from the constraints of the phosphorylating system, resulting in increased mitochondrial activity and thus brown fat heat generation (Lindberg et al, 1967; Prusiner et al, 1968a; 1968b). In the lamb, the perirenal adipose tissue resembles brown adipose tissue from other species with respect to its morphological appearance and pattern of fatty acid oxidation; however, it differs in that it has a high mitochondrial ATP synthetic capacity (Cannon et al, 1977).

Of enormous interest in these experiments was the observation that hypoxemic lambs continued to exhibit two of the thermoregulatory effector responses to bacterial pyrogen seen in the normoxemic condition. These responses included shivering thermogenesis and peripheral vasoconstriction (Figure 5). The sustained shivering thermogenic response as a mechanism of heat production during hypoxemia in these lambs is consistent with the observations that shivering is remarkably resistant to hypoxemia (Blatteis, 1964; Hemmingway & Birzis, 1956; Hemmingway & Nahas, 1952). Shivering thermogenesis, however, in itself as an effector mechanism for heat production was not sufficient to raise the body-core temperature of the hypoxemic lambs. In attempting to analyze these findings in terms of the relative importance of shivering versus nonshivering thermogenesis in young lambs following administration of a bacterial pyrogen, we would speculate that not only does hypoxemia alter the nonshivering thermogenic component of the febrile response, but that this component may be the essential effector mechanism of heat production for fever development in newborn mammals.

In order to explore the possible explanations for the attenuated febrile response, we also investigated the changes in oxygen utilization and transport in the hypoxemic condition. We found that in the absence of any

measurable increased metabolic oxygen demand, there were no changes in the cardiac output, total body oxygen extraction, and systemic oxygen transport following administration of SAE. However, the baseline values of cardiac output and total body oxygen extraction were significantly elevated in the hypoxemic condition. The baseline values of systemic oxygen transport, on the other hand, was significantly reduced in these lambs. Similar to these findings, it has been shown that when hypoxemia is induced in the intact newborn subject (such as the lamb), oxygen extraction and cardiac output increase although the net result is often a decrease in systemic oxygen transport (Sidi et al, 1983a; Sidi et al, 1983b; Lister, 1984a; 1984b). Although our values of total body oxygen extraction and systemic oxygen transport are consistent with values reported elsewhere for young lambs (Moss et al, 1987; Sidi et al, 1983a) with no concurrent fall in baseline total body oxygen consumption, there is a possibility that a reduced oxygen reserve during hypoxemia may have limited oxygen delivery to the thermogenic tissue 'brown fat'. As a result, the extra thermogenic metabolism required for fever production (Harris et al, 1985) may have been reduced. In fact, Sidi et al (1983b) reported that hypoxemic lambs exposed to a cool environment demonstrated a decreased reserve for oxygen extraction (ie, decreased mixed-venous P_{O2} and oxygen saturation), resulting in a

large decrease in oxygen consumption and body-core temperature. Thus we can speculate that since the baseline values of mixed-venous oxygen saturations were reduced and the baseline values of total body oxygen extraction elevated in the hypoxemic lambs, there was likely to be a decreased reserve for further oxygen extraction. Therefore, unlike the normoxemic lambs following SAE administration, these lambs could not have extracted more oxygen to meet the increased oxygen requirements for increased nonshivering thermogenic functioning of the brown adipose tissue. With more severe limitations in cardiac output (and thus systemic oxygen transport), total body oxygen consumption has been found to decrease and tissue hypoxia does occur (Moss et al, 1987; Adams et al, 1982).

In addition, our lambs demonstrated a respiratory alkalosis during the hypoxemic condition (Table 3). This would result in a leftward shift of the oxygen hemoglobin saturation curve such that a decrease in the P_{50} (ie, the P_{O2} at which the hemoglobin is 50% saturated) would occur. However, we measured the hemoglobin oxygen saturation directly and thus a shift in the oxygen hemoglobin curve would not affect our oxygen content.

As a final note, it is of importance to discuss the role of arginine vasopressin (AVP) as a possible antipyretic substance responsible for the attenuated febrile response in the hypoxemic lambs. The plasma

concentrations of AVP were not measured in these lambs, but there is sufficient evidence to suggest that AVP levels increase during hypoxemia (Daniel et al, 1984; Rurak, 1978). Although several lines of evidence have strongly suggested that the neuropeptide AVP has a role as an endogenous antipyretic substance (Kasting et al, 1978; Kasting et al, 1980), we would speculate that it is unlikely to be responsible for the attenuated febrile response in these hypoxemic lambs based on two observations. These include the elicitation of two effector responses to the bacterial pyrogen -- shivering thermogenesis and peripheral vasoconstriction. These observations suggest that a shift in the thermoregulatory set-point still occurred in the hypoxemic state. Thus, based on the findings that the antipyretic effects of AVP are centrally-mediated (Kasting et al, 1979a; Ruwe et al, 1985; Kasting & Wilkinson, 1987), we probably should not have expected to see these effector responses to the bacterial pyrogen in the hypoxemic lambs. From our findings, it is clear that further experimentation is required to fully elucidate the mechanism(s) responsible for the attenuated febrile response occurring during hypoxemia in young lambs.

III. Implications

Although our findings allow us to draw the conclusion the hypoxemia attenuates the febrile response in young lambs to a small dose of bacterial pyrogen, the implications of such findings are far from being clear. These findings seem to raise the question of whether fever (ie, the regulation of body temperature at an elevated level) is beneficial for the newborn. If we consider the effects of fever on mortality and morbidity, several reports have shown that newborn mammals infected with a variety of viruses have higher survival rates when febrile (Carmichael et al, 1969; Haahr & Mogensen, 1977). Furthermore, numerous other studies have demonstrated that small elevations in body temperature, similar to those observed during fever, result in an enhancement of the immune response. These include increased mobility and activity of the white blood cells (Nahas et al, 1971), stimulation of interferon production and function (Heron & Berg, 1978), and activation of the T-lymphocytes (Duff & Durum, 1982).

In spite of these beneficial outcomes, it is important to acknowledge that a detrimental component associated with fever may be its "metabolic cost". That is, there is an increased metabolic oxygen demand associated with elevating and then maintaining body temperature (demonstrated in the present experiments). Our data have shown , however, that

even in a state of limited oxygen reserve (ie, hypoxemia) young lambs "attempted" to develop a fever (ie, they demonstrated both shivering thermogenesis and peripheral vasoconstriction in response to the bacterial pyrogen), but were unable to do so possibly because of the limited reserve for oxygen delivery to the thermogenic tissue 'brown fat'. Thus these findings might provide further evidence that fever is in fact beneficial to the newborn.

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