

## **Abstract**

**Henderson, R.A. THERMOREGULATORY RESPONSES FOLLOWING HEMORRHAGE IN CONSCIOUS RATS. (Under the direction of R.G. Carroll, Ph.D. and G.W. Kalmus, Ph.D.) Departments of Physiology and Biology, East Carolina University. April, 1997.**

Body temperature in endothermic animals reflects the delicate balance between heat production and heat loss. Following hemorrhage, there is a disruption of the thermoregulatory balance inducing a drop in core temperature. Current patient management guidelines suggest that resuscitation from hemorrhagic shock should be aimed at prevention of hypothermia. However, as a physiological response to hypoxia, mild hypothermia reduces mortality and improves neurological outcomes. Following hemorrhagic shock states, mild hypothermia may possess similar protective characteristics. Male Sprague-Dawley rats (300 - 350 gm) were used to (a) establish a hemorrhage-induced hypothermia model, (b) determine the time course of hypothermia, (c) determine if heat loss from cutaneous blood flow mediates the hypothermic event, and (d) determine if a decreased metabolic rate accompanies the drop in body core temperature. Moderate hemorrhage (16 cc/kg) caused a significant drop in core temperature, stabilizing  $-0.7 \pm 0.3^{\circ}\text{C}$  below control seventy minutes after the hemorrhage. Animals hemorrhaged at elevated room temperature showed an attenuated drop in core temperature which stabilized 180 minutes after the hemorrhage at  $-0.5 \pm 0.15^{\circ}\text{C}$  below control values. Additionally, after eliminating autonomic control of cutaneous blood flow with an  $\alpha_1$  receptor antagonist,

hemorrhage induced a  $-3.1 \pm 0.21^{\circ}\text{C}$  drop in core temperature, suggesting that following hemorrhage, intrinsic regulatory mechanisms may actually protect body heat content. Measurements of oxygen consumption revealed that the hypothermic event which follows hemorrhage was accompanied by a significantly reduced ( $-0.21 \pm 0.05 \text{ cc O}_2/\text{gm hr}^{-1}$ ) metabolic rate. The drop in oxygen consumption was not reproduced in animals which were hemorrhaged and immediately retransfused with their aspirated blood or in animals given the  $\alpha_1$  specific antagonist. These data suggest that: (a) hemorrhage produces a drop in body temperature, (b) dry heat loss from cutaneous blood flow does not mediate the hypothermic event, (c) intrinsic regulation of blood distribution following hemorrhage may serve to protect against severe hypothermia, and (d) the drop in core temperature is preceded by a decreased metabolic rate suggesting that a reduction in metabolic heat production may contribute to the hypothermia which follows hemorrhage.



**THERMOREGULATORY RESPONSES  
FOLLOWING HEMORRHAGE  
IN CONSCIOUS RATS**

A THESIS  
PRESENTED TO  
THE FACULTY OF THE DEPARTMENT OF BIOLOGY  
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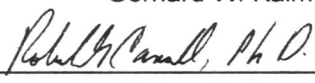
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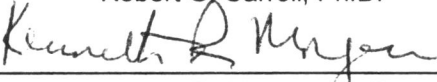
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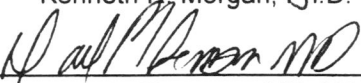
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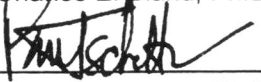
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FAR BEYOND MY VERY EXISTENCE, I AM FOREVER INDEBTED  
FOR THE SUPPORT MY PARENTS GAVE ME THROUGHOUT THIS PROJECT.  
AS SUCH, I DEDICATE THIS WORK TO MY PARENTS:

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## **Preface**

Following hemorrhage, regulation of tissue perfusion requires dynamic interaction between the cardiovascular, pulmonary, nervous, renal, and endocrine systems. Homeostatic principles direct both local and global modifications in each of the systems promoting a return to baseline status. Early in the hemorrhage, these compensatory responses are classically represented by increases in heart rate and peripheral resistance. As the severity of the shock state worsens, compensatory modifications can no longer maintain adequate tissue perfusion resulting in decreased cardiac performance parameters, altered mental states, and if left untreated, death.

Hemorrhage induces an alteration in the balance between heat production and heat loss that results in a drop in body temperature. Current patient management guidelines advocate a three-fold approach to resuscitation of hemorrhagic shock patients which addresses the body's normal cardiovascular, pulmonary, and thermoregulatory responses; including the prevention of hypothermia. However, recent studies have suggested that hypothermia is an adaptive response to hypoxemia and that hypothermia improves survival following hypoxic and shock states. The hypothermia which accompanies hemorrhagic shock may provide similar protective characteristics.

This paper will focus on the expected physiological and thermoregulatory responses following hemorrhage in conscious animals in an effort to elucidate

the underlying unknown mechanism mediating the thermoregulatory imbalance between heat production and heat loss.

## **Introduction**

### **Hemorrhagic Shock**

#### **A Historical Perspective**

Historically, war provided the bulk of observational and interventional hemorrhage research. The term *shock* first appeared in 1743 in Ledran's description of battlefield wounds leading to several hypothesis, most of which suggested presence of a circulating toxin liberated by the initial injury (in Geller, 1993). In World War I, Cannon provided the most comprehensive studies of traumatic hemorrhage to date and concluded that toxins and acidosis contributed to reduced vascular tone (in Geller, 1993). In the period before World War II, Blalock, along with other researchers, suggested that the reduction in circulating plasma, not toxins, was the underlying pathological cause of mortality (in Geller, 1993). The advent of transfusion therapy restored circulating oxygen carrying capacity for victims of traumatic hemorrhage in World War II: however, complications from whole blood product substitution plagued resuscitation outcomes. Efforts to limit immunogenic reactions were evaluated and it was determined that the administration of fractions of blood products reduced the prevalence of transfusion reactions. In the 1974 paper, Moss stated: "the era of whole blood substitution is probably coming to an end" (Moss et al., 1974). Simultaneously, a second line of volume resuscitation was being evaluated which suggested crystalloid and colloid products be given immediately after

hemorrhage. These products restored circulating volume but did not enhance oxygen carrying capacity. In the 1930's, prototype synthetic hemoglobin solutions, in theory, offered solutions to the allergenic as well as oxygen carrying problems; however, the difficulty in synthesizing a stable artificial hemoglobin molecule has prevented its clinical use (Dietz et al., 1996). Currently, volume resuscitation itself is controversial having been implicated as a factor contributing to increased mortality in penetrating trauma patients (Capone et al., 1995). The direction of hemorrhage research has historically been aimed at resuscitation modalities only recently redirecting to evaluate mechanistic properties.

### **Physiological Responses to Hemorrhage**

The body responses following hemorrhage follow a predictable pattern which involve dynamic interactions between the cardiovascular, autonomic nervous, endocrine, and pulmonary systems. The goals of this cascade of responses are to maintain perfusion to the critical organs such as the brain and heart, restore systemic tissue perfusion, optimize oxygen and nutrient delivery, and maintain waste removal.

#### **Cardiovascular System**

The cardiovascular responses are directed at maintaining adequate blood supplies to the heart and brain. Sympathetic efferent activity causes an increase in heart rate and contractility (Bond et al., 1985c). In this early phase, cardiac performance parameters are increased. From the onset of hemorrhage,

resistance increases linearly in the cutaneous and mesenteric microvasculature shunting blood to the central circulation (Bond et al., 1985a). As the severity of hemorrhage progresses, heart rate continues to increase as mean arterial pressure decreases. By maintaining effective perfusion pressures to the critical organs, the body has “compensated” for the hemorrhage. With continued uncontrolled hemorrhage, cardiovascular compensation is less effective in maintaining adequate perfusion pressures to the critical organs; therefore, the cardiovascular response begins to “decompensate”. As a result, the initial sympathetic excitatory phase is followed by a sympathetic inhibited phase characterized by relative bradycardia and severe hypotension. First characterized in 1962, the mechanism driving the reduced heart rate may lie in vagal afferents, cardiac mechanical receptors, or other “depressor responses” (Skoog et al., 1985). Meanwhile, mean arterial pressure continues to decline. Aggressive intervention early in the decompensated phase can reverse the shock state; however, as the shock state progresses, the body responds by entering the second decompensated sympathetic excitatory phase. Resuscitation efforts in this phase are often futile. In this final phase, sympathetic activity again rebounds to an excitatory state. Heart rate, epinephrine, and norepinephrine levels elevate. Maximal peripheral resistance values are observed. However, blood flow to the brain and heart is inadequate resulting in mental status changes, poor diastolic filling, low ejection fractions,

and low stroke volumes. In this terminal stage, the mean arterial pressure continues to decline until death.

As mentioned, total peripheral resistance increases linearly in response to hemorrhage. Acting primarily on arterial resistance vessels and secondarily on venous capacitance vessels, the combined effect of innervated and non-innervated alpha receptors provide for the redistribution of blood away from the cutaneous and mesenteric microvasculature. Maintenance of mean perfusion pressures during compensated hemorrhage is thought to be the synergistic effect of circulating catecholamines interacting with non-innervated  $\alpha_2$  receptors and innervated  $\alpha_1$  receptors. Specific blockade of  $\alpha_1$  receptors will drop mean arterial pressure before hemorrhage; however, following hemorrhage, the ability to compensate for hemorrhage is prolonged, thus refuting the hypothesis that  $\alpha_1$  receptors provide any significant long-term compensation (Bond et al., 1984b). The time that an individual can compensate appears to be a function of extra-synaptic  $\alpha_2$  receptors stimulated by circulating catecholamines or by close proximity presynaptic norepinephrine release (Bond et al., 1984b). In decompensated hemorrhage, the drop in perfusion pressures is predominately relative to bradycardia although maximal peripheral vasoconstriction is observed (Bond et al., 1985d).

### **Autonomic Nervous System**

The cardiovascular response to hemorrhage is at the direction of the autonomic nervous system. Sympathetic activity enhances myocardial performance and provides for vasoconstriction directly via sympathetic efferents and indirectly via release of catecholamines. Minute quantities of blood loss are sensed by carotid baroreceptors as poor cardiac filling reduces cardiac output (Bond et al., 1984b). Noting the pressure change, afferent activity is transmitted to the reticular substance of the medulla and lower pons (Harkema et al., 1993). Here the information is integrated and sympathetic efferents transmit the vasoconstrictive message to the local tissues. In cardiac tissue, it is thought that the increase heart rate is a direct result of sympathetic efferent activity and not the result of circulating catecholamines as non-specific beta adrenergic blockade does not influence the chronotropic response (Bond et al., 1984c). Renal sympathetic inhibition is thought to be mediated by arginine vasopressin (Edwards et al., 1994), or serotonin (Morgan et al., 1988). Furthermore, renal sympathetic inhibition does not appear dependent on renal artery blood pressure (Hasser et al., 1992). However, renal sympathetic inhibition may be uncoupled from observed decreases in heart rate and not be the effect of the above mechanisms. Animals, in which the receptors for arginine vasopressin in the area postrema were excised, demonstrated a recovery of renal sympathetic activity following administration of arginine vasopressin. However, bradycardia

was not attenuated with the hormone. Specific lesions of the area postrema alone enhanced the bradycardic effect (Edwards et al., 1994).

### **Endocrine System**

The predominate endocrine agents contributing to recovery following hemorrhage are the catecholamines. In addition to being released from sympathetic efferents upon baroreceptor afferent unloading, the sympathetic neurotransmitter, norepinephrine, directly imparts its stimulatory effect on heart rate, contractility, and induces local vasoconstriction. The vasoconstrictive effect is augmented by autocrine and paracrine diffusion stimulating alpha receptors in near proximity (Bond et al., 1984b). The principle site of catecholamine release is from the adrenal medulla. Circulating epinephrine and norepinephrine stimulate alpha and beta receptors in the heart and microvasculature; although, the relative vasoconstrictive properties of the individual subclasses of receptors is controversial (Bond et al., 1984b,c). Early in the hemorrhage, epinephrine levels are the first to respond to adrenal sympathetic nerve activity. As the severity of hemorrhage increases, norepinephrine levels rise parallel to epinephrine levels (Haggendal, 1986). Release of catecholamines do not appear to be affected by adrenal sympathetic inhibition as other release stimulus are employed (Haggendal, 1986).

In addition to circulating catecholamines, other contributions of the endocrine system are substantial following hemorrhage. The synergistic effect of



low renal perfusion, stimulation of the macula densa receptors, and renal sympathetic efferent activity, stimulates the release of renin from the juxtaglomerular cells. Renin, through a cascade of reactions, stimulates the formation of angiotensin II. The response to angiotensin II is: (a) promote water retention via enhanced sodium reabsorption in the kidney, (b) potent vasoconstrictor, (c) positive chronotropic effects on the heart, and (d) stimulate the release of aldosterone (Harkema et al., 1993), which promotes fluid retention in the kidney. Finally, the role of arginine vasopressin, as a potent vasoconstrictor, appears to be significant following severe hemorrhage. With mild to moderate hemorrhage, vasopressin enhances fluid retention by increasing the permeability to water in the distal tubule and collecting duct. However, in severe hemorrhage serum levels of vasopressin increase out of proportion to the maximal water retention capability and unlike the observation in mild to moderate hemorrhage, imparts potent vasoconstrictive properties within the microvasculature (Fejes-toth et al., 1988).

### **Pulmonary System**

The pulmonary response to hemorrhage is aimed at optimizing alveolar oxygen uptake, facilitating CO<sub>2</sub> release by the lungs, and maintaining an adequate buffer response to acute perfusion changes in the tissues. Using central control and chemosensitivity for compensation, immediate responses in gas exchange are evident.

Immediately following hemorrhage, there is an observed increase in respiratory rate driven by tissue ischemia and the resultant increase in carbon dioxide waste. In the carotid bodies, microvascular arterioles under sympathetic control vasoconstrict from the onset systemic hypoperfusion. As compensatory mechanisms correct global hypoperfusion, this local event produces an immediate relative hypoxic ischemia and tissue acidemia in the carotid body which is sensed by the type one cells. Although the transmitter that excites discharge is unknown (Nye, 1994), action potentials, via the nerve of Hering and the glossopharyngeal nerve, are transmitted to the respiratory centers of the dorsal and ventral respiratory group in the medulla (Whipp, 1994). Efferent activity then increases the respiratory rate, enhancing gas exchange. Increases in respiration also influence the thermoregulatory balance by accentuating heat loss through evaporative mechanisms. Directly related to the moisture content and temperature of the ambient air, respiratory insensible water loss has been shown to contribute up to 16% of the total heat loss for rats in ambient temperatures of 23 - 35°C (Gordon, 1993).

The effect relative tachypnea imparts on blood gas dynamics can be characterized following hemorrhage. Oxygen pressures rise modestly due to the increased frequency "fresh" air is exchanged at the alveolar level. Equilibration of oxygen levels occurs in the pulmonary capillaries and then is reflected in systemic arterial blood. By the similar mechanism, CO<sub>2</sub> removal is enhanced

resulting in lower systemic arterial  $P_{CO_2}$  levels. The immediate effect on acid-base balance is a relative respiratory alkalosis.

As the hemorrhage severity progresses, the effect of peripheral tissue hypoperfusion becomes evident. Combined sympathetic vasoconstriction and low perfusion pressures limit oxygen delivery to the peripheral tissues which results in local hypoxemia simultaneous with the accumulation of  $CO_2$  waste. The effect of this hypoperfused state is first evident in venous blood and later becomes recognizable in arterial blood analysis.  $PO_2$  values remain relatively unchanged however, increasing  $P_{CO_2}$  levels from the tissues produce a metabolic acidosis. Bicarbonate buffer levels decline in response to the acidemia.

### **Body Temperature Regulation**

Body temperature is a reflection of the balance between heat production and heat loss. Most often in the literature, clinical studies which focus on body temperature use rectal or esophageal probes to collect data because it provides easily obtainable, hard evidence for the investigators to analyze. The refutable concept of this method in collecting and reporting thermoregulatory data is that the complexity of the temperature balance is often lost and may lead to erroneous interpretation (Webb, 1995).

## **Temperature Sensation and Control**

The central control of heat regulation in endothermic animals lies chiefly within the two active temperature centers of the hypothalamus. The principle site of information procurement is the preoptic area of the hypothalamus: the anterior thermal sensitive center. Arterial blood flow located in close proximity to this thermal sensitive center provides optimal core temperature sensing (Benzinger, 1969). The anterior center is then responsible for temperature regulation by enhancing heat loss or heat production / conservation mechanisms (Gordon, 1993). The posterior thermal insensitive center of the hypothalamus is responsible for integration of impulses and directing them to the appropriate effector site.

In addition to central thermal sensitive sites, peripheral heat sensitive neurons provide local temperature information. Located in and immediately below cutaneous tissue, thermal information is transmitted via the dorsal horn of the spinal cord to the preoptic area of the hypothalamus for integration (Gordon, 1993). Similar thermosensitive neurons can be found in the spinal cord and in the peritoneal viscera.

## **Thermoregulation and Metabolism**

Certain endothermic animals, including rats and humans, employ thermogenic mechanisms enabling them to produce quantities of heat sufficient to maintain a core temperature within a narrow range. For animals in

thermoneutral ambient temperatures, the primary site of heat production is the oxidative processes of cellular metabolism. Oxidation of a single mole of glucose yields 673 kcal, or or, 4.7 - 4.8 kcal per liter of oxygen consumed (Bartholmew, 1977). Approximately 35% of the total energy from food will be evolved as heat. After ATP utilization, a higher percentage of total food energy will be lost as heat (Guyton et al., 1996).

### **Cutaneous Blood Flow**

Within a narrow range of ambient temperatures, the thermoneutral zone, animals are able to maintain a constant core temperature by autoregulation of cutaneous blood flow alone. (Savage et al., 1996). Through  $\alpha_2$  mechanisms, cutaneous blood flow resistance is increased when modest heat conservation is necessary (Ekenvall et al., 1988). As the ambient temperature drops below the animals lower critical temperature, cutaneous microvasculature resistance continues to increase dropping blood flow 60-70% below control; therefore, minimizing heat loss (Ekenvall et al., 1988). At the upper critical temperature, passive vasodilation allows modest enhancements of heat loss to ambient surrounding, although the mechanism is unknown (Savage et al., 1996). As the endothermic animal is exposed to ambient temperatures beyond the upper critical temperature, heat transfer is maximized as bradykinins mediate active vasodilation increasing cutaneous blood flow to over  $20 \text{ cc min}^{-1} 100 \text{ ml tissue}^{-1}$  (Savage et al 1996). The efficiency of heat loss from cutaneous blood flow can

be accentuated by sweating. Under hypothalamic control, sweat glands are stimulated by sympathetic cholinergics to release massive amounts of the sodium rich solution, significantly increasing evaporative heat loss (Guyton et al., 1996). For the rat, heat transfer from cutaneous blood flow occurs from the eyes, ears, nose, feet, and tail (Gordon, 1993). Because the tail is well vascularized with a high number of arteriovenous anastomoses and comprises approximately 7% of the total body surface area, it provides the animal with an efficient heat transfer method: yet, the tail is highly maneuverable and can be protected from cold exposure (Gordon, 1993).

### **Other Thermogenic Mechanisms**

Another site of heat production for endotherms below their lower critical temperature is skeletal muscle thermogenesis. Efferent motor activity from the hypothalamus upon cold stimulation produces oscillatory movements primarily of the limbs and jaws (Stainer et al., 1984), enhancing heat production four to five times normal (Guyton et al., 1996). For the rat, skeletal muscle thermogenesis is the primary source for extra-metabolic heat production: however, it is replaced by non-shivering thermogenesis earlier than in larger animals (Gordon, 1993). This observation may benefit the rat by: (a) heat is generated peripherally and due to high surface to volume ratios, transfer to the core structures is relatively limited, (b) shivering is uncomfortable and limits skeletal muscle activity that may be

needed to perform another task, and (c) body movement can enhance convective heat loss especially in smaller rodents (Jansky, 1979).

Heat production can also occur from non-shivering thermogenesis. Controlled by sympathetic stimulation from the hypothalamus, brown adipose tissue, which is dense with mitochondria, can increase heat production enormously to  $400 \text{ W kg}^{-1}$  (Gordon, 1993). For the human neonate, the uncoupling of oxidative phosphorylation by brown adipose tissue can increase metabolic oxygen consumption by over 100% with relatively no increase in ATP production (Guyton et al., 1996). As previously mentioned, rodents use brown adipose tissue as a source for heat production. Interestingly, the relative amount of brown adipose tissue is inversely proportional to the body mass. This is presumably an adaptive allometric response due to increased heat loss with decreased body mass (Gordon, 1993). The effect of tissue hypoperfusion and subsequent hypoxia following hemorrhage on the thermogenic efficiency of brown adipose tissue has not been studied. Indeed, in the setting of hemorrhage, tissue hypoxia may inhibit any significant thermogenic contribution of brown adipose tissue (Stanier et al., 1984). Another area of non-shivering thermogenesis is the transport and assimilation of food by the action of gastric smooth muscle, the physical forces of friction, the food composition (Guyton et al., 1996) or by accentuating brown adipose tissue formation (Gordon, 1993, and

Stanier et al., 1984). Diet-induced thermogenesis has not been studied following hemorrhage.

### **Thermoregulation Following Hemorrhage**

Recently, in humans the hypothermia which follows hemorrhagic shock was found to be associated with the hypotensive period prior to resuscitation (Bergstein et al 1996). However, the mechanism which disturbs the balance between heat production and heat loss following hemorrhage is poorly understood. Several plausible mechanisms which may individually or synergistically contribute to the hypothermic event include: (a) a reduction in frictional forces between blood and the vascular walls, (b) an hypoxic mediated reduction in cellular metabolism (Malvin et al., 1994), (c) an increase of evaporative losses from the respiratory system, or (d) an increase in dry heat loss from cutaneous blood flow.

### **Statement of Purpose**

The purpose of this study is to: (a) establish a conscious model of hemorrhage-induced hypothermia, (b) examine the time course of the hypothermic event, (c) determine if cutaneous blood flow mediates the drop in body temperature, and (d) to evaluate metabolic heat production following hemorrhage.



## **Materials and Methods**

### **Cutaneous Blood Flow Studies**

All of the following outlined experiments follow the protocol approved by the Animal Care and Use Committee of East Carolina University. Following delivery from the vendor, male Sprague - Dawley rats (300-350 gm: Charles River, Raleigh NC) were utilized after being allowed two or more days to acclimate to their new environment.

On the day of instrumentation, the animals were weighed and anesthetized with a combination ketamine (stock solution 90 mg/ml given 9 mg/100gm body weight) and xylazine (stock solution 10 mg/ml given 1 mg/100gm body weight) administered intra-peritoneally. The anesthetic dose was supplemented with one quarter of the initial dose as needed throughout the procedure.

Following skin preparation with a betadine skin wash, the right femoral triangle was draped in sterile fashion. Through a skin incision, the femoral artery and vein were identified. Tygon<sup>®</sup> catheters (ID 0.01", OD 0.03") were inserted into the vein and artery then advanced into the inferior vena cava and abdominal aorta, respectively. Post-mortem exam revealed the arterial catheter tip was placed just distal to the renal artery and the venous catheter was placed near the common iliac bifurcation. The catheters were secured with a non-absorbable suture and tunneled subcutaneously to the suprascapular region, exiting through

a small skin incision. Here the catheters were clamped and secured with stitches. Throughout the same incision, a thermocouple wire was tunneled subcutaneously and placed at the base of the tail on the dorsal side to obtain cutaneous temperature. Through a mid-line ventral incision, just inferior to the xyphoid process, a previously calibrated Mini-Mitter® (Mini-Mitter Co. Sunriver OR) transmitter was placed inside the peritoneal cavity. All incisions were closed with surgical staples. The animals were given five milliliters isotonic sterile saline intra-peritoneally, in addition to the antibiotic Durapen® (50000 u /ml; dose 0.1 ml / 100gm b.w. i.p. ) and the analgesic Buprenex® (0.03 mg / ml; dose 0.1ml / 100gm b.w. i.p.). The animals were then returned to their cage and observed at twenty minute intervals until conscious. After complete recovery, the animals were returned to the Department of Comparative Medicine for forty-eight hours with free access to food and water.

Experimental groups were formed to systematically examine the consequences of hemorrhage, including the role of cutaneous blood flow and metabolic rate, on hemorrhage-induced hypothermia. Table 1 depicts the summary of the experimental group assignments.

On the day of the experiment, the animal was placed inside an acrylic chamber (5"x 5"x 6") for control of ambient temperature. The temperature inside was maintained at 22° or 28°C by a water jacket. The circulating water was maintained at this temperature by a fluid circulator (VWR Scientific).

Table 1.

**Consequence of Hemorrhage**

| Group Number | Chamber Temperature (C) | Drug Therapy | Hemorrhage |
|--------------|-------------------------|--------------|------------|
| 1            | 22                      | no           | no         |
| 2            | 22                      | NaCl         | yes        |

**Cutaneous Blood Flow Studies**

| Group Number | Chamber Temperature (C) | Drug Therapy | Hemorrhage |
|--------------|-------------------------|--------------|------------|
| 3            | 38                      | NaCl         | yes        |
| 4            | 22                      | Prazosin     | no         |
| 5            | 22                      | Prazosin     | yes        |
| 6            | 28                      | Prazosin     | yes        |

**Oxygen Consumption Studies**

| Group Number | Chamber Temperature (C) | Drug Therapy | Hemorrhage         |
|--------------|-------------------------|--------------|--------------------|
| 7            | 22                      | no           | no                 |
| 8            | 22                      | no           | yes                |
| 9            | 22                      | no           | yes (retransfused) |
| 10           | 22                      | Prazosin     | no                 |

Temperatures within the chamber were monitored by a thermocouple wire placed at the center of the chamber top. A normal  $\text{FiO}_2$  was maintained within the chamber by purging room air at a rate of 2 L/min with a mechanical ventilator (Harvard Apparatus: South Natick, MA).

The arterial catheter was connected to a Gould-Statham (Oxnard, CA) pressure transducer, and the blood pressure displayed on a Grass polygraph (Model 7D, Grass Instruments Co. Quincy, MA). Heart rate was determined from the blood pressure waveform, and displayed on another channel of the polygraph. The thermocouple wire was monitored continuously using a hand-held temperature meter (Model 4, Atkins Technical Inc. Gainesville, FL) and cutaneous temperature was recorded at five minute intervals. Core temperature was monitored continuously and displayed every five minutes on a computer monitor (Mini-Mitter Co. Sunriver OR).

Once connected to the monitoring equipment and placed inside the chamber, the animal was allowed to acclimate for thirty minutes. Following this, the animal received an  $\alpha_1$  specific antagonist, Prazosin® (Sigma Co. St. Louis MO.) or a similar volume placebo of 0.9% NaCl solution and was then observed for an additional thirty minutes.

Animals were then subjected to a controlled hemorrhage induced by aspirating blood (16 cc/kg b.w or 20% total blood volume) through the femoral arterial line. Following the hemorrhage, the animal's mean arterial pressure,

heart rate, core and cutaneous temperature were monitored continuously for the next four hours.

For blood gas analysis, the first 0.25 cc of arterial blood aspirated was placed inside a heparinized syringe. Venous blood samples were simultaneously collected. The volume of blood collected in the control samples was subtracted from the calculated total hemorrhage volume. Following near completion of the controlled hemorrhage, both arterial and venous blood samples were collected. The samples were placed on ice and immediately delivered to the Department of Comparative Medicine for analysis of pH,  $P_{CO_2}$ ,  $P_{O_2}$ ,  $HCO_3^-$ , oxygen saturation, and base excess. A recovery sample of 0.2 cc was obtained from both the arterial and venous catheters one hour after the hemorrhage and analyzed in similar fashion.

Complete blood count samples were obtained from the arterial catheter at the initiation of the hemorrhage. These samples were placed inside ethylenediaminetetracetic acid coated tubules, agitated gently, and delivered to the Department of Comparative Medicine for analysis of white blood cell count, red blood cell count, hemoglobin, hematocrit, and platelet count. At the end of the experimental protocol, a second sample was obtained and analyzed in similar fashion.

The consequence of hemorrhage was evaluated with the thermally jacketed chamber controller set at room temperature (22°C). This temperature,

which falls on the lower end of the expected thermoneutral range for the Sprague-Dawley rat, allows for a maximal observed hypothermic response from hemorrhage while not inducing shivering thermogenesis (Gordon, 1993), non-shivering thermogenesis, reduction of cutaneous blood flow, or observable heat conserving behavior. Animals assigned to evaluate this aspect of hypothermia served as the “standard” to which all other comparisons were made. As control, animals (group 1) were placed inside the thermally jacketed chamber and core temperature was observed for four hours.

This study evaluated cutaneous blood flow as the possible mediator of hemorrhage-induced hypothermia using two approaches to minimize the role cutaneous blood flow plays as a thermoregulatory mechanism. In the first series of experiments, (group 3) animals were hemorrhaged at an elevated ambient temperature (28°C), which will block heat loss to ambient surroundings (Gordon, 1993). This temperature falls at the upper end of the thermoneutral range: therefore, the animal maintains a constant core temperature through modest cutaneous vasculature dilation. Therefore any physiologically induced increase in cutaneous blood flow would only minimally enhance radiant, convective, or conductive heat losses. The second method used to minimize the role of cutaneous blood flow during post-hemorrhage hypothermia, was to eliminate sympathetic adrenergic control. Using an alpha<sub>1</sub> adrenergic receptor specific antagonist, Prazosin<sup>®</sup>, circulating catecholamines were competitively inhibited

from binding with their ligand in the vasculature walls. Although the contribution of cold-induced sympathetic tone by the  $\alpha_2$  adrenergic receptors in cutaneous vascular beds has been documented (Ekenvall et al., 1988 and Nielsen et al., 1989), inhibition of the  $\alpha_1$  receptors in peripheral vessels will attenuate catecholamine driven vasoconstriction and prevent the autoregulatory redistribution of blood away from the region following hemorrhage. By maximizing cutaneous blood flow without intrinsic control, heat loss from the skin could also be maximized. With the chamber at 22°C, animals (group 4) were hemorrhaged as previously; however, they were also premedicated with the  $\alpha_1$  antagonist. The same protocol was repeated with the chamber temperature set at 28°C (group 5). The response to the drug was evaluated (group 3) with animals receiving the drug followed by sham hemorrhage.

### **Metabolism Studies**

Metabolic heat production contributes significantly to the overall heat balance equation, serving as the principle heat contributor for non-stressed, adult endotherms. This study evaluated the role of metabolic heat production following hemorrhage. Animals assigned to these groups were instrumented with the femoral arterial line and telemetry device as before; however, they did not receive a cutaneous thermocouple wire in the tail.

To evaluate the effect of hemorrhage on metabolic rate, minimal oxygen consumption values were obtained within a control period. These animals were

removed from the calorimetry chamber and hemorrhaged (16 cc/kg b.w.) by aspirating blood from the vascular access line. The animals were then promptly returned to the respirometry chamber and their oxygen consumption was measured continuously for the next 180 minutes. Control for these experiments was achieved by incorporation of a sham hemorrhage (group 7). Animals were removed from the chamber following the control period, handled for 10 minutes, and returned to the calorimetry chamber for the 180 minute observation period. Additionally to control for the hemorrhage itself, animals (group 9) were hemorrhaged the appropriate volume and immediately retransfused with their aspirated blood. Lastly, to determine the effects of Prazosin<sup>®</sup> on metabolic rate, animals (group 11) were observed for minimal oxygen consumption following administration of the  $\alpha_1$  antagonist (0.1 mg/kg b.w.).

As previously described, this study utilized oxygen consumption as a tool for measuring metabolic rate. House air is directed at constant pressure to a dehumidifying unit. This tubular column was filled with Drierite<sup>®</sup> which reduced humidity to <0.01%. From here, the air was directed to a previously calibrated rotameter (Manostat. New York, NY) for constant non-corrected flow rates of 830 cc/min. Flow rate was established using the allometric relationship for oxygen consumption:  $VO_2 = 3.9(\text{wt grams})^{.778}$ , then increased 20% to enhance gas mixing. Air then entered an acrylic respirometry chamber which was placed inside a temperature control cabinet. The acrylic material has been shown not to affect



metabolic rate, evaporative water loss, thermal conductance, cutaneous or core temperature when ambient temperature is within the rat thermoneutral zone (Gordon et al., 1994). The temperature control cabinet was adjusted to produce similar chamber temperatures (23.5°C) as were observed during the cutaneous blood flow experiments. The design of the chamber allowed maximal gas mixing equally dispersing both inflow and outflow gas samples with only the animal preventing the achievement of complete gas mixing. The result of this is to minimize the effective volume of the chamber thus producing accelerated washout characteristics. Air exiting the calorimetry chamber was sampled by a oxygen analyzer (Ametek Pittsburgh PA) at a rate of 100 cc/min. Voltages from the oxygen analyzer were transformed through a analog-to-digital converter and displayed as a continuous graph on a computer monitor. Conversion to oxygen consumption was facilitated by respirometry software (Sable Systems. Salt Lake City, UT) and reported as (cc O<sub>2</sub> / gm hr<sup>-1</sup>). Oxygen consumption values of pre-instrumentation and control periods were achieved by searching for the minimal observed values over a consecutive ten minute period. Similarly, analysis of post-interventional oxygen consumption, searched for the minimal observed values which occurred within the first, second, and third 60 minute time blocks, respectively. Temperature analysis was achieved by playing the telemetry transmission over a common AM radio. This was recorded continuously on a hand-held cassette tape recorder. This sound was played into a Macintosh

840AV computer and the frequency determined using sound analysis software (Macromedia. San Francisco, CA). The frequency was converted to real-time temperature by comparing pre-implantation and post-implantation control temperature / frequency relationships. By obtaining both pre and post-implantation calibration controls, we could then correct for any voltage drift of the transmitter battery.

### **Statistical Methods**

Utilizing analytical software (DataMost Co. Salt Lake City, UT), intra-group core and cutaneous temperature recordings, mean arterial pressure, heart rate, and oxygen consumption were analyzed for significance by one-way analysis of variance followed with Student-Newman-Keuls post-hoc test for inter-group significance ( $p < 0.05$ ). Where multiple control points were taken, a significant increase was determined from maximal observed control values; conversely, significant decrease was determined from minimal observed control values. The oxygen consumption data was normalized to eliminate data noise from a change in chamber design. All data are expressed as mean  $\pm$  standard error with  $p < 0.05$  significance displayed with an asterisk on the figures.

## **Results**

### **Consequence of Hemorrhage**

The response to hemorrhage was evaluated with groups 1 and 2 of the experimental protocol and summarized in figures 1a - 1f. The goals of this series of experiments was to determine the heart rate, blood pressure, temperature, blood gas, and complete blood count change following hemorrhage.

#### **Sham Hemorrhage, Group 1**

The core temperature of sham hemorrhaged animals (N=4) placed inside the jacketed chamber was monitored (control  $37.9 \pm 0.21$  °C) for 240 minutes without significant change. These animals were not, however, monitored for hemodynamic or cutaneous temperature changes throughout the period.

#### **Hemorrhage at 22°C, Group 2**

Administration of a NaCl placebo followed by moderate hemorrhage (N=6), induced a significant reduction in core temperature seventy minutes after the hemorrhage (control  $38.5 \pm 0.2$  °C) which stabilized at  $-0.7 \pm 0.3$  °C 150 minutes after the insult (fig. 1a). This hypothermic response persisted throughout the remainder of the observational period.

Cutaneous temperature (control  $34.3 \pm 0.81$  °C) of these animals dropped significantly ten minutes following hemorrhage, stabilized, and sporadically reached significance for the remainder of the observational period. Because no

cutaneous temperature was obtained from the sham hemorrhaged animals, no inter-group comparisons were possible.

Hemorrhage produced an expected drop in heart rate and blood pressure. Sympathetic inhibition induced by moderate hemorrhage caused an immediate reduction in heart rate (control  $358 \pm 15.9$  beats/min) to  $306.7 \pm 36.9$  beats/min; significantly below control, recovering within sixty minutes to control values (fig. 1b). Similarly, mean arterial pressure (control  $135.8 \pm 5.5$  mm Hg) fell immediately following hemorrhage to  $100 \pm 17.0$  mm Hg and remained significantly below control until the sixty minute observation point.

Arterial blood gases are summarized in figure 1c. Control samples confirmed normal ventilation status ( $P_{aO_2}$   $91.7 \pm 4.0$  mm Hg:  $S_{aO_2}$   $95.8 \pm 0.4$  %) coupled with mild metabolic acidosis (pH  $7.31 \pm 0.06$ :  $P_{aCO_2}$   $42.1 \pm 2.3$  mm Hg:  $HCO_3^-$   $20.3 \pm 2.3$  mEq/L: BE  $-5.5 \pm 3.3$  mEq/L). Following hemorrhage, we observed expected compensatory mechanisms producing a relative respiratory alkalosis (pH  $7.44 \pm 0.01$ :  $P_{aCO_2}$   $30.7 \pm 1.8$  mm Hg:  $HCO_3^-$   $20.3 \pm 1.4$  mEq/L: BE  $-1.8 \pm 1.1$  mEq/L) without change in the ventilatory status. In the recovery sample taken sixty minutes after hemorrhage, no significant change in the overall ventilatory status was observed: however, these animals displayed a more severe metabolic acidosis than control (pH  $7.31 \pm 0.06$ :  $P_{aCO_2}$   $35.5 \pm 1.9$  mm Hg:  $HCO_3^-$   $17.2 \pm 1.3$  mEq/L: BE  $-8.0 \pm 2.5$  mEq/L).

Venous blood gas samples were evaluated in similar fashion for the same parameters (fig. 1d). Due to the limited availability of venous blood samples in this group, presumed a consequence of the wall pliability of the inferior vena cava (N=1), only raw data is presented. Of specific interest, oxygen delivered to the tissues remained above adequate levels (control  $P_{vo_2}$  44.9 mm Hg) dropping to  $P_{vo_2}$  39.7 mm Hg immediately after the hemorrhage.

Complete blood count of the control sample were evaluated for cell count, hemoglobin, hematocrit, and platelet levels. Leukocyte ( $7.9 \pm 1.6$  k/ $\mu$ l), erythrocyte ( $7.32 \pm 0.5$  m/ $\mu$ l), hemoglobin ( $13.6 \pm 0.4$  g/dl), hematocrit ( $38.1 \pm 2.9$  %), and platelet ( $690 \pm 53.5$  m/ $\mu$ l) levels were all within normal limits for the species (fig. 1e). Following the 20% total blood volume hemorrhage, we observed the expected anemia ( $5.72 \pm 0.8$  m/ $\mu$ l) with reduced hemoglobin ( $8.8 \pm 1.0$  gm/dl) and hematocrit ( $24.6 \pm 3.3$  %) levels, confirming >20% total blood volume hemorrhage.

Figure 1a.

Core (-●-) and cutaneous (-■-) temperature expressed as °C following moderate hemorrhage in conscious rats (N=6). Water bath circulating the chamber set at 22° C. Hemorrhage period identified with filled bar. Data expressed as mean  $\pm$  standard error. \* identifies intra-group  $p < 0.05$  significance between minimal observed control and post-hemorrhage temperatures.

Figure 1a. **Body Temperature Response Following Hemorrhage**

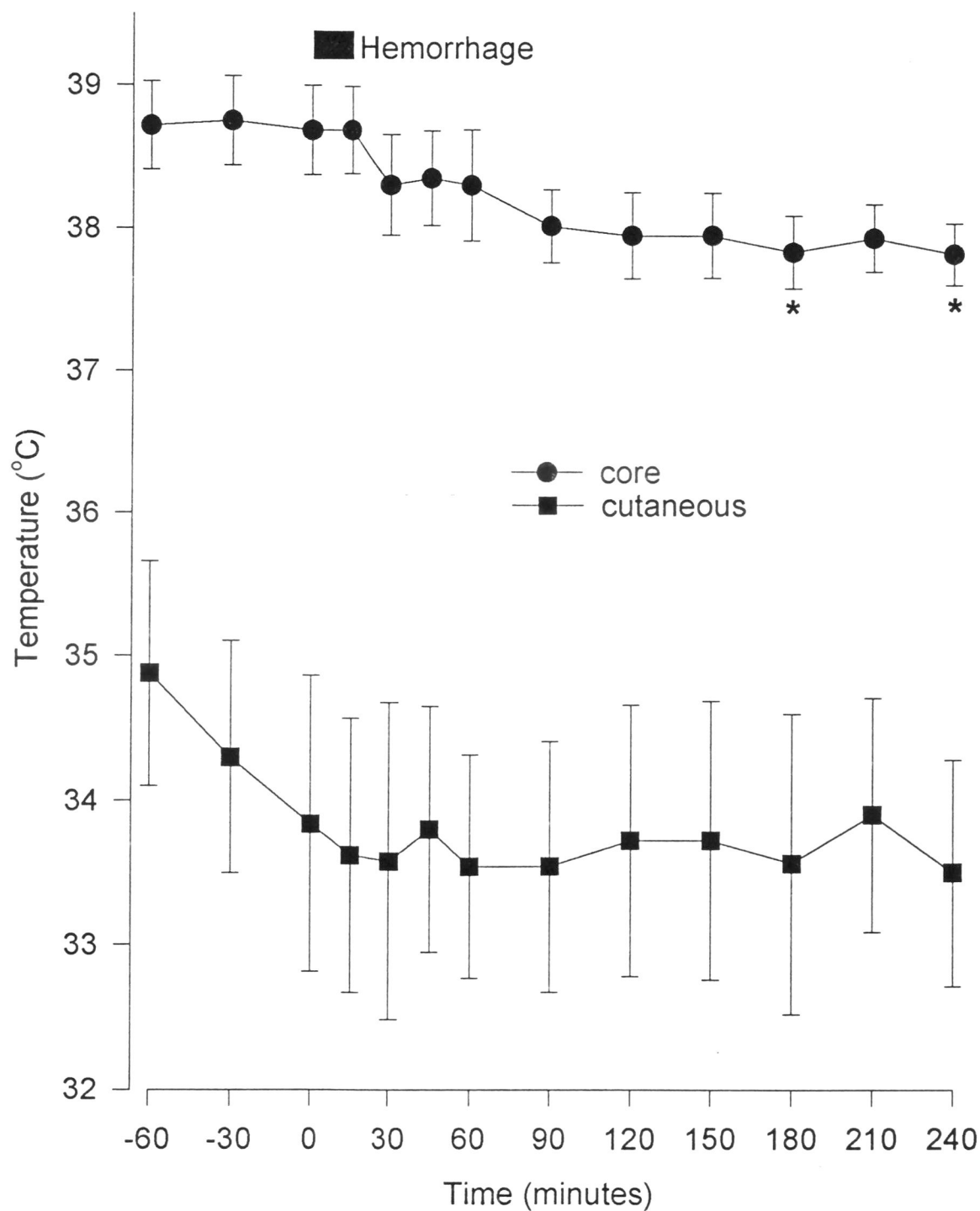


Figure 1b.

Heart rate (-●-) and mean arterial pressure (-■-) response over time to moderate hemorrhage (N=6). Chamber bath set to a temperature of 22° C. Hemorrhage period identified with filled bar. Data expressed as mean  $\pm$  standard error. \* identifies  $p < 0.05$  intra-group significance of control minimal observed heart rates and mean arterial pressures when compared with post-hemorrhage values.



Figure 1b.

## Cardiovascular Response Following Hemorrhage

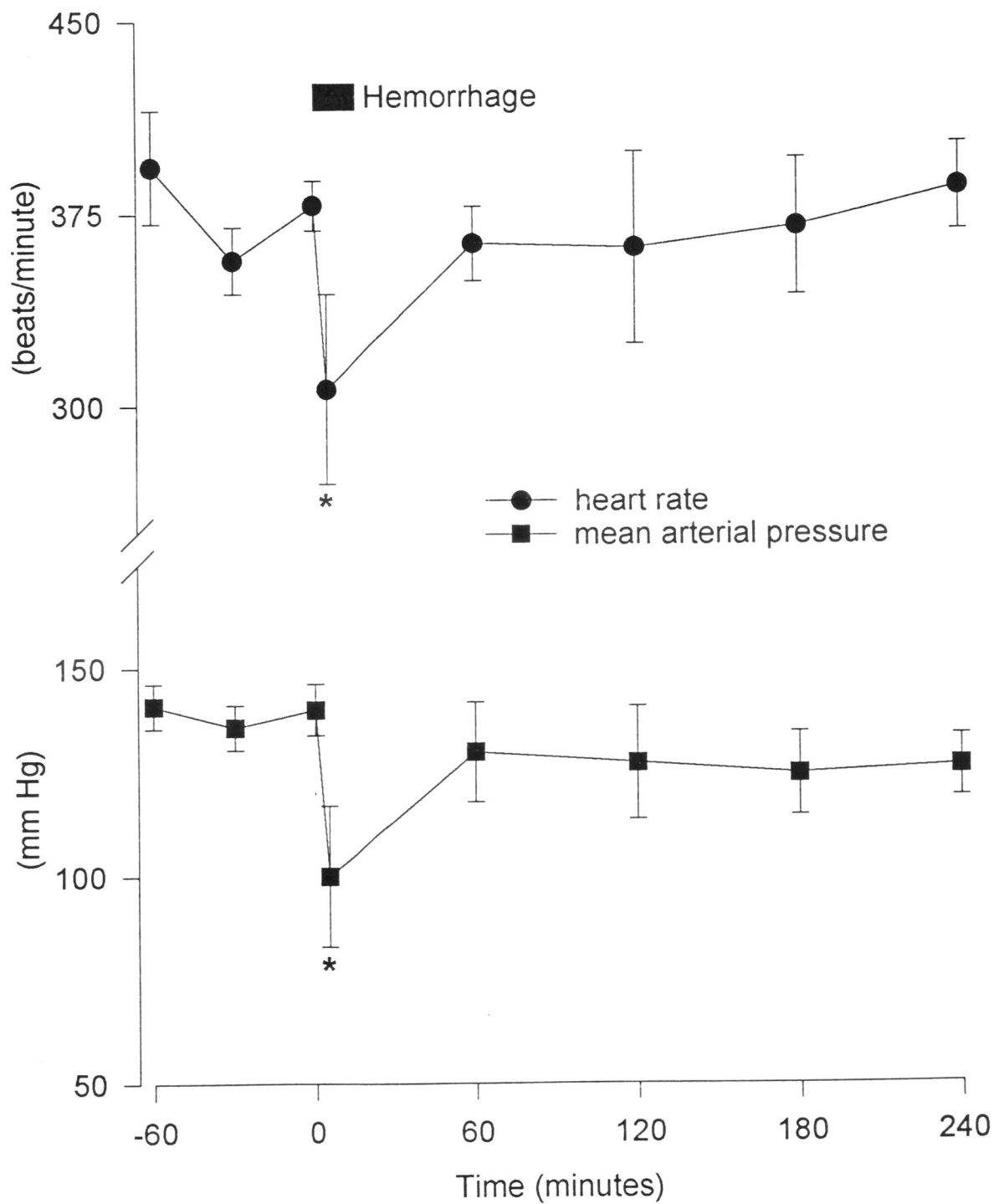


Figure 1c.

Arterial blood gas analysis following moderate hemorrhage (N=6). **A.** pH ( $-\log[H^+]$ ) values on left Y axis,  $Pao_2$  (mm Hg) and  $Paco_2$  (mm Hg) values are on right Y axis. **B.**  $HCO_3^-$  (mEq/L), and Base Excess (mEq/L) *Open bars:* control samples. *Filled bars:* immediately post-hemorrhage samples. *Hatched bars:* hour post-hemorrhage recovery samples. Data expressed as mean  $\pm$  standard error with intra-group significance ( $p < 0.05$ ) identified with an \*.

Figure 1c.

## Arterial Blood Gas Analysis Following Hemorrhage

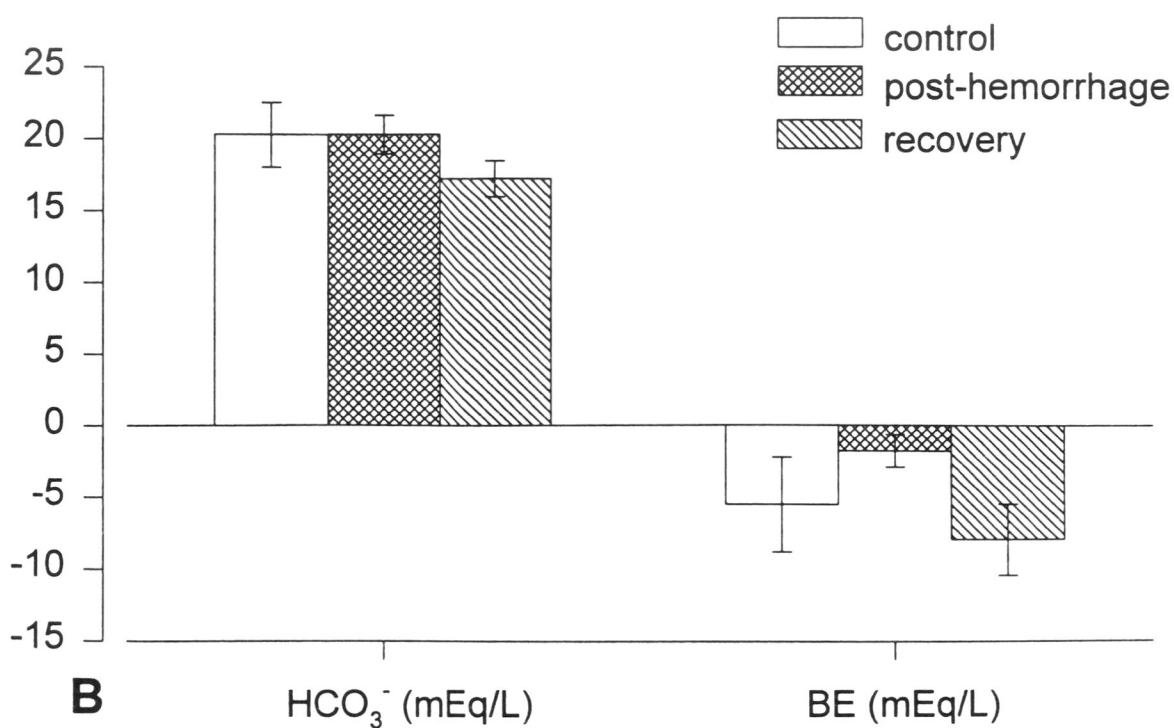
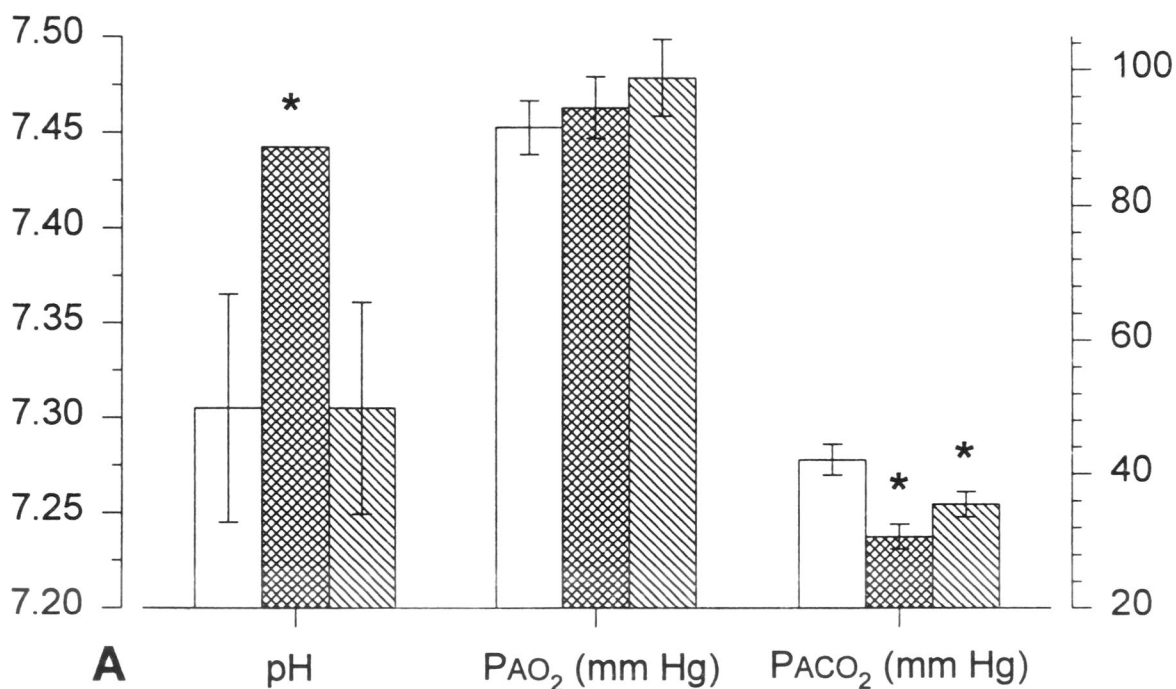


Figure 1d.

Venous blood gas analysis following moderate hemorrhage (N=1). Ambient chamber temperature 22° C. **A.** pH ( $-\log[H^+]$ ) values on left Y axis,  $PvO_2$  (mm Hg) and  $PvCO_2$  (mm Hg) values are on right Y axis. **B.**  $HCO_3^-$  (mEq/L), and Base Excess (mEq/L) *Open bars*: control samples. *Filled bars*: immediately post-hemorrhage samples. *Hatched bars*: hour post-hemorrhage recovery samples. Data expressed as mean.

Figure 1d.

## Venous Blood Gas Analysis Following Hemorrhage

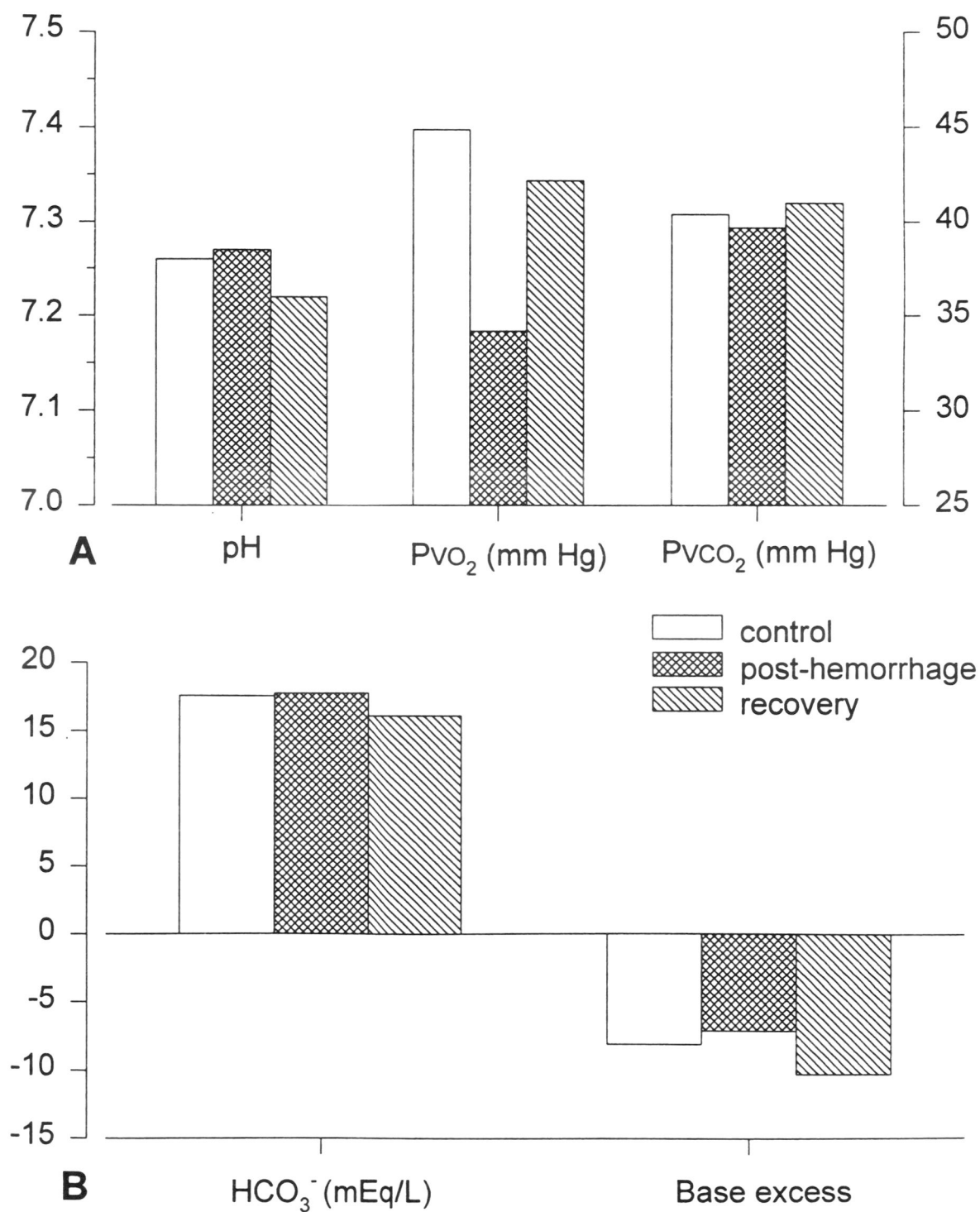


Figure 1e.

Complete blood count analysis following hemorrhage (N=6). **A.** white blood cells (k/ $\mu$ L), red blood cells (m/ $\mu$ L) on left Y axis. Platelet count (k/ $\mu$ L) on right Y axis. **B.** Hemoglobin (g/dl) and hematocrit (%). *Open bars:* control sample. *Hatched bars:* post-hemorrhage sample. Data expressed as mean  $\pm$  standard error with \* identifying  $p < 0.05$  intra-group significance before and after hemorrhage.

Figure 1e.

## Complete Blood Count Response to Hemorrhage

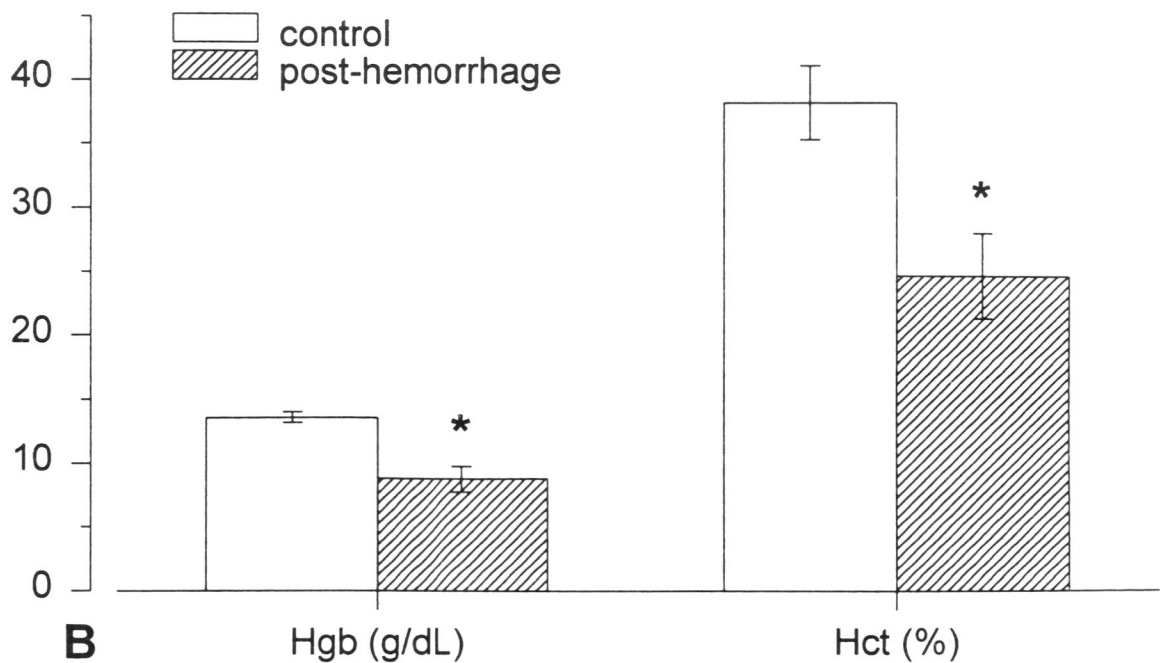
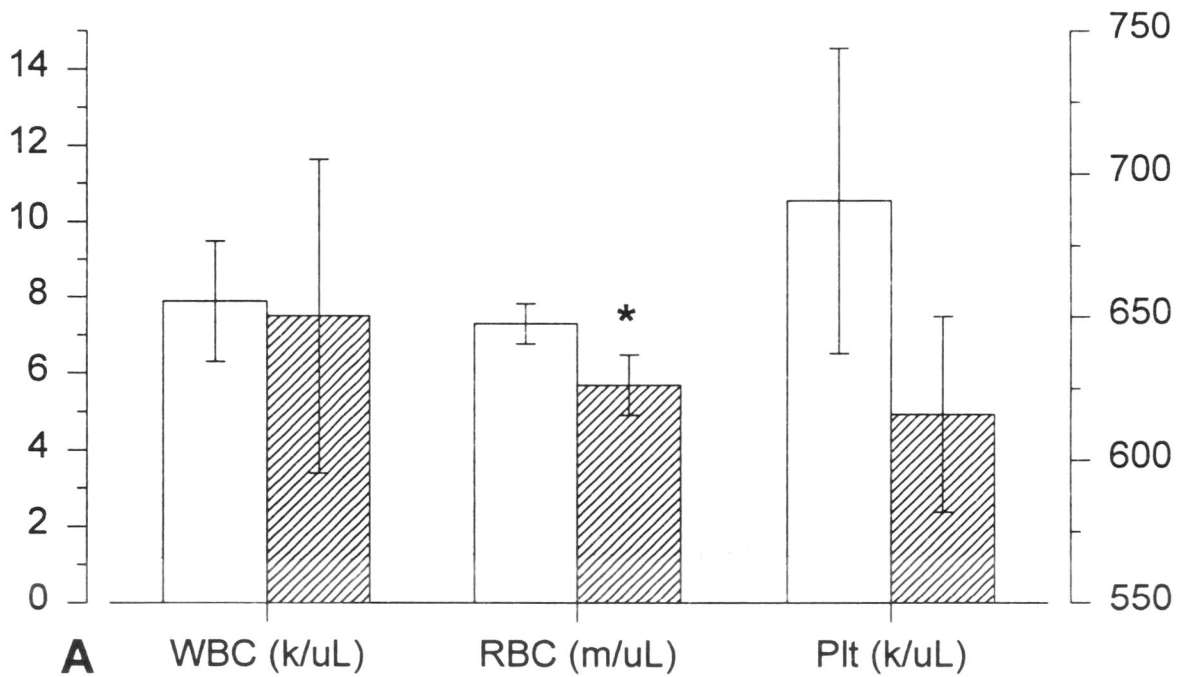


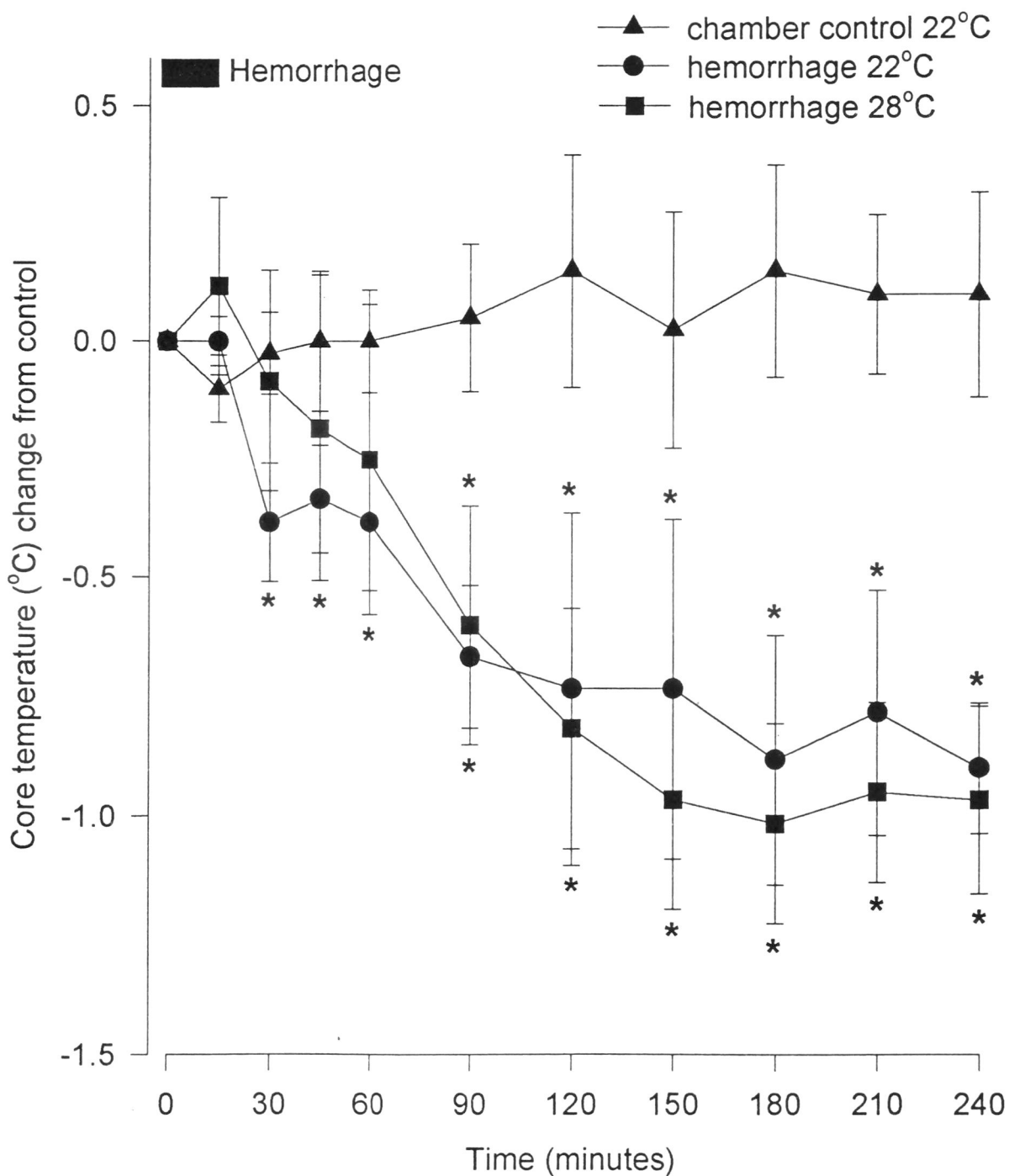
Figure 1f.

The change in core temperature expressed in °C observed in (N=4) sham hemorrhaged (-▲-), (N=6) hemorrhage at 22° C chamber bath (-●-), and (N=6) hemorrhage at 28° C chamber bath (-■-). Hemorrhage period identified with filled block. Data expressed as mean change  $\pm$  standard error with \* identifying  $p < 0.05$  intra-group significance from control temperatures.



Figure 1f.

## Core Temperature Following Moderate Hemorrhage



### **Cutaneous Blood Flow I**

Heat loss from cutaneous blood flow was evaluated in groups 3 - 6 and the results are shown in figures 2a - 3h. The specific aim of this series of experiments is to evaluate to what extent, if any, cutaneous blood flow contributes to the observed hypothermic event.

#### **Hemorrhage at 28°C, Group 3**

In the first series of experiments (N=6), animals were hemorrhaged inside a chamber with an elevated ambient temperature (28°C). With conductive, convective, and radiant heat loss mechanisms minimized, core temperature (control  $38.4 \pm 0.1$  °C) dropped significantly below intra-group control temperatures to  $38.0 \pm 0.2$  °C 150 minutes after the insult, stabilized, but never recovered to control levels (fig. 2a). The control temperatures for this group were similar to group 2: however, it initially rose above intra-group control before decreasing through baseline thirty minutes after the hemorrhage. Compared to group 2, the hypothermic response was parallel with no inter-group significance obtained.

Evidence suggesting the chamber's higher ambient temperature was effective in minimizing heat loss was found in the cutaneous data. Here, cutaneous temperature (control  $34.0 \pm 1.06$  °C) did not fall significantly following the insult suggesting enhanced adrenergic activity resulting in redistribution of local blood flows did not contribute to further heat loss. It is plausible that the

elevated ambient temperature contributed to the initial increase in core temperature before cutaneous blood flows were adrenergically reduced.

The cardiovascular response was similar to animals hemorrhaged at the lower ambient temperature (fig. 2b). Sympathetic inhibition affected the heart rate (control  $368.6 \pm 9.1$  beats/min) as seen in group 2, dropping the heart rate to  $338.6 \pm 35.1$  beats/min before recovery in the first hour. The control mean arterial pressure was  $134.3 \pm 7.2$  mm Hg which dropped to  $90 \pm 10.8$  mm Hg following hemorrhage before returning to control within ten minutes.

Blood gas analysis, specifically  $P_{aO_2}$  (control  $97.9 \pm 3.3$  mm Hg), of this group revealed no significant change related to the chamber's higher ambient temperature (fig. 2c). Similarly, the single venous sample (control  $P_{vO_2}$  33.7 mm Hg) remained above adequate  $O_2$  delivery levels at  $P_{vO_2}$  32.5 mm Hg following the hemorrhage but dropped to a  $P_{vO_2}$  28.8 in the recovery sample (fig. 2d).

Figure 2a.

Core and cutaneous temperatures, expressed in °C, of animals hemorrhaged (N=6) with chamber water temperature at 28° C (-●-) and animals hemorrhaged with chamber water temperature at 22° C (-□-). Hemorrhage period identified with filled bar. Data presented as mean  $\pm$  standard error with \* identifying  $p < 0.05$  intra-group significance from minimal observed control values.

Figure 2a. **Body Temperature Response to Hemorrhage in Different Ambient Temperature**

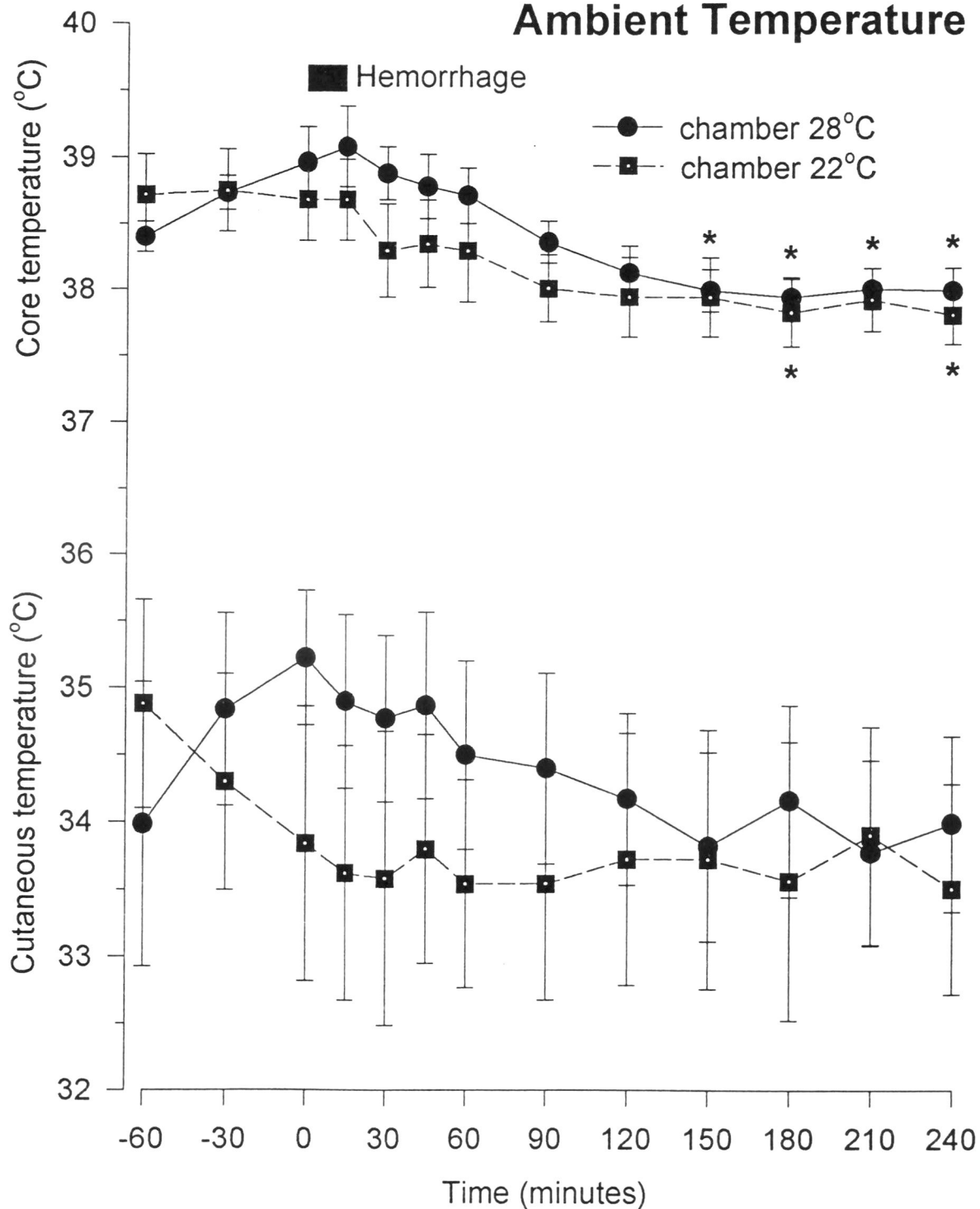


Figure 2b.

Heart rate (beats per minute) and mean arterial pressures (mm Hg) for animals (N=6) hemorrhaged inside chambers with circulating water set at 28° C (-●-) and for animals (N=6) hemorrhaged at 22° C (-□-). The hemorrhage is depicted by a filled bar. Data are mean  $\pm$  standard error with an \* identifying a  $p < 0.05$  level of significance from minimal observed control values.

Figure 2b.

### Heart Rate and MAP Response to Hemorrhage at Different Ambient Temperatures

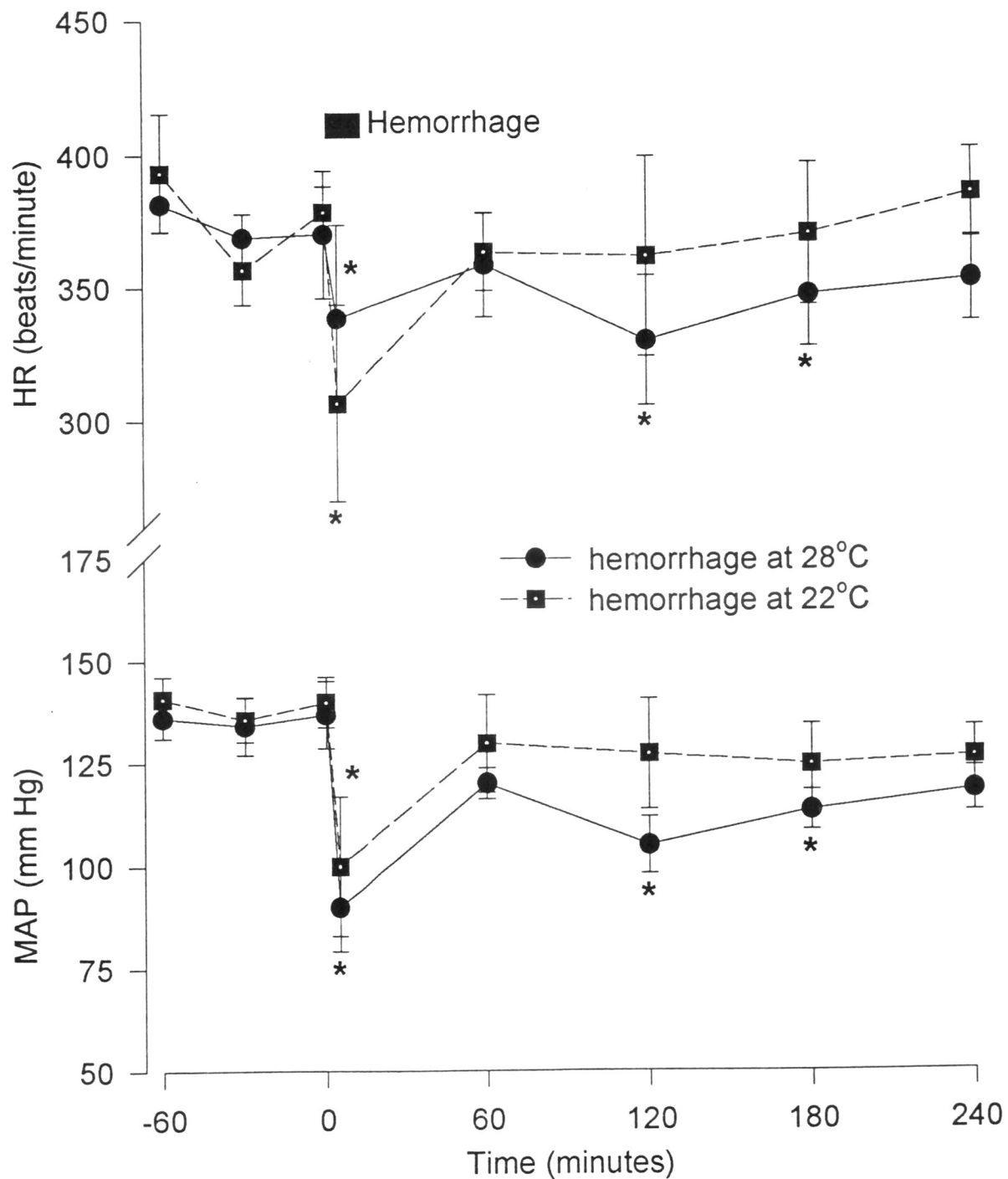


Figure 2c.

Arterial  $\text{Po}_2$ , expressed in mm Hg, response to hemorrhage at different ambient temperatures from control, post-hemorrhage, and hour post-hemorrhage recovery samples. *Hatched bars*:  $\text{Pao}_2$  of animals (N=6) hemorrhaged inside chambers with the circulating water temperature set at 22° C. *Open bars*:  $\text{Pao}_2$  of animals (N=6) hemorrhaged inside chambers with ambient temperature set at 28° C. Values expressed as mean  $\pm$  standard error.



Figure 2c.

## Arterial $P_{O_2}$ Response to Hemorrhage at Different Ambient Temperature

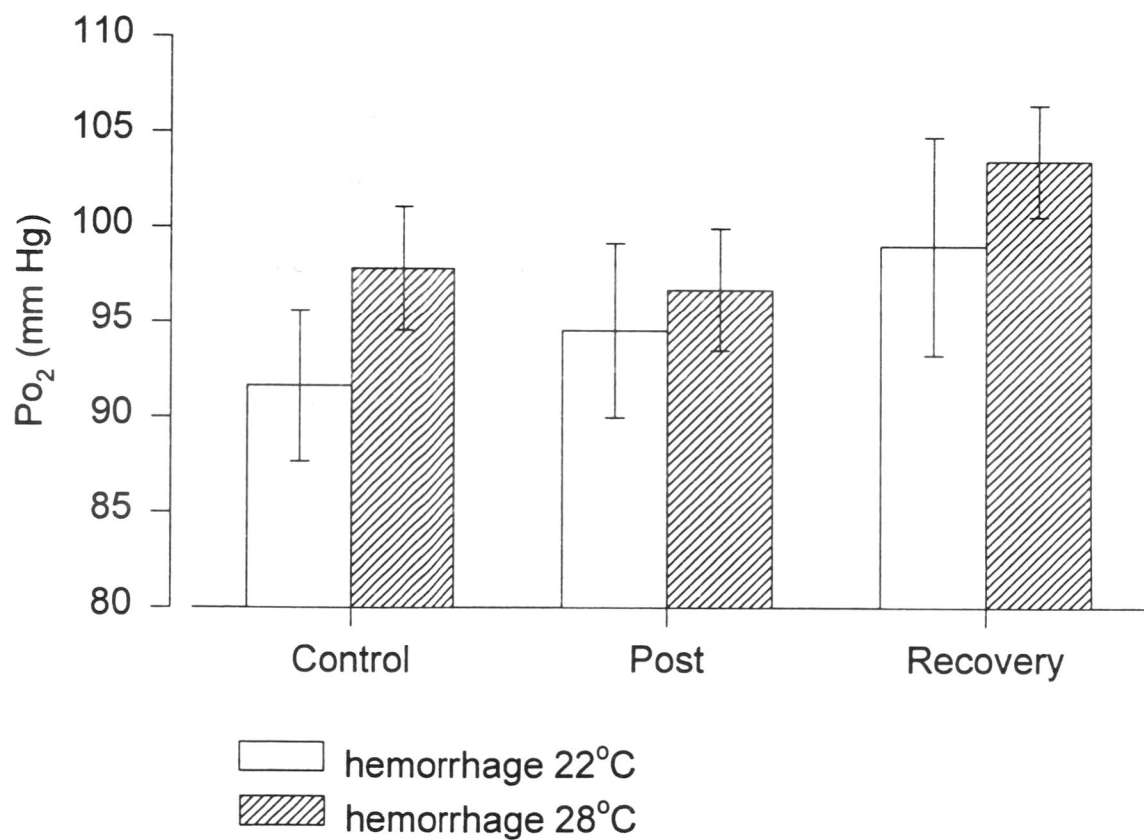
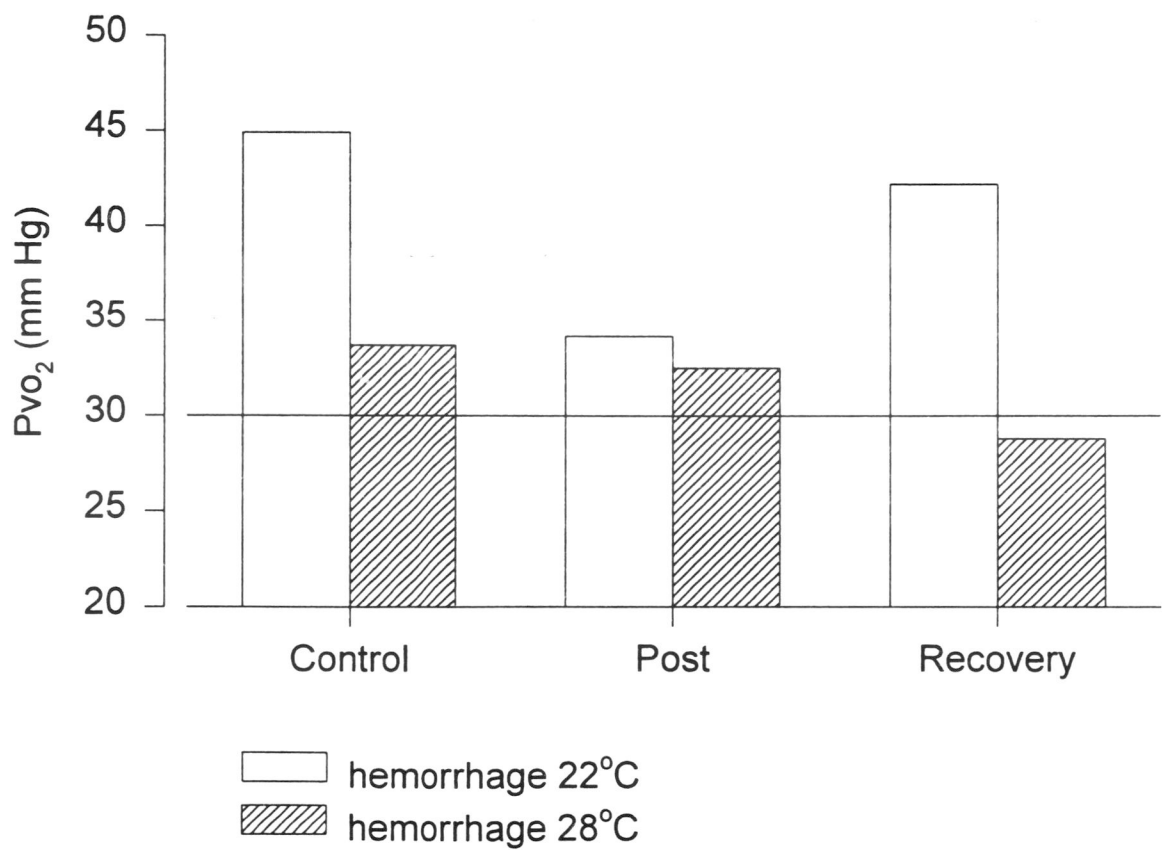


Figure 2d.

Venous  $\text{Po}_2$  levels, expressed in mm Hg, of an animal (N=1) hemorrhaged at 22° C (*hatched bars*) and an animal (N=1) hemorrhaged at 28° C (*open bar*) during control, immediately post-hemorrhage, and hour post-hemorrhage recovery samples. Reference line marks 30 mm Hg. Data expressed are actual values.

Figure 2d.

## Venous $P_{O_2}$ Following Hemorrhage at Different Ambient Temperatures



## **Cutaneous Blood Flow II**

### **Alpha Adrenergic Blockade: Drug Response, Group 4**

The second method this study used to evaluate the role of cutaneous blood flow in the hemorrhage mediated hypothermia was to inhibit adrenergic mediated control to the cutaneous vascular beds. With ambient chamber temperature set at 22°C (N=6), animals (group 4) were pre-treated with the  $\alpha_1$  specific adrenergic antagonist Prazosin® followed by sham hemorrhage. The core temperature of these animals (control  $37.9 \pm 0.1$  °C ) dropped to  $36.8 \pm 0.3$  °C beginning at the onset of observation to before stabilizing, but never returned to control values for the remainder of the observational period (fig. 3a).

Cutaneous temperature data (control  $34.2 \pm 0.6$  °C), revealed no change during the observational period after administration of the drug.

With  $\alpha_1$  specific blockade, the compensatory mechanisms, in response to loss of adrenergic control of peripheral vasculature beds (maximal control  $398.3 \pm 22.6$  beats/min), elevated the heart rate to  $465 \pm 20.5$  beats/min and remained significantly above control until the second hour of observation (fig. 3b). The mean arterial pressure (control  $140.2 \pm 5.6$  mm Hg) fell immediately after drug delivery to  $104.5 \pm 3.1$  mm Hg and remained below control until the second hour of observation.

Blood gas analysis of this group, performed only at the “recovery” time, revealed adequate oxygen transport with an arterial  $P_{aO_2}$  of  $97.9 \pm 1.4$  mm Hg (fig. 3c).

### **Alpha Adrenergic Blockade and Hemorrhage at 22°C, Group 5**

Animals who received the  $\alpha_1$  antagonist followed by moderate hemorrhage (N=6), reduced their core temperature (control  $38.1 \pm 0.3$  °C) within sixty minutes to  $36.1 \pm 0.2$  °C before stabilizing 150 minutes after the insult (fig. 3d). Compared to the drug response animals, which at sixty minutes following hemorrhage had dropped  $-1.1 \pm 0.3$  °C, these animals surpassed this hypothermic response dropping their core temperature  $-2.0 \pm 0.2$  °C at the same observational point.

Similarly, the cutaneous temperature of this group dropped below control (control  $32.6 \pm 0.5$  °C) forty-five minutes after the hemorrhage before stabilizing 130 minutes into the observation period.

Combination of both  $\alpha_1$  adrenergic blockade with moderate hemorrhage, produced the most marked hemodynamic changes (fig. 3e). Although the increased post- $\alpha_1$  blockade heart rate (control  $386.7 \pm 9.2$  beats/min) compensated for the inhibited vascular auto-regulation (post- $\alpha_1$  antagonist  $511.7 \pm 11.9$  beats/min), there was no evidence that the rat, in response to decreased tissue perfusion, could overcome sympathetic inhibition by enhancing chronotropic compensation (post-hemorrhage  $276.7 \pm 57.2$

beats/min) as compared to animals who did not receive the  $\alpha_1$  antagonist. The control mean arterial pressure was  $139.2 \pm 5.7$  mm Hg which fell to  $99.6 \pm 5.1$  mm Hg following  $\alpha_1$  blockade. This pressure was further dropped to  $55.2 \pm 2.5$  mm Hg following moderate hemorrhage and remained below control for the duration of the experiment.

At this ambient chamber temperature, oxygen transport was not effected by the combination drug and hemorrhage (fig. 3c). Post-hemorrhage  $Pao_2$  levels was  $99.1 \pm 5.7$  mm Hg and were similar to both control and recovery samples.  $Pvo_2$  levels following hemorrhage (58.6 mm Hg) did not suggest tissue hypoxemia: however with only one sample, conclusive determinations of oxygen delivery can not be made (fig. 3f).

#### **Alpha Adrenergic Blockade and Hemorrhage at 28°C, Group 6**

With the ambient chamber temperature increased to 28°C, the core temperature (N=6) of group 6 animals (control  $38.2 \pm 0.2$  °C) dropped below control ninety minutes after the hemorrhage to  $37.5 \pm 0.4$  °C, stabilized, but did not recover to control (fig. 3g). Throughout the observational period, these did not differ significantly from drug response animals (group 4).

Cutaneous temperatures, (control  $34.8 \pm 0.5$  °C), were not significantly affected by the  $\alpha_1$  antagonist with elevated ambient chamber temperatures.

The effect of alpha blockade produced a similar cardiovascular response as seen in animals hemorrhage at 22°C (group 5). The chronotropic

compensation of hypoperfusion increased the post-drug heart rate to  $452.9 \pm 21.5$  beats/min; however, hemorrhage-induced sympathetic inhibition reduced the heart rate to  $301.4 \pm 25.1$  beats/min (fig.3h). The mean arterial pressure (control  $134.7 \pm 3.7$  mm Hg) fell to  $103.9 \pm 3.9$  mm Hg immediately after the drug was administered. The hypoperfused state was more severe after hemorrhage with perfusion pressures dropping to  $80.0 \pm 10.8$  mm Hg.

Analysis of arterial blood oxygen content revealed no changes related to the administration of the  $\alpha_1$  antagonist: however, the  $Pao_2$  (control  $108.8 \pm 5.9$  mm Hg) was significantly elevated ( $Pao_2$   $122.3 \pm 4.1$  mm Hg) in the recovery sample of animals hemorrhaged inside the chamber at  $28^\circ\text{C}$  (fig. 3c).

Figure 3a.

Core (-●-) and cutaneous (-■-) temperature expressed in °C of animals (N=6) given the  $\alpha_1$  adrenergic receptor antagonist, Prazosin® followed by sham hemorrhage inside chambers with the circulating water temperature set at 22° C. Time of drug administration and duration of pharmacological effect are identified with a filled bar. Data are expressed as mean  $\pm$  standard error with an \* identifying the  $p < 0.05$  intra-group significant drop in core temperature.



Figure 3a. **Body Temperature Responds to Alpha<sub>1</sub> Adrenergic Blockade**

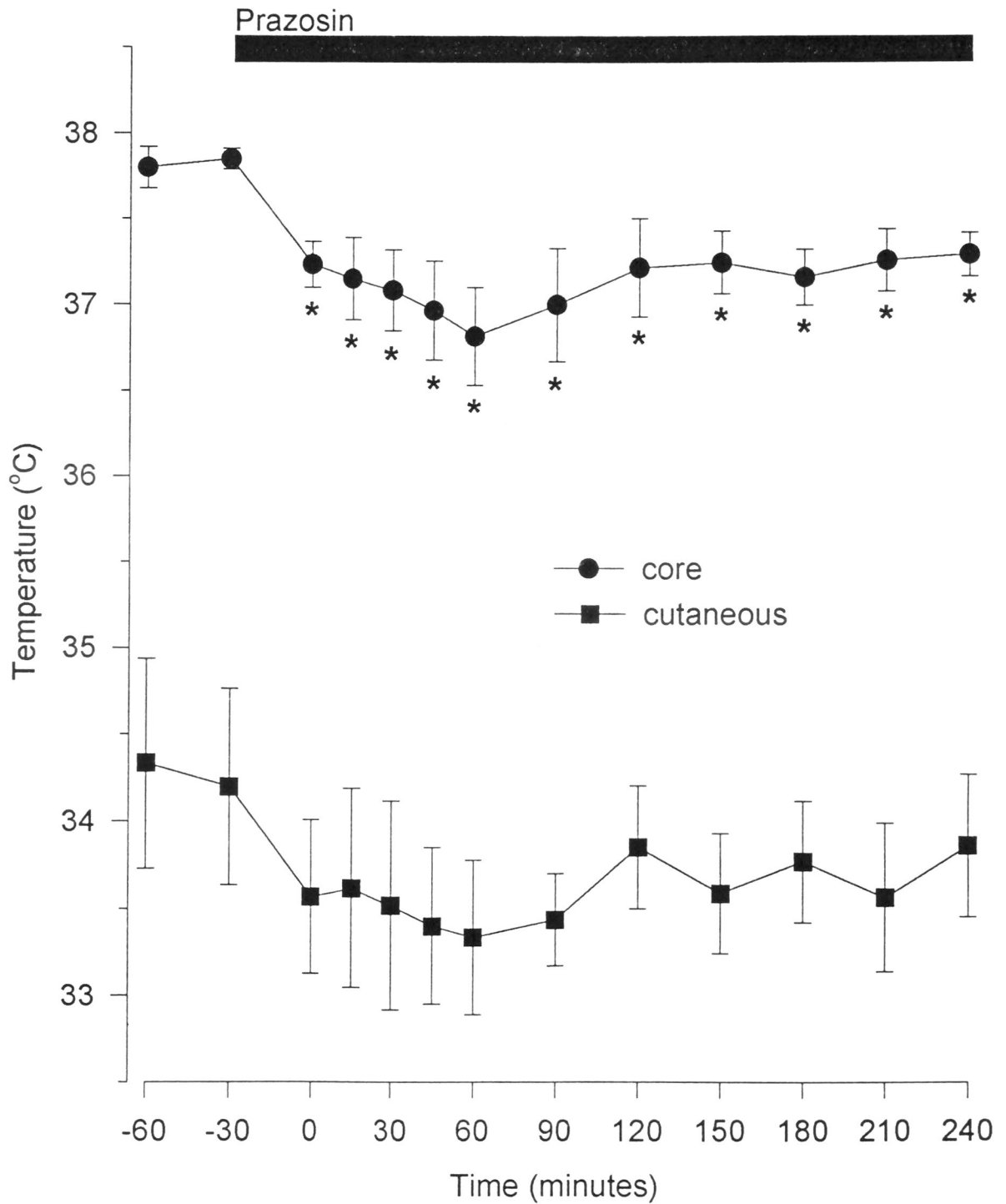


Figure 3b.

The heart rate (-●-) and mean arterial pressure (-■-) for animals (N=6) who received Prazosin® followed by sham hemorrhage inside chambers with the circulating water temperature set at 22° C. Data are expressed as mean  $\pm$  standard error with an \* depicting  $p < 0.05$  levels of intra-group significant changes from control values.

Figure 3b.

# Cardiovascular Response Following $\alpha_1$ Adrenergic Blockade

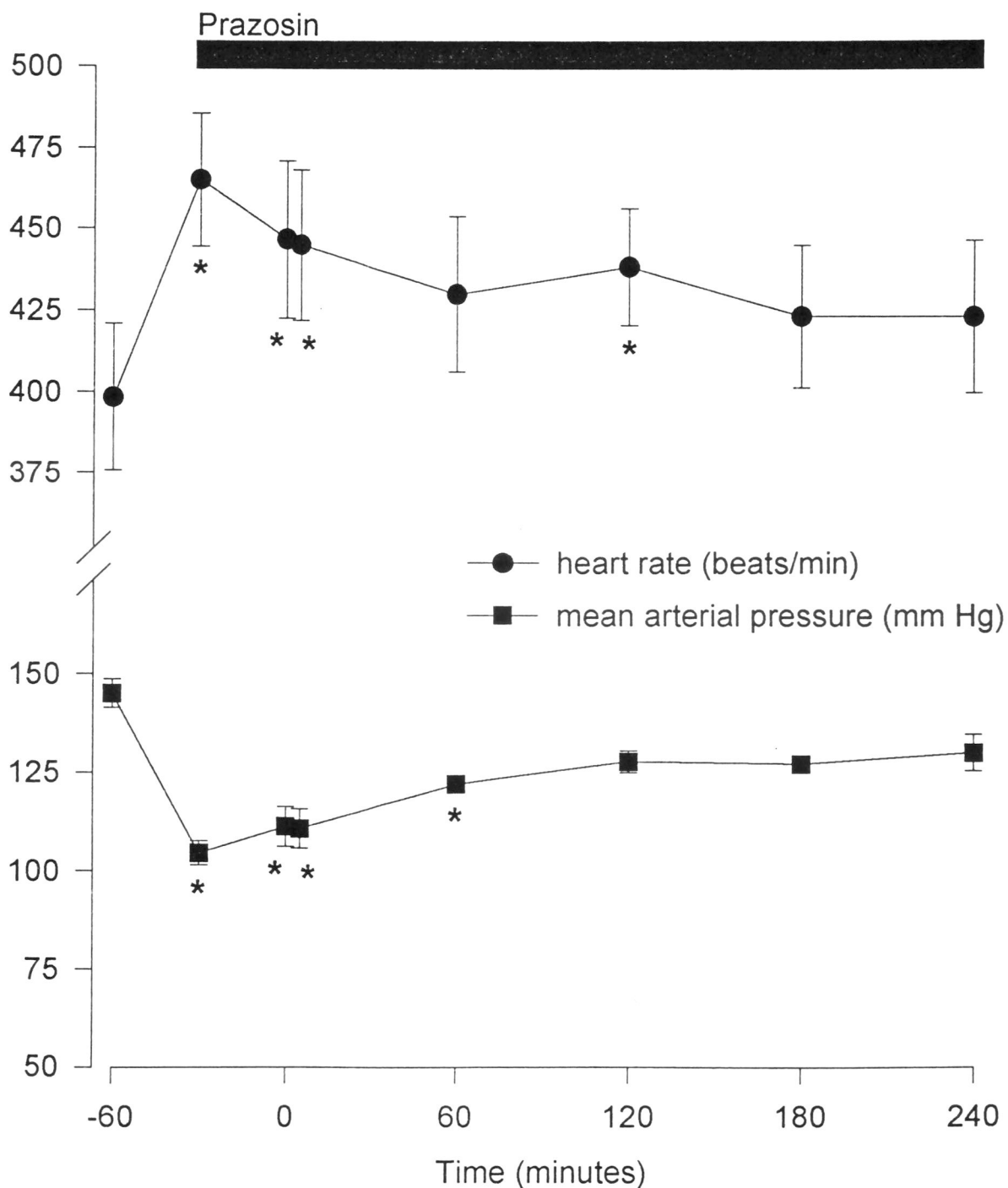


Figure 3c.

Arterial  $\text{Po}_2$ , expressed in mm Hg, values for animals (N=6) hemorrhaged at 22° C (*open bars*), animals (N=6) hemorrhaged at 28° C (*hatch bars to lower left*), and animals (N=6) given the  $\alpha_1$  antagonist followed by sham hemorrhage (*hatch bars to lower right*) for the control, immediately post-hemorrhage, and hour post-hemorrhage recovery sample period.  $\text{Pao}_2$  samples were not taken from drug response animals during the sham hemorrhage phase. Data expressed are mean  $\pm$  standard error with an \* identifying  $p < 0.05$  significance between inter-group values.

**Figure 3c. Hemorrhage Induced Changes in Arterial Blood Gas Analysis of Alpha<sub>1</sub> Adrenergic Blocked Animals**

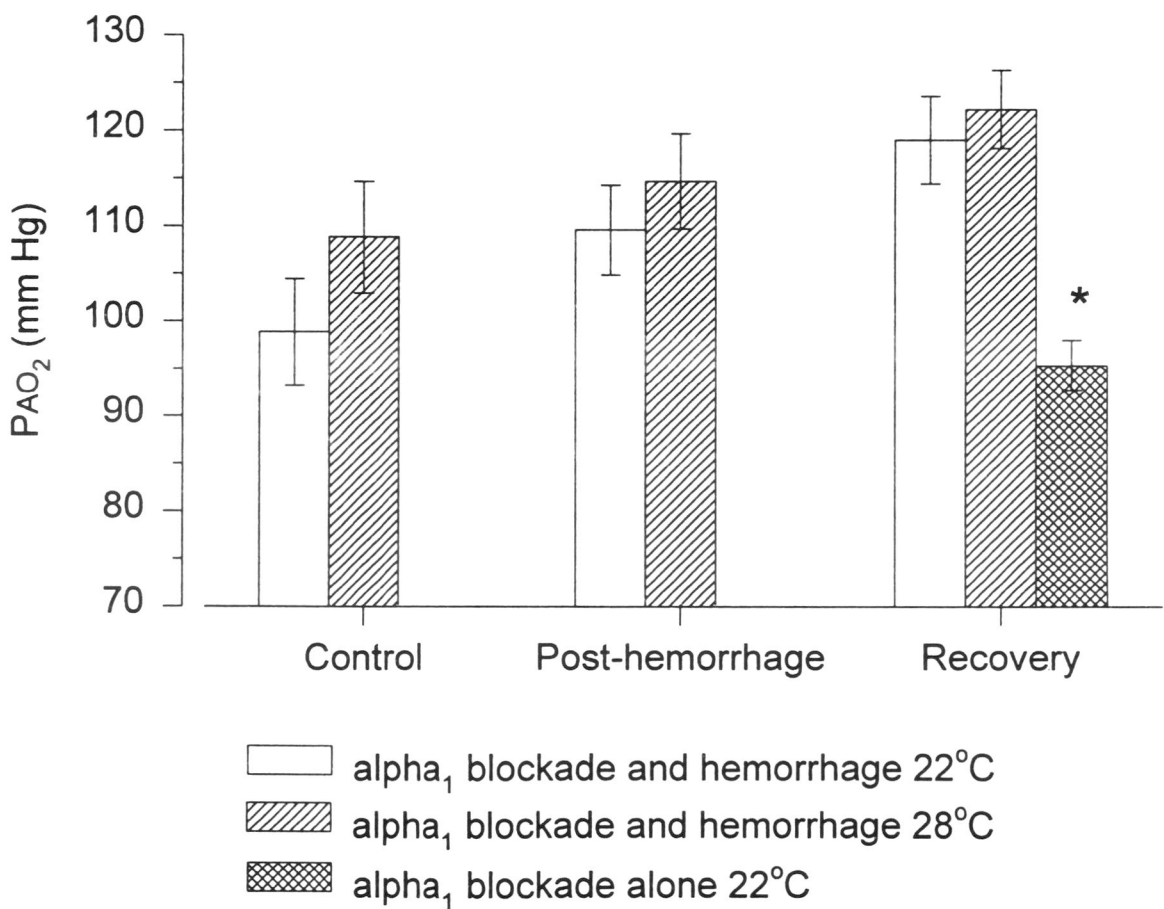


Figure 3d.

The core and cutaneous temperatures ( $^{\circ}\text{C}$ ) of animals ( $N=6$ ) who received  $\alpha_1$  blockade followed by hemorrhage inside chambers with the circulating water temperature set at  $22^{\circ}\text{C}$  (-●-) are compared to animals ( $N=6$ ) who were given the  $\alpha_1$  antagonist alone (-□-). The time and duration of pharmacological effect as well as the hemorrhage period are depicted by filled bars. Data are expressed as mean  $\pm$  standard error. An \* indicates  $p<0.05$  levels of significance observed between minimal observed and post-hemorrhage temperatures.

Figure 3d. **Body Temperature Following Alpha<sub>1</sub> Blockade and Hemorrhage**

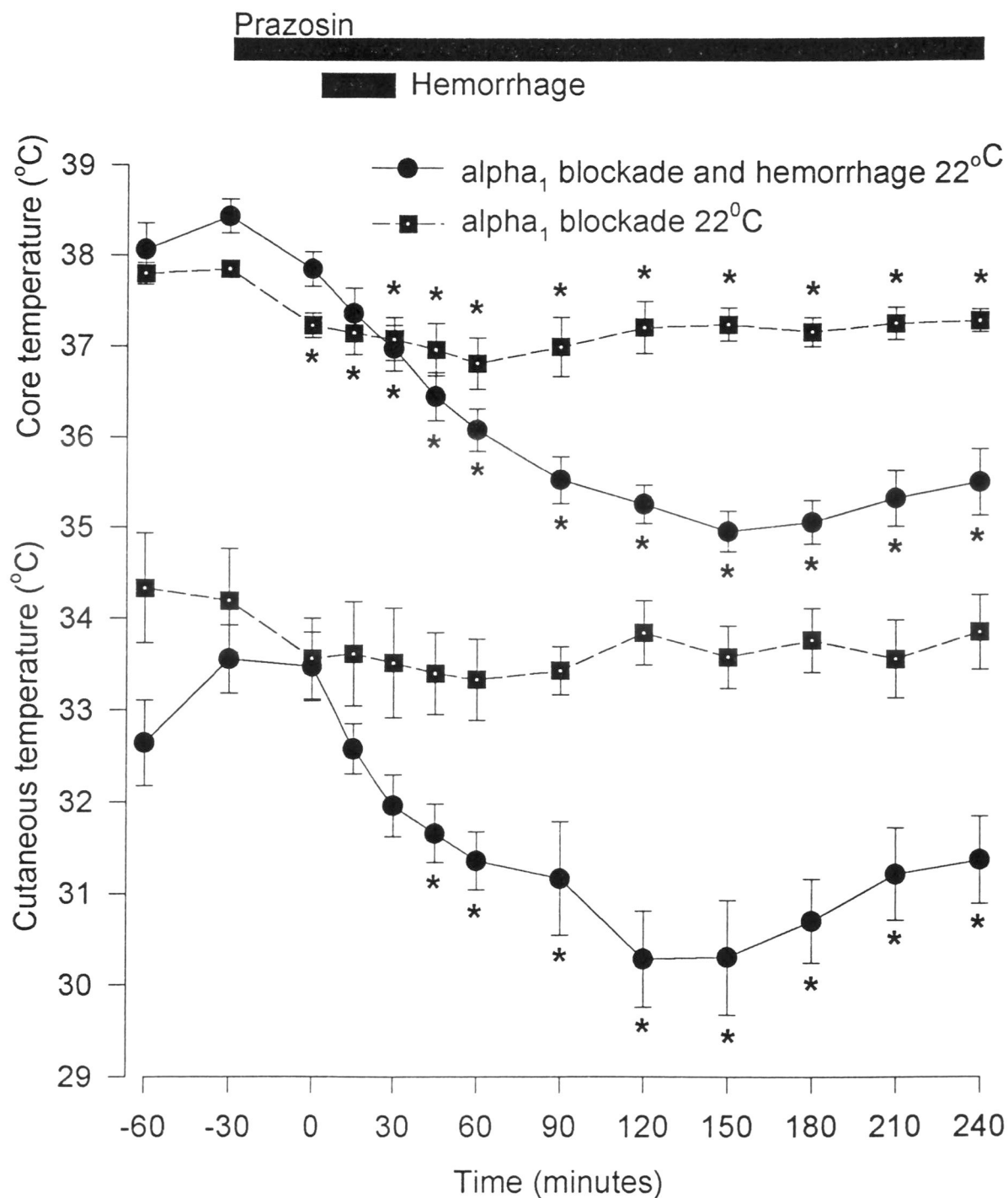


Figure 3e.

The heart rate (beats per minute) and mean arterial pressure (mm Hg) for animals (N=6) who received Prazosin® followed by hemorrhage inside chambers with the circulating water temperature set at 22° C (-●-) are compared to animals (N=6) who received the  $\alpha_1$  antagonist alone (-□-). Data are expressed as mean  $\pm$  standard error with an \* depicting  $p < 0.05$  levels of intra-group significant changes from control values.



Figure 3e. **Alpha<sub>1</sub> Adrenergic Blockade  
Preceding Hemorrhage Influences  
Both Heart Rate and MAP**

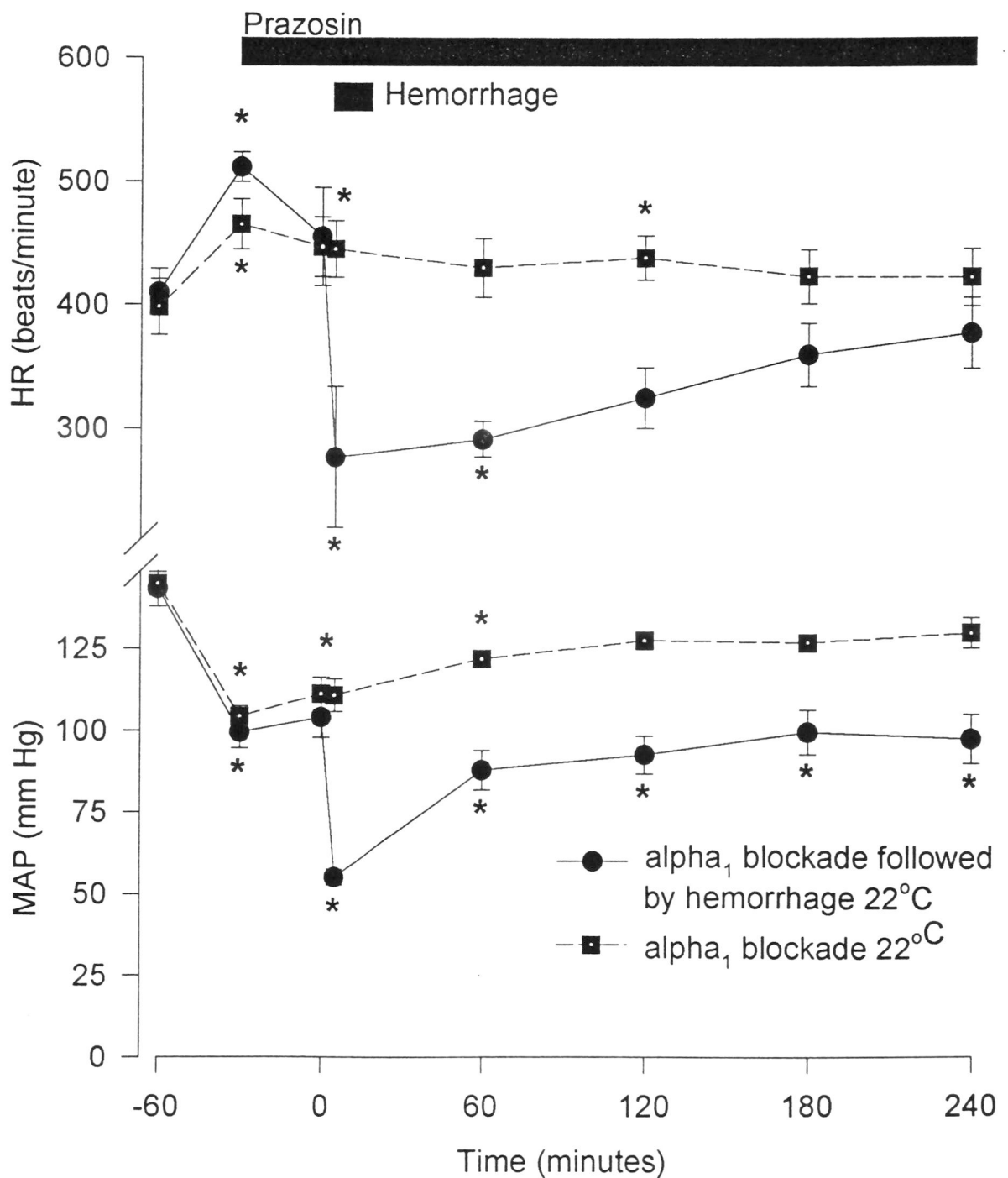


Figure 3f.

Venous  $\text{Po}_2$  levels of an animal (N=1) treated with Prazosin<sup>®</sup> followed by hemorrhage inside jacketed chambers set at 22° C are compared to a similarly treated animal at 28° C. (*Open bars*): control samples, (*hatch line to lower left*) immediately post-hemorrhage sample, (*hatch line lower right*): hour post-hemorrhage recovery sample. Actual data are presented.

Figure 3f.

## Venous $P_{O_2}$ Following $\alpha_1$ Adrenergic Blockade and Moderate Hemorrhage

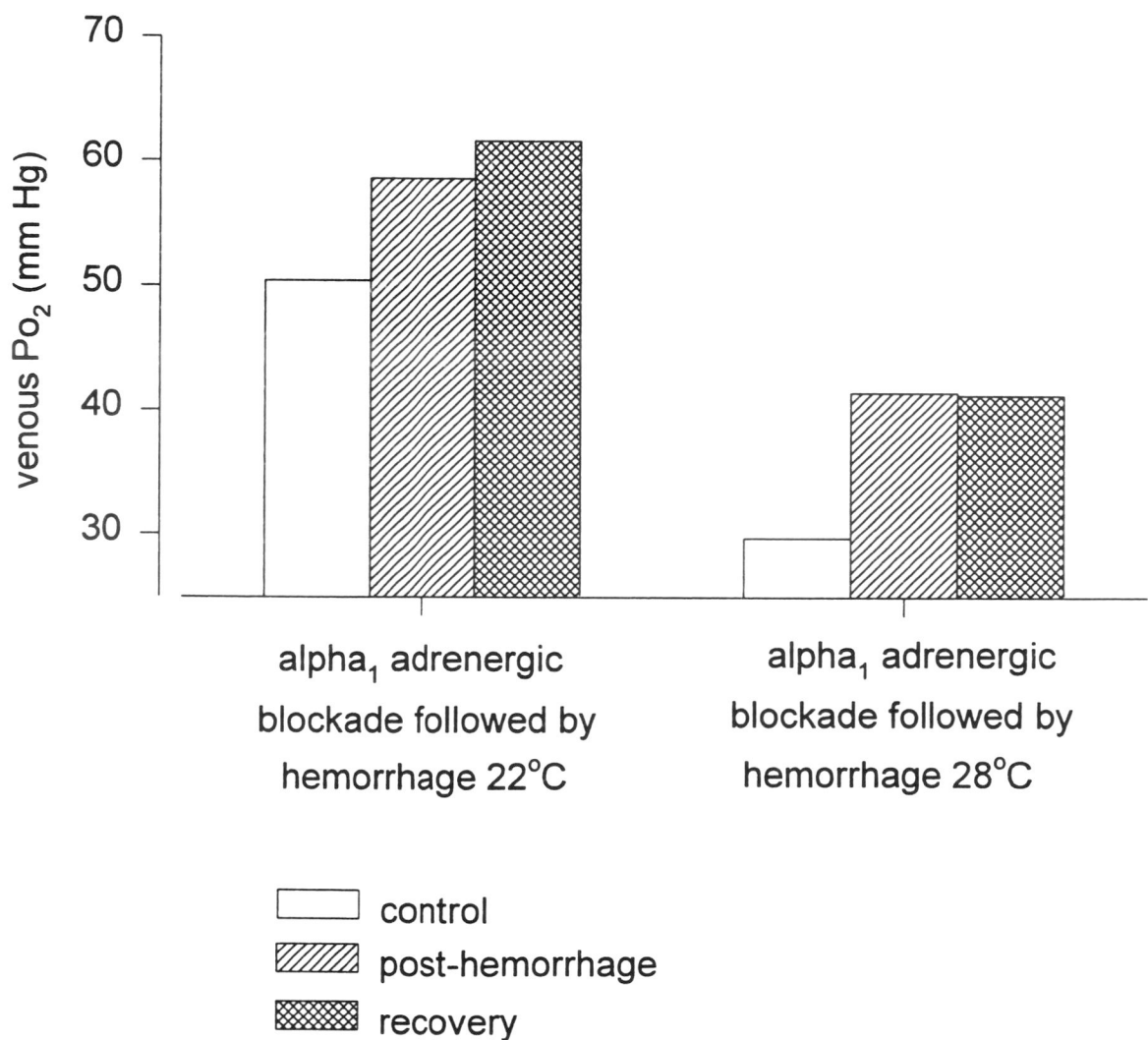


Figure 3g.

Core and cutaneous temperatures ( $^{\circ}\text{C}$ ), of animals ( $N=6$ ) given the  $\alpha_1$  adrenergic receptor antagonist, Prazosin<sup>®</sup> followed by sham hemorrhage inside chambers with circulating water temperature set at  $28^{\circ}\text{C}$  (-●-). Similar temperature recordings are expressed of animals who received the  $\alpha_1$  antagonist followed by hemorrhage ( $N=6$ ) in similar chamber conditions (-□-). Time of drug administration and duration of pharmacological effect as well as hemorrhage time are identified with a filled bar. Data are expressed as mean  $\pm$  standard error with an \* identifying the  $p<0.05$  intra-group significant drop both core temperatures.

**Figure 3g. Body Temperature Responds to Hemorrhage in  $\alpha_1$  Blocked Animals at Different Ambient Temperatures**

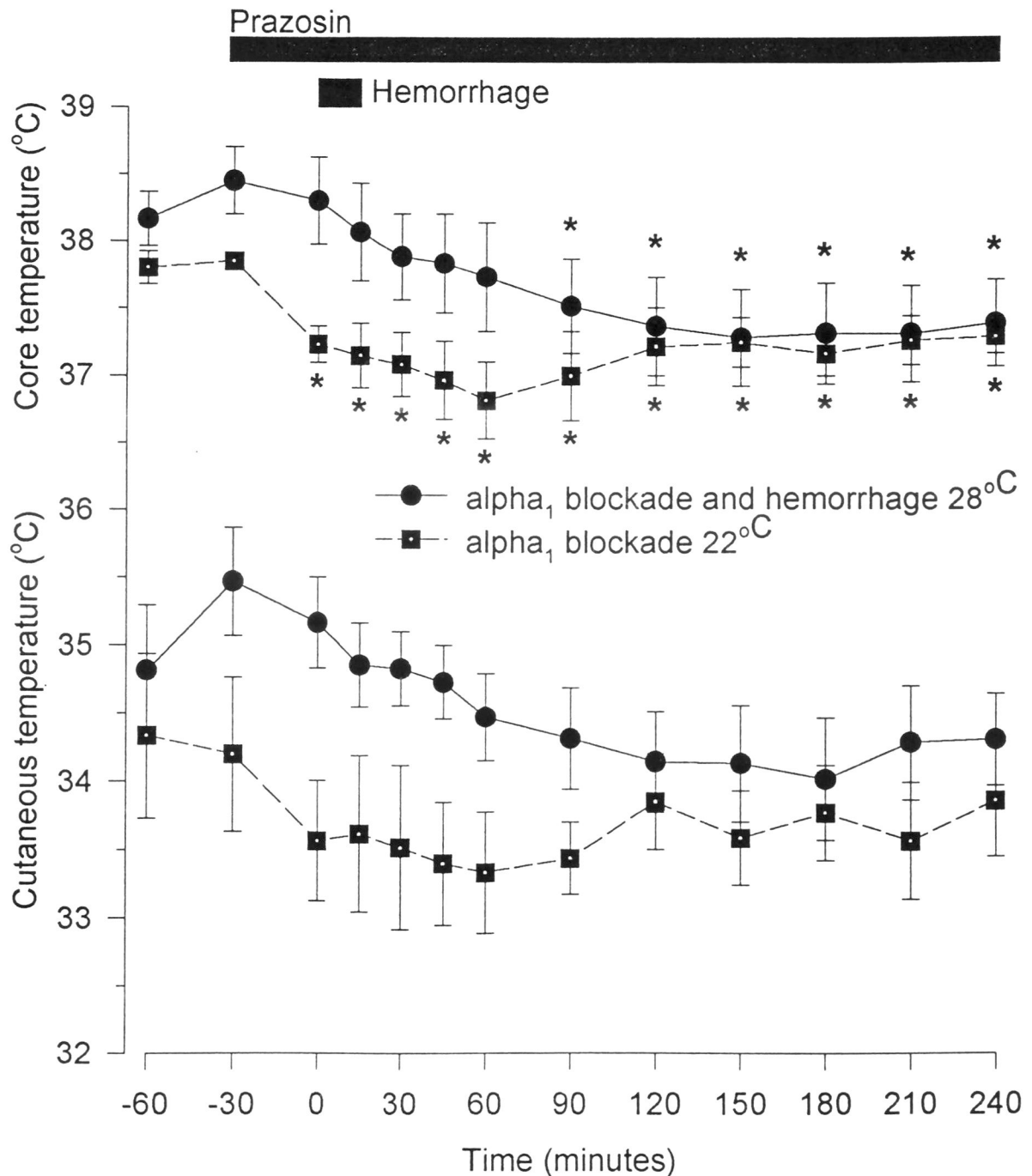
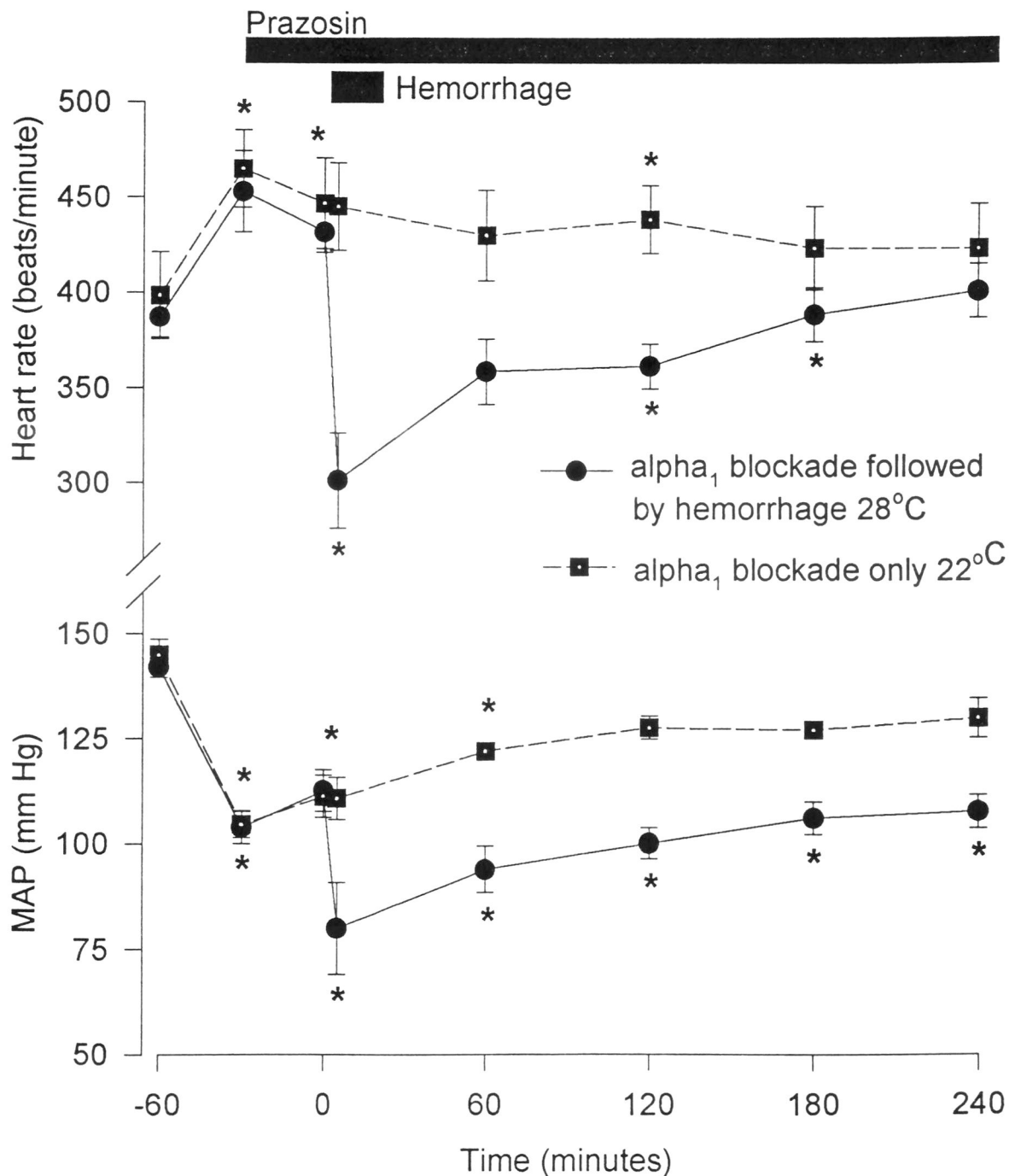


Figure 3h.

The heart rate (beats per minute) and mean arterial pressure (mm Hg) for animals (N=6) who received Prazosin<sup>®</sup> followed by hemorrhage inside chambers with the circulating water temperature set at 28° C (-●-) are compared to animals (N=6) who received the  $\alpha_1$  antagonist alone (-□-). Data are expressed as mean  $\pm$  standard error with an \* depicting  $p < 0.05$  levels of intra-group significant changes from control values.

Figure 3h. **Cardiovascular Responses with  
Alpha<sub>1</sub> Adrenergic Blockade  
Followed by Hemorrhage at 28°C**



### **Metabolic Rate and Hemorrhage**

Hemorrhage mediated changes of metabolic rate were evaluated in groups 7 - 10 and are summarized on figures 4a - 4c. The specific aim of this series of experiments was to determine if decreased oxygen consumption and associated decreased metabolic heat production potentially could have a causal relationship with hemorrhage-induced hypothermia.

#### **Sham Hemorrhage at 22°C, Group 7**

Recovered, instrumented animals (N=6), were subjected to sham hemorrhage and resting oxygen consumption was determined. Oxygen consumption in the control period was  $1.09 \pm 0.06$  cc O<sub>2</sub>/gm hr<sup>-1</sup> and remained stable for the three hour observation period (fig. 4a).

Core temperature (control  $37.5 \pm 0.1$  °C) of the sham hemorrhaged group did not change throughout the study period (fig. 4b).

#### **Hemorrhage at 22°C, Group 8**

Oxygen consumption of animals subjected to moderate hemorrhage (N=8) dropped to  $-0.21 \pm 0.05$  cc O<sub>2</sub>/gm hr<sup>-1</sup> in the first hour following hemorrhage and values remained significantly below control before returning to baseline in the third hour (fig. 4a).

Moderate hemorrhage (control  $37.6 \pm 0.1$  °C), induced a decreased body temperature to  $37.0 \pm 0.3$  °C within the first fifteen minutes. The core temperature dropped -1.3° C sixty minutes after the hemorrhage, before



recovering to control in the third hour, parallel to oxygen consumption values (fig. 4b).

#### **Hemorrhage and Prazosin Control, Groups 9 and 10**

Neither transient hypoperfusion and re-transfusion of aspirated blood (N=5) (control  $1.24 \pm 0.09$  cc O<sub>2</sub>/gm hr<sup>-1</sup>) or administration of Prazosin® (N=5) (control  $1.11$  cc O<sub>2</sub>/gm hr<sup>-1</sup>) significantly influenced oxygen consumption (fig. 4c).

Figure 4a.

Normalized oxygen consumption ( $\text{cc O}_2/\text{gm hr}^{-1}$ ) values for sham hemorrhaged animals ( $N=6$ ) and for hemorrhaged animals ( $N=6$ ) for both control and three hour observation periods. Sham hemorrhaged animals (-●-). Hemorrhaged animals (-■-). Both groups were evaluated in similar ambient chamber temperatures. Data are derived from the minimal observed oxygen consumption rate for ten consecutive minutes during each sixty minute sample period. Values are expressed as mean  $\pm$  standard error with an \* identifying the  $p<0.05$  inter-group significant drop in oxygen consumption.

Figure 4a.

## Oxygen Consumption Following Hemorrhage

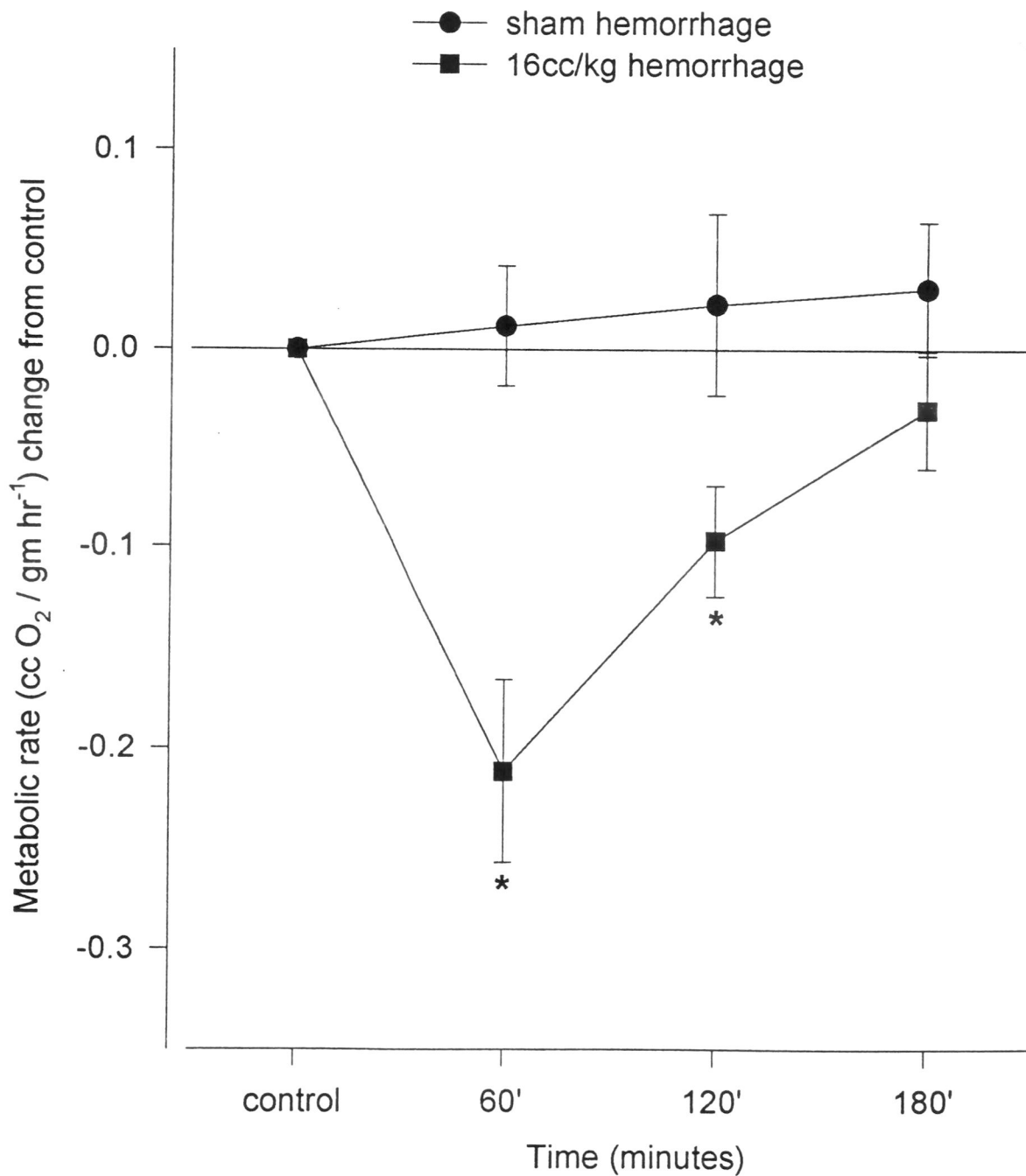


Figure 4b.

Core temperature (°C) of animals (N=6) inside the calorimetry chamber who were sham hemorrhaged (-●-) compared to animals (N=6) bled 20% total blood volume (-■-). Data expressed as mean  $\pm$  standard error with an \* indicating a  $p < 0.05$  significant drop in core temperature.

Figure 4b.      **Core Temperature Responds to Hemorrhage Inside Calorimetry Chamber**

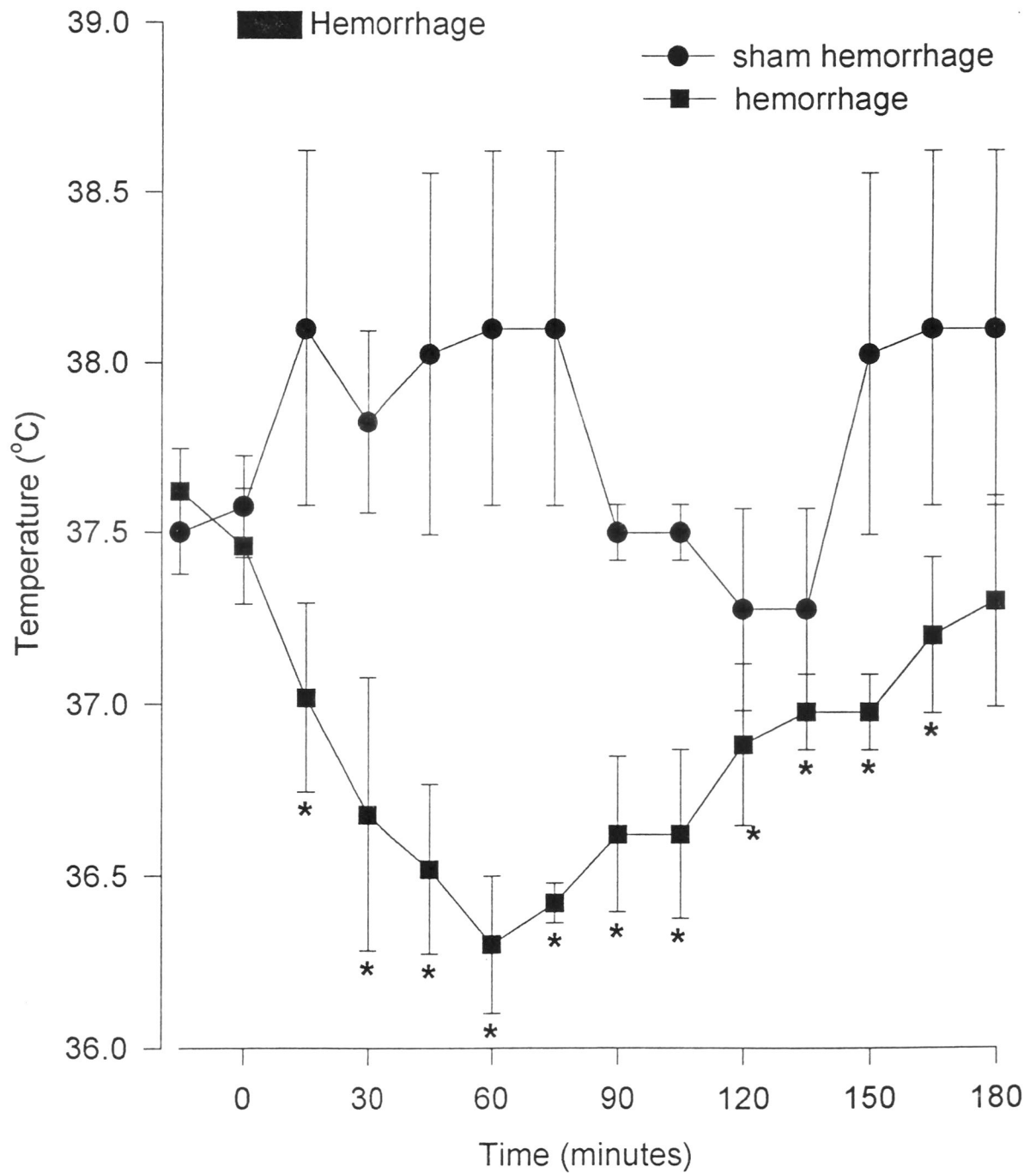
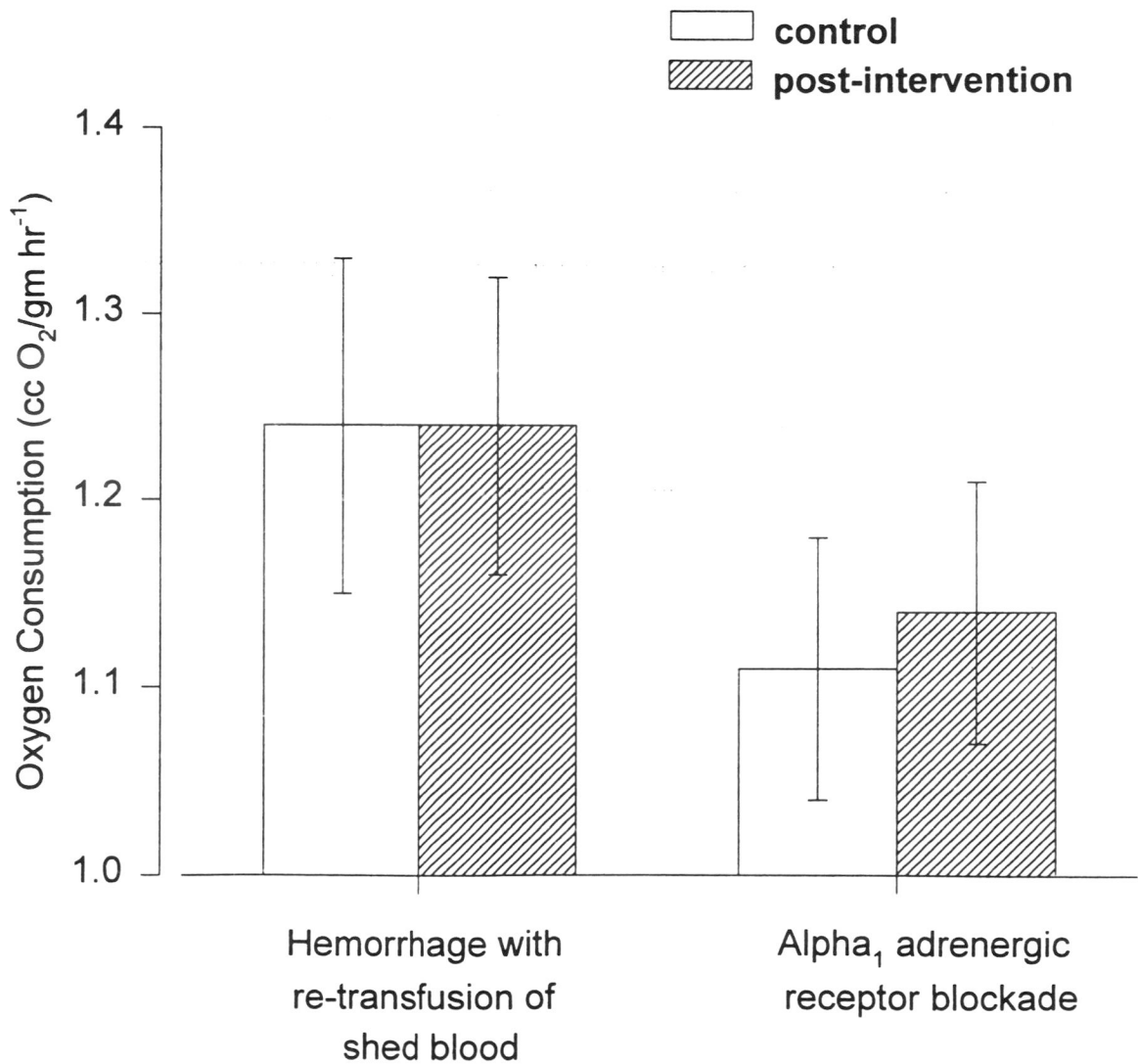


Figure 4c.

Pre (*open bars*) and post-interventional (*hatched bars*) oxygen consumption values ( $\text{cc O}_2/\text{gm hr}^{-1}$ ) of animals which were hemorrhaged followed by immediate re-transfusion of the aspirated blood. Additionally, oxygen consumption values of animals who were given Prazosin<sup>®</sup> are depicted. Chamber temperature for both groups was similar compared to hemorrhaged and sham hemorrhaged groups. Data are expressed as mean  $\pm$  standard error.

Figure 4c.

## Metabolic Rate Following Transient Hypotension or $\alpha_1$ Adrenergic Blockade



## **Discussion**

The most important finding from this study was that the drop in body core temperature following hemorrhage, which is a direct result of an alteration in the balance between heat production and heat elimination, is preceded by a reduction in metabolic heat production. Furthermore, this drop in core temperature does not appear to be mediated by enhanced dry heat loss mechanisms of the skin. This discussion, will examine the cardiovascular, body temperature, and pulmonary responses following moderate hemorrhage. This information, combined with data on cutaneous heat loss and metabolic heat production, will explain the mechanism by which hemorrhage alters the thermoregulatory balance of a conscious rat.

### **Cardiovascular Response to Hemorrhage**

Following hemorrhage, the cardiovascular system compensates to maintain adequate perfusion to the heart and brain. The cascade of events, initiated by the drop in arterial pressure, leads to increases in both sympathetic outflow as well as catecholamine release from the adrenal medulla. The increased sympathetic activity and catecholamine secretion, enhance myocardial performance and increase total peripheral resistance. The duration of the systemic compensation appears to be determined by  $\alpha_2$  adrenergic receptors predominately located in the larger central arteries with peripheral  $\alpha_1$  adrenergic receptor activity being the principle short-term compensatory contributor (Bond et



al., 1984)<sup>6</sup>. Following hemorrhage, negative feedback mechanisms may inhibit the sympathetic response resulting in lowered heart rate and peripheral resistance: the decompensated phase of shock (Bond et al., 1985)<sup>8</sup>. While the compensated and decompensated phases of shock follow similar mechanisms as humans, the rat enters a sympathetically inhibited phase earlier than the human (Haggendal, 1986).

Consistent with previously mentioned work, we observed the expected cardiovascular response to hemorrhage (fig. 1b). Immediately following hemorrhage, sympathetic inhibition induced a significant drop in heart rate. This paralleled a drop in perfusion pressure but did not appear to have a causative relationship. Similarly, animals hemorrhaged at higher ambient temperatures displayed the relative bradycardia and hypotension which did not significantly differ from that of animals hemorrhaged at 22°C.

The cardiovascular response to  $\alpha_1$  blockade produced expected results (fig. 3b). Immediately following administration, mean perfusion pressures declined as a result of the loss of peripheral arteriole tone. To compensate, via enhanced sympathetic  $\beta_1$  innervation, combined with decrease vagal tone, the heart rate increased. The cardiovascular response to combined  $\alpha_1$  blockade and hemorrhage gave insight to the relative power of compensatory mechanisms following perfusion status changes. Following administration of the  $\alpha_1$  antagonist we observed the compensatory tachycardic response which maintained

adequate perfusion pressures. However, sympathetic inhibition following moderate hemorrhage overcame this and produced the previously observed relative bradycardia. As expected, the mean arterial pressure dropped well below both intra and inter-group controls. The cardiovascular responses to hemorrhage at the elevated chamber temperatures were comparable that seen at the lower temperatures.

Hemorrhage produced the expected changes in complete blood count analysis (fig. 1e). Calculating the total blood volume empirically as contributing 8% to the animal's total body weight, a 20% blood volume hemorrhage produced a significant relative anemia. Hemoglobin and hematocrit levels revealed 20 - 30% reductions from control values, concurring with the method used to calculate total blood volume. Neither higher ambient temperature nor  $\alpha_1$  blockade produced significant changes in the complete blood count analysis.

### **Pulmonary Response to Hemorrhage**

Following hemorrhage alveolar minute ventilation increases to maximize oxygen procurement and delivery. This compensation is modest, as post-hemorrhage arterial oxygen pressures were comparable to values obtained in the control period (fig.1c). Because alveolar  $PO_2$  reaches equilibrium with capillary  $PO_2$  just before the expiratory phase begins, only minimal  $Pao_2$  increases are to be expected as the oxygen concentration of inspired air remained 21% throughout the period. Oxygen uptake by the lungs was modestly

enhanced, but not significantly, in the 28°C environment. Similarly, the effect of  $\alpha_1$  adrenergic blockade did not appear to augment oxygen loading at the pulmonary capillary site. For animals who received the  $\alpha_1$  antagonist, their recovery samples revealed  $P_{aO_2}$  levels similar to those of animals who did not receive the  $\alpha_1$  antagonist. No blood was obtained in the hemorrhage phase of the drug response group; therefore, no intra-group comparisons were possible.  $P_{aO_2}$  pressures were enhanced following combined  $\alpha_1$  blockade and hemorrhage compared to the drug response animal's recovery samples.

However, the relative increase in respiratory rate does allow enhanced removal of  $CO_2$  from the blood. With each breath, the  $P_{CO_2}$  pressure reaches equilibrium with alveolar  $P_{CO_2}$  pressures just before expiration. Given relative consistence in the depth (gas mixing) of respiration with ambient  $CO_2 < 0.5\%$ , the resultant allows each exchange to be equally effective in lowering  $P_{CO_2}$  levels. Arterial pH is coupled directly to  $P_{CO_2}$  levels via carbonic acid. We observed the expected relative respiratory alkalosis accompanying post-hemorrhage hyperventilation which returned to control values within the first hour.

Baseline concentrations of  $HCO_3^-$  were not significantly changed immediately following hemorrhage. However, as the hypoperfused state persists,  $HCO_3^-$  levels are significantly reduced as the demand for acid buffers increases.

Base excess estimates, which reflect the amount of titrateable base necessary to correct a tissue acidemia to standard conditions, suggest

respiratory compensation in the acute phase is sufficient; however, with continued hypoperfusion, base concentrations necessary to correct tissue acidemia rose above control.

Our experimental protocol directed the collection of venous blood gases to assure adequate tissue oxygenation and to document subsequent tissue hypoxic conditions. Due to presumed inferior vena cava wall pliability, venous blood samples were rarely available. This prohibits determination of the possible role of hypoxia as a contributing factor in the observed hypothermic event. The single venous sample obtained from each group revealed  $P_{vo_2}$  levels above 30 mm Hg with exception in the recovery sample of animals who were hemorrhaged at 28°C, where  $P_{vo_2}$  was 28.8 mm Hg (fig.1e). While no inference can be made from this single observation, the drop in core temperature of this animal over the entire observation period was  $-0.85 \pm 0.17$  °C while for the other animals it was  $-0.53 \pm 0.14$  °C. It will be of specific interest to determine if this observation persists in a larger sample of animals.

### **Body Temperature Responses to Hemorrhage**

Following moderate hemorrhage there is an observed hypothermic event evident in both core and cutaneous temperature. Animals hemorrhaged in jacketed chambers with water circulating set 22°C, revealed an intra-group steady decline in body core temperature beginning fifteen minutes after the hemorrhage and continuing until reaching a  $p < 0.05$  level of significance 180 minutes after the

insult (fig.1a). Similarly, normalized data revealed that core temperature fell significantly thirty minutes after the hemorrhage and remained below control for the remainder of the observation period (fig.1f). Cutaneous temperature of this group, declined parallel to the core temperature, but due to high individual variance, no intra or inter-group significance was found.

In the first series of experiments, one specific aim was to determine to what extent the dry heat loss mechanism of cutaneous blood flow contributed to the hypothermic event following hemorrhage. For all animals, principle heat transfer mediators follow standard laws of thermodynamics. Maintaining a delicate balance, endothermic animals transfer a thermal load via conductive, convective, evaporative, and radiant mechanisms. By adjusting ambient and chamber wall temperatures to the upper critical temperature, conductive, convective and radiant heat loss mechanisms can be minimized. To prevent evaporative loss, however, the air would have to be hypersaturated with moisture. In conscious active animals, with all other heat loss mechanisms minimized, to eliminate the only other available avenue of heat loss, would quickly result in an hyperthermic load leading to unconsciousness and death. This, however, leaves an uncontrolled avenue for evaporative heat loss from the respiratory system as well as cutaneous sweat glands.

Animal hemorrhaged while inside the chamber at 28°C, displayed an initial increase in core temperature which was preceded by an increase in cutaneous

temperature (fig. 2a). Because animals placed in chambers with ambient temperatures closer to their home cage did not demonstrate an initial increase in core temperature, this increase can be explained by the quantitative heat gain achieved by removing the animals from their cage at room temperature and placing them in chambers at the higher upper critical temperature. Following hemorrhage, the core temperature declined parallel with cutaneous temperature, gradually reaching intra-group significance 150 minutes after the hemorrhage. This hypothermic response was similar to the core temperature response of animals hemorrhaged inside chambers with circulating water set at 22°C. The change in core temperature was also delayed and did not fall significantly until ninety minutes after the hemorrhage as compared to thirty minutes in the 22°C group. This delay in core temperature response to hemorrhage may be a function of the initial increase due to heat influx. More importantly, however, with three of the four conventional heat transfer methods “clamped” by elevated ambient temperature, following hemorrhage, animals continued to display the hypothermic event. This suggest that an initial unloading of heat does not contribute to the hypothermia.

A second approach to inhibit heat loss from cutaneous blood flow is to block the  $\alpha_1$  adrenergic receptors which mediate sympathetic nervous system regulation of cutaneous blood flow. Willette et al. found the principle contributor to cutaneous vascular tone was post-junctional  $\alpha_1$  adrenoceptors

stimulated by circulating catecholamines and not significantly influenced by post-ganglionic sympathetic nerves (Willette et al., 1991). Alpha adrenergic receptors of the  $\alpha_1$  subclass were pharmacologically antagonized by vascular administration of Prazosin<sup>®</sup>. By blocking this subclass of receptors located primarily in cutaneous arteriole walls, we were able to uncouple cutaneous blood flow from any centrally mediated redistributory or thermoregulatory input.

The response of  $\alpha_1$  antagonist without hemorrhage was evaluated in jacketed chambers set at 22°C (fig. 3a). By eliminating the subcutaneous vascular neuronal control, resulting in enhanced heat loss, animals dropped their core temperature significantly within thirty minutes until the drop was offset via enhanced endogenous heat production mechanisms, ninety minutes post-administration. The observed increase metabolic rate of animals following administration of Prazosin<sup>®</sup> may mediate this stabilization of core temperature (fig 4c). The cutaneous temperature dropped then rose parallel with the core temperature; however, again due to individual variance, no significance was obtained.

The combined effect of  $\alpha_1$  adrenergic receptor blockade and hemorrhage produced the most pronounced hypothermia observed in this study. Intra-group evaluation revealed the core temperature of animals who received the antagonist dropped significantly fifteen minutes after the hemorrhage and remained well below control before stabilizing 150 minutes after the insult. This drop in core

temperature was significantly greater than that of the drug response group only forty-five minutes into the observation period (fig. 3d). At the same time, the cutaneous temperature of these animals dropped significantly below those of the drug response group and remained below control throughout the observation period. This suggested that cutaneous blood flow following hemorrhage is reduced not only to redistribute blood volume to critical areas but also this shunting of blood away from the cooler periphery serves to protect the core structures from severe hypothermia.

Another way to demonstrate that heat loss is blocked from cutaneous blood flow is to maximize regional cutaneous blood flow while inside chambers with temperatures set to the animal's upper critical temperature. The avenue by which the animal's heat loss can therefore be maximized; yet, without a thermal gradient, no heat transfer can occur. To demonstrate this,  $\alpha_1$  blocked animals were hemorrhage inside chambers set at 28°C (fig.3g). As before, prior to hemorrhage we observed a rise in core temperature from heat influx of the relatively warmer chamber. Following hemorrhage, the core temperature declined steadily to significance ninety minutes after the insult. This suggest that despite heat loss mechanisms being blocked, the hypothermic response persists. Individual variance of the cutaneous temperature prohibited significance; however, declining cutaneous temperatures paralleled the core temperature observation.



### **Metabolic Responses Following Hemorrhage**

With no evidence suggesting that the heat loss mechanisms of cutaneous blood flow was a significant contributor to the drop in body temperature following hemorrhage, we turned our attention to the heat production side of the balance: cellular metabolism. The animals oxygen consumption rate provides an accurate index of metabolic rate and therefore heat production. As the principle heat source for non-stressed endothermic animals, within their thermoneutral zone, oxidative phosphorylation maintains the heat production side of the thermoregulatory balance.

Instrumented, fully recovered animals increased O<sub>2</sub> consumption from basal levels. This observation may be due to discomfort of the surgical site, allergenic properties of the implants, or food assimilation as the animals were allowed free access to food and water following surgery. All observations were obtained during the photophase of these nocturnal animals thus eliminating diurnal activity variation.

With post-instrumentation metabolic rate as control, sham hemorrhaged animals did not significantly alter their metabolic rate throughout the three hour observational period (fig. 4a). However, animals that were subjected to a moderate hemorrhage experienced a  $-0.21 \pm 0.05$  cc O<sub>2</sub>/gm hr<sup>-1</sup> reduction of oxygen consumption in the sixty minute period following hemorrhage which remained significantly below control until the third hour. Activity significantly

impacts metabolic rate. This influence was minimized by selecting the lowest oxygen consumption values for ten consecutive minutes within each sixty minute sampling period. Activity therefore limited demonstration of causality between the reduced metabolic rate and the hypothermic event. To show causality by decreasing the sampling period to fifteen minutes, would yield highly variable results.

No causative factors were found to exist between perceived hemorrhage and immediate retransfusion of the aspirated blood. In contrast, transient hypoperfusion revealed modest increase of oxygen consumption in the post-interventional observations (fig. 4c).

The reduced metabolic rate was accompanied by the previously observed hypothermic event occurring within fifteen minutes after the hemorrhage (fig. 4b). Animals dropped their core temperature well below control and did not recover until 180 minutes post-hemorrhage. For fasting animals, with an RQ of 0.79, energy production can be rounded to 4.7 to 4.8 kcal/L of O<sub>2</sub> consumed (Bartholomew, 1977). Prior to hemorrhage, animals consumed  $1.229 \pm 0.051$  cc O<sub>2</sub>/gm hr<sup>-1</sup> (2.08 kcal/hr) to maintain an average core temperature of  $37.6 \pm 0.1$  °C, compared to  $0.996 \pm 0.061$  cc O<sub>2</sub>/gm hr<sup>-1</sup> (1.69 kcal/hr) following hemorrhage, which accompanied an average core temperature of  $36.3 \pm 0.2$  °C. The reduced energy utilized in the metabolic process can easily account for the reduced body temperature. However, as previously mentioned, the hypothermic

event that accompanies the reduced metabolic heat production could not be coupled successively with causality: i.e. (a) did the reduced metabolic rate cause the hypothermia or, (b) did the hypothermia cause the reduced metabolic rate? This question may be addressed in the future by continual oxygen consumption measurements obtained paralleled with a quantitative estimate of activity.

### **Conclusions: Thermoregulation Following Hemorrhage**

For endothermic animals, there exists a balance between heat production and heat loss. Factors such as ambient temperature, activity, hypoxia, anesthesia, immunologic status, and shock alter this balance resulting in hyper or hypothermic events. As demonstrated previously (Wood, 1996), the alteration of thermogenic characteristics can be adaptive and serve to protect the organism from potential adverse outcomes.

In hemorrhagic shock, thermoregulatory control is altered. The clinical implication of this hypothermic event and its potential protective or conversely detrimental effects, is controversial. Evolutionary processes have directed a proportion of species to respond to hemorrhage in similar fashion. With a better understanding of the underlying mechanism of hemorrhage-induced hypothermia, insightful investigators can proceed to search for the causal stimulus. Once this has been elucidated, one can then assign this drop in core temperature to being either a protective adaptive process or the coincidental result of regulation gone bad.

Cutaneous blood flow does not appear to significantly contribute to the drop in core temperature following hemorrhage. Circulating catecholamine concentrations increase accompanied with an increase of sympathetic efferent activity resulting in the local redistribution of blood away from the skin. The limited flow directed to the skin therefore has relatively little capacity to transfer heat from the core to the subcutaneous beds where it can be lost. The lag time from the onset of hemorrhage until the compensatory translocation of cutaneous blood can potentially offer a time window through which heat loss can exceed production: a debt from which the body cannot recover following the hemorrhage. By eliminating conventional heat loss mechanisms of the skin, excluding evaporative loss from sweat, animals displayed a significant drop of their core temperature. Furthermore, with skin blood flow regulation blocked, the hypothermic event was more severe. This suggest that cutaneous blood flow is not the mediating factor of the drop in core temperature rather serves to prevent exaggerated heat loss.

Conversely, the hypothermic event following hemorrhage is accompanied by a significantly reduced metabolic rate despite sympathetic stimulatory effects. As mentioned the net compensatory effect from the autonomic nervous, endocrine, and renal systems is to increase systemic resistance, increase heart rate and contractility, and increase respiratory gas exchange. Despite this overwhelming stimulatory input, we observed a net decrease in metabolic

oxygen consumption following hemorrhage. Do the localized tissues also contribute to the compensatory mechanism by lowering their individual energy demand throughout this stressful period? If so, the net result would be an observable decrease in metabolic oxygen consumption and a decrease in a indirect measurement of heat production: core temperature.

### **Study Limitations**

The first limitation I wish to address in the cutaneous blood flow studies, control group animals were not handled identically to experimental groups. Once inside the chamber, the control group animals were not attached to the hemodynamic or cutaneous temperature monitoring cables resulting in less psychological stress placed upon the control animals. However, these animals did not exhibit a chamber-induced reduction in core temperature (fig 1f).

A second related limitation involves skeletal muscle activity. Throughout the study, we observed that activity varied from individual to individual throughout the different phases of the experiment. The typical response to attachment to the monitoring equipment was one of enhanced skeletal muscle activity, while immediately following hemorrhage, the animals became somewhat lethargic. To quantify activity would allow us to control for the thermogenic contribution of skeletal muscle activity following hemorrhage; however, this information is not critical given the individual patient response is also variable.

Thirdly, evaporative heat loss from the respiratory system and skin could not be quantified. To hypersaturate the air with water to prevent evaporative loss, combined with the other three heat loss mechanisms being minimized, would result in an untimely death. Enhanced water loss from compensatory respiratory rate increases, as well as sweat gland stimulation by sympathetic cholinergics, can contribute to heat loss; although, the latter is implausible in hypothermic situations. Further study to quantify evaporative heat transfer from insensible fluids is indicated.

Lastly, the lack of venous blood gas samples does not preclude individuals from experiencing combined hypoxic and hemorrhage-mediated hypothermia. Although of the four venous samples, only one had a  $P_{vo_2} < 30$  mm Hg. The mean drop in core temperature was significantly greater in this individual compared to the remainder of the group. It would be of particular interest to sample from a pulmonary artery catheter providing a true mixed venous sample while allowing for conscious evaluation.

### **Clinical Implications**

Advanced Trauma Life Support guidelines suggest resuscitation for patients suffering from hemorrhagic shock focus on: (a) maximizing oxygen delivery by supplementing inspired  $O_2$ , (b) restoring circulating volume by administration of crystalloid, colloid, or blood products, and (c) prevention of exposure mediated hypothermia (ATLS, 1993). Patients presenting to pre-

hospital care providers often exhibit the classical symptoms of shock, including: altered levels of consciousness, tachycardia, and “cold and clammy” skin.

Presentation vital signs suggest that many patients suffer from relatively mild to moderate hypothermia following traumatic blood loss. It has been established that mild hypothermia as a adaptive response to hypoxia (Wood, 1996). In the setting of hypoxia, the potential benefits of hypothermia include: (a) decreased metabolic oxygen demand due to the Q10 effect, (b) decreased energy cost of responding to the hypoxia, and (c) improved oxygen loading in the lung due to a right-ward shift of the oxygen-hemoglobin dissociation curve (Wood, 1996). Furthermore, it has been shown that hypothermia in itself improves the neurological outcome and survival in rats (Yager et al., 1993: Sori et al., 1987: Crippen et al., 1991: Wood, 1996) and dogs (Tisherman et al., 1991). At the cellular level, post-ischemic hypothermia reduces neuronal histopathologic findings (Yager et al., 1993: Towfighi et al., 1994: Minamisawa et al., 1990), reduces cerebral oxygen consumption (Hagerdal et al., 1975 and Yager et al., 1996), limits lactate (Berntman et al., 1981), and excitatory amino acid release (Nakashima et al., 1996). Following hemorrhage, hypothermia attenuates the decline of serum glucose as well as the increase of serum potassium (Johnson et al., 1996), maintains neuronal ATP and phosphocreatine levels (Berntman et al., 1981), maintains liver ATP levels and prevents

elevations in liver enzymes (Johannigman et al., 1991), and protects the relative impermeability of the blood-brain barrier (Smith et al., 1996).

Conversely, combined hemorrhage and hypothermia is also associated with severe coagulopathy (Brenneman et al., 1994; Sharp et al., 1992; Bruch et al., 1992; Oung et al., 1993). Our data from figure 1e show that hemorrhage induces a reduction in platelet concentrations which alone will prolong bleeding times (Gubler et al., 1994). Aside from hypovolemic-induced thrombocytopenia, hypothermia will promote platelet sequestration by the liver (Villalobos et al., 1958). Furthermore, hypothermia slows the enzyme kinetics necessary to produce a clot (Reed et al., 1990). Both thrombocytopenia and hypothermic coagulopathies can be mechanistically uncoupled (Gubler et al., 1994). Identification of a threshold temperature clinicians could theoretically aim for in passive or active cooling, that would prevent severe coagulopathies and possible death, has not been established .

### **Future Directions**

The protective attributes hypothermia imparts at the cellular, tissue, system, and organismal levels cannot be ignored. Future studies should continue to focus on the mechanisms underlying hypothermia induced by hemorrhage to ascertain if this observation is indeed an adaptive, protective response. Specifically, metabolism studies need to be coupled with activity quantification to control the skeletal muscle thermogenesis which occurs before



the animal relaxes in the calorimetry chamber. Additionally, evaporative heat loss can be obtained simultaneously by plethysurography.

Artificially-induced hypothermia is currently under evaluation. Clinical application of active cooling techniques of cerebral and myocardial tissues are utilized in the controlled setting of operating rooms. Ice baths, are inexpensive and quite effective in quickly dropping core temperature; however, limiting global application of this method is the uncontrollable rate and degree of the hypothermic response. Under current investigation are methods to use crystalloid intravenous therapy to induce a controlled hypothermic event. Additionally, administration of hypothermic blood products coupled with active external cooling have been shown to prolong the resuscitation time window and improve neurologic outcome following hypotensive hemorrhage (Simon et al., 1995).

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