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SKIN AND SKELETAL MUSCLE VASCULAR RESPONSES

TO HYPOTHERMIA

presented by

Terry Charles Major

has been accepted towards fulfillment
of the requirements for

Master degree in Physiology

James A. Schwieghamer
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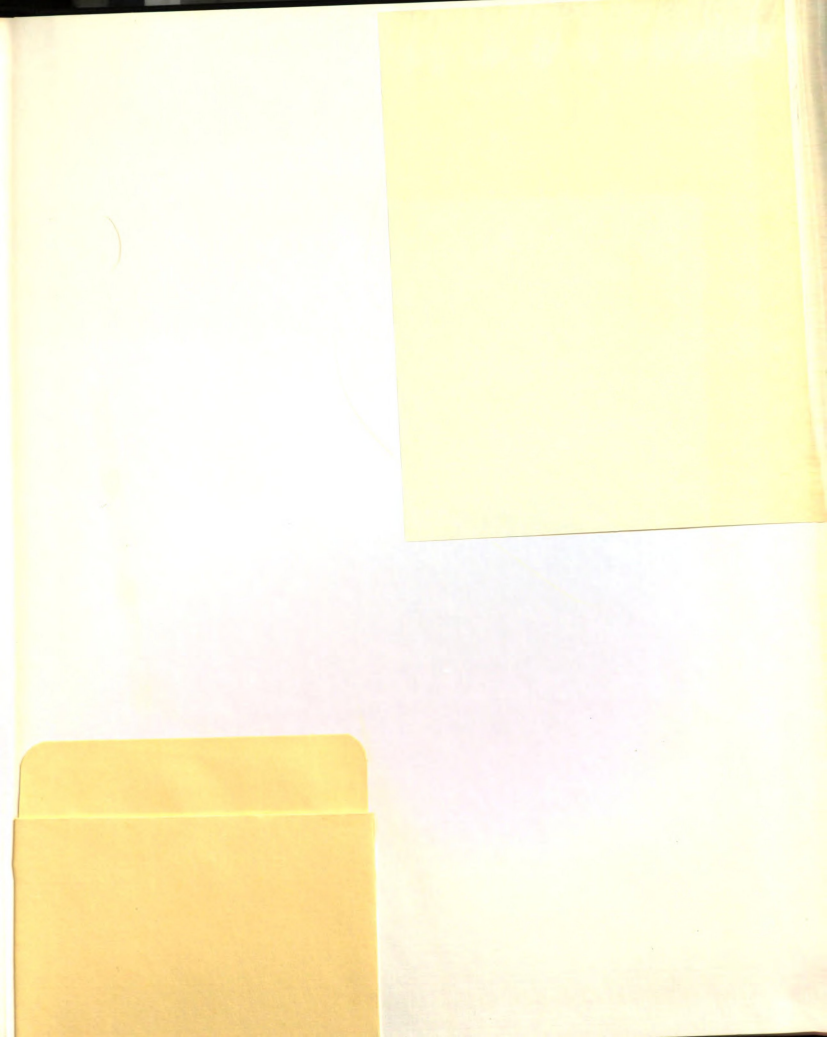

SKIN AND SKELETAL MUSCLE VASCULAR RESPONSES
TO HYPOTHERMIA

By

Terry Charles Mayer

A thesis

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

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Vascular resistance and capacitance as well as transcapillary fluid partitioning were studied in innervated or denervated forelimbs of 37 pentobarbitalized dogs. Hypothermia (38° C to 28° C) was induced systemically, by external cooling of blood which returned to the right heart, or locally, by cooling blood perfusing the forelimb. Whole-body cooling to 33° C and then to 28° C elicited significant decreases in limb weight with substantial increases in both skin and skeletal muscle vascular resistances and blood flow decreases. Acute denervation of forelimbs attentuated both the fall in limb weight and increase in skin vascular resistance.

Local cooling elicited skin and skeletal muscle vascular dilation at 33° C in both innervated and denervated forelimb whereas a slight increase in skin and skeletal muscle blood vessel resistance resulted upon local cooling to 28° C; perhaps due to a rise in blood viscosity.

Terry Charles Major

Cutaneous vasoconstriction during systemic cooling was mediated primarily by sympathetic nerves, whereas skeletal muscle vasoconstriction was mediated primarily by circulating hormones. The locally-induced vasodilation apparently resulted from a direct inhibition of skin and skeletal muscle vasomotor tone and was attenuated by increases in blood viscosity.

To My Parents

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INTRODUCTION

The circulatory responses to whole-body cooling have long interested physiologists and clinicians. Hypothermia is an important tool for studying temperature regulation and hibernation, and recently advances in our understanding of the effects of lowered temperature on metabolic activity and oxygen consumption have led to the use of hypothermia in certain medical and surgical procedures. Metabolic activity is reduced at subnormal temperatures associated with either hypothermia or hibernation. In the case of hypothermia, depression of body temperature is an imposed condition from which the subject may not recover without respiratory and/or circulatory support (2,3,4,7,8). In contrast, animals in deep hibernation maintain homeostasis at body temperatures of 5°C for prolonged periods of time (77). Homeothermic, adult humans and nonhibernating laboratory animals cannot be cooled safely to a body temperature much below 20°C (4).

Total metabolic activity as indicated by oxygen consumption is reduced at subnormal body temperatures. In dogs, the total body oxygen consumption falls to 50 or 60% of the normothermic control value when rectal temperature is lowered to 28°C. According to Thauer (65), oxygen consumption in

nonthermoregulating humans may be lowered by as much as 50% at a rectal temperature of 30°C.

When prolonged periods of circulatory or cardiac arrest are desirable, particularly in neuro, cardiac, or transplantation surgery (3,16,40,41,62), mild hypothermia has been used to temporize the effects of ischemia. The reduced oxygen requirement at subnormal temperatures permits interruption of the circulation for longer periods of time than would be possible at normal tissue temperatures.

Whole-body hypothermia affects hemodynamics in all organs and vascular beds including skin and skeletal muscle circulations. The latter two beds comprise about 55% of the total body tissue mass. Hypothermia elicits both remote (i.e., neurohumoral) and local (i.e., direct effect of the cold) vasomotor responses which are known to influence the skin and muscle circulations. The characteristics of these two vascular control mechanisms can be examined separately by comparing responses to whole-body (systemic) hypothermia (15,16,18,19,32) with those of local cooling (58,68-72). Recent investigations have attempted to elucidate more precisely the neural, humoral, and local vascular components of the peripheral circulatory response to whole-body cooling (19). Methods used to induce hypothermia include surface and internally (i.e., blood) cooled procedures (11,39). The study reported in this thesis compares skin and muscle

circulatory response to local and to systemic cooling, induced sequentially and independently, in the same animal.

The experimental preparations and protocol described here represent an attempt to identify the neural, humoral, and direct effects of cooling on the skin and skeletal muscle blood vessels.

LITERATURE REVIEW

I. General Considerations

Tissue perfusion is the primary function of the cardiovascular system. Since maintenance of an adequate blood supply to systemic organs depends on both systemic arterial pressure and local vascular resistance, it is appropriate to begin this review with an analysis of the cardiac and peripheral vascular responses to hypothermia. It should be noted that the commonly used anesthetics influence overall cardiovascular response to cooling (3,47,50).

A. Cardiac Output

There is general agreement that anesthetized, non-thermoregulating animals exhibit a large reduction in cardiac output as body temperature decreases (65). Three factors are involved in decreasing the cardiac output: a) the direct effect of cold onto the cardiac excitatory tissue which results in a decreased heart rate, b) the cold-induced decrease in cardiac metabolism which decreases myocardia contractility, and c) the cold-induced increase in blood viscosity (8). Thauer (65) reports that in rats, dogs, and man the cardiac output may be decreased to anywhere from 30 to 70% of normothermic control values when core temperature

is lowered to 25°C. The decrease in cardiac output has been reported by some to display a direct linear relationship to temperature (2,8,51). However, Thauer (65) also cites evidence that the decrease in cardiac output may be exponentially related to temperature because of an exponential decrease in whole-body oxygen consumption. The reason for this variability in the slope of cardiac output curves reported in the literature is most likely the result of different types and degrees of anesthesia which cause varying levels of thermoregulatory inhibition process.

Rittenhouse et al. (52) observe an 82% decrease in the cardiac output of dogs cooled to 20°C. Reissman and Kapoor (57) note a 35% decrease in cardiac output in dogs cooled to 25°C. Hegnauer and D'Amato (12) reduced cardiac output to 11% of control by reducing the rectal temperature of dogs to 17°C. Bullard (8) reduced the body temperature of unanesthetized rats to 16°C and observed that cardiac output fell to 16% of the normothermic control value. He reports a direct, linear relationship between cardiac output and rectal temperature (60% of the normothermic cardiac output at a rectal temperature of 27°C and 20% at 15°C). Fisher et al. (16) reduced rectal temperature to 23°C for twenty-four hours in anesthetized dogs. They report a 33% decrease in cardiac output after completion of the cooling and a further reduction to 6% of control after twenty-four hours of sustained hypothermia.

Zarins and Skinner (77) observed an 83% decrease in the cardiac output of anesthetized dogs cooled to 5°C. This report agrees with Thauer that the cardiac output decreases exponentially with the reduction in body temperature. Evonuk (15) also reported an exponential decrease in cardiac output during cooling in warm-acclimatized, anesthetized dogs. He reports an average 57% reduction in cardiac output from control during the first 3°C drop in rectal temperature.

B. Heart Rate and Stroke Volume

The two immediate determinants of cardiac output are heart rate and stroke volume. Reissman and Kapoor (51), Rittenhouse et al. (52), Hegnauer et al. (23), and Thauer (65) agree that there is little, if any change in stroke volume in animals cooled to rectal temperatures as low as 17°C. Therefore, changes in cardiac output are due primarily to changes in heart rate. The previously described linear decreases in cardiac output can be shown to be due to a linear decrease in heart rate (Bullard (8) and Andrews et al. (2) in unanesthetized rats and Rittenhouse et al. (52) in anesthetized dogs). Rittenhouse observes a decrease in heart rate from a mean of 152 beats/min. at 38°C to 60 beats/min. at 25°C, while Bullard (7) observes that the heart rate of rats decreased from 424 beats/min. at 36°C to 51 beats/min. at 16°C. Hook and Stormont (26) demonstrated that the

heart rate fell from 163 beats/min. at 38°C to 22 beats/min. at 18°C.

An exponential relationship between heart rate and rectal temperature has been described by Bigelow (4) and Thauer (65). Hegnauer et al. (23) report a sigmoid decrease in heart rate of anesthetized dogs starting at above 170 beats/min. at a rectal temperature of 37°C, decreasing to 120 beats/min. at 28°C and further decreasing to a minimum of 15 beats/min. at 20°C. Evonuk (15) reports that heart rate decreased from 100 beats/min. to 90 beats/min. as rectal temperature was reduced from normal to 32°C in warm-acclimatized, anesthetized dogs.

Reissman et al. (51) using a canine heart-lung preparation confirmed the parallel decreases in cardiac output and heart rate. In these preparations heart rate decreased from 130 to 80 beats/min. when cardiac tissue temperature was lowered to 27°C. Mohri et al. (41) report reductions in heart rate from 175 to 30 beats/min. in human infants cooled from 37°C to between 22 and 19°C while Hicks et al. (24) note a similar progressive decrease in the heart rate of humans, ages three months to thirty-six years; reporting average reductions of heart rate from 120 beat/min. at 37°C to about 45 beats/min at a rectal temperature of 28°C.

Thauer (65) reports that the temperature related fall in heart rate is not influenced by vagotomy or atropine.

This suggests that the bradycardia observed in hypothermia results from a direct effect of lowered temperature on cardiac electrogenic tissue rather than from increased vagal tone. Electrocardiographic evidence (24,26) suggests that hypothermic bradycardia results from a depression of both diastolic depolarization in the S-A node and conduction velocity in the His-Purkinje system. The EKG tracings during progressive reduction in body temperature (38°C and 25°C) shows gradual prolongation of the P-R interval, QRS complex, and QT segment. Also, marked inversion of the T wave is seen with lowered body temperature (24,40). Hicks et al. (24) reports that during hypotheria a greater portion of the cardiac cycle is spent in systole even though diastole is also lengthened. Hegnauer et al. (23) report a 70% increase in systolic duration when core temperature is reduced to 28°C, and a 6-fold increase above normothermic control at 18°C.

C. Arterial Blood Pressure

The two immediate determinants of mean systemic arterial blood pressure are cardiac output and total peripheral resistance. Thauer (65) has reviewed data which indicate that the reduced blood pressure during hypothermia is almost exclusively due to a bradycardia-induced reduction in cardiac output.

As rectal temperature is lowered from normal to approximately 28°C, heart rate and cardiac output fall relatively more than does blood pressure, indicating that total peripheral resistance is increased. As rectal temperature is reduced below 25°C, heart rate and blood pressure fall approximately equally with mean systemic arterial pressure reaching 40 to 50 mm Hg at 16 to 18°C rectal temperature. Mohri et al. (41) report a decrease in mean pressure of human infants during cooling with the pattern of reduction dependent upon the disease being treated. Hegnauer et al. (23) and Andrews et al. (2) also report that arterial pressure falls slightly when rectal temperature is lowered from 37°C to between 29 and 23°C, but when rectal temperature is reduced below 23°C, a proportional reduction in arterial pressure and heart rate is observed. Hook and Stornant (26) report that blood pressure in anesthetized dogs did not decrease significantly with moderate reductions of body temperature. However, when rectal temperature fell below 26°C, these investigators report large reductions in blood pressure (50 mm Hg at 22°C and 31 mm Hg at 18°C).

Evonuk (15) and Rittenhouse et al. (52) report no change in mean arterial pressure when rectal temperature was lowered from normal to 32°C. At rectal temperature below 32°C a gradual decline in blood pressure was reported by Rittenhouse et al. (112 mm Hg at 25°C and 75 mm Hg at 20°C).

Hegnauer and D'Amato (12) report that systemic blood pressure of rats decreased from 126 mm Hg at 38°C to 54 mm Hg at 17°C (pressure response pattern like that observed in dogs). Bullard's study (8) in unanesthetized rats showed a 10% fall in mean arterial pressure when rectal temperature was reduced to 22°C, after which the fall began to parallel the decrease in cardiac output. He attributes the slight initial fall in blood pressure to the fact that the decrease in cardiac output was largely counteracted by an increase in total peripheral resistance. Several other investigators also demonstrated that hypothermia causes a proportionately greater fall in cardiac output than in blood pressure (15, 49).

D. Total Peripheral Resistance

According to Thauer's review (65), lowering body temperature to 25°C causes total peripheral resistance to increase anywhere from 2.1 to 2.3 fold. Bullard (8) reports that total peripheral resistance in rats increases three-fold when rectal temperature is lowered from 37 to 16°C. Rittenhouse et al. (52) observed a gradual increase from 67 PRU (peripheral resistance units) to 79 PRU as body temperature was reduced from 38 to 30°C; further reductions in rectal temperature produced relatively much larger increases in resistance so that the value for PRU reached 212 at 20°C. Zarin and Skinner (76) lowered rectal temperature of

anesthetized dogs from 37 to 5°C and observed that total peripheral resistance increased from 80 to 238 mm Hg per liter per minute.

Thauer suggests that the inverse relationship between rectal temperature and total peripheral resistance may be due to: 1) a passive reduction in cross-sectional area of the vasculature due to the fall in transmural pressure; 2) the anesthesia (especially barbiturate anesthesia) which according to Olmsted et al. (46) and to Priano et al. (50) increases the peripheral resistance of dogs with normal body temperature. The increasing depth of anesthesia during cooling could then lead to increased peripheral resistance; 3) active vasoconstriction unrelated to changing depth of anesthesia; and 4) increased blood viscosity.

II. Control of Peripheral Vascular Resistance During Hypothermia

Peripheral vascular resistance is regulated centrally by neural reflexes and circulating vasoconstrictors and locally by various conditions or stimuli in and around the small arteries and arterioles, precapillary sphincters, and venules. Interplay between these two regulatory systems determines vascular resistance in each of the major, parallel-coupled systemic organs. The study described here focuses on cutaneous and skeletal muscle circulations of the

dog forelimb. These vascular networks were chosen for study because of the relatively large quantity of tissues they supply (skin and skeletal muscle together comprise approximately 60% of the total body mass), because they have been shown to play an important role in reflex compensatory response to systemic hypotension (a condition which accompanies hypothermia), and because they are responsive to changes in temperature.

A. Systemic Hypothermia: Neural Control

Neural regulation of the cutaneous and skeletal muscle vasculatures is accomplished primarily by the sympathetic vasoconstrictor fibers which exert prominent α-adrenergic influences on skin and skeletal muscle vessels. Keller (7), concludes that exposure to subnormal environmental temperatures brought about increased impulse traffic in vasoconstrictor fiber networks, especially those supplying the cutaneous vasculature. His data suggest that the "heat loss center" of the anterior hypothalamus is of primary importance in regulating core temperature through vasoconstrictor fiber activation. Haddy et al. (12) demonstrate that arterial resistance in the canine forelimb increased significantly when ambient temperature was reduced from 20 to 0°C. Juhasz-Nagy and Kudasz (28), describe an increased total peripheral resistance when the brain is locally cooled to

25°C. However, below 25°C, they report that blood pressure drops below normothermic values due to a progressive inhibition of the centrally mediated vasomotor tone as well as to decreased cardiac output.

Cold elicited vasodilaton in the skin and muscle circulations could result either from withdrawal of α-adrenergic neural stimulation or from activation of sympathetic cholenergic vasodilator fibers which have been shown to supply resistance vessels in skeletal muscle (5,6).

Brooks (7) demonstrated that cooling slows conduction velocity in peripheral nerves and spinal pathways, due to a reduction in the rate of depolarization (spike slope). However, when nerves are cooled to a moderate degree (38 through 25°C) the action potentials evoked are of increased amplitude and duration, a condition which could account for increased central nervous system responsiveness in the cold. These findings show that the central nervous system excitation may be established by electrotonic current triggered by cooling (6). According to Thauer (65), the baroreceptor reflex becomes progressively weaker with cooling and is completely abolished between 26 and 20°C. Therefore, cardiovascular reflexes are probably increased during moderate nerve cooling (38 to 26°C) but when temperatures are reduced from 25 to 20°C, the reflexes (including the baroreceptor reflex) are decreased substantially.

B. Humoral Control

Holobut (25) reports that the blood plasma levels of both epinephrine and norepinephrine rose significantly during whole-body cooling to 25°C in anesthetized dogs. Rapid cooling causes small increases in plasma levels of catecholamines, whereas a slow decrease in body temperature causes relatively larger increases.

The effect of cooling on vascular smooth muscle sensitivity to catecholamines is somewhat controversial. Rodgers et al. (54), using an isolated perfused mesenteric artery preparation, demonstrate that the pressor responses elicited by intraarterially injected catecholamines increased as temperature decreased from 37 to 27°C. The mean pressor responses more than doubled as temperature is reduced from 45 to 29°C in contrast, other investigators have found that resistance vessel sensitivity to plasma catecholamines is reduced with lowered temperatures (33). Despite the increased plasma catecholamine levels, Smith (61) demonstrates that the reactivity time (an index of the rate constriction) in dog femoral arteries was greatly prolonged at the reduced temperatures and this may indicate a reduced contractile response to pressor agents. Keatings (30,31) reports that moderate cooling (37 to 28°C) of isolated ulnar arteries usually reduced their contractile response to epinephrine and also showed that the response to epinephrine and

norepinephrine was completely abolished at temperatures of 10°C and below. Resting tension became low with moderate cooling in the isolated arteries but this was not permanent because the control contractile response was re-established when the arteries were warmed. Iar et al. (29) show that the pressor responses to norepinephrine and epinephrine fall by 1-7 mm Hg per degree fall in rectal temperature.

The effect of cold on vessel responses to other agents has also been considered. According to Smith (61) vascular response time to acetylcholine was prolonged at 27°C. Nayor (43) also reports that cooling decreased vascular sensitivity to acetylcholine. Smith (61) reports that cooling to 15°C produced a progressive increase in vascular reactivity time to histamine. Keatinge (31) shows that vasopressin reactivity of ulnar arteries was attenuated at 28°C and nearly abolished at 17°C.

C. Local Hypothermia: Direct Effect on Vessel Musculature

Local cold exposure has been reported to cause vasodilation of the skin and skeletal muscle circulations (56). Scott et al. (58) reports that resistance in precapillary vessels of the dog forelimb decreased in response to local cooling despite an increased blood viscosity. They suggest that the dilation resulted from a direct effect of cold upon smooth muscle cells. In their study vascular responses to

local cold exposure were not greatly affected by denervation or adrenergic blockade. Brodie et al. (6) found that a decrease in temperature reduces the tone of aortic strips. Glover and Wallace (17) also demonstrate that in rabbit ear arteries, smooth muscle tone is reduced by cooling. Finally, Smith (61) found that the rate of cooling influences the response of arterial segments from dogs and pigs. They observed constriction when the cooling rate was 4 to 6°C per minute, and no response at cooling rates of 7°C per minute and above.

D. Effect of Cold on Blood Rheology

The direct effect of cold on physical characteristics of blood and the resulting influence on resistance to blood flow has been studied recently. The two properties that will be examined here are blood viscosity and red cell concentration (hematocrit).

Hypothermia has been reported to decrease the circulating blood and plasma volumes without producing a significant increase in plasma-protein concentration. In studies on cooled chicks and rabbits, Rodbard et al. (53) demonstrated that the circulating plasma volume decreased 31% (41.2 to 25.6°C) in chicks and 36% (39.1 to 29.1°C) in rabbits. Hematocrits increased about 3% in chicks and 3% to 15% in rabbits. The relatively small increases in hematocrit suggest that whole blood is being lost from the circulating

portion of the cardiovascular system. D'Amato and Hegnauer (12) and Shukla et al. (60) have published data which supports Rodbard's conclusion that whole blood is lost from the circulatory system, perhaps by "locking" or sequestration in the microcirculation (12,53,60).

The increased hematocrits described above might cause a small increase in blood viscosity and hence resistance. Shorrock and Hillman (59), however, observed no significant difference in the hematocrit of cooled rats (35 to 10°C) but did observe a 47% increase in hematocrit when the same blood was cooled to 2°C. The relationship of viscosity to hematocrit has also been described by Marty et al. (35). The yield shear stress (an index of viscosity) of whole blood was observed to increase in children who were cooled from 38 to 27°C during an operative procedure. This increase occurred only when the hematocrit value was over 50%. Reissman and Kapoor (48) report increases in viscosity of 15, 42, and 60% at temperatures of 30, 25, and 22°C, respectively when compared to a 37°C control viscosity (hematocrit was 50, 57, and 51.5%, respectively). Merrill et al. (37) confirmed the findings of Marty et al. that the yield shear stress remains constant at a given hematocrit level, independent of temperature (up to hematocrit 35). At higher hematocrits yield stress increases as temperature drops.

Even a slight increase in blood viscosity due to hypothermia can influence the effect of cold on peripheral resistance if the other parameters in the Poiseville's equation are constant. As long as hematocrit and body temperature are normal, the viscous component of resistance to blood flow is quite constant. During moderate hypothermia, blood viscosity increases because of the effects of cold on hematocrit and yield shear stress. These effects result in at least a slight increase in the viscous component of resistance.

E. Effect of Cold on Na^+-K^+ ATPase Activity

Na^+-K^+ dependent adenosine triphosphatase (Na^+-K^+ ATPase) is an enzyme which resides, among other places, in the membranes of red blood cells and vascular smooth muscle cells and whose activity has been shown to be temperature dependent (14). Ellory et al. (14) showed that erythrocyte membrane Na^+-K^+ ATPase activity was halved by reducing incubation temperature from 38 to 30°C. A further dramatic decrease (97.5%) was observed when the temperature was lowered to 10°C.

The cold-induced decrease in Na^+-K^+ ATPase activity in red blood cells might be expected to be associated with a decreased membrane flexibility. Weed et al. (73) reduced ATPase activity by substrate deprivation and observed that due to water influx the red cell membrane became turgid and

less able to deform. This condition was shown to inhibit smooth movement of the cell through the microcirculation due to the swollen state of the cells. This swollen state also causes an increase in hematocrit. The same effect might occur in red blood cells subjected to cold.

Vascular smooth muscle tone has been shown to be dependent on the Na^+-K^+ ATPase activity (1). As has been previously discussed, cold temperature inhibits Na^+-K^+ ATPase activity. The lowered activity of the electrogenic Na^+-K^+ "pump" leads to a decreased net transfer of Na^+ ions from the vascular smooth muscle cells into the extracellular fluid and hence cellular depolarization. Ouabain, a well-known inhibitor of Na^+-K^+ ATPase, substantiates this observation in that upon the addition of ouabain, vascular smooth muscle cells rapidly depolarize while the resistance progressively increases (1). Therefore, the evidence suggests that the response to cold blood perfusion would be a decrease in blood flow via active vasoconstriction.

III. Peripheral Vasculature Capacitance During Hypothermia

The veins of skin and skeletal muscle contribute relatively little to the total vascular resistance in these circuits, but instead play an important role as capacitance vessels. Approximately 80 percent of the total blood volume

within a given vascular network is contained in the venous system. Furthermore, by actively constricting or dilating, veins are able to mobilize or sequester relatively large amounts of blood. It is a consistent observation that cold exposure, be it local or systemic, elicits venous constriction (19,58,68-72). Scott et al. (58) found that resistance to flow through small superficial and deep veins of the dog forelimb rose to 313% of control when perfused with 13°C blood. They speculated that increased viscosity contributed to the resistance response. Webb-Peploe and Shepherd (70) reported that locally or centrally cooling the blood perfusing superficial dog forelimb veins caused them to constrict. Webb-Peploe (69) reduced central body temperature from 39 to 35°C and observed constriction of saphenous but not of splenic veins. Wood and Echstein (75) observed a 34% decrease in venous volume of intact human forearms when ambient temperature was reduced to between 21 and 18°C. They also found that local cooling of the forearms caused a 31% decrease in venous volume. According to Webb-Peploe (69), the available evidence concerning superficial venous function during reduced temperatures demonstrates a specialized thermoregulatory function for these vessels but not for visceral veins.

A. Systemic Hypothermia: Neural Control of Capacitance Vessels

According to Ross (55) sympathetic nerve stimulation of veins in the dog's hindpaw produces a relatively slight increase in resistance, compared to that of their arterial counterparts. Furthermore, he found that cutaneous veins in the dog displayed intense constriction during sympathetic nerve stimulation, whereas muscle veins in the same animal showed only a very slight constriction. Webb-Peploe and Shepherd (69) also found little or no adrenergic innervation in muscle veins of the hindlimb, but reported that lowering central body temperature from 40 to 33°C elicited intense constriction of canine saphenous veins (70). Since the veins in their preparation were perfused with normothermic blood from a donor dog, they reasoned that the observed venoconstriction was not mediated either humorally or via the direct effect of cold on venous smooth muscle, but rather resulted from a neural reflex triggered by the systemic cooling. In a previous study, Webb-Peploe and Shepherd (70) showed that after sympathectomy, the maximal venoconstriction and thus the maximal blood volume reduction in response to cooling was decreased by about 60%. Webb-Peploe (69) found that reduced body temperature resulted in saphenous venoconstriction which was abolished by lumbar sympathectomy. However, hypothermia did not increase tension in the splenic veins.

Therefore, he concluded that the venoconstrictor response to the cooling was dependent on an intact sympathetic nervous system. In contrast, Haddy et al. (19) demonstrated that local nerve blockade did not change the venous resistance response to cold air exposure. In Haddy's (19) experiments cold-induced venous constriction was apparently mediated by circulating catecholamines. His findings differ strikingly from those of Webb-Peploe (69) because of the difference in the type of cooling technique used. One of the objectives of the study described in this thesis is to quantify the role of the sympathetic nervous system in mediating venous constriction during systemic cooling.

B. Humoral Control

Weideman (74) points out that venous smooth muscle shows a great temperature sensitivity in its responsiveness to epinephrine so that a 1-2°C fall in temperature causes a 20-fold increase in responsiveness; contrary to that observed in arterial smooth muscle. Webb-Peploe and Shephard (70) as mentioned earlier, reduced the maximal saphenous venoconstriction at 27°C by 60% with sympathectomy; subsequent α-receptor blockade with phenoxybenzamine abolished the remaining 40% of the constriction response. Haddy et al. (19) also showed that denervation plus α-adrenergic blockade abolished the venoconstriction normally elicited by cold exposure.

C. Local Hypothermia: Direct Effect on Venous Musculature

Scott (58) reported that the venous resistance in the canine forelimb increased during local cooling to 13°C. This response was either absent or greatly attenuated after local nerve and α-adrenergic receptor blockade. Similarly, Webb-Peploe (68) abolished the venoconstrictor response associated with local cooling to 27°C by denervation and α-adrenergic receptor blockade. Haddy et al. (19) also found, that after nerve block and α-adrenergic blockade, there was no significant venoconstrictor response at an ambient temperature of 0°C.

D. Passive Venous Responses

Precapillary dilation, such as that elicited by cooling (58), will raise the flow of blood into downstream veins and thereby tend to raise transmural pressure and cause passive venous distention. This passive effect of cold exposure on venous resistance has not been carefully evaluated.

IV. Transcapillary Flux During Hypothermia

The capillary system is one of the major regulators of body fluid homeostasis. Therefore, it is important to understand how net transcapillary fluid movement is influenced by cold-induced hemodynamic changes.

Since capillaries do not contain a smooth muscle layer, active changes in their radii are not possible; blood flow in the microcirculation is dependent on the pre- to post-capillary resistance ratio and the pressure gradient. Therefore, cold-induced changes in arterial or venous resistance can affect net transcapillary fluid movement by altering capillary blood volume and hence capillary hydrostatic pressure.

A. Systemic Hypothermia

Systemic cold stress triggers neural and humoral responses which help to buffer the fall in core temperature. The hemodynamic consequences included a reduced microcirculatory blood flow due to increased precapillary resistance. Precapillary sphincters have adrenergic innervation which is more prominent in cutaneous than in skeletal muscle vascular networks (45). Precapillary sphincters in muscle are regulated primarily by tissue metabolites. PaO_2 during systemic cooling increased from 64 mm Hg at 38°C to 82.5 mm Hg at 28°C rectal temperature.

Increased small vessel resistance during whole-body cooling has been investigated and related to arteriolar and precapillary sphincter constriction (17,58), increased blood viscosity (55,59), and erthrocyte aggregation (59). Haddy et al. (19) attributed the increased small vessel resistance which they observed at an air temperature of 0°C to

precapillary constriction caused by increased levels of circulating epinephrine and norepinephrine. In this study, acute denervation does not significantly reduce the precapillary constriction. Svanes et al. (64) observed that capillary flow in the hypothermic rabbit omentum was reduced even though the arterioles were dilated. Mean arterial pressure showed little change despite the fact that body temperature was lowered to 25°C (82 mm Hg at 38°C to 70 mm Hg at 25°C). Therefore, the decreased flow was due to an increase in blood viscosity. When rectal temperature is reduced to 22°C and below, blood flow in the rabbit ear microcirculation is no longer laminer and the red blood cells appear granular.

B. Local Hypothermia

The effect of local cooling on transcapillary fluid movement has been indirectly studied by Scott et al. (58), who report that precapillary dilation along with venous constriction results in a decreased pre/post capillary resistance ratio which would predictably increase capillary hydrostatic pressure and lead to a net fluid filtration.

Svanes et al. (64) found significant increases in plasma colloid osmotic pressure in the hypothermic rabbit omentum during active cooling. They suggest that despite elevated plasma protein concentration, fluid movement across single capillaries was about the same as that observed under normothermic conditions. Therefore, capillary hydrostatic

pressure was calculated to be somewhat higher at 25 than at 37°C. During a five-hour period of sustained hypothermia, their microcirculation studies showed little or no change in fluid movement across capillary wall (64).

Several independent investigations have demonstrated that progressive reductions in heart rate, systemic arterial blood pressure, and circulating blood volume occurs with systemic cooling. This blood volume reduction could result from "locking" or sequestration in microcirculatory channels, expansion of the venous vascular segment, or net transcapillary fluid filtration. Contradictory evidence is shown for the responsiveness of arterial and venous smooth muscle to systemic as well as local cooling. The majority of the investigators agree that vascular responsiveness is greatly reduced during either systemic or local cooling to neural and/or humoral inputs. Both systemic and local cooling has been found to influence vascular resistance by means of increased blood viscosity. This increased viscosity is possibly due to swollen red blood cells, net transcapillary fluid filtration and/or the increased yield shear stress (friction between particles of fluid).

The present study is designed to examine the relative contributions of neural, humoral, and direct effects of local and systemic cooling on skin and skeletal muscle vascular resistances, capacitances, and net transcapillary fluid fluxes.

METHODS

Adult mongrel dogs of either sex, weighing 20-25 Kg, were anesthetized with sodium pentobarbital (30 mg/Kg i.v.), intubated and ventilated with a mechanical respirator (Harvard Apparatus Co. model 607). Supplements of sodium pentobarbital were given as necessary during the surgical and experimental procedures. Rectal temperature and in some experiments, central arterial temperature (aortic arch via left common carotid artery) were monitored continuously with thermistor probes connected to a telethermometer (YSI model B-40431). The animals were maintained normothermic (rectal temperature of 37.5°C to 38.5°C) throughout surgery with a heating pad (The Walker Co., Inc., Middleboro, Mass.).

Skin of the right forelimb was sectioned 3-5 cm above the elbow. The brachial artery was isolated as were the brachial and cephalic veins and the muscles and remaining connective tissue were sectionalized by electro-cautery. The forelimb nerves (median, ulnar, radial, and musculo-cutaneous) were isolated and coated with an inert silicone spray (Antifoam A, Dow Corning, Midland, Michigan) to prevent drying. The humerus was cut and the ends of the narrow cavities packed with bone wax (Ethicon, Inc., Sommerville,

New Jersey). Blood entered the forelimb only through the brachial artery and exited only through the brachial and cephalic veins. When surgery was completed, sodium heparin (The Upjohn Co., Kalamazoo, Michigan) was administered in an initial dose of 1000 USP units/kg body weight followed by hourly supplements of 300 USP units/kg body weight.

Intravascular pressures were measured from saline filled, polyethylene (P.E.) cannulae (Intra-medic tubing, Clay Adams) inserted into the following sites (Figure 1): 1) brachial artery via the collateral ulnar artery just above the level of the elbow (P.E. 50; outside diameter (o.d.) = 0.038"); 2) skin small artery from the third superficial volar metacarpal artery (P.E. 60; o.d. = 0.048")*; 3) muscle small artery from a vessel supplying a flexor muscle on the upper portion of the forelimb (P.E. 50)*; 4) skin small vein from the second superficial dorsal metacarpal vein (P.E. 60)*; 5) muscle small vein from one of the deep vessels draining a flexor muscle in the middle portion of the forelimb (P.E. 10, o.d. = 0.024")*; 6) skin venous outflow pressure from the cephalic vein via a side branch at the level of the elbow (P.E. 60); and 7) muscle venous outflow pressure from the brachial vein via a side branch at the level of the elbow (P.E. 60). The small vessel cannulation techniques originally described by Haddy was used for

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*In Series I and II.

Figure 1. A schematic of the isolated canine forelimb preparation (skin removed in drawing to better illustrate vessel cannulations).

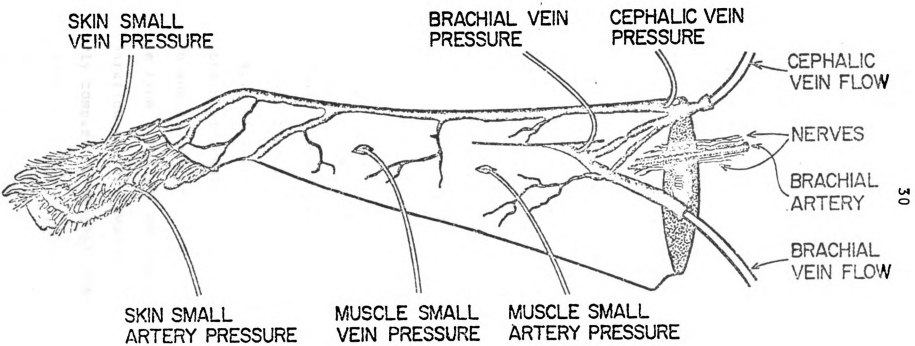
SKIN SMALL
VEIN PRESSURE
BRACHIAL VEIN
PRESSURE
CEPHALIC VEIN
PRESSURE
CEPHALIC
VEIN FLOW
NERVES
BRACHIAL
ARTERY
BRACHIAL
VEIN FLOW
SKIN SMALL
ARTERY PRESSURE
MUSCLE SMALL
VEIN PRESSURE
MUSCLE SMALL
ARTERY PRESSURE

Figure 1

all small vessel pressures (18). All pressures were measured with low volume displacement Statham transducers (model no. P23Gb, Statham Laboratories, Hato Rey, Puerto Rico) and recorded on a Hewlett-Packard direct-writing oscillograph (model no. 7784A, Hewlett-Packard Co., Waltham, Massachusetts).

The brachial and cephalic veins were partially transected 3-5 cm downstream from the sites of venous outflow pressure measurements and each was cannulated with a 10-15" section of P.E. 320 tubing (o.d. = 0.138"). Flow from both veins was directed into an open reservoir which was in series with a variable speed, roller pump that returned blood to the left external jugular vein. Volume of blood in the reservoir was maintained at 100 $\pm$ 10 cc by manually adjusting the speed of the roller pump. Brachial and cephalic flows were determined by timed collections of the two venous outflows. The median cubital vein, which represents the major anastonmatic channel between forelimb skin and skeletal muscle circulations, was ligated in all experiments so that brachial venous flow was predominately from forelimb muscle whereas cephalic flow was predominately from skin. Evidence from a variety of sources indicates that blood flow separation in the parallel skin and muscle circulation is nearly complete with this preparation (12,18,34, 57).

The limb was placed on a plastic mesh platform attached to a weighing device which consisted of a strain gauge balance connected to the direct-writing oscillograph. The balance was calibrated periodically during the course of the experiment by adding a known weight to the limb. Mean systemic arterial pressure was measured in all experiments from a P.E. 240 (o.d. = 0.095") catheter positioned in the lower abdominal aorta via the right femoral artery. Heart rates were determined by counting the number of arterial systolic peaks which appeared on the oscillogram per unit of time, excluding extra systolic peaks.

For Series I and II, total and segmental vascular resistances in muscle were calculated by dividing the appropriate pressure gradient by the brachial venous flow. The total and segmental vascular resistances in skin were calculated by dividing the appropriate pressure gradients by the cephalic venous flow. In Series III and IV, only the total muscle and total skin resistances were calculated. Appendix A contains a more complete description of the resistance calculations.

Series I: Naturally Perfused, Innervated Forelimbs; Whole-body Cooling and Hypothermia

In 10 dogs, the forelimb nerves were left intact and whole-body cooling was produced with a Sigma-motor pump which

diverted blood from the left carotid artery through a silicone-lined, copper tube (extracorporeal heat exchanger) into the right femoral vein. The rate at which the dog's rectal temperature fell could be controlled by regulating the flow rate through the heat exchanger. Duplicate pressure and flow determinations were made while the animals were normothermic and the heat exchanging, copper coil was then suspended in an ice bath. Rate of flow through the heat exchanger was adjusted so that the temperature of blood entering the right femoral vein was between 10 and 15°C. This procedure--blood flow through this heat exchanger--caused rectal temperature to fall at an average rate of 1°C per 7 minutes. Limb weight change was recorded continuously throughout the experiment. Determinations of heart rate, hematocrit, intravascular pressures and blood flows were made at 0.5°C increments as rectal temperature fell from 38°C to 36°C. From 36 to 28°C these same data were taken at 1.0°C increments and thereafter every fifteen minutes throughout a two hour hypothermic period during which rectal temperature was maintained at 28°C. Respiratory rate was adjusted to maintain arterial pH within a normal range (7.3 to 7.5) during the hypothermic period.

Series II: Naturally Perfused, Innervated Forelimb; Normothermic Controls

Five dogs were prepared as described in Series I except that the extracorporeal cooling circuit was not included. These animals were wrapped in a thermal blanket connected to a temperature controlling unit (Aquamatic-K thermia, Gorman-Rupp Industries) and maintained with $\pm$ 0.2°C of their respective, initial rectal temperatures. Pressure and flow determinations as well as limb weight changes were obtained at points in time corresponding to those in Series I (0,5,10, 15,20,25,30,40,50,60,70,80,90,100,110,125,140,155,170,185, 200,215, and 230 minutes).

Series III: Constant Pressure Perfusion, Innervated Forelimbs; Local and Whole-body Hypothermia

The forelimbs of 13 dogs were prepared as described above except that the small vessel cannulations were omitted. Figure 2 presents a schematic drawing of the experimental preparation. The limbs were mechanically perfused at controlled pressure by a servo-regulated roller pump which diverted blood from the right femoral artery into the right brachial artery. Brachial artery perfusion pressure was set equal to mean systemic arterial pressure by adjusting the set-point control (Figure 3) of the servosystem. Mechanical perfusion of the brachial artery made it possible to

Figure 2. A schematic drawing of the extracorporeal preparation.

----- = arterial and venous pressures.
····· = temperature measurements.
xxxxxx = brachial and cephalic venous flows.

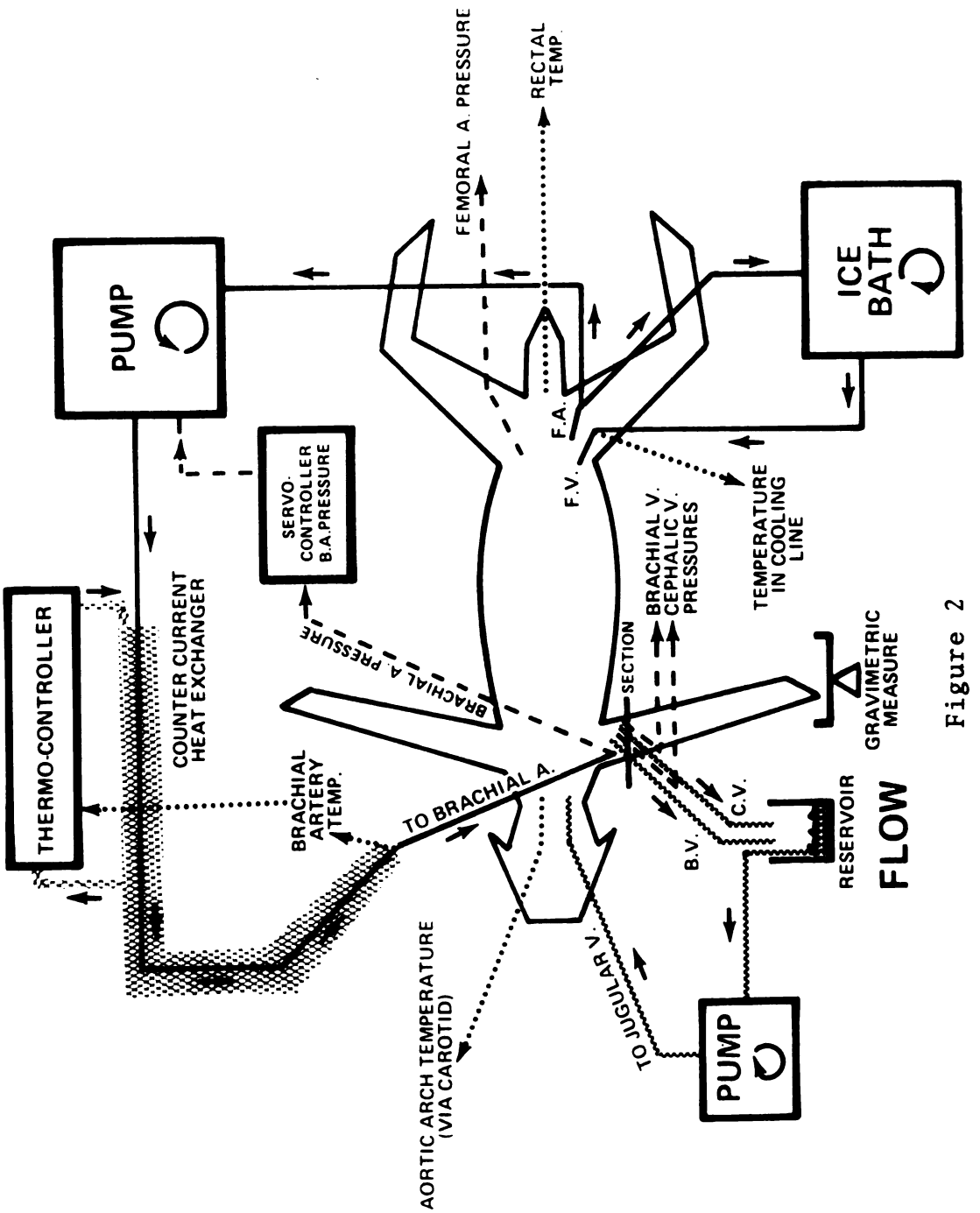
PUMP
ICE BATH
FEMORAL A. PRESSURE
RECTAL TEMP.
F.A.
F.V.
SERVO CONTROLLER B.A.PRESSURE
THERMO-CONTROLLER
COUNTER CURRENT HEAT EXCHANGER
BRACHIAL A. PRESSURE
BRACHIAL V. CEPHALIC V. PRESSURES
TEMPERATURE IN COOLING LINE
SECTION
GRAVIMETRIC MEASURE
BRACHIAL ARTERY TEMP.
TO BRACHIAL A.
C.V.
B.V.
RESERVOIR
FLOW
AORTIC ARCH TEMPERATURE (VIA CAROTID)
TO JUGULAR V.
PUMP

Figure 2

Figure 3. Schematic of the servosystem used to control brachial artery pressure.

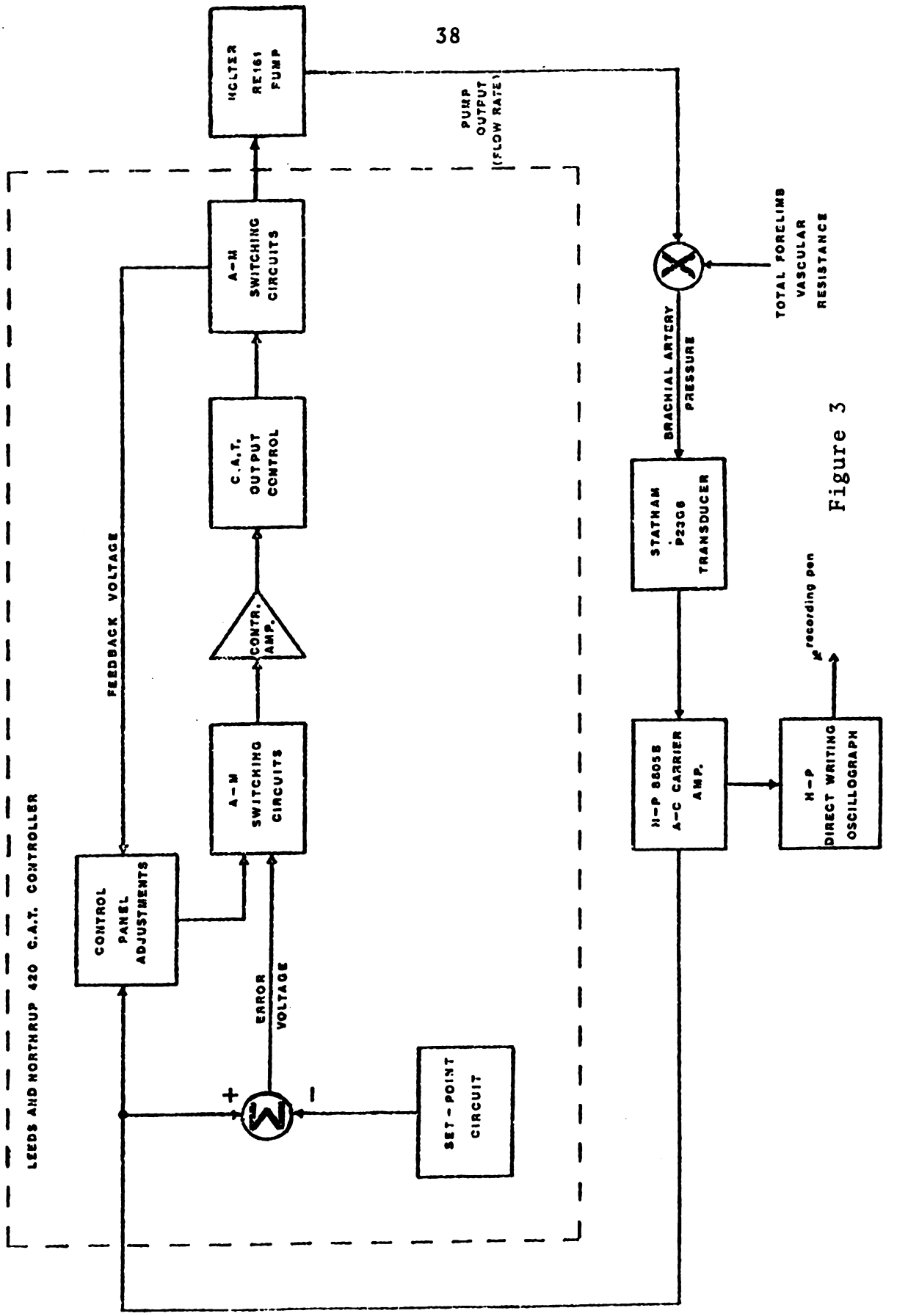
LEEDS AND NORTHRUP 420 C.A.T. CONTROLLER
CONTROL PANEL ADJUSTMENTS
FEEDBACK VOLTAGE
A-M SWITCHING CIRCUITS
CONTR. AMP.
C.A.T. OUTPUT CONTROL
A-M SWITCHING CIRCUITS
HGLTER RE161 PUMP
PUMP OUTPUT (FLOW RATE)
ERROR VOLTAGE
SET-POINT CIRCUIT
TOTAL FORELIMB VASCULAR RESISTANCE
BRACHIAL ARTERY PRESSURE
STATHAM P23GB TRANSDUCER
H-P 8805B A-C CARRIER AMP.
recording pen
H-P DIRECT WRITING OSCILLOGRAPH

Figure 3

selectively cool blood that entered the limb while the dog's rectal and central arterial temperatures remained normal. Local cooling and rewarming of the forelimb was accomplished as follows: the tubing which carried femoral arterial blood from the roller pump to the brachial artery was surrounded by a fluid filled, counter current heat exchanger connected to a thermocontrolling unit (Aquamatic-K-thermia, Gorman-Rupp Industries). The temperature of blood entering the brachial artery was monitored continuously by a thermistor probe and adjusted according to the protocol described in Table 1, Sections I, III, and V.

Systemic cooling (whole-body hypothermia) was accomplished by pumping blood from the right femoral artery through an iced, silicone-lined, copper heat exchange coil into the right femoral vein (Figure 2). The temperature of this diverted blood was measured at the point where it returned to the dog (femoral vein). Rectal temperature and central arterial temperature (from the aortic arch via the carotid artery) were monitored continuously. The protocol (Table I) was divided into the following sections: 1) normothermic dog-hypothermic limb, 2) hypothermic dog-normothermic limb, and 3) hypothermic dog-hypothermic limb. As described in Series I, changes in forelimb weight were recorded continuously and duplicate measurements of intravascular pressures and blood flow were obtained after

Table 1. The experimental protocol.

Section I:	Normothermic Dog; Hypothermic Limb	
1.	Control	38°C Dog, 38°C Limb
2.	Local cooling	38°C Dog, 33°C Limb
3.	Local cooling	38°C Dog, 28°C Limb
4.	Post control	38°C Dog, 38°C Limb
Section II:	Hypothermic Dog; Normothermic Limb	
5.	Systemic cooling	37°C Dog, 38°C Limb
6.	Systemic cooling	36°C Dog, 38°C Limb
7.	Systemic cooling	35°C Dog, 38°C Limb
8.	Systemic cooling	34°C Dog, 38°C Limb
Section III:	Hypothermic Dog; Hypothermic Limb	
9.	Sustained Hypothermia	33°C Dog, 38°C Limb
10.	Local cooling	33°C Dog, 33°C Limb
11.	Post control	33°C Dog, 38°C Limb
Section IV:	Hypothermic Dog; Normothermic Limb	
12.	Systemic cooling	32°C Dog, 38°C Limb
13.	Systemic cooling	31°C Dog, 38°C Limb
14.	Systemic cooling	30°C Dog, 38°C Limb
15.	Systemic cooling	29°C Dog, 38°C Limb
Section V:	Hypothermic Dog; Hypothermic Limb	
16.	Sustained Hypothermia	28°C Dog, 38°C Limb
17.	Local cooling	28°C Dog, 28°C Limb
18.	Post control	28°C Dog, 38°C Limb

stability* was reached at each of the points listed in Table 1. After stable control values were obtained, local cooling was initiated. As listed in Section I of Table 1, the temperature of blood perfusing the forelimb was reduced to 33°C $\pm$ 0.5°C and then to 28°C. The limb was then rewarmed and postcontrol data were obtained. The dog's rectal temperature was lowered from 38°C to 33°C (Section III of protocol) at an average rate of 1°C every 7.5 minutes while the limb was maintained normothermic. Pressure and flow data were collected at each degree centigrade reduction in rectal temperature. Section III of the protocol describes the steps involved in the local cooling of the forelimb via the reduction of the brachial arterial blood temperature to 33°C and then the rewarming of the limb to control. Systemic cooling was reinstituted as shown in Section IV of protocol so that rectal temperature was reduced to 28°C at an average rate of 1°C every 7.5 minutes while the limb was kept normothermic. Again data were obtained at each degree centigrade increment and when a rectal temperature of 28°C was reached, the limb blood temperature was reduced to 28°C (Section V of protocol), pressure and flow data were collected, and the limb rewarmed to control.

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*Stability was defined by three criteria: 1) unchanging limb temperature (+ 0.5°C), 2) constant or nearly constant limb weight, and 3) constant blood flow.

Series IV: Constant Pressure Perfusion, Denervated Forelimb; Local and Whole-body Hypothermia

The limbs of 9 dogs were prepared as described above except that the nerves (median, ulmar, radial, and musculocutaneous) were severed 15 minutes before the beginning of data collection. The protocol was identical to that described in Series III.

DATA ANALYSIS

Series I and II: The Student's "t" test for paired difference was made between each point of Series I (experimentally cooled) and Series II (time control group) on a time basis. Statistical significance was considered to be $P \leq 0.05$.

Series III and IV: Due to the unstability of the original data variances, the resistance data were stabilized by the natural logarithm transformation before any statistical methods were utilized. The mean logarithmic resistance values of skin, muscle and total forelimb vasculatures were analyzed by a two-way analysis of variance. Control means (vascular resistances and change in forelimb weight) were compared with experimental means (produced by local and systemic hypothermia) by using Dunnett's paired-t test for multiple comparisons. Differences between innervated and denervated forelimb vascular resistance and weight responses to hypothermia were analyzed with the t-test for unpaired observations and unequal variances. For all comparisons referred to in Results, differences between means were considered significant only if the probability of making

a type -I error (α) was less than 0.05. Appendix C has a more detailed description of the statistical methods used in analyzing the data.

RESULTS

I. Series I and Series II (normothermic controls): Naturally Perfused, Innervated Forelimbs; Effects of Whole-body Cooling and Hypothermia

A. Heart Rate

Figure 4 shows the cardiac chronotropic response to cooling and sustained hypothermia. Data are mean values ± standard errors for eight experiments. As rectal temperature was lowered from 37.5 to 32.0°C, heart rate fell from 163 ± 15.7 to 116 ± 6.8 beats/minute. This apparently substantial decrease in heart rate was not statistically significant when compared to that of the normothermic controls during the same time period (mins. 0 to 55). Heart rate was significantly reduced from normothermic control values at rectal temperatures of 31°C and below.

B. Arterial Hematocrit

Figure 5 reports the effect of cooling and hypothermia on arterial hematocrit. Systemic cooling (37.5 to 28°C) elicited a progressive increase in hematocrit from 43.9 ± 2.4 to 48.6 ± 2.2%; a significant difference from normothermic period there was an additional, slower increase in hematocrit to a maximum value of 51.6 ± 2.2 vol. percent at the end of the hypothermic period.

Figure 4. Effects of whole-body cooling and sustained hypothermia (28°C) on heart rate (bottom graph). Normothermic controls are shown in top graph. Ordinate shows heart rate beats per minute. Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values + standard errors from 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

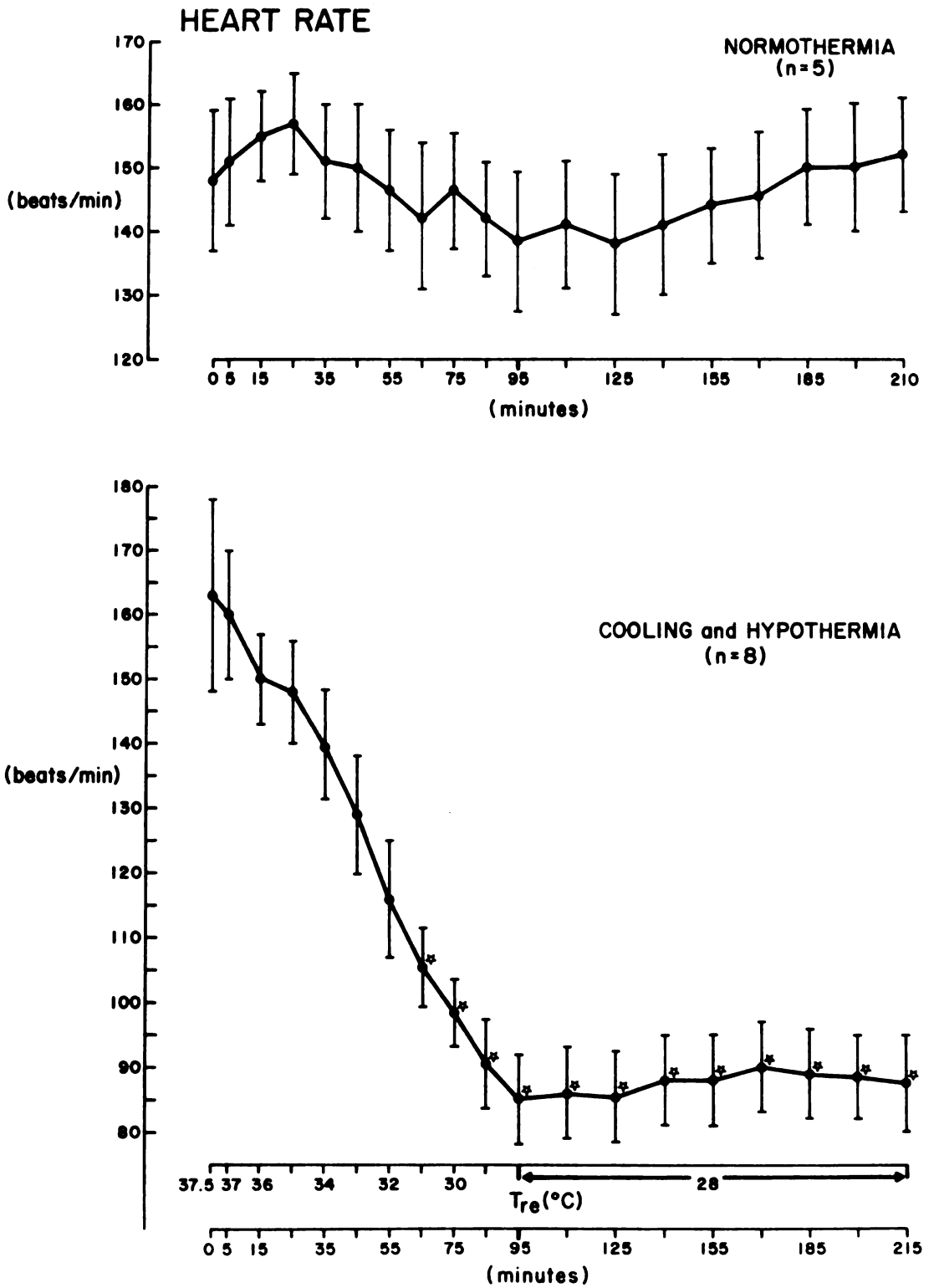
HEART RATE
NORMOTHERMIA
(n=5)
(beats/min)
170
160
150
140
130
120
0 5 15 35 55 75 95 125 155 185 210
(minutes)
COOLING and HYPOTHERMIA
(n=8)
(beats/min)
180
170
160
150
140
130
120
110
100
90
80
37.5 37 36 34 32 30
28
T_{re}(°C)
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 4

Figure 5. Effects of whole-body cooling and sustained hypothermia (28°C) on arterial hematocrit (bottom graph). Normothermic controls are shown in top graph. Ordinate shows hematocrit (Volume percent). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values ± standard errors from 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

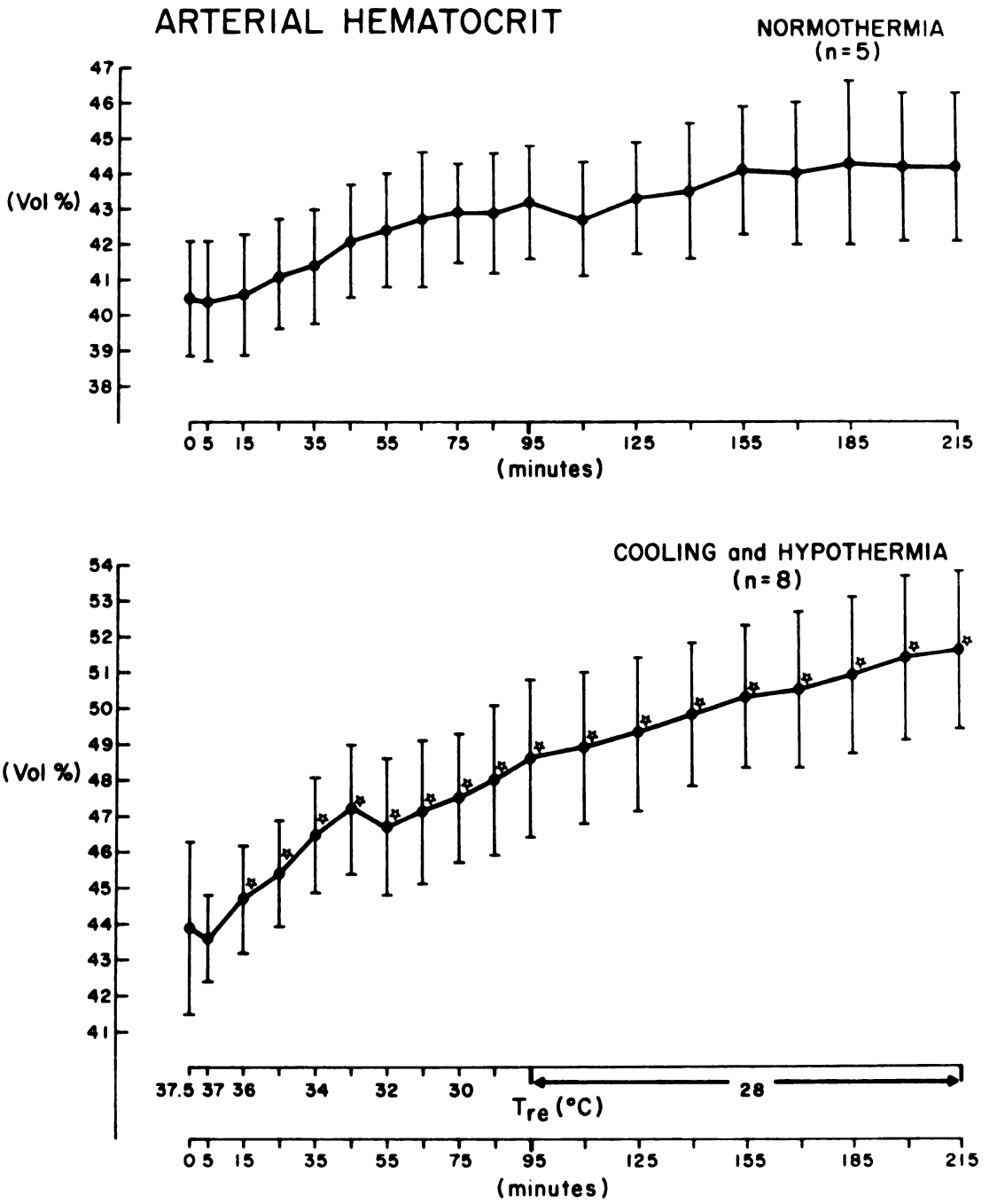
ARTERIAL HEMATOCRIT
NORMOTHERMIA
(n=5)
(Vol %)
47
46
45
44
43
42
41
40
39
38
0 5 15 35 55 75 95 125 155 185 215
(minutes)
COOLING and HYPOTHERMIA
(n=8)
(Vol %)
54
53
52
51
50
49
48
47
46
45
44
43
42
41
37.5 37 36 34 32 30
28
Tre (°C)
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 5

C. Intravascular Pressures

As shown in Figure 6, mean systemic arterial blood pressure decreased throughout the cooling period. Blood pressure in the hypothermic dogs was significantly lower than that of the normothermic controls at a rectal temperature of 35°C and below. Mean systemic arterial pressure averaged 121.0 ± 8.3 mm Hg at 37.5°C and fell to 6.1 ± 6.9 mm Hg at 28°C. The normothermic controls (Figure 6) for the same time period showed no change in blood pressure. During the two hour hypothermic period, blood pressure also does not change.

Results shown in Tables 2 and 3 are the mean intravascular pressures for both forelimb skin and skeletal muscle during whole-body cooling and hypothermia. Cooling from 37.5 to 28°C caused all forelimb intravascular pressures to fall significantly. In the skin circulation, all of the measured pressures showed no change during the two hour hypothermic period. In all cases, however, cutaneous intravascular pressures were significantly below the normothermic control values at min. 215. Intravascular pressures in muscle also showed no change during the two hour hypothermia period. However, all muscle vascular pressures were significantly below their respective normothermic controls at the end of the hypothermic period.

Figure 6. Effects of whole-body cooling and hypothermia (28°C) on systemic arterial blood pressure (bottom graph). Normothermic controls are shown in top graph. Ordinate shows blood pressure (mm Hg). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values ± standard errors in 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

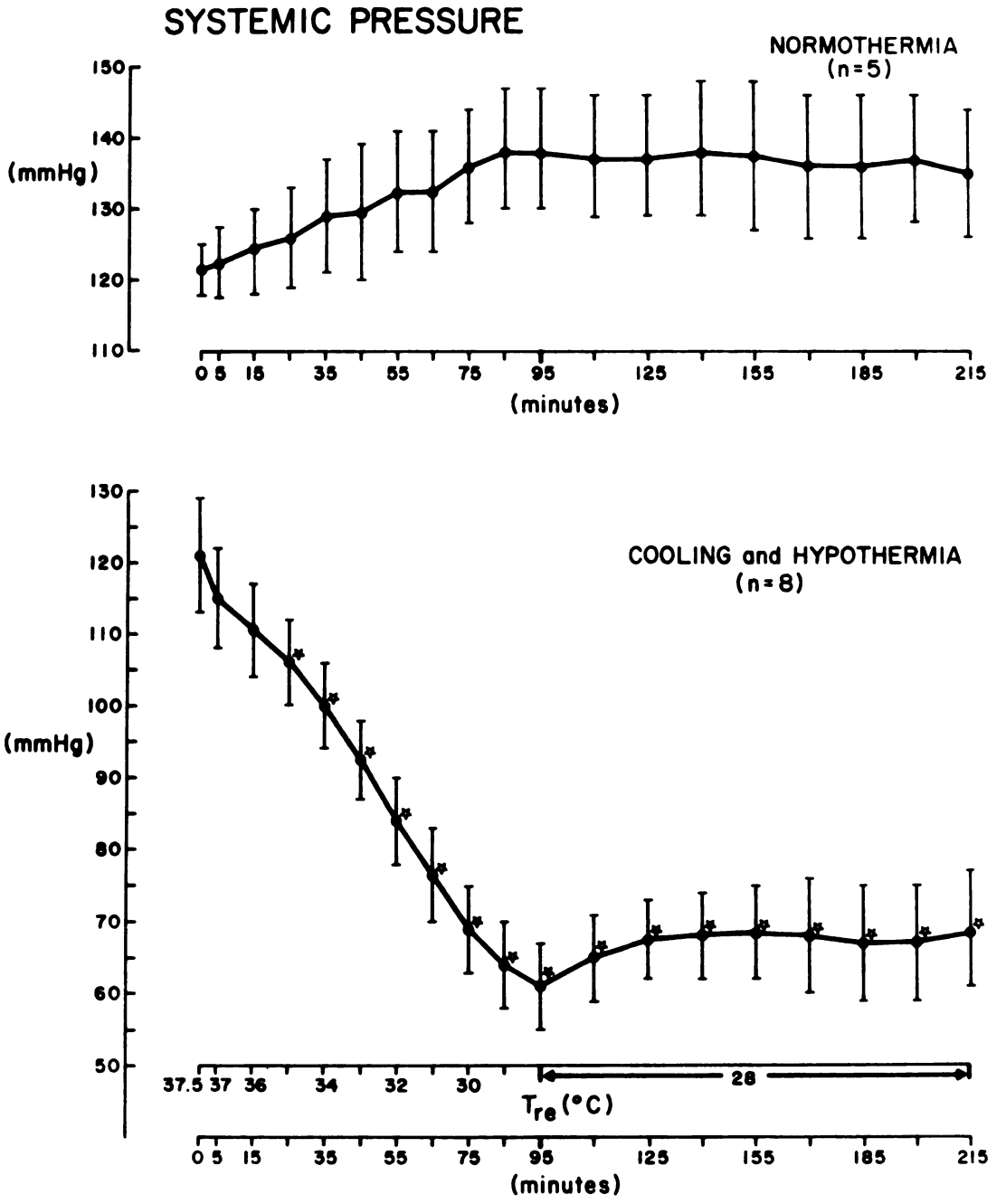
SYSTEMIC PRESSURE
NORMOTHERMIA
(n=5)
150
140
130
120
110
(mmHg)
0 5 15 35 55 75 95 125 155 185 215
(minutes)
130
120
110
100
90
80
70
60
50
(mmHg)
COOLING and HYPOTHERMIA
(n=8)
37.5 37 36 34 32 30
28
T_{re} (°C)
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 6

Table 2. Effects of whole-body cooling and sustained hypothermia on mean intravascular pressure ($\bar{P}$) in innervated skin arteries (SA), small skin vein (SSV), and cephalic vein (CV). P_{BA} = brachial artery pressure. Values in mm Hg are means ± standard errors from 8 experiments. Brackets = normothermic controls with $\bar{T}_{RE}$ 37.7°C.

Temp. (°C)	P_{BA}		SA		SSV		CV	
37.5	[117.2±4.2]	116.2±7.7	[102.2±4.1]	87.7±12.7	[7.4± .6]	9.1± .4	[3.1±.7]	4.2±1.3
37.0	[118.0±5.1]	107.0±8.7	[102.0±5.0]	82.1±8.7	[7.3± .7]	11.8±1.5	[3.3±.7]	4.9±1.6
36.5	[118.8±5.4]	106.3±8.1	[104.2±4.3]	83.1±8.0	[7.3± .7]	10.6±1.5	[3.3±.7]	4.2±1.3
36.0	[119.2±6.0]	102.5±7.3	[104.8±4.6]	79.1±7.9	[7.0± .8]	11.8±1.8	[3.0±.7]	5.1±1.6
35.0	[122.0±7.0]	98.6±5.5	[107.2±5.7]	75.9±6.0	[7.1± .8]	11.3±1.9	[3.0±.7]	4.9±1.7
34.0	[125.2±8.2]	93.6±5.6	[110.6±7.1]	71.1±5.6	[7.4± .7]	10.6±2.0	[3.2±.7]	4.6±1.7
33.0	[126.4±8.3]	86.6±5.3	[111.0±7.4]	65.6±6.2	[7.7± .8]	10.0±1.6	[3.2±.7]	4.2±1.9
32.0	[129.6±9.2]	80.4±6.2	[114.0±7.6]	63.1±6.5	[7.3± .7]	8.1±1.4	[3.0±.6]	2.6±1.4
31.0	[129.4±9.2]	73.9±5.3	[113.4±8.1]	59.9±6.3	[7.7± .6]	7.3±1.3	[2.9±.7]	2.1±1.2
30.0	[131.8±7.6]	66.1±6.4	[115.0±7.0]	54.5±7.6	[7.6± .8]	6.8±1.1	[2.9±.7]	1.9±1.0
29.0	[133.6±8.3]	61.3±6.5	[116.4±6.7]	52.9±8.2	[7.5± .9]	6.4±1.1	[2.9±.7]	1.6± .9
28.0 @ 0 mins.	[133.6±8.5]	57.9±6.6	[118.2±7.8]	50.2±8.5	[7.3± .9]	6.5±1.1	[2.8±.7]	1.5± .9
28.0 @ 15 mins.	[132.6±8.8]	62.7±5.9	[118.4±8.0]	54.2±7.8	[7.3± .9]	6.6±1.0	[2.8±.7]	1.4±1.0
28.0 @ 30 mins.	[132.4±8.6]	64.2±6.1	[118.4±7.1]	55.7±8.4	[7.2±1.0]	7.3±1.0	[2.7±.7]	1.7±1.2
28.0 @ 45 mins.	[134.0±9.5]	65.2±6.2	[118.8±8.2]	55.7±7.8	[7.6±1.0]	7.3±1.1	[2.8±.7]	1.7±1.2
28.0 @ 60 mins.	[133.4±10.7]	65.7±6.5	[117.2±9.2]	56.1±8.0	[7.5± .9]	7.6±1.2	[2.8±.6]	1.8±1.4
28.0 @ 75 mins.	[132.4±9.5]	65.7±7.1	[116.6±9.1]	55.2±8.5	[7.3± .8]	7.8±1.1	[2.6±.5]	1.8±1.4
28.0 @ 90 mins.	[132.4±9.8]	64.6±7.3	[116.0±9.0]	53.4±8.6	[7.5± .8]	8.3±1.0	[2.8±.6]	1.9±1.3
28.0 @ 105 mins.	[133.6±8.4]	63.8±7.2	[116.2±8.3]	53.2±8.5	[7.7± .8]	7.9±1.0	[2.9±.6]	1.8±1.3
28.0 @ 120 mins.	[131.0±8.1]	65.1±7.4	[114.2±9.2]	54.1±8.5	[7.7± .7]	7.8± .7	[2.8±.7]	1.7±1.2

Table 3. Effects of whole-body cooling and sustained hypothermia on mean intravascular pressures ($\bar{P}$) in innervated muscle arteries (MA), small muscle vein (MSV), and brachial vein (BV). P_{BA} = brachial artery pressure. Values in mm Hg are means ± standard errors from 8 experiments. Brackets = normothermic controls with $\bar{T}_{RE}$ 37.7°C.

Temp. (°C)	P_{BA}		MA		MSV		BV	
37.5	[117.2±4.2]	116.2±7.7	[102.6±4.1]	101.2±6.5	[9.6±1.4]	6.8±1.7	[4.2±.4]	3.8±.8
37.0	[118.0±5.1]	107.0±8.7	[102.8±4.7]	92.1±6.3	[9.3±1.3]	7.6±1.3	[4.1±.4]	4.2±.7
36.5	[118.8±5.4]	106.3±8.1	[102.6±6.2]	92.3±5.6	[9.2±1.3]	6.9±1.2	[4.0±.5]	3.6±.8
36.0	[119.2±6.0]	102.5±7.3	[103.6±6.5]	88.5±4.9	[9.1±1.4]	7.1±1.3	[4.0±.5]	4.0±.9
35.0	[122.0±7.0]	98.6±5.5	[106.6±6.9]	84.9±3.1	[9.3±1.4]	7.0±1.2	[4.0±.5]	3.4±.8
34.0	[125.2±8.2]	93.6±5.6	[108.8±7.7]	81.6±3.2	[9.6±1.2]	7.0±1.1	[4.1±.3]	3.6±.8
33.0	[126.4±8.3]	86.6±5.3	[108.6±8.7]	73.7±3.4	[9.6±1.2]	6.6±1.0	[4.3±.2]	3.5±.8
32.0	[129.6±9.2]	80.4±6.2	[108.6±9.6]	70.6±4.5	[10.7±2.5]	5.6± .8	[4.3±.3]	2.9±.8
31.0	[129.4±9.2]	73.9±5.3	[108.8±9.6]	65.4±4.2	[9.8±1.6]	5.4± .8	[4.2±.3]	2.7±.7
30.0	[131.8±7.6]	66.1±6.4	[112.4±9.7]	57.9±5.4	[9.6±1.4]	5.5± .8	[4.1±.4]	2.3±.6
29.0	[133.6±8.3]	61.3±6.5	[114.6±8.1]	55.1±6.4	[9.1±1.3]	5.2± .8	[4.0±.4]	2.2±.5
28.0 @ 0 mins.	[133.6±8.5]	57.9±6.6	[114.4±9.8]	53.0±6.9	[8.8±1.4]	5.2± .8	[3.8±.4]	2.3±.5
28.0 @ 15 mins.	[132.6±8.8]	62.7±5.9	[114.6±10.2]	57.1±6.5	[8.4±1.0]	5.1± .8	[3.5±.4]	2.2±.5
28.0 @ 30 mins.	[132.4±8.6]	64.2±6.1	[116.0±9.4]	58.0±6.4	[8.0± .9]	5.5±1.0	[3.5±.4]	2.4±.5
28.0 @ 45 mins.	[134.0±9.5]	65.2±6.2	[117.0±11.1]	59.1±6.1	[7.8± .8]	5.3± .9	[3.4±.5]	2.4±.6
28.0 @ 60 mins.	[133.4±10.7]	65.7±6.5	[117.0±10.8]	60.4±6.0	[7.4± .5]	5.7±1.0	[3.4±.5]	2.4±.6
28.0 @ 75 mins.	[132.4±9.5]	65.7±7.1	[113.2±11.3]	58.0±6.6	[7.6± .6]	5.5±1.0	[3.4±.6]	2.3±.6
28.0 @ 90 mins.	[132.4±9.8]	64.6±7.3	[116.2±10.1]	57.4±7.0	[8.0± .6]	5.6± .8	[3.5±.8]	2.5±.6
28.0 @ 105 mins.	[133.6±8.4]	63.8±7.2	[114.6±9.6]	57.4±6.9	[8.0± .5]	5.3± .8	[3.5±.7]	2.5±.5
28.0 @ 120 mins.	[131.0±8.1]	65.1±7.4	[113.8±10.5]	57.1±7.0	[8.0± .5]	5.5± .9	[3.9±.5]	2.3±.5

D. Forelimb Weight

In Series I limb weight was significantly reduced at a rectal temperature of 30°C and below (Figure 7). The rate of limb weight loss was greater during the active cooling process than during the two hour hypothermic period.

E. Skin Total and Segmental Vascular Resistances

Figures 8, 9, and 10 illustrate the effects of cooling and sustained hypothermia on total and segmental resistances in the forelimb cutaneous vascular segment. Data are mean values ± standard errors. Total vascular resistance in skin (Figure 8), skin small vessel resistance (Figure 9), and skin venous resistance (Figure 10) were not significantly increased until rectal temperature had decreased to 30°C (veins) and to 29°C (small vessel and total resistance). During the cooling period, total skin vascular resistance rose from 2.1 ± 0.5 at 37.5°C to 11.3 ± 2.1 mm Hg·min/ml at 28°C, skin small vessel resistance increased from 1.6 ± 0.6 to 8.3 ± 1.6 mm Hg·min/ml at 28°C while the skin vein resistance rose from 0.1 ± 0.1 to 1.1 ± 0.4 mm Hg·min/ml. During the two hour hypothermic period all vascular resistances increased significantly in relation to their respective normothermic controls with skin total, small vessel, and venous resistances rising to 21.7 ± 5.6, 16.0 ± 4.3, and 2.4 ± 0.9 mm Hg·min/ml, respectively.

Figure 7. Effects of whole-body cooling and sustained hypothermia (28°C) on forelimb weight change (bottom graph). Normothermic controls are shown in top graph. Ordinate shows limb weight change (grams). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values $\pm$ standard errors in 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

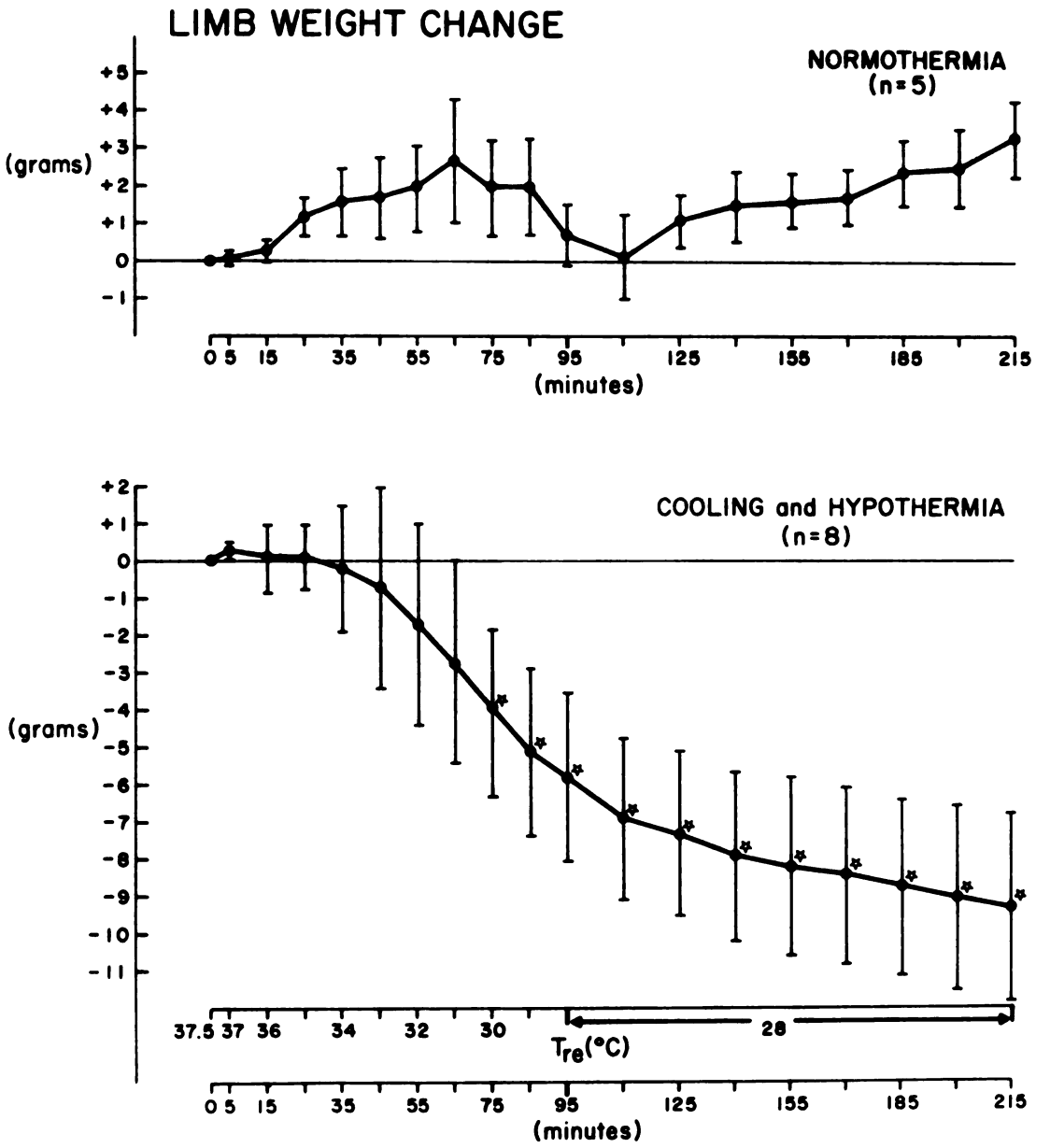
LIMB WEIGHT CHANGE
NORMOTHERMIA
(n=5)
(grams)
+5
+4
+3
+2
+1
0
-1
0 5 15 35 55 75 95 125 155 185 215
(minutes)
COOLING and HYPOTHERMIA
(n=8)
(grams)
+2
+1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
37.5 37 36 34 32 30
T_{re}(°C)
28
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 7

Figure 8. Effects of whole-body cooling and sustained hypothermia (28°C) on total skin vascular resistance (bottom graph). Normothermic controls are shown in top graph. Ordinate shows skin vascular resistance (mm Hg·minute per milliliter). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values $\pm$ standard errors in 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

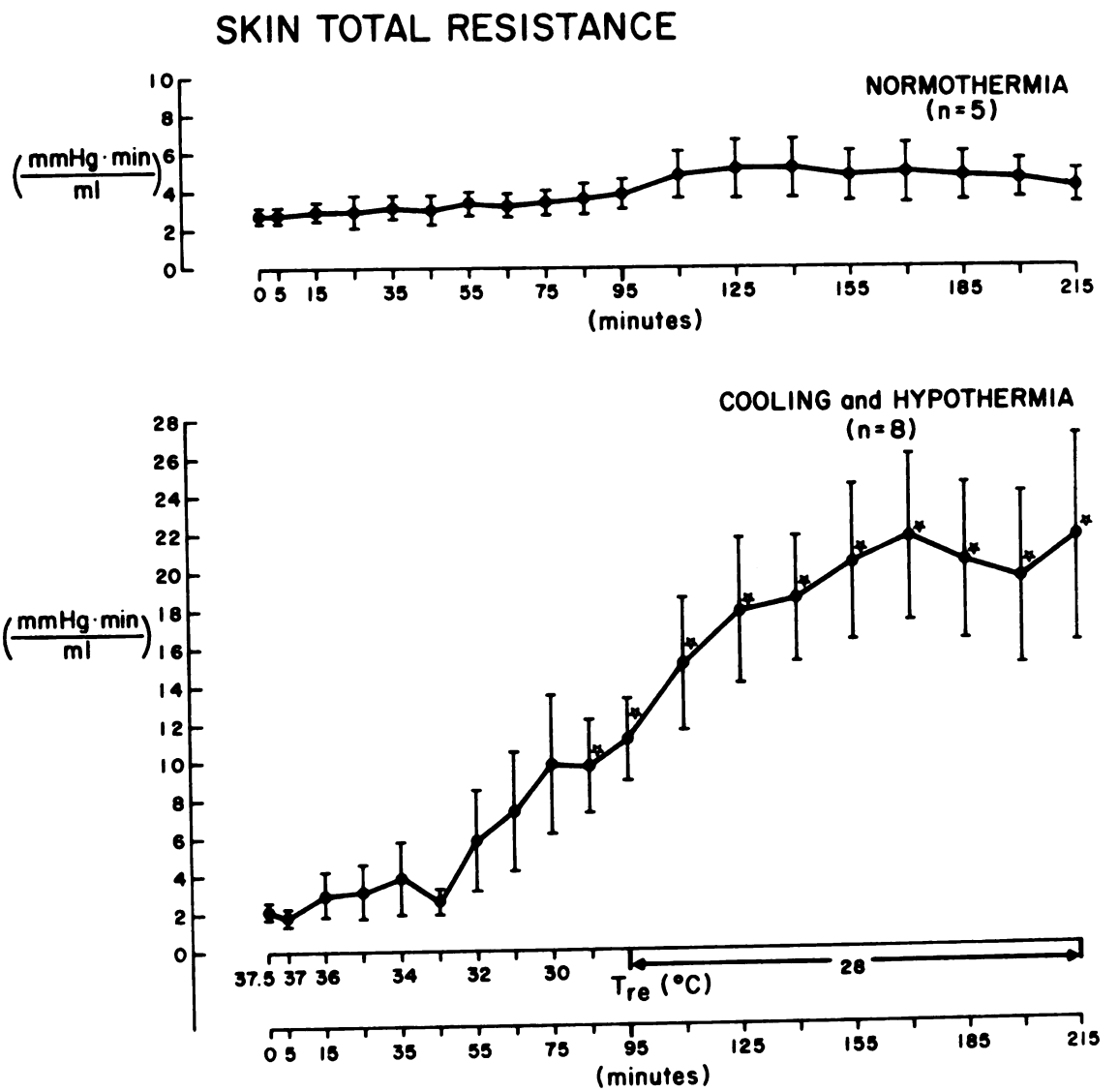
SKIN TOTAL RESISTANCE
NORMOTHERMIA
(n=5)
(mmHg·min/ml)
0 2 4 6 8 10
0 5 15 35 55 75 95 125 155 185 215
(minutes)
COOLING and HYPOTHERMIA
(n=8)
(mmHg·min/ml)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
37.5 37 36 34 32 30 28
Tre (°C)
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 8

Figure 9. Effects of whole-body cooling and sustained hypothermia (28°C) on skin small vessel resistance (bottom graph). Normothermic controls are shown in top graph. Ordinate shows skin small vessel resistance (mm Hg·minute per milliliter). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values $\pm$ standard errors in 8 experiments. (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

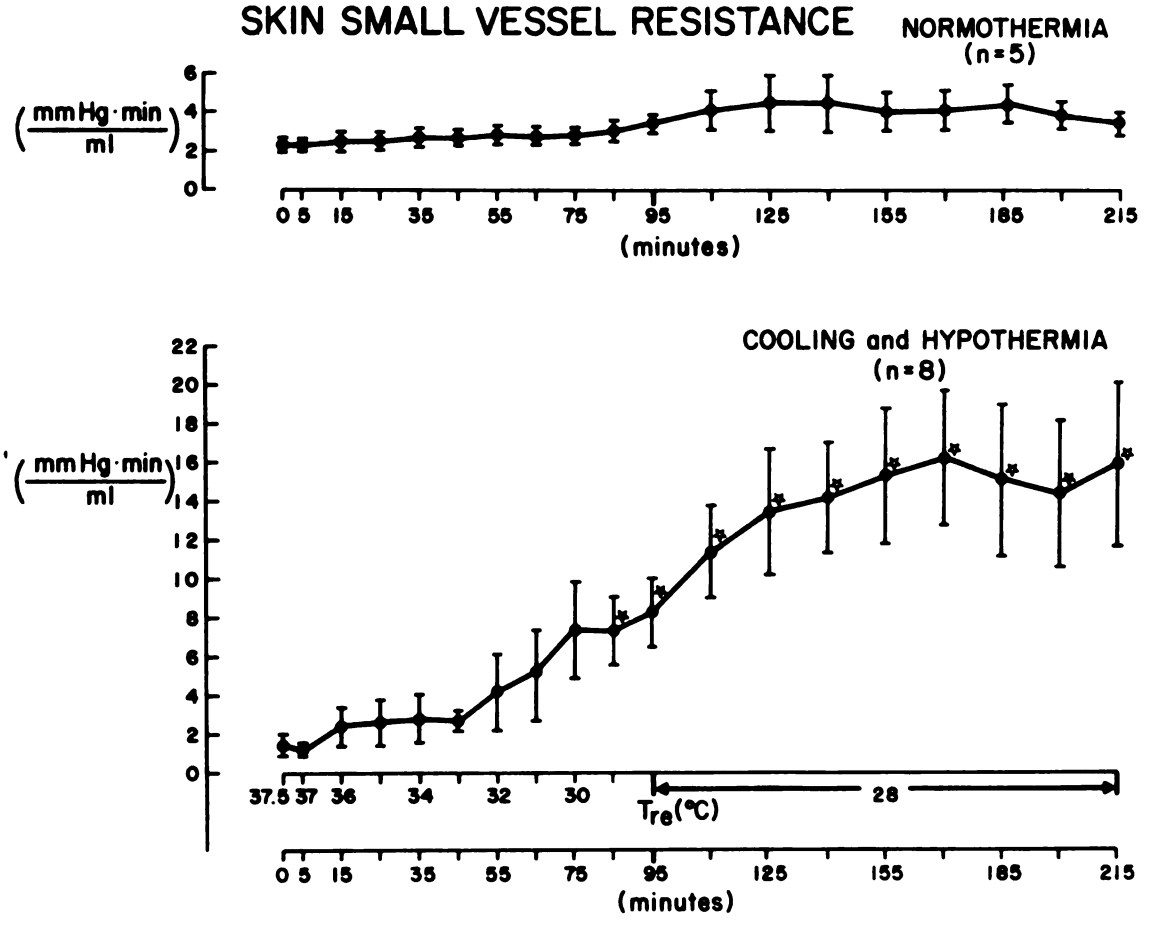
SKIN SMALL VESSEL RESISTANCE
NORMOTHERMIA
(n=5)
(mmHg·min/ml)
0
2
4
6
0 5 15 35 55 75 95 125 155 185 215
(minutes)
COOLING and HYPOTHERMIA
(n=8)
(mmHg·min/ml)
0
2
4
6
8
10
12
14
16
18
20
22
37.5 37 36 34 32 30
T_{re}(°C)
28
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 9

Figure 10. Effects of whole-body cooling and sustained hypothermia (28°C) on skin venous resistance (bottom graph). Normothermic controls are shown in top graph. Ordinate shows skin venous resistance (mm Hg·minute per milliliter). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values ± standard errors in 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

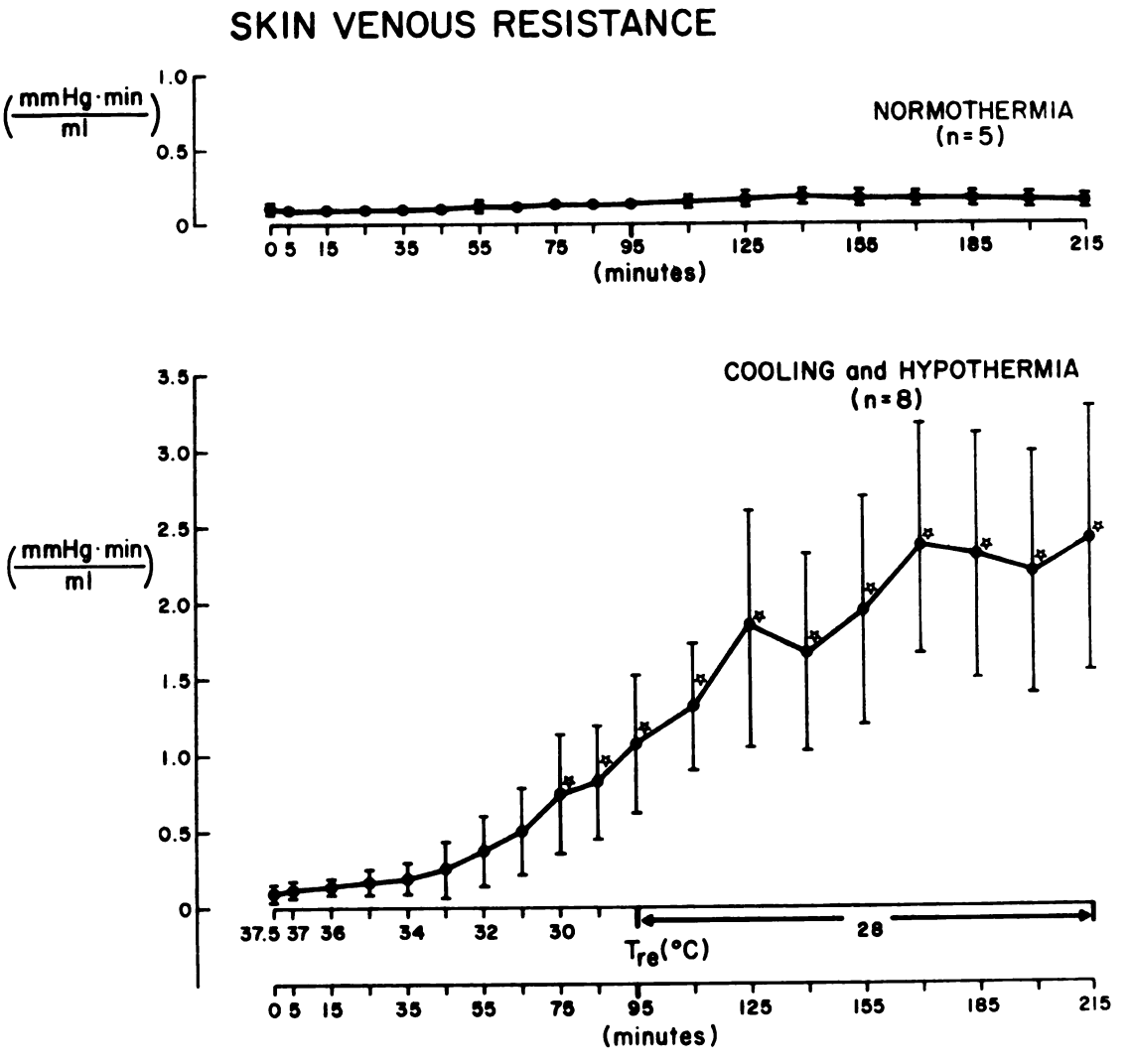
SKIN VENOUS RESISTANCE
(mmHg·min / ml)
1.0
0.5
0
NORMOTHERMIA
(n=5)
0 5 15 35 55 75 95 125 155 185 215
(minutes)
COOLING and HYPOTHERMIA
(n=8)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
37.5 37 36 34 32 30
28
Tre(°C)
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 10

F. Muscle Total and Segmental Vascular Resistances

Figures 11, 12, and 13 report the effects of cooling and hypothermia on muscle total, small vessel, and venous resistances in the eight isolated canine forelimbs. Total and segmental vascular resistances in muscle were increased significantly at rectal temperatures 31°C and below. During the cooling period, total muscle vascular resistance rose from 3.7 $\pm$ 0.6 at 37.5°C to 14.1 $\pm$ 2.1 mm Hg·min/ml at 28°C. Muscle small vessel resistance increased from 3.1 $\pm$ 0.6 at 37.5°C to 12.1 $\pm$ 2.0 mm Hg·min/ml at 28°C while the muscle vein resistance rose from 0.1 $\pm$ 0.02 to 0.8 $\pm$ 0.2 mm Hg·min/ml. During the two hour hypothermic period, all the forelimb muscle vascular resistances increased significantly in relation to the corresponding normothermic controls with muscle total, small vessel, and venous resistances rising to 20.6 $\pm$ 5.1, 61.7 $\pm$ 4.1, and 1.2 $\pm$ 0.4 mm Hg·min/ml, respectively.

Figure 11. Effects of whole-body cooling and sustained hypothermia (28°C) on total muscle vascular resistance (bottom graph). Normothermic controls are shown in top graph. Ordinate shows muscle vascular resistance (mm Hg·minute per milliliter). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values $\pm$ standard errors in 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

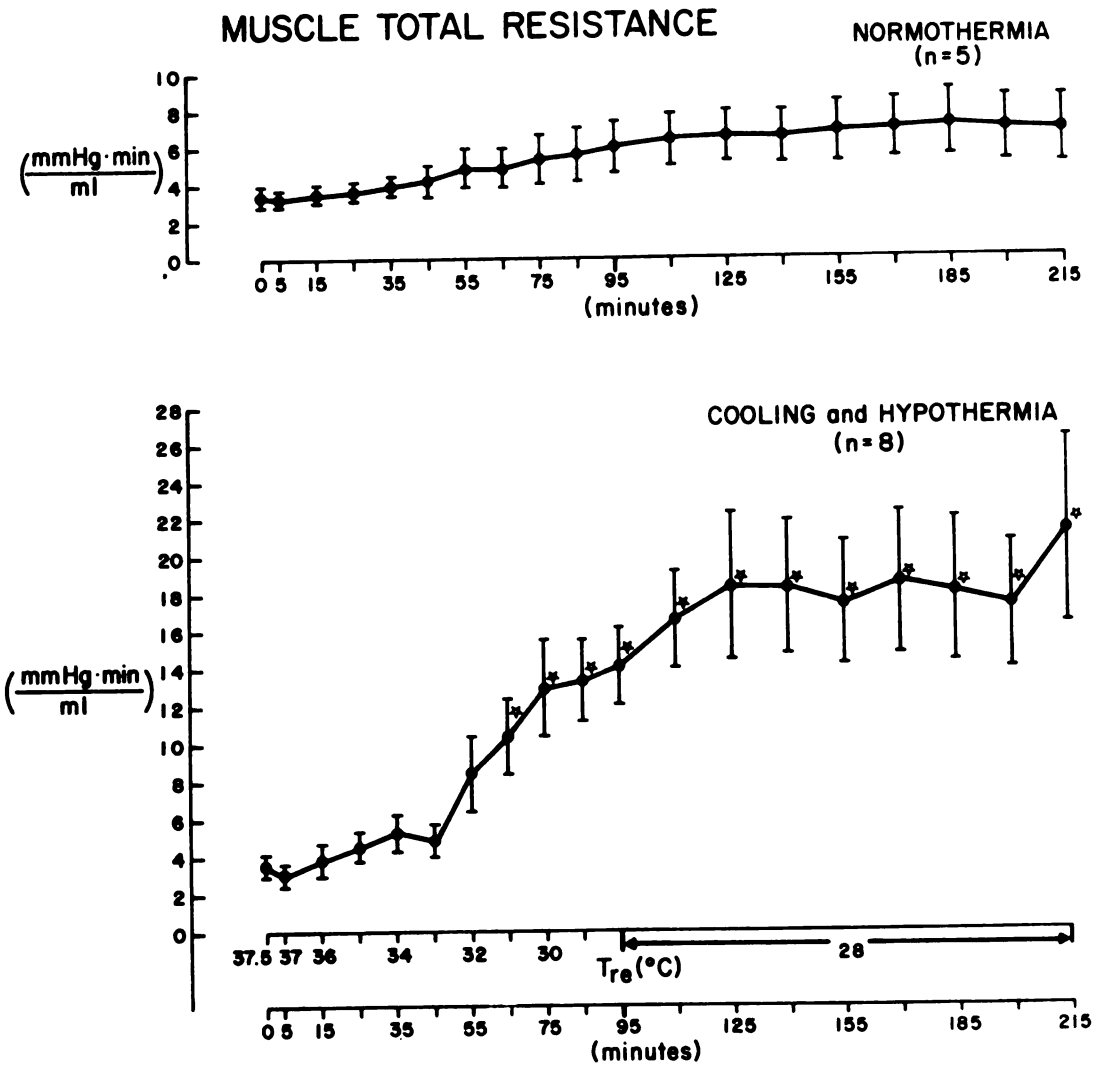
MUSCLE TOTAL RESISTANCE
NORMOTHERMIA
(n=5)
(mmHg·min / ml)
0 2 4 6 8 10
0 5 15 35 55 75 95 125 155 185 215
(minutes)
COOLING and HYPOTHERMIA
(n=8)
(mmHg·min / ml)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
37.5 37 36 34 32 30 T_{re}(°C) 28
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 11

Figure 12. Effects of whole-body cooling and sustained hypothermia (28°C) on muscle small vessel resistance (bottom graph). Normothermic controls are shown in top graph. Ordinate shows muscle small vessel resistance (mm Hg·minute per milliliter). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values $\pm$ standard errors in 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

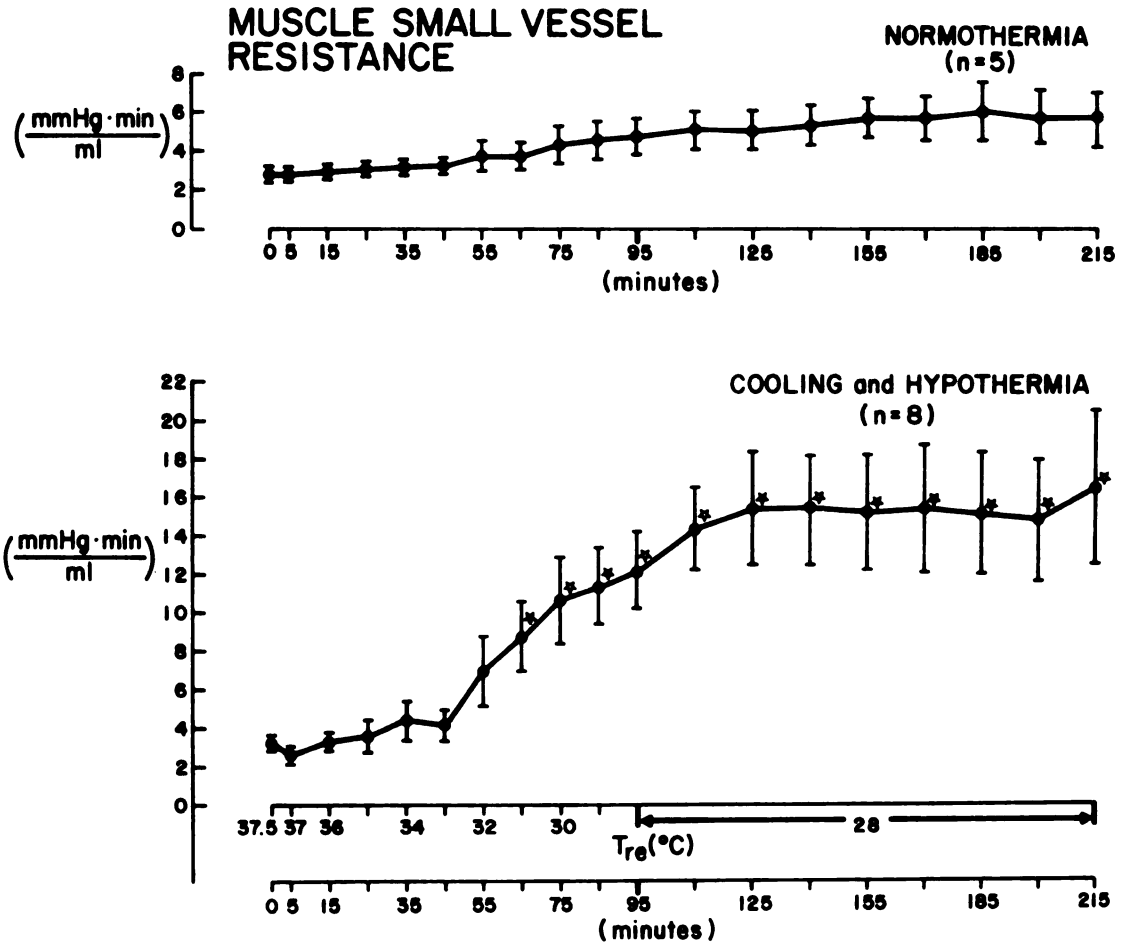
MUSCLE SMALL VESSEL RESISTANCE
NORMOTHERMIA (n=5)
(mmHg·min/ml)
0 2 4 6 8
0 5 15 35 55 75 95 125 155 185 215
(minutes)
COOLING and HYPOTHERMIA (n=8)
(mmHg·min/ml)
0 2 4 6 8 10 12 14 16 18 20 22
37.5 37 36 34 32 30 28
T_{re}(°C)
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 12

Figure 13. Effects of whole-body cooling and sustained hypothermia (28°C) on muscle venous resistance (bottom graph). Normothermic controls are shown in top graph. Ordinate shows muscle venous resistance (mm Hg·minute per milliliter). Abcissae show rectal temperature (°C) and time in minutes. Data represent mean values $\pm$ standard errors in 8 experiments (n=5 for normothermic controls). Asterisk denotes type I error less than .05.

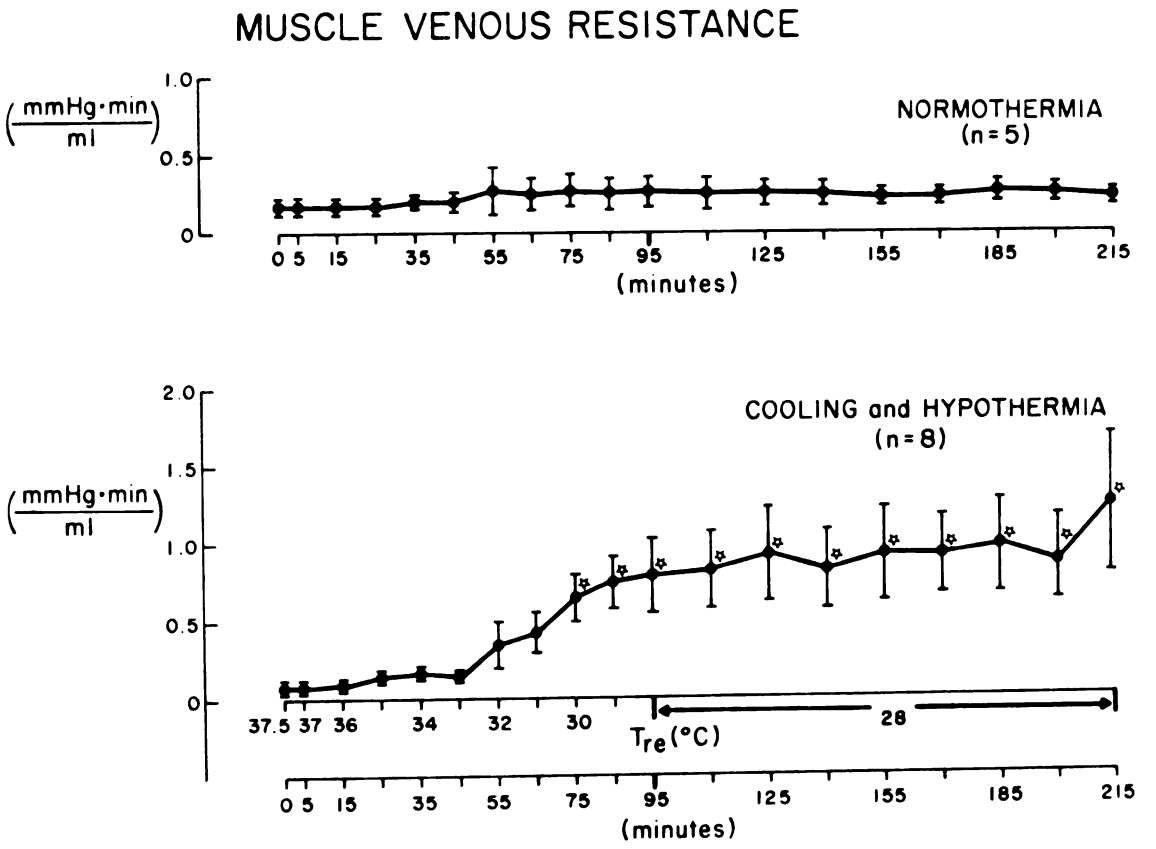
MUSCLE VENOUS RESISTANCE
(mmHg·min / ml)
1.0
0.5
0
NORMOTHERMIA
(n=5)
0 5 15 35 55 75 95 125 155 185 215
(minutes)
2.0
1.5
1.0
0.5
0
COOLING and HYPOTHERMIA
(n=8)
37.5 37 36 34 32 30
Tre(°C)
28
0 5 15 35 55 75 95 125 155 185 215
(minutes)

Figure 13

II. Series III: Constant Pressure Perfusion, Innervated Forelimbs; Local and Whole-body Hypothermia

A. Forelimb Weight

Figure 14 reports the effects of local and whole-body cooling on limb weight change for 13 experiments. The limbs were isogravimetric throughout the initial control period. In normothermic dogs, cooling arterial blood supply to the forelimb elicited a significant ($P < 0.05$) weight gain which averaged 9.6 $\pm$ 1.8 gms when brachial artery temperature reached 33°C. The limbs remained isogravimetric at this elevated weight as long as perfusate temperature was held at 33°C. Further cooling from 33 to 28°C caused a small, additional weight gain which averaged 2.4 $\pm$ 0.7 grams and was statistically significant ($P < 0.05$). Rewarming brachial artery blood to 38°C caused limb weight to return to and stabilize at a value not significantly different from the initial control weight.

Whole-body cooling with brachial artery blood maintained normothermic elicited a progressive fall in limb weight. When rectal temperature reached 33°C, limb weight had decreased 8.3 $\pm$ 0.7 grams. Rectal temperature was maintained at 33°C and forelimb perfusion temperature was reduced from 38 to 33°C. This maneuver caused a significant ($P < 0.05$) increase in limb weight which averaged 5.0 $\pm$ 1.0 grams. Rewarming brachial artery blood to 38°C while rectal

Figure 14. Effects of local and systemic cooling on weight change in innervated, pump-perfused forelimbs. Ordinate shows limb weight change (grams). Abcissae show rectal and forelimb temperatures (°C). Data represent mean values ± standard errors for 13 experiments.

a* is significant from a at 5% level.
b* is significant from b at 5% level.
c* is significant from c at 5% level.

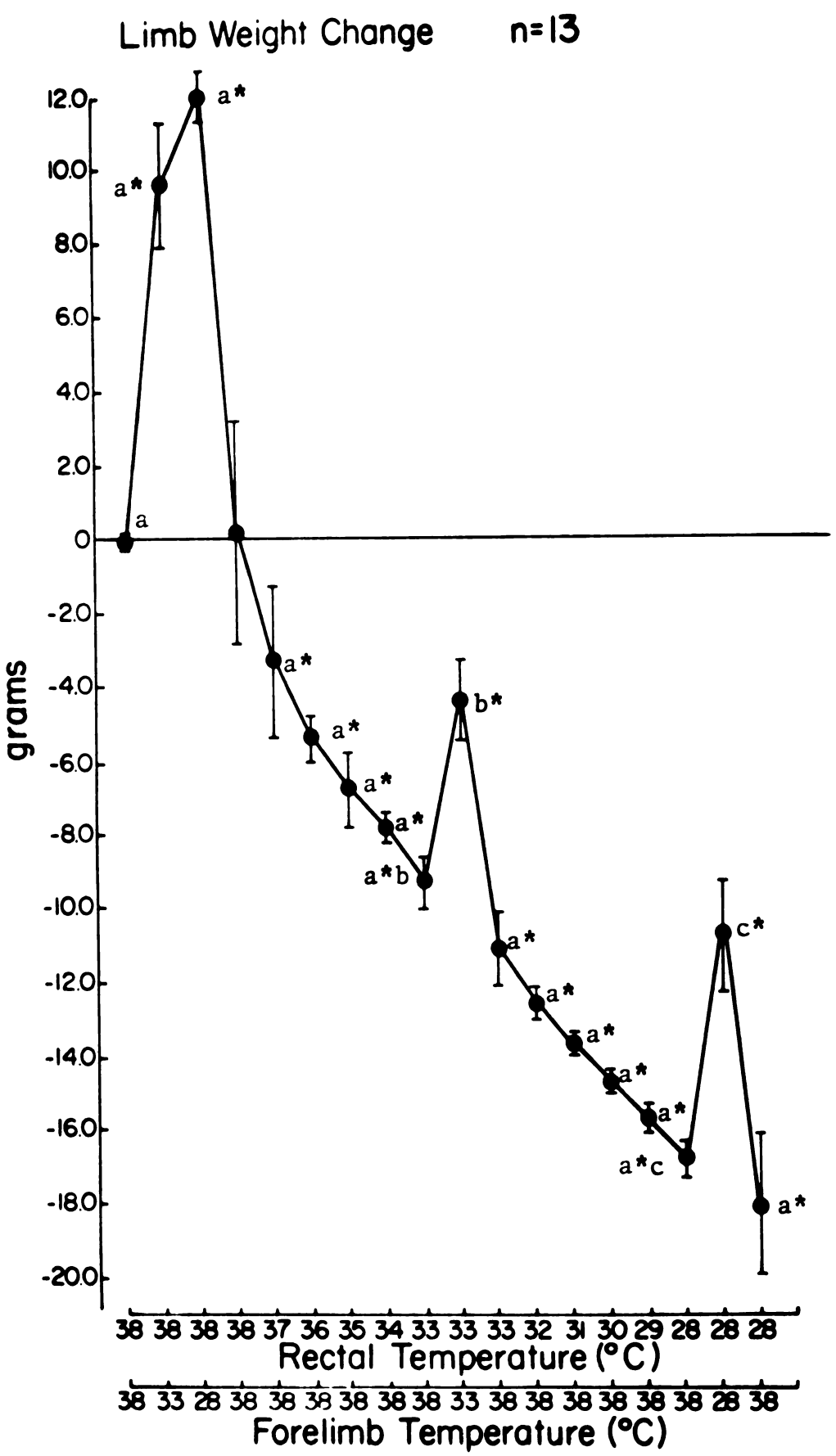
Limb Weight Change n=13
12.0
10.0
8.0
6.0
4.0
2.0
0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
-18.0
-20.0
grams
a
a*
a*
a*
a*
a*
a*b
b*
a*
a*
a*
a*
a*
a*c
c*
a*
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 14

temperature remained at 33°C caused limb weight to fall 6.3 ± 1.1 grams (11.1 ± 1.0 gms below the normothermic control weight) to a value not significantly different from that immediately before the local cooling procedure.

Forelimb perfusion temperature was maintained at 38°C and the animals were subjected to further systemic cooling which elicited an additional, progressive weight loss. When rectal temperature reached 28°C, the normothermic limbs had lost a total of 16.8 ± 0.5 grams. Rectal temperature was maintained at 28°C and forelimb perfusion temperature was reduced from 38 to 28°C. This maneuver caused a significant increase in limb weight ($P < 0.05$) which averaged 6.1 ± 1.5 grams. Rewarming brachial artery blood to 38°C while rectal temperature remained at 28°C caused limb weight to fall an average of 7.4 ± 2.0 grams, to a value which was not significantly different from that immediately before the local cooling procedure. Therefore, at the end of the experiment rectal temperature was 28°C, forelimb perfusion temperature 38°C and limb weight 18.1 ± 2.0 grams below the original control value.

B. Cutaneous Vascular Resistance

Figure 15 reports the effects of local and whole-body hypothermia on vascular resistance in the segment supplied by the brachial artery and drained by the cephalic vein.

Figure 15. Resistance response of the cutaneous circulation to local and systemic cooling in innervated, pump-perfused forelimbs. Ordinate shows ln total skin vascular resistance and abcissae show rectal and forelimb temperature (°C). Data represent mean logarithmic values ± standard errors from 13 experiments.

a* is significant from a at 5% level.
b* is significant from b at 5% level.
c* is significant from c at 5% level.

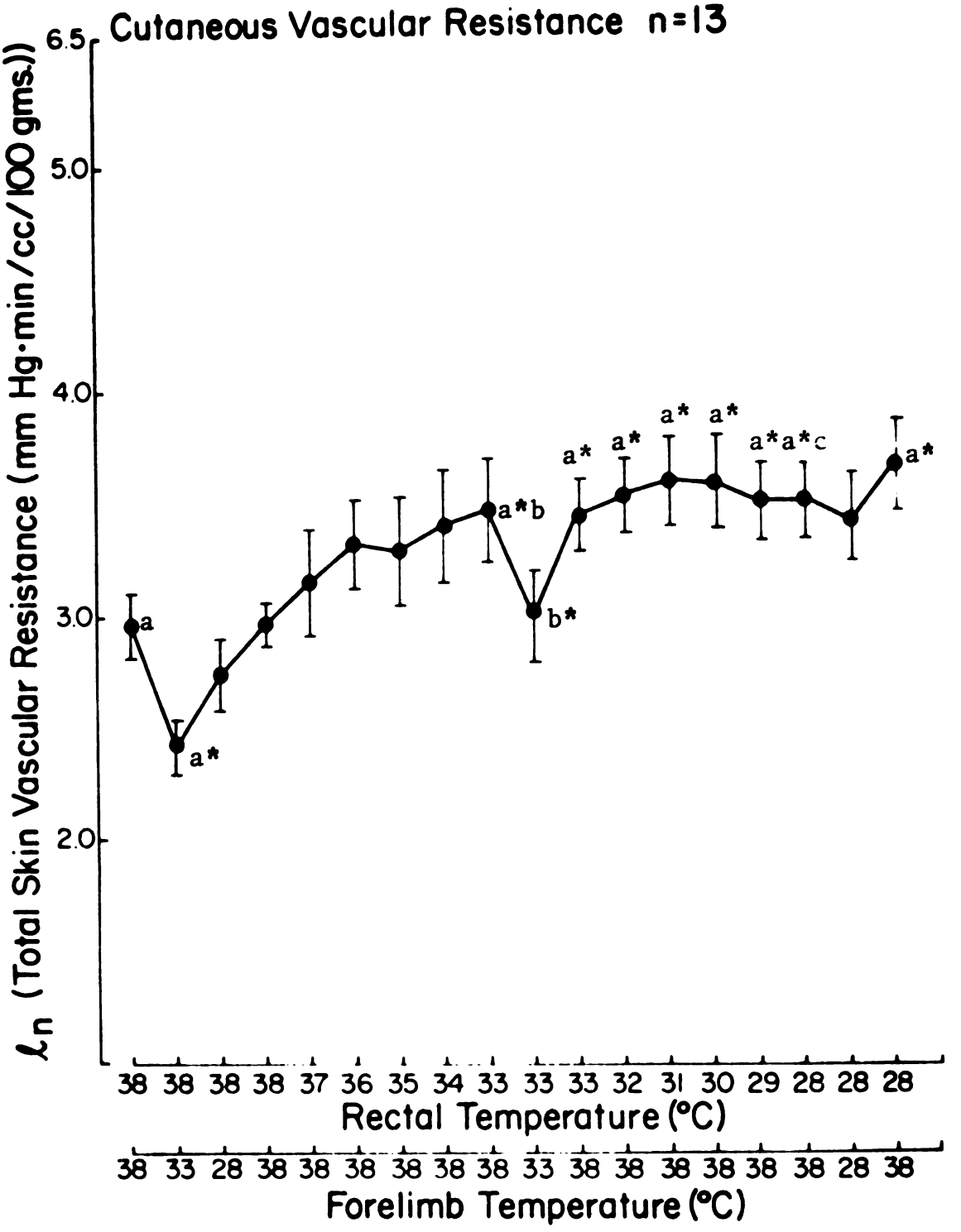
Cutaneous Vascular Resistance n=13
6.5
5.0
4.0
3.0
2.0
ln (Total Skin Vascular Resistance (mm Hg·min/cc/100 gms.))
a
a*
a*b
b*
a*
a*
a*
a*
a*a*c
a*
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 15

Cutaneous vascular resistance was calculated by dividing cephalic vein flow in ml per min per 100 gm forelimb weight, into the pressure head (brachial artery minus cephalic vein pressure). Data are expressed as logarithmic means $\pm$ standard errors (see Appendix C). In normothermic dogs, cooling arterial blood supply to the forelimb elicited a progressive fall in cutaneous vascular resistance which was statistically significant ($P < 0.05$) when brachial artery temperature reached 33°C. At this temperature, resistance in the skin vasculature was 18.3% below the normothermic control value and blood flow increased from 6.65 $\pm$ 0.78 to 11.06 $\pm$ 1.32 ml/min/100 gm. When blood perfusing the forelimb was locally cooled from 33 to 28°C there was a 11.5% increase in skin vascular resistance and blood flow at 8.44 $\pm$ 1.17 ml/min/100 gm. This change was not statistically significant (i.e., resistance at 28°C was not significantly different than that at 33°C) warming brachial artery blood to 38°C caused cutaneous vascular resistance to increase to a value not significantly different from the initial control resistance.

Whole-body cooling with brachial artery blood maintained normothermic elicited a progressive increase in skin vascular resistance which became statistically significant when rectal temperature reached 33°C. At this point vascular resistance in skin was 117% above the normothermic control value and cutaneous blood flow at 3.66 $\pm$ 0.70 ml/min/100 gm.

Rectal temperature was maintained at 33°C and forelimb perfusion temperature was reduced from 38 to 33°C. This maneuver caused cutaneous vascular resistance to decrease an average 13.7% ($P < 0.05$) and increased blood flow to 5.36 $\pm$ 1.05 ml/min/100 gm. Rewarming brachial artery blood to 38°C while rectal temperature remained at 33°C caused skin vascular resistance to return to a value not significantly different from that immediately prior to local cooling.

Forelimb perfusion temperature was maintained at 38°C and the animals were subjected to further systemic cooling which lowered rectal temperature to 28°C. This procedure elicited a slight but significant ($P < 0.05$) additional increase in forelimb cutaneous vascular resistance and decreased skin blood flow to 2.34 $\pm$ 0.39 ml/min/100 gm. Rectal temperature was maintained at 28°C and forelimb perfusion temperature was reduced from 38 to 38°C. This maneuver caused a slight decrease in the skin vascular resistance which was not statistically significant and increased blood flow to 2.84 $\pm$ 0.60 ml/min/100 gm. Rewarming brachial artery blood to 38°C while rectal temperature remained at 28°C caused a slight, insignificant increase in cutaneous vascular resistance. Therefore, local cooling of brachial artery blood causes active dilation in the forelimb cutaneous vascular segment. The magnitude of this dilation is greatest at a normal rectal temperature, is reduced significantly when rectal temperature has been lowered to 33°C, and

is essentially abolished at a rectal temperature of 28°C. Data described in the preceding paragraphs and reported in Figure 15 also indicate that systemic cooling elicits reflexly mediated, active vasoconstriction in the cutaneous vasculature of the normothermic forelimb.

C. Skeletal Muscle Vascular Resistance

Figure 16 reports the effects of local and whole-body hypothermia on vascular resistance in the segment supplied by the brachial artery and drained by the brachial vein. Procedures for normalizing and expressing the data are identical to those described for Figure 15. In normothermic dogs, cooling brachial artery blood elicited a progressive fall in skeletal muscle vascular resistance which was statistically significant ($P < 0.05$) when brachial artery temperature reached 33°C. At this temperature, resistance in the skeletal muscle vasculature was 11.6% below the normothermic control value and blood flow increased from 6.62 ± 0.76 to 9.01 ± 1.04 ml/min/100 gm. When brachial artery blood temperature was locally reduced from 33 to 28°C there was a 6.7% increase in muscle vascular resistance; this response was not statistically significant and blood flow at 7.41 ± 0.82 ml/min/100 gm. Rewarming brachial artery blood to 38°C caused forelimb skeletal muscle vascular resistance to return to a value not significantly different from the initial control resistance.

Figure 16. Effects of local and systemic cooling on total muscle vascular resistance in innervated, pump-perfused forelimbs. Ordinate shows ln total muscle vascular resistance and abcissae show rectal and forelimb temperature (°C). Data represent mean logarithmic values ± standard errors from 13 experiments.

a* is significant from a at 5% level.
b* is significant from b at 5% level.
c* is significant from c at 5% level.

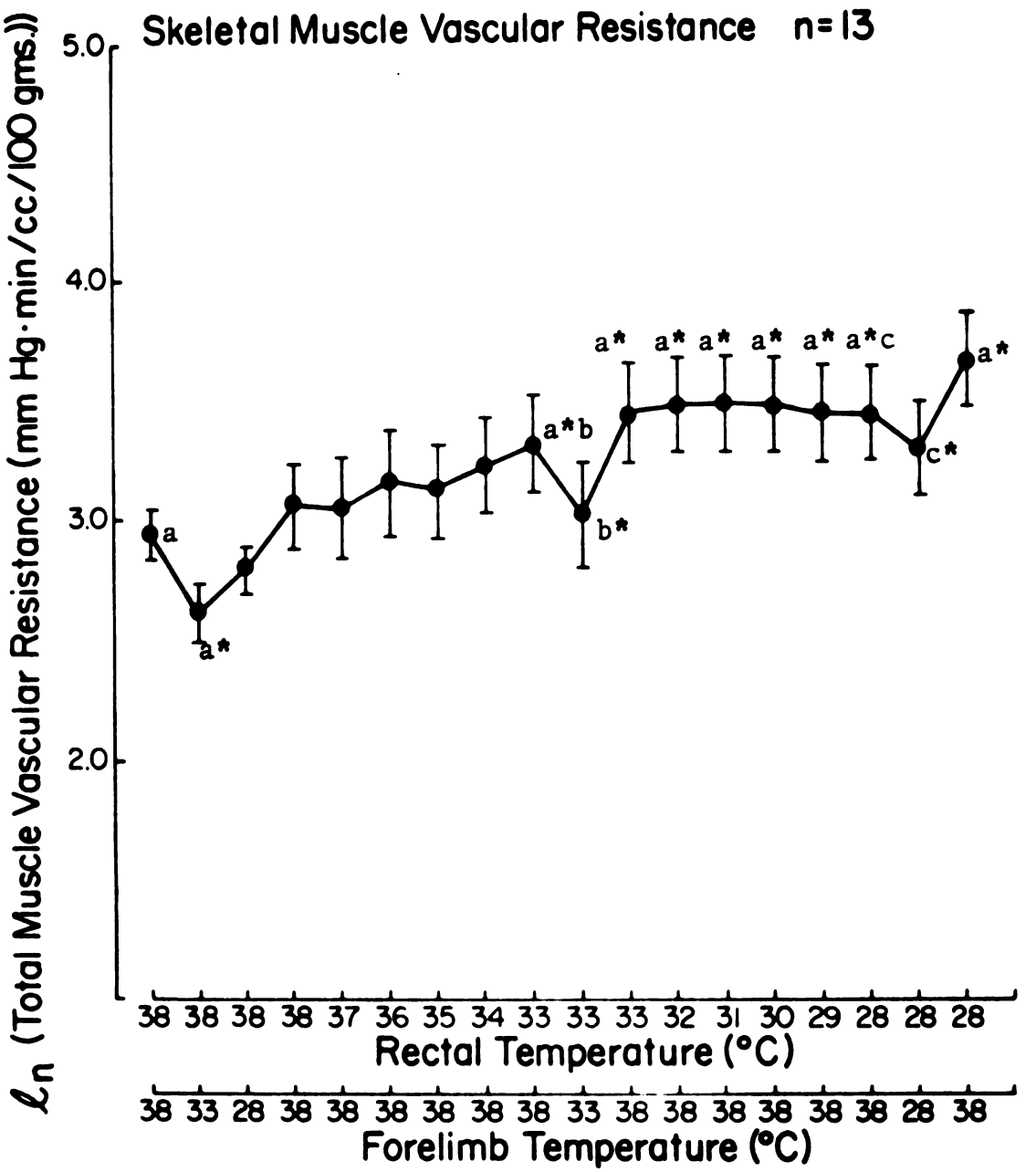
Skeletal Muscle Vascular Resistance n=13
ℓn (Total Muscle Vascular Resistance (mm Hg·min/cc/100 gms.))
5.0
4.0
3.0
2.0
a
a*
a*b
b*
a* a* a* a* a* a*c
c*
a*
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 16

Whole-body cooling with brachial artery blood maintained normothermic caused a progressive increase in muscle vascular resistance, with a maximum value observed at a rectal temperature of 33°C. This response was statistically significant ($P < 0.05$) and represented a 113% increase over the normothermic control value and brachial blood flow at 3.69 $\pm$ 0.56 ml/min/100 gm. Rectal temperature was maintained at 33°C and forelimb perfusion temperature was reduced from 38 to 33°C. This procedure caused a significant ($P < 0.05$) 8.9% decrease in muscle vascular resistance and increased blood flow to 5.17 $\pm$ 0.89 ml/min/100 gm. Rewarming the brachial artery blood to 38°C while rectal temperature remained at 33°C caused muscle vascular resistance to return to a value not significantly different from that immediately prior to local cooling.

Forelimb perfusion temperature was maintained at 38°C and the animals were subjected to further systemic cooling which lowered rectal temperature to 28°C. During this period, skeletal muscle vascular resistance remained significantly above the normothermic control value but did not display an additional increase (i.e., resistance at 32, 31, 30, 29 and 28°C was not significantly different from that at 33°C) and decreased muscle blood flow to 2.47 $\pm$ 0.35 ml/min/100 gm. Rectal temperature was maintained at 28°C and forelimb perfusion temperature was reduced from 38 to 28°C.

This maneuver caused the muscle vascular resistance to decrease significantly ($P < 0.05$) and increased blood flow to 3.20 ± 0.63 ml/min/100 gm. When brachial artery blood was rewarmed to 38°C while rectal temperature remained at 28°C, muscle vascular resistance increased to a value not significantly different from that immediately prior to local cooling. Therefore, local cooling of brachial artery blood causes active dilation in the forelimb skeletal muscle vascular segment. The magnitude of this dilation is greatest when at normal rectal temperature, and is reduced significantly when rectal temperature has been lowered to 33 and to 28°C. Data described in the preceding paragraphs and reported in Figure 16 also indicate that systemic cooling elicits reflexly mediated, active vasoconstriction in the skeletal muscle vasculature of the normothermic forelimb.

D. Total Forelimb Vascular Resistance

Figure 17 reports the effects of local and whole-body hypothermia on total forelimb vascular resistance; procedures for normalizing and expressing data are identical to those described for Figure 15. When brachial artery blood temperature was reduced to 33°C in normothermic dogs, there occurred a statistically significant decrease in total forelimb vascular resistance which averaged 19.6% of the normothermic control value. When brachial artery blood temperature was locally reduced from 33 to 28°C there was a

Figure 17. Effects of local and systemic cooling on total forelimb vascular resistance in innervated, pump-perfused forelimbs. Ordinate shows ln total forelimb vascular resistance and abcissae show rectal and forelimb temperatures (°C). Data represent mean logarithmic values ± standard errors from 13 experiments.

a* is significant from a at 5% level.
b* is significant from b at 5% level.
c* is significant from c at 5% level.

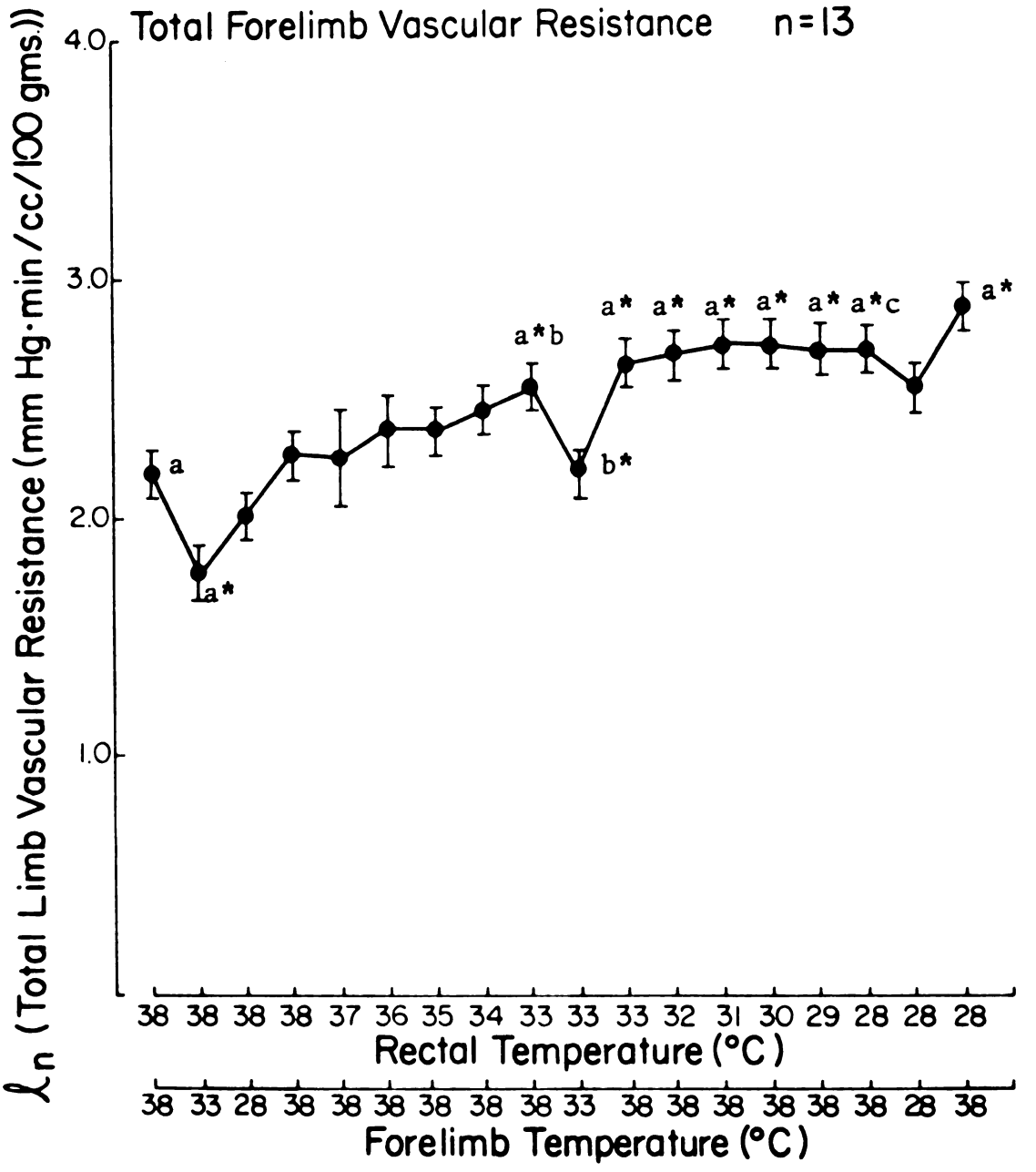
Total Forelimb Vascular Resistance n=13
4.0
3.0
2.0
1.0
ℓ_n (Total Limb Vascular Resistance (mm Hg·min/cc/100 gms.))
a
a*
a*b
b*
a* a* a* a* a* a*c
a*
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 17

12.2% increase in limb vascular resistance. This increase in resistance associated with cooling from 33 to 28°C was not statistically significant. Rewarming brachial artery blood to 38°C caused the total resistance in the forelimb circulation to return to a value not significantly different from the initial control resistance.

Whole-body cooling to 33°C while brachial artery blood was maintained normothermic caused a progressive increase in total forelimb vascular resistance. This response represented a 116% increase from the normothermic control value and was statistically significant. Rectal temperature was maintained at 33°C and brachial artery blood was cooled from 38 to 33°C. This procedure produced a 14.3% decrease in total forelimb resistance ($P < 0.05$). Rewarming brachial artery blood while rectal temperature was maintained at 33°C caused vascular resistance to return to a value not significantly different from that immediately prior to local cooling.

Further systemic cooling with the forelimb blood temperature maintaindd at 38°C elicited a slight additional increase in total forelimb vascular resistance with a maximum value observed when rectal temperature reached 31°C. At this point, total forelimb resistance was 124% above the normothermic control value. As rectal temperature was lowered from 31 to 28°C the limb vascular resistance

remained elevated and relatively constant. Rectal temperature was maintained at 28°C and brachial artery blood was cooled from 38 to 28°C. This procedure caused total forelimb vascular resistance to decrease by 5.9%, a response which was not statistically significant. Rewarming brachial artery blood to 38°C while rectal temperature remained at 28°C caused a similarly small, insignificant increase in total forelimb vascular resistance.

Table 4 reports the effects of local and systemic cooling on resistance in the skin, skeletal muscle and total forelimb vascular circuits. Data appear as logarithmic means ± standard errors for 13 experiments.

III. Series IV: Constant Pressure Perfusion, Denervated Forelimbs; Local and Whole-body Hypothermia

A. Forelimb Weight

Figure 18 reports the effects of local and of whole-body cooling on limb weight change for 9 experiments. In normothermic dogs, cooling the arterial blood supply to the forelimb elicited a significant ($P < 0.05$) weight gain which averaged 7.0 ± 2.0 gms when brachial artery temperature reached 33°C. The limbs remained isogravimetric at this elevated weight as long as brachial artery blood temperature was held at 33°C. Further local cooling from 33 to 28°C caused a small additional weight gain which averaged

Table 4. Effects of local and whole-body hypothermia on total skin and muscle vascular resistances as well as total limb vascular resistances in innervated forelimbs. R_{ST} = total skin resistance. R_{MT} = total muscle resistance. R_{FT} = total limb resistance. P_{BA} = brachial artery pressure in mm Hg. All vascular resistances are reported as logarithmic means ± S.E. from 13 experiments.

	Protocol	P_{BA}	R_{ST}	R_{MT}	R_{FT}
1.	38°C dog; 38°C limb (control)	121.7±3.7	2.963±0.140	2.947±0.121	2.192±0.058
2.	38°C dog; 33°C limb	121.3±3.8	2.421±0.122	2.606±0.133	1.763±0.070
3.	38°C dog; 28°C limb	121.0±3.7	2.736±0.155	2.793±0.114	2.010±0.078
4.	38°C dog; 38°C limb (post control)	118.3±3.6	2.957±0.131	3.080±0.148	2.257±0.087
5.	37°C dog; 38°C limb	103.1±2.7	3.153±0.230	3.049±0.220	2.250±0.157
6.	36°C dog; 38°C limb	110.6±3.0	3.322±0.232	3.160±0.210	2.369±0.152
7.	35°C dog; 38°C limb	96.4±5.7	3.286±0.252	3.130±0.166	2.356±0.100
8.	34°C dog; 38°C limb	94.2±5.7	3.400±0.258	3.234±0.158	2.450±0.090
9.	33°C dog; 38°C limb (control)	92.6±5.6	3.471±0.233	3.318±0.162	2.542±0.080
10.	33°C dog; 33°C limb	90.8±6.2	2.995±0.191	3.022±0.220	2.178±0.120
11.	33°C dog; 38°C limb (post control)	90.2±6.2	3.447±0.178	3.436±0.161	2.641±0.089
12.	32°C dog; 38°C limb	81.5±7.5	3.541±0.208	3.476±0.158	2.674±0.086
13.	31°C dog; 38°C limb	80.0±7.8	3.605±0.201	3.481±0.158	2.713±0.081
14.	30°C dog; 38°C limb	77.7±8.0	3.594±0.202	3.477±0.159	2.709±0.090
15.	29°C dog; 38°C limb	76.0±8.5	3.510±0.178	3.441±0.156	2.678±0.104
16.	28°C dog; 38°C limb (control)	72.9±7.9	3.516±0.171	3.444±0.166	2.689±0.111
17.	28°C dog; 28°C limb	72.6±7.9	3.434±0.214	3.287±0.204	2.530±0.132
18.	28°C dog; 38°C limb (post control)	71.9±8.0	3.673±0.194	3.656±0.168	2.862±0.120

Figure 18. Weight changes (grams) to local and systemic cooling in denervated, pump-perfused forelimbs. Ordinate shows limb weight changes (grams) and abcissae show rectal and forelimb temperatures (°C). Data represent mean values ± standard errors in 9 experiments.

a* is significant from a at 5% level.
b* is significant from b at 5% level.
c* is significant from c at 5% level.

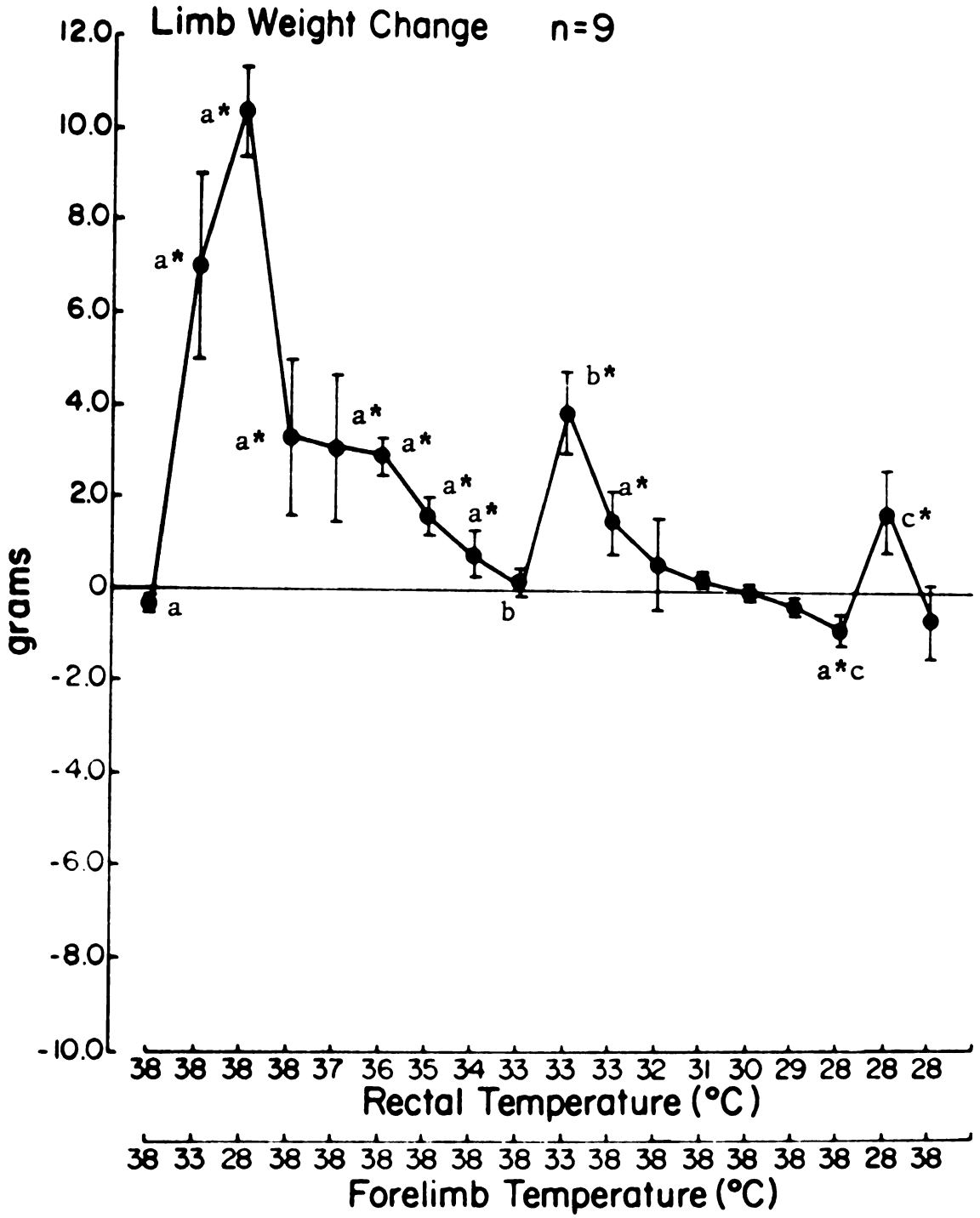
Limb Weight Change n=9
12.0
10.0
8.0
6.0
4.0
2.0
0
-2.0
-4.0
-6.0
-8.0
-10.0
grams
a*
a*
a*
a*
a*
a*
a*
b*
a*
c*
a
b
a*c
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 18

3.4 ± 1.0 grams and was not statistically significant. Rewarming brachial artery blood to 38°C caused limb weight to return toward but stabilize at a value significantly ($P < 0.05$) above the initial control weights (venous pressures and blood flows were significantly elevated at this point as shown in Appendix B_2).

Whole-body cooling with brachial artery blood maintained normothermic elicited a progressive fall in limb weight. When rectal temperature reached 33°C, limb weight had fallen 5.5 ± 0.3 grams and was significantly ($P < 0.05$) below the value recorded immediately before the onset of whole-body cooling. It was not, however, different from the original normothermic control weight. Rectal temperature was maintained at 33°C and forelimb perfusion temperature was reduced from 38 to 33°C. This maneuver caused a significant ($P < 0.05$) weight gain which averaged 3.7 ± 0.5 grams. Rewarming brachial artery blood to 38°C while rectal temperature remained at 33°C caused forelimb weight to fall 2.4 ± 0.7 grams to a value not significantly different from that immediately before local cooling.

Forelimb perfusion temperature was maintained at 38°C and the animals were subjected to further systemic cooling which elicited an additional, progressive weight loss. When rectal temperature reached 28°C, weight of the normothermic limbs was significantly (3.9 grams; $P < 0.05$) below the value

recorded immediately before the onset of whole-body cooling. Limb weight at rectal temperature 28°C and limb temperature 38°C was not, however, different from the original normothermic control value. Rectal temperature was maintained at 28°C and forelimb perfusion temperature was reduced from 38 to 28°C. This maneuver caused a significant increase ($P < 0.05$) in limb weight which averaged 2.6 $\pm$ 0.9 grams. Rewarming brachial artery blood to 38°C while rectal temperature remained at 28°C caused limb weight to fall an average of 2.4 $\pm$ 0.8 grams, to a value not different from that immediately before the local cooling procedure. Therefore, at the end of the experiment, rectal temperature was 28°C, forelimb perfusion temperature 28°C and limb weight was significantly below that recorded immediately before the onset of whole-body cooling, but was not significantly different from the original normothermic control weight.

B. Cutaneous Vascular Resistance

Figure 19 reports the effects of local and whole-body hypothermia on vascular resistance in the segment supplied by the brachail artery and drained by the cephalic vein. Cutaneous vascular resistance was calculated by dividing cephalic vein flow in ml per min per 100 gm forelimb weight, into the pressure head (brachial artery minus cephalic vein pressure). Data are expressed as logarithmic means $\pm$ standard errors (see Appendix C). In normothermic dogs,

Figure 19. The resistance response of the cutaneous circulation to alterations in local and systemic cooling in denervated, pump-perfused forelimbs. Ordinate shows ln total skin vascular resistance and abcissae show rectal and forelimb temperature (°C). Data represent mean logarithmic values ± standard errors from 9 experiments.

b* is significant from b at 5% level.

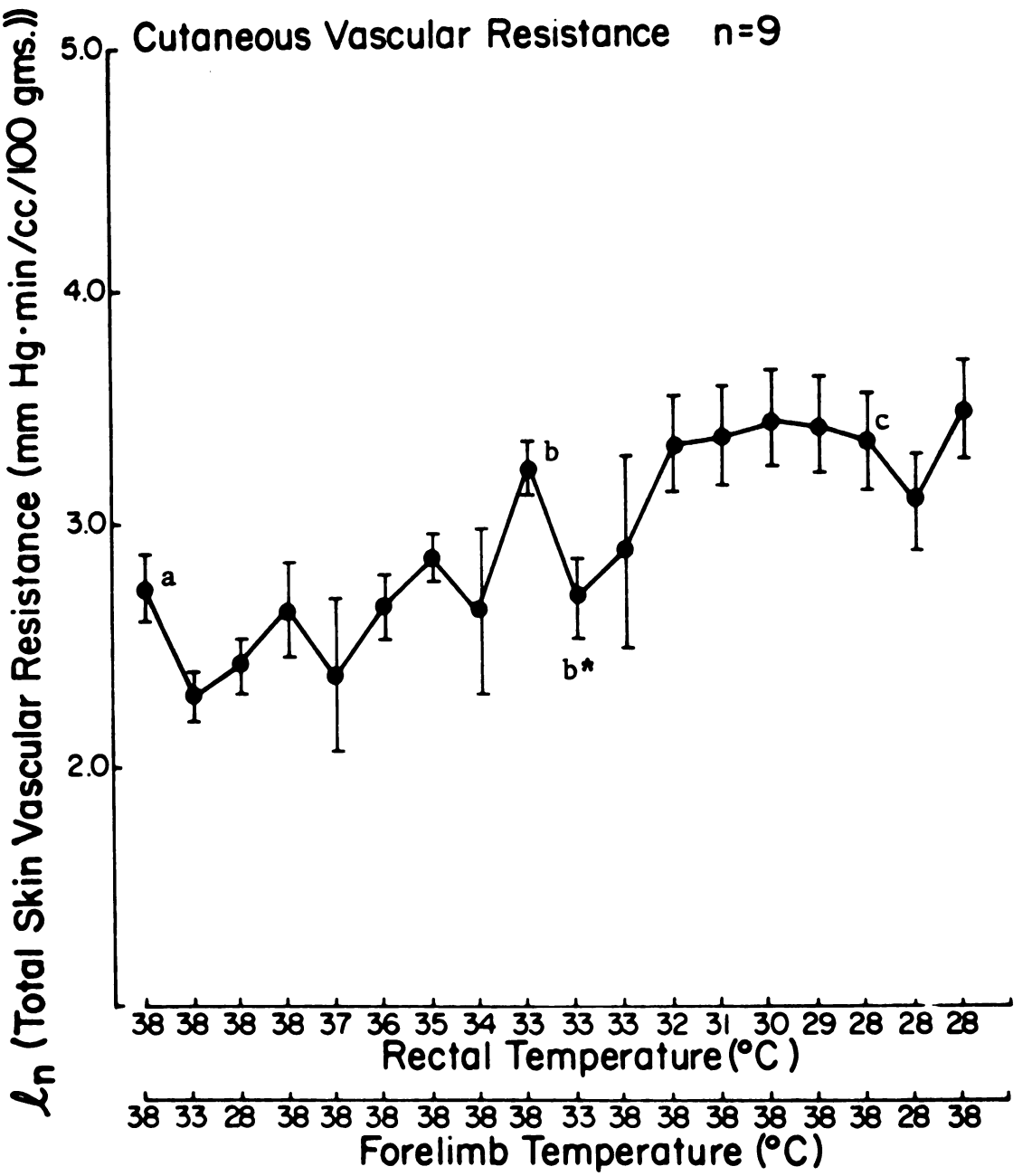
Cutaneous Vascular Resistance n=9
5.0
4.0
3.0
2.0
$\mathcal{L}_n$ (Total Skin Vascular Resistance (mm Hg·min/cc/100 gms.))
a
b
b*
c
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 19

cooling brachial artery blood to 33 and then to 28°C elicited a slight fall in cutaneous vascular resistance which was not statistically significant and skin blood flow increased from 7.95 ± 1.12 to 11.67 ± 1.43 at 33°C and then to 10.40 ± 1.57 ml/min/100 gms at 25°C. Rewarming brachial artery blood to 38°C caused a correspondingly small increase in cutaneous vascular resistance.

Whole-body cooling with brachial artery blood maintained normothermic elicited a slight increase in cutaneous vascular resistance which was not statistically significant at any point in the hypothermia protocol. While rectal temperature was maintained at 33°C, forelimb perfusion temperature was reduced from 38 to 33°C. This maneuver caused cutaneous vascular resistance to decrease by an average 16.7% and blood flow increased from 3.17 ± 0.38 to 5.95 ± 1.27 ml/min/100 gms. This response, although quite small, was statistically significant ($P < 0.05$). Rewarming brachial artery blood to 38°C while rectal temperature remained at 33°C caused skin vascular resistance to return to an average value not signifidantly different from that immediately before local cooling.

Forelimb perfusion temperature was maintained at 38°C and the animals were subjected to further systemic cooling. Rectal temperature was then maintained at 28°C and forelimb perfusion temperature was reduced from 38 to 28°C.

This maneuver caused a slight decrease in cutaneous vascular resistance which was not statistically significant and skin blood flow increased slightly from 2.21 ± 0.30 to 2.92 ± 0.53 ml/min/100 gms. Rewarming brachial artery blood to 38°C while rectal temperature remained at 28°C elicited a corresponding small increase in cutaneous vascular resistance. Therefore, local cold exposure appears to elicit a slight dilation of the cutaneous vascular segment in denervated forelimbs. This response is appreciably less than that observed for innervated limbs subjected to the same stimulus. Data reported in Figure 19 also indicate that acute denervation eliminates the cutaneous vasoconstriction observed in innervated limbs during whole-body hypothermia.

C. Skeletal Muscle Vascular Resistance

Figure 20 reports the effects of local and whole-body hypothermia on vascular resistance in the segment supplied by the brachial artery and drained by the brachial vein. Procedures for calculating, normalizing, and expressing data are identical to those described for Figure 19. In normothermic dogs, cooling brachial artery blood to 33 and then to 28°C elicited a slight fall in skeletal muscle vascular resistance which was not statistically significant and muscle blood flow increased from 5.21 ± 0.68 to 8.14 ± 1.33 at 23°C and then to 7.64 ± 7.17 ml/min/100 gms at 28°C. Rewarming brachial artery blood to 38°C caused a corresponding small increase in skeletal muscle vascular resistance.

Figure 20. Effects of local and systemic cooling on total muscle vascular resistance of denervated pump-perfused forelimbs. Ordinate shows in total muscle vascular resistance and abcissae show rectal and forelimb temperature (°C). Data represent mean logarithmic values ± standard errors from 9 experiments.

a* is significant from a at 5% level.

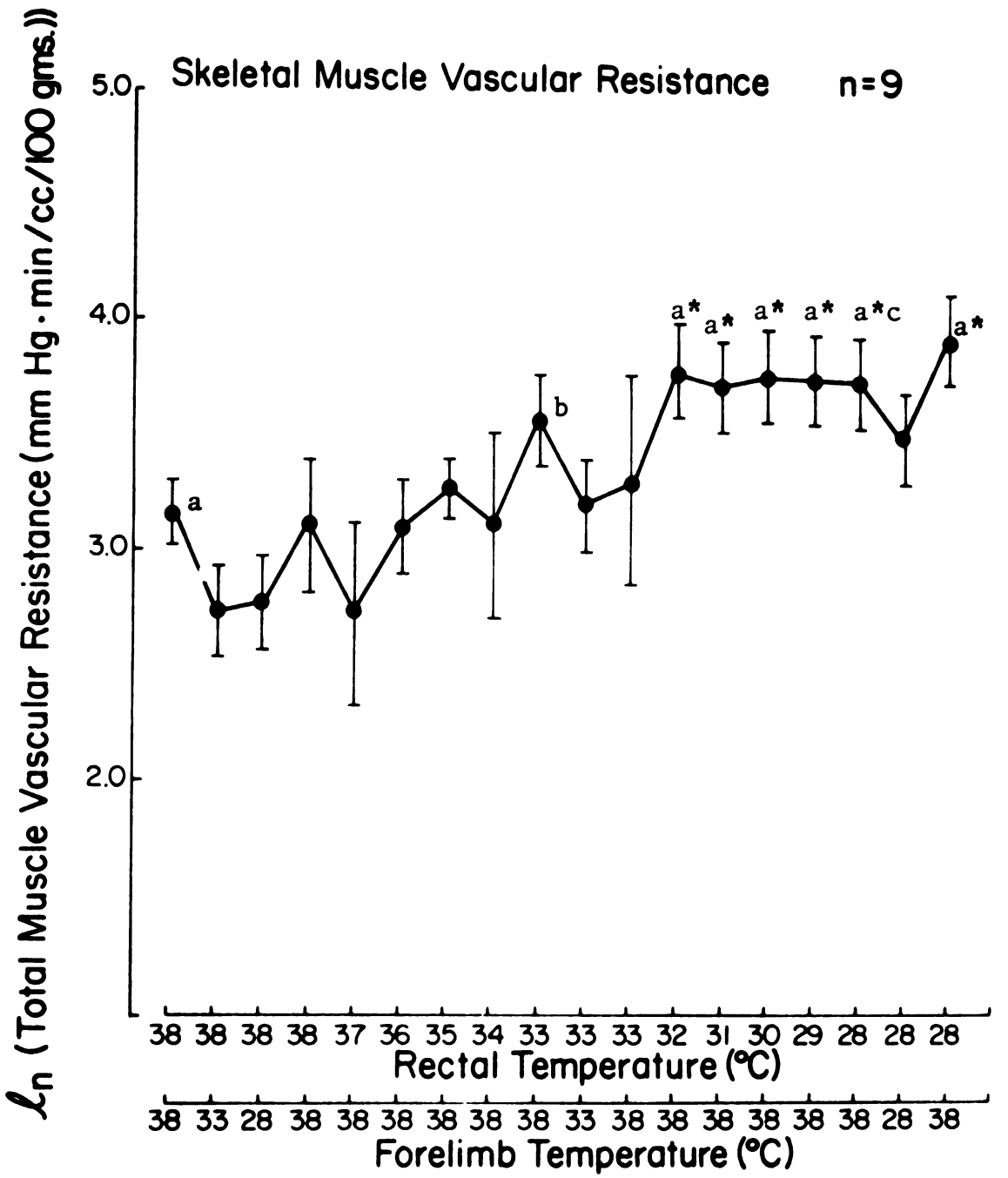
Skeletal Muscle Vascular Resistance n=9
$\mathcal{L}_n$ (Total Muscle Vascular Resistance (mm Hg · min/cc/100 gms.))
5.0
4.0
3.0
2.0
a
b
a* a* a* a* a*c a*
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 20

Lowering rectal temperature to 33°C while brachial artery blood was maintained at 38°C elicited a slight increase in skeletal muscle vascular resistance which was not statistically significant. Rectal temperature was maintained at 33°C and forelimb perfusion temperature was reduced from 38 to 33°C. This procedure caused a 10.5% decrease in muscle vascular resistance and increased blood flow from 2.35 $\pm$ 0.30 to 3.64 $\pm$ 0.66 ml/min/100 gms. This change was not statistically significant and rewarming brachial artery blood to 38°C caused a correspondingly small increase in resistance.

Core temperature was reduced further while brachial artery blood was maintained at 38°C. This procedure elicited an increase in skeletal muscle vascular resistance which was statistically significant ($P < 0.05$) at a rectal temperature of 32°C and below (i.e., skeletal muscle vascular resistance was above the original normothermic control value) and decreased blood flow to 1.56 $\pm$ 0.23 ml/min/100 gms. Rectal temperature was maintained at 28°C and forelimb perfusion temperature was reduced from 38 to 28°C. This maneuver caused a slight decrease in muscle vascular resistance which was not statistically significant and muscle blood flow increased to 2.16 $\pm$ 0.49 ml/min/100 gms. Rewarming brachial artery blood to 38°C while rectal temperature remained at 28°C caused a correspondingly small increase in muscle vascular resistance.

Therefore, acute denervation essentially eliminates the vasodilatory effect of local cold exposure to forelimb skeletal muscle. The data described in the preceding paragraphs and reported in Figure 20 also suggest that systemic cooling elicits humorally mediated vasoconstriction since resistance in the denervated skeletal muscle vasculature increased significantly at rectal temperatures below 33°C.

D. Total Forelimb Vascular Resistance

Figure 21 reports the effects of local and whole-body hypothermia on total forelimb vascular resistance; procedures for normalizing and expressing data are identical to those described for Figure 19. Locally cooling brachial artery blood to 33°C and then to 28°C elicited a slight decrease in total forelimb vascular resistance which was not statistically significant. Rewarming brachial artery blood to 38°C caused a correspondingly small increase in total forelimb vascular resistance.

Lowering rectal temperature from 38 to 33°C while brachial artery blood was maintained normothermic failed to elicit a significant change in total forelimb vascular resistance. Rectal temperature was maintained at 33°C and forelimb perfusion temperature reduced from 38 to 33°C. This procedure produced an 18.9% decrease in total forelimb vascular resistance ($P < 0.05$). Rewarming of brachial artery

Figure 21. Effects of local and systemic cooling on total vascular resistance in denervated, pump-perfused forelimbs. Ordinate shows in total forelimb vascular resistance and abscissae show rectal and forelimb temperatures (°C). Data represent mean logarithmic values ± standard errors from 9 experiments.

a* is significant from a at 5% level.
b* is significant from b at 5% level.

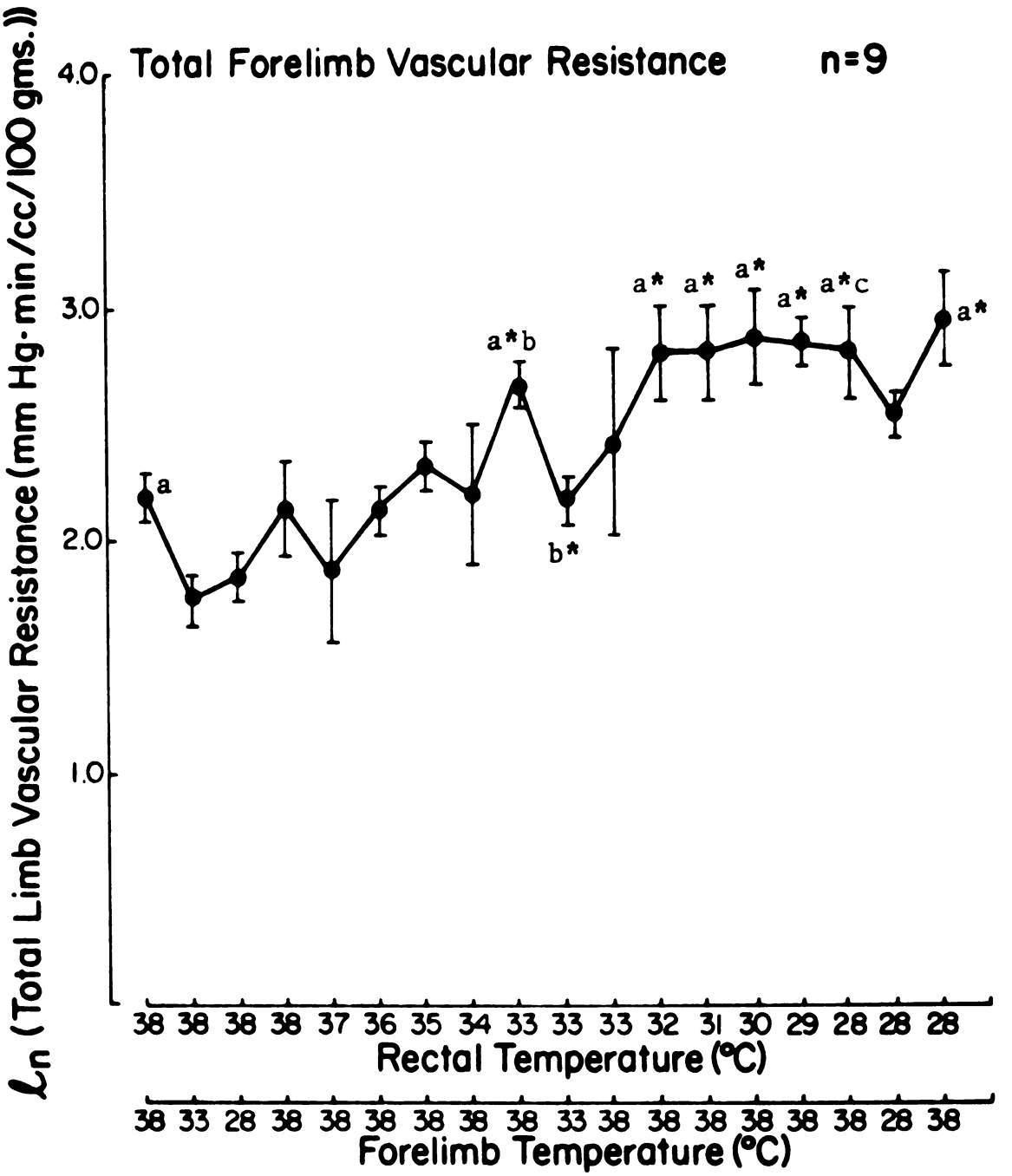
Total Forelimb Vascular Resistance n=9
L_n (Total Limb Vascular Resistance (mm Hg·min/cc/100 gms.))
4.0
3.0
2.0
1.0
a
a*b
b*
a* a* a* a* a*c a*
38 38 38 38 37 36 35 34 33 33 33 32 31 30 29 28 28 28
Rectal Temperature (°C)
38 33 28 38 38 38 38 38 38 33 38 38 38 38 38 38 28 38
Forelimb Temperature (°C)

Figure 21

blood while rectal temperature was maintained at 33°C caused total forelimb vascular resistance to return to a value not significantly different from that immediately prior to local cooling.

Further systemic cooling with brachial artery blood temperature maintained at 38°C elicited a significant ($P < 0.05$) increase in total forelimb vascular resistance. Rectal temperature was maintained at 28°C and brachial artery blood was cooled from 38 to 28°C. This procedure caused a slight decrease in total forelimb vascular resistance which was not statistically significant. Rewarming brachial artery blood to 38°C while rectal temperature remained at 28°C caused a correspondingly small increase in vascular resistance of the circuit supplied by the brachial artery and drained by the brachial and cephalic veins.

Table 5 shows the logarithmic values of the denervated total skin and muscle vascular resistances as well as total forelimb vascular resistance as influenced by local and systemic hypothermia.

Table 5. Effects of local and whole-body hypothermia on total skin and muscle vascular resistances as well as total limb vascular resistances in innervated forelimbs. R_{ST} = total limb resistance. P_{BA} = brachial artery pressure in mm Hg. All vascular resistances are reported as logarithmic means ± S.E. from 13 experiments.

	Protocol	P_{BA}	R_{ST}	R_{MT}	R_{FT}
1.	38°C dog; 38°C limb (control)	118.2± 2.6	2.739±0.145	3.165±0.131	2.193±0.083
2.	38°C dog; 33°C limb	115.6± 3.4	2.292±0.104	2.725±0.187	1.752±0.095
3.	38°C dog; 28°C limb	115.3± 3.3	2.424±0.128	2.768±0.182	1.848±0.111
4.	38°C dog; 38°C limb (post control)	118.8± 2.6	2.667±0.230	3.123±0.263	2.141±0.182
5.	37°C dog; 38°C limb	97.5±14.2	2.363±0.325	2.734±0.366	1.873±0.246
6.	36°C dog; 38°C limb	101.4± 6.7	2.669±0.140	3.103±0.148	2.128±0.089
7.	35°C dog; 38°C limb	96.1± 6.0	2.872±0.112	3.277±0.133	2.323±0.065
8.	34°C dog; 38°C limb	77.5±11.5	2.643±0.346	3.115±0.418	2.199±0.285
9.	33°C dog; 38°C limb (control)	80.3± 6.4	3.248±0.127	3.583±0.164	2.671±0.101
10.	33°C dog; 33°C limb	80.0± 6.3	2.706±0.174	3.207±0.218	2.167±0.133
11.	33°C dog; 38°C limb (post control)	67.8±10.2	2.898±0.421	3.320±0.460	2.421±0.347
12.	32°C dog; 38°C limb	76.4± 7.0	3.337±0.194	3.785±0.184	2.799±0.151
13.	31°C dog; 38°C limb	72.2± 7.8	3.373±0.192	3.731±0.194	2.798±0.153
14.	30°C dog; 38°C limb	71.1± 7.9	3.451±0.206	3.780±0.177	2.867±0.160
15.	29°C dog; 38°C limb	70.0± 7.6	3.422±0.180	3.769±0.173	2.846±0.140
16.	28°C dog; 38°C limb (control)	65.3± 8.9	3.358±0.185	3.755±0.185	2.808±0.153
17.	28°C dog; 28°C limb	64.7± 8.8	3.113±0.166	3.507±0.190	2.539±0.132
18.	28°C dog; 38°C limb (post control)	67.8± 5.7	3.485±0.210	3.951±0.236	2.949±0.175

DISCUSSION

I. Series I and II: Naturally Perfused, Innervated Forelimbs

A. Forelimb Weight

The fall in forelimb weight that accompanied hypothermia appears to be due primarily to decreases in skin and skeletal muscle vascular capacity. This conclusion is supported by the concomitant increase in forelimb venous resistance. An additional, slow reduction in forelimb weight occurred throughout the two-hour hypothermia period (28°C rectal temperature) and can likely be attributed to net transvascular fluid reabsorption caused by a fall in capillary hydrostatic pressure. This interpretation is supported by the reduced small vein pressures observed throughout the two-hour hypothermia period. Furthermore, the pre- to post-capillary resistance ratio almost certainly was increased during this period as evidenced by a marked elevation in small vessel segment resistance as shown in Figures 9 and 12.

Haddy *et al.* (19) report that whole-body cold exposure elicits venous constriction. However, they observed that cooling caused a net gain in limb weight which apparently resulted from the mode of forelimb perfusion. Haddy *et al.*

(19) studied limbs which were pump-perfused at constant flow so that any increase in venous resistance would cause capillary hydrostatic pressure to rise even though precapillary resistance also increased. The weight gain which they observed appears, therefore, to have been an experimental artifact. In our study (Series I), the limbs were naturally perfused so that capillary hydrostatic pressure was determined by arterial and venous pressure and by the pre- to postcapillary resistance ratio as described by Pappenheimer and Soto-Rivera (48):

$$Pc = \frac{\frac{rv}{ra} \cdot Pa + Pv}{1 + \frac{rv}{ra}}$$

Where: P = pressure
r = resistance
a = precapillary
v = postcapillary

Data obtained in Series I demonstrate conclusively that whole-body cooling and sustained hypothermia cause fluid to be relocated from peripheral to more central parts of the circulation due to reductions in venous capacity and capillary hydrostatic pressure. Since forelimbs in Series I were innervated, these responses were presumably mediated by both adrenergic nerves and circulating vasoconstrictors.

B. Forelimb Vascular Resistance

Cooling and sustained hypothermia elicited increased resistance in all forelimb vascular segments. For both skin and skeletal muscle, these increases in resistance did not become statistically significant until rectal temperature decreased from 37.5 to 33°C at which point mean systemic arterial blood pressure had fallen from 121 to 90 mm Hg (Figure 6). Active as well as passive vasodilation may have occurred. Under normothermic conditions, the baroreceptor reflex initiates vasoconstriction much sooner than observed in this study (i.e., a much smaller fall in blood pressure causes reflexly mediated constriction of the forelimb vasculature). Apparently the baroreceptor reflex is attenuated by hypothermia, an effect which could be due to: 1) cold-induced suppression of pressure receptors and/or neurons as has been suggested by Thauer (65), 2) cold-induced vasodilation, 3) or both. As rectal temperature was reduced below 33°C, blood pressure continued to fall, and associated with this fall was a rapid rise in skin and muscle vascular resistance which we attributed primarily to a sympathico-adrenal induced increase in vessel hinderance rather than to increased blood viscosity. However, elevated blood viscosity, due to the effect of cooling on shear stress (35) may have contributed somewhat to the increased resistances observed during this period.

II. Series III and Series IV: Constant Pressure Perfusion, Innervated and Denervated Forelimbs; Local and Whole-body Hypothermia

A. General Considerations

Series III and IV investigated the relative contributions of local cold-induced vasodilation, sympathetic nerve activity, and circulating vasoactive substances in controlling forelimb hemodynamics at lowered body temperature. Results from Series I revealed that whole-body cooling elicited an increase in skin and skeletal muscle vascular resistance and a decrease in limb weight. The experiment did not, however, provide direct information regarding mechanisms responsible for these changes.

B. Forelimb Weight

Figures 14 and 18 report the effects of local and systemic cooling on weight change in innervated and denervated forelimbs. The weight gain associated with local cooling is ascribed primarily to increased intravascular capacity rather than to net transcapillary filtration. If the opposite were true, the limbs would, predictably, continue to gain weight throughout local hypothermia. The period of gain was, in fact, relatively brief so that limb weight plateaued soon after brachial artery blood temperature reached 33 or 28°C. Therefore, local hypothermia appears to dilate both arterial and venous segments causing an increase

in blood flow and vascular capacity. Capillary hydrostatic pressure (Pc) apparently is not altered during local, cold-mediated, dilation. This conclusion can be supported by comparing the blood flow and limb weight responses which occurred when brachial artery temperature was reduced from 38 to 33°C. In contrast, the limbs gained weight during the period when arterial perfusate temperature was falling but became isogravimetric soon after temperature plateaued at 33°C. The condition of elevated blood flow and unchanging limb weight prevailed as long as the temperature was held at 33°C indicating that mean Pc in the forelimb had not increased or increased mean Pc could cause filtration to increase but was accompanied by an equal increase in lymph flow. Several factors probably contributed to the maintenance of a constant Pc in the face of increased blood flow. First of all, the effect of a fall in precapillary resistance on Pc was at least partly counteracted by postcapillary dilation. Secondly, some of the fall in precapillary resistance may have been due to a relaxation of sphincters, which would cause part of the extra blood flow to be channeled through additional, parallel-coupled capillaries, thereby buffering the rise in Pc normally associated with precapillary dilation. Finally, local cold exposure may have opened arterial-venous anastomoses to provide a route for part of the extra blood flow to by-pass microcirculatory exchange vessels.

Some combination of these three responses apparently kept average Pc in the forelimb constant during the period of increased blood flow caused by local cold exposure. It is possible that cold temperature reduces capillary permeability thereby influencing Pc.

There was no significant difference between the amount of weight gained by innervated and denervated forelimbs during any of the local cooling procedures, an observation which suggests that the cold-induced increase in forelimb vascular capacity is due to a direct inhibition of venomotor tone by lowered temperature. Two other possible explanations appear to be ruled out; namely, that cold exposure causes venous dilation by: a) inhibiting resting, neurally mediated venoconstrictor tone, b) activating a neurally mediated vasodilator reflex or c) activating an axon reflex. The responses at rectal 33°C and limb 33°C as well as rectal 28°C and limb 28°C reveal that cold elicited venodilation is attenuated by the increased sympathico-adrenal activity which accompanies whole-body hypothermia but without constant flow strong evidence for active venodilation is not apparent.

Since the fall in limb weight associated with systemic hypothermia was attenuated by denervation, we concluded that this response is due, in part, to sympathetically mediated vasoconstriction which acts to decrease intravascular

capacity and lower Pc by increasing the pre- to postcapillary resistance ratio. Webb-Peploe and Shephard (72) reported that 60% of the saphenous vein constriction elicited by systemic hypothermia could be abolished by sympathectomy. The remaining 40%, which was judged to be humorally mediated, could be eliminated by alpha adrenergic blockade. Our findings appear to be quite similar since denervation attenuated but did not abolish the reduction in vascular capacity caused by systemic hypothermia.

C. Forelimb Vascular Resistance

Systemic cooling caused skin vascular resistance to increase significantly in innervated but not in denervated forelimbs. This observation indicates that adrenergic nerves rather than vasopressor hormones are the primary mediators of hypothermia-induced cutaneous vasoconstriction.

Systemic cooling to 33°C and below caused a significant elevation in the skeletal muscle vascular resistance of both innervated and denervated forelimbs. This observation indicates that vasopressor hormones are important mediators of the skeletal muscle vascular constriction elicited by whole-body cooling.

In normothermic dogs with innervated forelimbs, part of the resting cutaneous vasomotor tone is apparently mediated by sympathetic nerves. Local cooling to 33°C decreases skin vascular resistance (Figure 15), perhaps by counteracting

neurally mediated constriction by acting as an anesthetic. It is also remotely possible that local cooling may activate a neural vasodilator reflex. When the forelimbs of these same normothermic dogs were cooled from 33 to 38°C, there was a tendency for cutaneous vascular resistance to increase slightly but not significantly. This response may have resulted from constriction of the skin vessels, but in view of the preceding dilatory response when limb temperature was reduced to 33°C, it appears more likely that a rise in blood viscosity was responsible for the slight rise in resistance associated with cooling from 33 to 28°C.

In normothermic dogs with denervated forelimb, initial cutaneous vascular resistance (Figure 19) was lower than that observed for the innervated limbs and local cooling to 33°C failed to elicit a significant dilation. This observation supports our contention that the local cold-induced dilation observed in innervated limbs results either from antagonism of neurally mediated vasoconstrictor tone, or less likely from activation of a neural vasodilator reflex. Thus, when the initial, neurally mediated constriction and the capacity for reflex dilation are removed before local cooling to 33°C, this maneuver no longer elicits dilation.

In innervated, normothermic limbs of dogs made hypothermic (rectal temperature = 33°C) there occurred an increased cutaneous vascular resistance (Figure 15) due to

neural and/or humoral vasoconstriction. Local cooling to 33°C caused forelimb cutaneous vascular resistance in these animals to decrease significantly. Therefore, when vasomotor tone is elevated, in this case by systemic hypothermia, local cooling is able to antagonize this extrinsically mediated constriction and produce a substantial decrease in cutaneous vascular resistance.

In denervated, normothermic limbs, systemic cooling did not elicit a significant rise in cutaneous vascular resistance (Figure 19). There was, however, a consistent tendency for resistance in the skin vasculature to increase as rectal temperature approached 33°C suggesting that vasomotor tone may have been elevated by this procedure. When limb temperature was reduced from 38 to 33°C there was a significant decrease in cutaneous vascular resistance which may have been at least partly due to cold antagonism of humorally mediated constriction.

With deeper hypothermia (rectal temperature = 28°C) local cooling from 38 to 28°C failed to elicit a significant dilation in either innervated or denervated limbs, perhaps because the effect of any increase in vessel radius which may have occurred was counteracted by a concomitant rise in blood viscosity.

In normothermic dogs, local cooling to 33°C caused a small dilation in the skeletal muscle vasculature of both

innervated and denervated forelimbs (Figure 16 and 20). This response may have been mediated by a direct effect of cold on the tonically active pacemaker cells which are known to be responsible for basal vascular tone in skeletal muscle. Local cooling from 33 to 28°C failed to elicit an additional fall in resistance, perhaps because any further reduction in vascular hinderance was offset by an increase in blood viscosity. Skeletal muscle vascular resistance did not increase significantly in the innervated or denervated, normothermic limbs of dogs made hypothermic (rectal temperature = 33°C). This observation suggests that moderate whole-body cooling elicits sympathetic stimulation of cutaneous but not of skeletal muscle blood vessels. The small dilatory response to local cooling did not appear to be modified when rectal temperature had been reduced to 33°C. When rectal temperature had stabilized at 28°C, lowering blood temperature from 38 to 28°C consistently elicited a very slight decrease in skeletal muscle vascular resistance again because any reduction in vascular hinderance caused by this procedure may have been largely offset by an increased blood viscosity.

SUMMARY AND CONCLUSIONS

1. The fall in forelimb weight that accompanied systemic hypothermia appears to be due primarily to decreases in skin and skeletal muscle vascular capacity. This conclusion is supported by the concomitant increase in forelimb venous resistance (Series I and III). Since the fall in limb weight associated with systemic hypothermia was attenuated by acute denervation (Series IV), we concluded that this response is due, in part, to sympathically mediated vasoconstriction which acts to decrease intravascular capacity and lower capillary hydrostatic pressure by increasing the pre- to postcapillary resistance ratio.

2. The weight gain associated with local cooling is ascribed primarily to increased intravascular capacity rather than to net ranscapillary filtration. The period of gain was relatively brief so that limb weight plateaued soon after brachial artery blood temperature reached 33 or 28°C. Therefore, local hypothermia appears to dilate both arterial and venous segments causing an increase in blood flow and vascular capacity. Since there was no significant difference between the amount of weight gain by innervated and denervated forelimbs during any of the local cooling procedures,

we conclude that the cold-induced increase in forelimb vascular capacity is due to a direct inhibition of venomotor tone by lowered temperature.

3. Whole-body cooling and hypothermia (Series I) elicited increased resistance in all forelimb vascular segments. The increase did not become significant until rectal temperature decreased from 37.5 to 33°C at which point mean systemic arterial blood pressure had fallen from 121 to 90 mm Hg. As rectal temperature was reduced below 33°C, blood pressure continued to fall, and associated with this fall was a rapid rise in skin and muscle vascular resistance which we attribute primarily to a sympathico-adrenal-induced increase in vessel hinderance. Acute denervation of the forelimb (Series IV) attenuated the increase in skin vascular resistance, an observation indicating that adrenergic nerves rather than vasopressor hormones are the primary mediators of hypothermis-induced cutaneous vasoconstriction. Systemic cooling to 33°C and below caused a significant elevation in the skeletal muscle vascular resistance of both innervated and denervated forelimbs. This observation indicates that vasopressor hormones are important mediators of the skeletal muscle vascular constriction elicited by whole-body cooling.

4. Local cooling elicited skin and skeletal muscle vascular dilation at 33°C in both innervated and denervated

forelimbs possibly due to a direct inhibition of vasomotor tone, whereas further local cooling to 28°C caused a slight increase in skin and skeletal muscle vascular resistance; perhaps due to a rise in blood viscosity. A neurally mediated increase in vascular tone in the skin upon systemic cooling to 33°C partially attenuated the dilation elicited by local cooling. Local cooling to 28°C while rectal temperature was 28°C failed to elicit a significant dilation in either innervated or denervated limbs, perhaps because the effect of any increase in vessel radius which may have occurred was offset by a concomitant rise in blood viscosity. The skeletal muscle vascular response at rectal 33°C and limb 33°C was a small dilation in both innervated and denervated limbs, due to the humorally mediated vasoconstriction which partially attenuated the local response. The response at rectal 28°C and limb 28°C was consistently a very slight decrease in skeletal muscle vascular resistance again because any reduction in vascular hinderance by this procedure may have been largely offset by an increased blood viscosity.

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APPENDICES

APPENDIX A

FORELIMB VASCULAR RESISTANCE CALCULATIONS

APPENDIX A

RESISTANCE CALCULATIONS

Series I and II: Total and segmental vascular resistances (arterial, small vessel, and venous) in muscle and skin were calculated by dividing brachial or cephalic flow into the corresponding pressure gradient:

1. Skin Total Resistance (R_{st}) = $\frac{Pba - Pcv}{Fc}$
2. Skin Arterial Resistance (R_{sa}) = $\frac{Pba - Pssa}{FC}$
3. Skin Small Vessel Resistance (R_{ssv}) = $\frac{Pssa - Pssv}{Fc}$
4. Skin Venous Resistance (R_{sv}) = $\frac{Pssv - Pcv}{Fc}$
5. Muscle Total Resistance (R_{mt}) = $\frac{Pba - Pbv}{Fb}$
6. Muscle Arterial Resistance (R_{ma}) = $\frac{Pba - Pmsa}{Fb}$
7. Muscle Small Vessel Resistance (R_{msv}) = $\frac{Pmsa - Pmsv}{Fb}$
8. Muscle Venous Resistance (R_{mv}) = $\frac{Pmsv - Pbv}{Fb}$
9. Total Forelimb Resistance (R_{tf}) = $\frac{Rst \cdot Rmt}{Rst + Rmt}$

All resistances are expressed as (mm Hg · min/ml), where:

Pba = brachial artery pressure

Pssa = small skin artery pressure

Pssv = skin small vein pressure

Pcv = cephalic vein pressure

Fc = cephalic vein flow

Pmsa = muscle small artery pressure

Pmsv = muscle small vein pressure

Pbv = brachial vein pressure

Fb = brachial vein flow

Series III and IV: Total vascular resistances in skeletal muscle and skin were calculated by dividing brachial or cephalic flow into the corresponding pressure gradient. The calculations used were numbers 1, 5, and 9.

APPENDIX B_1

HEMODYNAMIC RESPONSES TO LOCAL AND SYSTEMIC COOLING
(Pressure, Flow, and Resistance Data)
(TABLES B-1 THROUGH B-4)

Table B-1. Systemic arterial blood pressure and brachial artery perfusion pressure (mmHg).

	Protocol	Blood Pressure	Perfusion Pressure
1.	38°C dog; 38°C limb	129.3 ± 4.8	121.7 ± 3.7
2.	38°C dog; 33°C limb	128.7 ± 4.1	121.3 ± 3.8
3.	38°C dog; 28°C limb	125.0 ± 3.8	121.0 ± 3.7
4.	38°C dog; 38°C limb	120.5 ± 4.1	118.3 ± 3.6
5.	37°C dog; 38°C limb	107.9 ± 7.8	102.9 ± 7.4
6.	36°C dog; 38°C limb	106.5 ± 7.1	99.5 ± 6.5
7.	35°C dog; 38°C limb	104.4 ± 6.3	96.4 ± 5.7
8.	34°C dog; 38°C limb	101.5 ± 6.4	94.2 ± 5.7
9.	33°C dog; 38°C limb	101.3 ± 6.1	92.6 ± 5.6
10.	33°C dog; 33°C limb	99.2 ± 6.6	90.8 ± 6.2
11.	33°C dog; 38°C limb	98.2 ± 7.2	90.2 ± 6.2
12.	32°C dog; 38°C limb	86.9 ± 8.4	81.5 ± 7.5
13.	31°C dog; 38°C limb	83.7 ± 9.2	80.0 ± 7.8
14.	30°C dog; 38°C limb	81.4 ± 8.7	77.7 ± 8.0
15.	29°C dog; 38°C limb	77.7 ± 8.3	76.0 ± 8.5
16.	28°C dog; 38°C limb	77.3 ± 7.7	72.9 ± 7.9
17.	28°C dog; 28°C limb	79.6 ± 6.8	72.6 ± 7.9
18.	28°C dog; 38°C limb	84.7 ± 6.8	71.9 ± 8.0

Table B-2. Cephalic and Brachial Flow (cc/min./100 gms.).

	Protocol	Cephalic	Brachial
1.	38°C dog; 38°C limb	6.65 ± 0.78	6.62 ± 0.74
2.	38°C dog; 33°C limb	11.06 ± 1.32	9.01 ± 1.04
3.	38°C dog; 28°C limb	8.44 ± 1.17	7.41 ± 0.82
4.	38°C dog; 38°C limb	5.33 ± 1.15	5.76 ± 1.16
5.	37°C dog; 38°C limb	6.56 ± 0.92	5.96 ± 0.82
6.	36°C dog; 38°C limb	4.71 ± 1.04	5.01 ± 1.02
7.	35°C dog; 38°C limb	4.80 ± 1.03	4.63 ± 0.66
8.	34°C dog; 38°C limb	4.28 ± 0.95	4.06 ± 0.60
9.	33°C dog; 38°C limb	3.66 ± 0.70	3.69 ± 0.56
10.	33°C dog; 33°C limb	5.36 ± 1.05	5.17 ± 0.89
11.	33°C dog; 38°C limb	3.36 ± 0.61	3.20 ± 0.47
12.	32°C dog; 38°C limb	2.84 ± 0.55	2.83 ± 0.45
13.	31°C dog; 38°C limb	2.58 ± 0.49	2.74 ± 0.44
14.	30°C dog; 38°C limb	2.46 ± 0.45	2.62 ± 0.39
15.	29°C dog; 38°C limb	2.47 ± 0.44	2.62 ± 0.41
16.	28°C dog; 38°C limb	2.34 ± 0.39	2.47 ± 0.35
17.	28°C dog; 28°C limb	2.84 ± 0.60	3.20 ± 0.63
18.	28°C dog; 38°C limb	2.08 ± 0.41	2.03 ± 0.30

Table B-3. Cephalic and Brachial Vein Pressures (mmHg).

	Protocol	Cephalic	Brachial
1.	38°C dog; 38°C limb	4.27 ± 0.74	4.98 ± 0.86
2.	38°C dog; 33°C limb	9.08 ± 1.35	7.72 ± 1.10
3.	38°C dog; 28°C limb	7.66 ± 1.33	7.62 ± 1.26
4.	38°C dog; 38°C limb	4.94 ± 0.81	4.69 ± 0.82
5.	37°C dog; 38°C limb	4.57 ± 0.88	5.21 ± 1.06
6.	36°C dog; 38°C limb	3.67 ± 0.79	4.58 ± 0.98
7.	35°C dog; 38°C limb	3.15 ± 0.87	3.55 ± 0.99
8.	34°C dog; 38°C limb	3.17 ± 0.87	3.45 ± 1.03
9.	33°C dog; 38°C limb	2.88 ± 0.81	3.16 ± 1.13
10.	33°C dog; 33°C limb	5.38 ± 1.20	5.17 ± 0.92
11.	33°C dog; 38°C limb	2.23 ± 0.71	3.19 ± 0.94
12.	32°C dog; 38°C limb	2.03 ± 0.74	2.72 ± 1.07
13.	31°C dog; 38°C limb	1.96 ± 0.81	2.54 ± 1.06
14.	30°C dog; 38°C limb	1.82 ± 0.78	2.40 ± 1.02
15.	29°C dog; 38°C limb	1.74 ± 0.77	2.60 ± 1.06
16.	28°C dog; 38°C limb	1.44 ± 0.72	2.30 ± 1.14
17.	28°C dog; 28°C limb	3.67 ± 0.94	3.64 ± 1.10
18.	28°C dog; 38°C limb	1.02 ± 0.87	2.45 ± 1.19

Table B-4. Heart Rate (beats/minute) and Aortic Blood Temperature (°C).

	Protocol	Heart Rate	Aortic Blood Temperature
1.	38°C dog; 38°C limb	116.9 ± 11.45	37.4 ± 0.39
2.	38°C dog; 33°C limb	116.9 ± 11.45	37.2 ± 0.33
3.	38°C dog; 28°C limb	116.9 ± 11.45	36.7 ± 0.29
4.	38°C dog; 33°C limb	116.9 ± 11.45	36.6 ± 0.31
5.	37°C dog; 38°C limb	117.8 ± 8.35	35.6 ± 2.47
6.	36°C dog; 38°C limb	107.0 ± 7.03	34.7 ± 2.20
7.	35°C dog; 38°C limb	102.3 ± 4.27	33.7 ± 0.19
8.	34°C dog; 38°C limb	99.2 ± 3.63	32.5 ± 0.21
9.	33°C dog; 38°C limb	83.1 ± 8.45	31.9 ± 0.25
10.	33°C dog; 33°C limb	83.1 ± 8.45	31.9 ± 0.23
11.	33°C dog; 38°C limb	83.1 ± 8.45	32.0 ± 0.26
12.	32°C dog; 38°C limb	82.3 ± 4.43	30.3 ± 0.19
13.	31°C dog; 38°C limb	74.6 ± 4.51	29.4 ± 0.21
14.	30°C dog; 38°C limb	65.4 ± 6.73	28.4 ± 0.20
15.	29°C dog; 38°C limb	66.9 ± 3.20	27.5 ± 0.22
16.	28°C dog; 38°C limb	56.2 ± 7.50	27.0 ± 0.28
17.	28°C dog; 28°C limb	56.2 ± 7.50	27.2 ± 0.29
18.	28°C dog; 38°C limb	56.2 ± 7.50	27.2 ± 0.27

APPENDIX B_2

HEMODYNAMIC RESPONSES TO LOCAL AND SYSTEMIC COOLING
(Pressure, Flow, and Resistance Data)
(TABLES B-5 THROUGH B-8)

Table B-5. Systemic arterial blood pressure and Brachial artery perfusion pressure (mmHg).

	Protocol	Blood Pressure	Perfusion Pressure
1.	38°C dog; 38°C limb	122.8 ± 2.7	118.2 ± 2.6
2.	38°C dog; 33°C limb	119.0 ± 4.3	115.6 ± 3.4
3.	38°C dog; 28°C limb	113.1 ± 4.4	115.3 ± 3.3
4.	38°C dog; 38°C limb	114.0 ± 10.1	118.8 ± 10.6
5.	37°C dog; 38°C limb	95.1 ± 13.8	97.5 ± 14.2
6.	36°C dog; 38°C limb	99.4 ± 5.6	101.4 ± 6.7
7.	35°C dog; 38°C limb	92.4 ± 6.2	96.1 ± 6.0
8.	34°C dog; 38°C limb	79.2 ± 12.4	77.5 ± 11.5
9.	33°C dog; 38°C limb	83.6 ± 8.8	80.3 ± 6.4
10.	33°C dog; 33°C limb	85.1 ± 8.1	80.0 ± 6.3
11.	33°C dog; 38°C limb	75.0 ± 11.6	67.8 ± 10.2
12.	32°C dog; 38°C limb	80.0 ± 8.5	76.4 ± 7.0
13.	31°C dog; 38°C limb	78.1 ± 9.0	72.2 ± 7.8
14.	30°C dog; 38°C limb	73.9 ± 8.6	71.1 ± 7.9
15.	29°C dog; 38°C limb	68.3 ± 8.4	70.0 ± 7.6
16.	28°C dog; 38°C limb	71.4 ± 10.3	65.3 ± 8.9
17.	28°C dog; 28°C limb	74.2 ± 9.1	64.7 ± 8.8
18.	28°C dog; 38°C limb	78.4 ± 12.7	67.8 ± 11.4

Table B-6. Cephalic and Brachial Flow (cc/min./100 gms.).

	Protocol	Cephalic	Brachial
1.	38°C dog; 38°C limb	7.95 ± 1.12	5.21 ± 0.68
2.	38°C dog; 33°C limb	11.67 ± 1.43	8.14 ± 1.33
3.	38°C dog; 28°C limb	10.40 ± 1.51	7.64 ± 1.17
4.	38°C dog; 38°C limb	8.75 ± 1.84	5.40 ± 1.08
5.	37°C dog; 38°C limb	7.26 ± 1.64	4.74 ± 0.97
6.	36°C dog; 38°C limb	7.33 ± 1.19	4.84 ± 0.74
7.	35°C dog; 38°C limb	5.47 ± 0.63	3.81 ± 0.60
8.	34°C dog; 38°C limb	4.03 ± 0.73	2.50 ± 0.49
9.	33°C dog; 38°C limb	3.17 ± 0.38	2.35 ± 0.30
10.	33°C dog; 33°C limb	5.95 ± 1.27	3.64 ± 0.66
11.	33°C dog; 38°C limb	3.03 ± 0.79	1.82 ± 0.44
12.	32°C dog; 38°C limb	3.06 ± 0.67	1.93 ± 0.36
13.	31°C dog; 38°C limb	2.76 ± 0.60	1.94 ± 0.38
14.	30°C dog; 38°C limb	2.46 ± 0.47	1.76 ± 0.31
15.	29°C dog; 38°C limb	2.38 ± 0.38	1.71 ± 0.26
16.	28°C dog; 38°C limb	2.21 ± 0.30	1.56 ± 0.23
17.	28°C dog; 28°C limb	2.92 ± 0.53	2.16 ± 0.49
18.	28°C dog; 38°C limb	1.88 ± 0.40	1.25 ± 0.28

Table B-7. Cephalic and Brachial Vein Pressures (mmHg).

	Protocol	Cephalic	Brachial
1.	38°C dog; 38°C limb	3.29 ± 1.17	3.93 ± 0.63
2.	38°C dog; 33°C limb	5.90 ± 1.51	5.46 ± 0.91
3.	38°C dog; 28°C limb	6.70 ± 1.77	5.86 ± 1.00
4.	38°C dog; 38°C limb	2.75 ± 0.83	1.95 ± 0.76
5.	37°C dog; 38°C limb	2.59 ± 1.14	3.24 ± 0.74
6.	36°C dog; 38°C limb	1.81 ± 1.01	2.97 ± 0.87
7.	35°C dog; 38°C limb	1.20 ± 0.94	2.69 ± 0.90
8.	34°C dog; 38°C limb	0.58 ± 0.97	1.48 ± 1.00
9.	33°C dog; 38°C limb	0.48 ± 0.79	1.78 ± 0.76
10.	33°C dog; 33°C limb	2.16 ± 1.23	3.09 ± 0.72
11.	33°C dog; 38°C limb	0.82 ± 0.80	1.16 ± 0.89
12.	32°C dog; 38°C limb	0.44 ± 0.75	1.67 ± 0.90
13.	31°C dog; 38°C limb	0.40 ± 0.85	1.48 ± 1.00
14.	30°C dog; 38°C limb	0.41 ± 0.77	1.63 ± 0.95
15.	29°C dog; 38°C limb	0.42 ± 0.86	1.53 ± 0.94
16.	28°C dog; 38°C limb	0.18 ± 0.85	1.33 ± 1.00
17.	28°C dog; 28°C limb	1.59 ± 1.45	2.37 ± 0.95
18.	28°C dog; 38°C limb	-0.25 ± 0.77	0.91 ± 0.99

Table B-8. Heart rate (beats/minute) and Aortic Blood temperature (°C).

	Protocol	Heart Rate	Aortic Blood Temperature
1.	38°C dog; 38°C limb	114.4 ± 15.42	40.2 ± 2.89
2.	38°C dog; 33°C limb	108.9 ± 15.76	35.6 ± 1.83
3.	38°C dog; 28°C limb	114.4 ± 15.42	36.7 ± 0.25
4.	38°C dog; 38°C limb	130.0 ± 11.56	36.6 ± 3.24
5.	37°C dog; 38°C limb	95.6 ± 19.77	31.8 ± 4.23
6.	36°C dog; 38°C limb	112.2 ± 5.80	34.8 ± 0.33
7.	35°C dog; 38°C limb	105.6 ± 5.34	33.8 ± 0.37
8.	34°C dog; 38°C limb	86.7 ± 12.12	28.8 ± 3.82
9.	33°C dog; 38°C limb	92.2 ± 3.86	31.9 ± 0.28
10.	33°C dog; 33°C limb	91.1 ± 4.13	31.8 ± 0.28
11.	33°C dog; 38°C limb	78.9 ± 10.96	28.4 ± 3.78
12.	32°C dog; 38°C limb	74.4 ± 10.17	31.0 ± 0.45
13.	31°C dog; 38°C limb	74.4 ± 1.86	29.7 ± 0.42
14.	30°C dog; 38°C limb	67.8 ± 2.95	28.9 ± 0.47
15.	29°C dog; 38°C limb	64.4 ± 3.58	28.1 ± 0.46
16.	28°C dog; 38°C limb	58.9 ± 2.13	27.5 ± 0.40
17.	28°C dog; 28°C limb	58.9 ± 2.13	27.3 ± 0.23
18.	28°C dog; 38°C limb	60.0 ± 3.65	27.3 ± 1.61

APPENDIX C

STATISTICAL METHODS

APPENDIX C

STATISTICAL METHODS

I. Series I and II

Intravascular pressures, blood flows, rate of forelimb change, and vascular resistances were determined for timed control periods and the corresponding experimentally cooled periods during whole-body cooling and hypothermia from 37.5°C to 28°C. For each period individual means ($\bar{x}$) were calculated for each parameter. The individual means were used to calculate a grand mean ($\bar{X}$), variance (s^2), standard deviation (S), and standard error of the mean ($SE_{\bar{x}}$) for each period during the hypothermia process as follows:

$$\bar{X} = \sum_{i=1}^{n} \frac{\bar{x}_i}{n}$$

$$s^2 = \frac{\sum_{i=1}^{n} \bar{x}_i^2 - \frac{(\sum_{i=1}^{n} \bar{x}_i)^2}{n}}{n - 1}$$

$$S = \sqrt{s^2}$$

$$SE_{\bar{x}} = S/\sqrt{n}$$

A. Comparisons of Control Means with Experimental Means During Whole Body Cooling and Hypothermia

The Student's "t" test for paired difference was made between each point of series I (experimentally cooled) and series II (time control group) on a time basis. If m independent comparisons among means are made, the probability of obtaining at least one significant comparison by chance is $1 - (1 - \alpha)^m$. The probability for making a type I error (α) for the collection of comparisons was set at .05. Pairing reduced the effect of animal to animal variability. To compute t for paired samples, the paired difference variable $D = X_1 - X_2$ is formed, where X_1 is the measurement of the cardiovascular parameter during the normothermic control period (series I) and X_2 the measurement of the same parameter during experimental cooling (series II). D is normally distributed with mean δ. The sample mean and variance ($\bar{d}$ and $s_{\bar{d}}^2$) are computed, and then

$$t = \frac{\bar{d} - \delta}{s_{\bar{d}}}$$

degrees of freedom = n - 1 where n is the number of pairs,

and $$s_{\bar{d}} = \sqrt{(s_1^2 + s_2^2 - \frac{2\Sigma X_1 X_2}{n - 1})/n}$$

$(\Sigma X_{1i} X_{2i})/(n - 1)$ is the covariance between X_1 and X_2. The null hypothesis for a paired t-test is that δ is some specific value, usually $H_0 : \delta = 0$. The alternative H_1 for a two-tailed test would be $H_1 : \delta \neq 0$ and the paired difference would be significant if the calculated t value was greater than the tabulated t value at the 5% level.

II. Series III and Series IV

The mean resistance values* of total skin and total muscle as well as forelimb weight change and blood pressure means were analyzed by a two-way analysis of variance in order to observe any significant change over any of the 18 experimental cold temperature variations. The dogs were classified as replications and the temperature

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*The mean resistance values as well as forelimb weight change and blood pressure values for Series III and IV were expressed as logarithmic means before any statistical analysis was done. The original data showed non-normality of the data variances and one criterion for the analysis of variance is that the data variances be stable. This stabilization was done by a natural logarithm transformation of the original data from which the mean logarithm values were calculated. The transformation would result in a gain in efficiency of the analysis of variance and would tend to produce the proper significant results in F- and t-tests.

variations as treatments. The classification of the dog variability in the replications made it possible to dissect this variability from that of the experimental treatments, indicating an improved accuracy in the experimental error. The two-way ANOVA table was set up as follows on page 143.

Source of Variation	Sum of Squares	d.f.	Mean Square	Expected Mean Square
Main Effects (total)	$\sum_{i=1}^{n_d} \sum_{j=1}^{h_j} \overline{y_{ij}} - n(\bar{y})^2$	$n - 1$	---	---
Temperature	$[n_d \Sigma(\bar{y}_{.j})^2] - n(\bar{y})^2$	$n_t - 1$	Temp.SS/n_t-1	$\sigma^2 + bK_A^2$
Dog	$[n_t \Sigma(\bar{y}_{i.})^2] - n(\bar{y})^2$	$n_d - 1$	Dog SS/n_d-1	$\sigma^2 + aK_B^2$
Residual (Experimental Error)	Total - Temp.SS - dog SS	$(n_t-1)(n_d-1)$	Residual SS/$(n_t-1)(n_d-1)$	σ^2

where: $y_{i,j}$ = observation on ith dog and jth temperature variation, n_d - number of dogs, n_t = number of temperature variations, $\bar{y}_{.j}$ = mean of all observations on jth temperature variation, $\{y_{1,j},\ldots,y_{nd}\}$, $\bar{y}_{i.}$ = mean of all observations on ith dog, $\{y_{i,1},\ldots,y_{nt}\}$, and $\bar{y}$ = mean of all observations. The null hypothesis for the ANOVA test is that the expected parameter, K_A^2, be $H_0 : K_A^2 = 0$. The alternative H_1 would be $H_1 : K_A^2 \neq 0$. If the F-test $(\frac{\text{Treatment MS}}{\text{Error MS}})$ at $P \leq .05$ supports H_1, then there are significant vascular responses to the 18 experimental temperature variations (systemic and local hypothermia).

A. Comparison of Means of Total Skin and Muscle Vascular Resistances, Systemic Blood Pressure and Change in Forelimb Weight During Local and Systemic Hypothermia

If the F-test from the ANOVA examination was significant for those vascular parameters tested, Dunnett's one-sided t-test ($P \leq 0.05$) was used to test significance on a paired basis of the individual controls and experimental values across the 18 temperature treatments. The Dunnett test was selected because it holds the error rate on any experimentwise basis. It uses a single range value, calculated as follows:

$$\bar{d}/_{s\bar{d}} \quad \text{where } s\bar{d} = \sqrt{\frac{2\ (\text{Error MS})}{n}}$$

for each particular seat of controls and experimental values compared, regardless of how many means are to be in a subset. Significance between the control and experimental treatment pairs was found when the calculated Dunnett's value was greater than the tabulated Dunnett's value (t_D, 0.05, k, v where k = no. of means less control and v = degress of freedom).

B. Comparison of the Resistance Responses of Innervated and Denervated Limbs During Hypothermia

The systemic blood pressure responses in the innervated and denervated forelimbs during hypothermia followed one another relatively close (statistically the same), which is a requirement for comparing resistance changes across the two series (appendix B_1 and B_2 reports pressure, flow, and temperature data for series III and IV, respectively).

Given populations (innervated and denervated forelimbs) with unequal variances, an approximation of t was calculated for the independent samples. The test statistic (ts) for each comparison at the corresponding experimental temperature variation was:

$$ts = \frac{\bar{X}_1 - \bar{X}_0}{\sqrt{\frac{s_I^2}{n_I} + \frac{s_D^2}{n_D}}}$$

where $\bar{X}_I$ and $\bar{X}_0$ = mean resistance in corresponding vascular segments of innervated and denervated forelimbs at corresponding temperature variation; s_I^2 and s_D^2 = variances of mean resistances in innervated and denervated forelimbs; n_I and n_D = number of observations. This statistic (ts) is not distributed as Student's t. However, the probability for ts can be approximated by treating it as t, but with degrees of freedom:

$$d.f. = \frac{[(s_I^2/n_I) + (s_D^2/n_D)]^2}{[(s_I^2/n_I)^2/(n_I-1)] + [s_D^2/n_D)^2/(n_D-1)]}$$

If ts exceeded the tabulated t value at 5% level, the null hypothesis ($\mu_I = \mu_D$) was rejected and the alternative hypothesis ($\mu_I \neq \mu_D$) was accepted.

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