LOCAL REGULATION OF SKELETAL MUSCLE BLOOD VESSELS: INFLUENCE OF PULSE PRESSURE AND VASOMOTOR TONE

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ABSTRACT

LOCAL REGULATION OF SKELETAL MUSCLE BLOOD VESSELS: INFLUENCE OF PULSE PRESSURE AND VASOMOTOR TONE

By

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The vascularly isolated gracilis muscles from 18 dogs anesthetized with sodium pentobarbital were exposed to alterations in local perfusion pressure before and during hemorrhage induced elevations of vasomotor tone to evaluate the influence of reflex constriction on muscle blood flow autoregulation. Using the same preparation, another series of 10 experiments were designed to examine the influence of pulse pressure on muscle blood flow autoregulation and the vascular responses to venous pressure elevation and increased mean vascular distending pressure.

In one group of natural flow experiments (Series I; n = 10), the gracilis nerve was left intact and muscle vascular responses to 4 sequential step reductions of gracilis artery pressure were studied while the animals were normovolemic and normotensive and during two periods of hemorrhage induced systemic arterial hypotension. Hemorrhage significantly reduced muscle blood flow and

elevated muscle vascular resistance. However, blood flow autoregulation was not affected since local reductions of gracilis artery pressure from approximately 120 to 70 mm Hg elicited statistically similar percent reductions in vascular resistance during both normovolemia and hypovolemia. These data indicate that steady state muscle blood flow autoregulation is maintained when vascular tone is elevated by hemorrhage.

In Series II, the innervated gracilis muscles of 8 dogs were perfused with the animals femoral arterial blood by a servocontrolled pump which maintained gracilis artery pressure at any set level by continually adjusting pump flow rate. Transient and steady state vascular responses to step changes in perfusion pressure to or from 140 mm Hg were examined while the animals were normovolemic and normotensive and while systemic arterial pressure was reduced to 100 mm Hg by hemorrhage. During normovolemia, all alterations in gracilis artery pressure below 140 mm Hg were associated with proportionately smaller changes in steady state blood flow. When vascular tone was significantly elevated by hemorrhage, the same local alterations in perfusion pressure elicited slightly smaller percent changes in flow. However, the rate of development of these autoregulatory responses were significantly prolonged during

hemorrhage. These data indicate that, when vascular tone is elevated by hemorrhage, steady state blood flow autoregulation is slightly improved, but some competition or interaction between remote vasoconstrictor influences and local control mechanisms delays the development of the autoregulatory response during hypovolemia.

To determine the influence of pulse pressure on local control of skeletal muscle blood vessels, vascular responses to local, graded hypotension, venous pressure elevation, and increased mean vascular distending pressure were examined using both pulsatile and non-pulsatile perfusion in the naturally perfused, denervated gracilis muscles of 10 dogs (Series III). Muscle vascular resistance and the vasoconstriction observed in response to venous and mean vascular distending pressure elevations were all significantly greater during pulsatile compared to non-pulsatile perfusion. These data indicate that myogenic mechanisms of blood flow control are present within the muscle vasculature and are sensitive to the transmural pressure changes associated with pulse pressure distension. When mean gracilis artery pressure was progressively lowered from 140 to 60 mm Hg during pulsatile perfusion both pulse pressure and muscle vascular resistance decreased significantly, whereas during non-pulsatile perfusion the same maneuver

elicited a progressive rise in vascular resistance. These data indicate that muscle blood flow autoregulation is mediated to a large extent by alterations in pulse pressure induced myogenic activity.

LOCAL REGULATION OF SKELETAL MUSCLE BLOOD VESSELS: INFLUENCE OF PULSE PRESSURE AND VASOMOTOR TONE

By

Brian John LaLone

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To Joan and Aimee

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INTRODUCTION

Blood flow distribution in the systemic circulation is regulated by control systems which adjust the contractile state of vascular smooth muscle. Alterations in smooth muscle tone are mediated by inhibitory and excitatory stimuli originating within and outside of the various tissues. In general, remote regulatory systems maintain a stable systemic arterial blood pressure whereas local circulatory control mechanisms establish optimal conditions for transvascular exchange. It is currently believed that maintenance of optimum exchange conditions results from local adjustments in arteriole and precapillary sphincter radius so that an appropriate regional blood supply and perfused capillary surface area is achieved.

One example of local blood flow control involves those factors which stabilize an organ's blood supply during variations in arterial pressure. When perfusion pressure to certain systemic vascular networks is varied over the range of approximately 70 to 180 mm Hg, there is a less than proportionate change in blood flow. This ability to maintain a relatively constant blood flow in the face of varying perfusion pressure is termed autoregulation and has

been defined by Johnson (64) as "the intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure". The results of extensive investigation over the last 25 years indicate that some combination of at least three different mechanisms accounts for blood flow autoregulation: 1) a tissue pressure mechanism; 2) a metabolic mechanism; and 3) a myogenic mechanism. Each of these is described below with emphasis on the experimental criteria used to determine which may be the predominant mediator of autoregulation in a given vascular bed.

The tissue pressure hypothesis states that alterations in vascular caliber responsible for blood flow autoregulation result from the effect of tissue fluid pressure on veins. An elevation of arterial pressure is hypothesized to cause net efflux of fluid from capillaries into the interstitial space so that tissue pressure increases, especially in organs surrounded by relatively incompliant capsules. The increased tissue pressure allegedly compresses veins causing mean vascular radius to decrease so that flow increases less than perfusion pressure.

Several aspects of the tissue pressure hypothesis can be tested experimentally. Because veins have the lowest internal pressure and wall rigidity, changes in tissue pressure should elicit correspondingly greater changes in venous as opposed to arterial resistance, Furthermore, an

increase in arterial pressure should, unless the capsule surrounding the organ is absolutely rigid, increase extravascular volume and consequently organ weight. Finally, since elevation of venous pressure should cause net efflux of fluid from capillaries into the interstitial space, the tissue pressure hypothesis predicts an increased precapillary resistance in response to venous pressure elevation (i.e., the so-called venous arteriolar response). Because the tissue pressure hypothesis does not involve active vasomotion, neither the venous arteriolar response nor autoregulation should be abolished by pharmacological agents which paralyze vascular smooth muscle.

The metabolic hypotheses of blood flow autoregulation state that a decreased blood flow results in vascular relaxation mediated either by dilator metabolites released from the surrounding tissues or by a decreased nutrient supply to these tissues. According to the metabolic hypothesis, reducing arterial pressure will decrease blood flow and cause accumulation of dilator metabolites, thereby eliciting a vascular relaxation which helps to restore flow. Decreased blood flow will also decrease interstitial pO_2 and conceivably alter tissue metabolism in a way that increases the production of vasodilator substances. In addition, a lowered pO_2 may reduce vascular tone directly. Therefore, the various metabolic mechanisms of autoregulation are characterized as being flow dependent responses

which act to maintain a constant flow to metabolism ratio.

The identification of a vasodilator substance in the venous effluent from organs perfused at low arterial pressure would provide strong experimental support for the metabolic hypothesis of blood flow autoregulation. Reducing the pressure head in a vascular network by elevating venous pressure should decrease flow and, according to the metabolic hypothesis, cause an accumulation of dilator substances. Organs exhibiting metabolically mediated autoregulation should therefore display a decreased vascular resistance when venous pressure is elevated (i.e., should not display a venous-arteriolar response).

The myogenic hypothesis of blood flow autoregulation states that intrinsic mechanisms in smooth muscle cells of arteries, arterioles, and precapillary phincters allows these vessels to respond to a decrease in wall tension with relaxation or to an increase in wall tension with contraction. Evidence has been presented to suggest that stretch of vascular smooth muscle evokes a contractile response by increasing pacemaker activity and eliciting more frequent bursts of action potentials which are propagated to neighboring cells. When vascular transmural pressure is increased, by increasing either arterial or venous pressure, or by decreasing pressure around an organ, the myogenic hypothesis predicts that the involved vessels will constrict. Studies of the response to venous pressure

elevation represent a critical experiment for demonstration of myogenically mediated autoregulatory responses. Elevation of venous pressure will produce precapillary constriction according to both the myogenic and tissue pressure hypotheses, whereas the metabolic hypothesis predicts dilation in response to venous pressure elevation. Precapillary constriction resulting from venous hypertension will be abolished by agents which paralyze vascular smooth muscle if this constriction is myogenic but not if it is due to increased tissue pressure.

The available data seem to indicate that myogenic mechanisms are primarily responsible for blood flow autoregulation in the intestine (48, 52, 62, 63, 65, 66, 69, 109), mesentery (67, 71), and liver (50, 53, 123), whereas metabolic mechanisms are probably most important in the myocardium (9, 10, 27, 41, 100, 103). The mechanism(s) responsible for cerebral (30, 81, 94), renal (16, 44, 46, 54, 89, 90, 120, 126), and particularly skeletal muscle blood flow autoregulation are less well defined. Since agents that paralyze vascular smooth muscle abolish both autoregulation and the venous arteriolar response in skeletal muscle (58, 84, 85, 111, 117), and since autoregulatory resistance responses to changes in perfusion pressure occur almost exclusively in precapillary vessels rather than veins (51, 58, 70, 88, 121), the tissue pressure mechanism for blood flow autoregulation appears to be relatively

unimportant in skeletal muscle vasculature. Rather, skeletal muscle blood flow autoregulation is probably mediated by myogenic and metabolic factors.

The uncertainty regarding the relative importance of these two autoregulatory mechanisms results in part from conflicting reports of the resistance responses to venous pressure elevation. In the skinned hindlegs (80% skeletal muscle) of reserpinized cats Folkow and Oberg (36) observed 16 to 42% increases in vascular resistance when venous pressure was elevated 10 mm Hg. Similar results were reported by Nagle et al. (88) in the denervated canine gracilis muscle where 30 mm Hg elevations in venous pressure consistently increased vascular resistance by about 40%. Hanson (49) found equivocal alterations in vascular resistance when venous pressure was elevated 20 mm Hg in the isolated canine hindlimb (50% muscle; 20% skin; 30% bone). In half of Hanson's experiments resistance at elevated venous pressure was slightly below control, while in others it either did not change or increased slightly. Jones and Berne (73) obtained similar results in a skinned canine thigh preparation; 20 mm Hg elevations of venous pressure elicited only slight increases in vascular resistance in about one third of the preparations. These disparate findings may be related to preparation differences. The canine hindlimb preparations of Hanson (49) and Jones and Berne (73) were removed from the animals by sectioning the muscles

under study whereas the skinned cat hindlimbs used by Folkow and Oberg (36) were isolated from the animals at the level of the lower abdominal aorta so that the muscles being studied were not sectioned. Furthermore, in the canine hindlimb preparations of Jones and Berne (73) resting blood flow per unit tissue weight was considerably lower than that reported by Folkow and Oberg (36) and Nagle et al. (88). This difference appears to be especially important since, in the few preparations which displayed relatively high control blood flows, Jones and Berne (73) did observe an increase in vascular resistance with elevated venous pressure. These results suggest that elevated vascular tone caused by preparation deterioration, surgical trauma, or hemorrhage may alter local control of skeletal muscle vasculature. Indeed, hemorrhage has been suggested by several investigators (63, 72, 80, 126) to be a factor contributing to the somewhat labile nature of blood flow autoregulation. It has been found, for example, that hemorrhage abolishes renal blood flow autoregulation (25, 126). However, Bond and Green (13, 43) reported that skeletal muscle blood flow autoregulation was more pronounced during hemorrhage.

Because alterations in arterial perfusion pressure will cause qualitatively similar changes in blood flow and vascular wall tension, it is difficult to determine whether the resistance responses elicited by this maneuver are metabolically or myogenically mediated. However, if

autoregulation is primarily a myogenic response, one might expect a different degree of autoregulation depending on the magnitude of pulse pressure. Two separate investigations (85, 99) have shown that pulse pressure distension contributes to the total basal vascular tone present in skeletal muscle. Since it is unlikely that variations in pulse pressure about a given mean pressure would alter the nutrient supply to skeletal muscle, potentiation of blood flow autoregulation by increased pulse pressure would suggest that myogenic mechanisms are important mediators of blood flow control in this tissue.

The purpose of this study is two-fold. Because the conflicting results described above may be due to elevation of vascular tone, experiments were designed to evaluate the effects of hemorrhage induced increases in vascular tone on skeletal muscle blood flow autoregulation. The effect of pulse pressure distension on muscle blood flow autoregulation was also studied to evaluate the contribution of myogenic mechanisms to autoregulatory responses in this tissue.

LITERATURE REVIEW

Local regulation of blood flow in skeletal muscle is believed to result from both metabolic and myogenic mechanisms (68). Because it is difficult to design experiments which clearly distinguish metabolic from myogenically mediated vascular responses, little definitive evidence exists regarding the relative importance of each of these mechanisms. The following sections analyze the role of these two mechanisms in muscle blood flow control. The first section discusses literature pertinent to the metabolic hypothesis, particularly as it pertains to autoregulation. Most investigations of metabolic blood flow control have focused on functional hyperemia (the blood flow increase accompanying skeletal muscle exercise) and reactive hyperemia (the blood flow increase after release of arterial occlusion), whereas relatively little attention has been directed at muscle blood flow autoregulation. Thus, in some instances it has been necessary to draw inferences from studies of functional and reactive hyperemia when assessing the role of certain vasodilator chemicals in autoregulation. The second section discusses vascular myogenic mechanisms and their possible importance in autoregulation. Since only a few investigators have examined myogenic mechanisms in skeletal

muscle vascular beds, some pertinent studies in other vascular networks have been included.

I. Metabolic Mechanisms of Skeletal Muscle Blood Flow Autoregulation

The metabolic hypothesis of blood flow autoregulation proposes that a decrease in flow caused by a fall in perfusion pressure will decrease the concentration of oxygen or increase the concentration of vasodilator metabolites in the tissue fluids, thereby resulting in active arteriolar dilation. Although active vasodilation has been observed in response to decreases in skeletal muscle blood flow (14, 36, 46, 49, 51, 58, 73-75, 105, 116, 117, 125), this response is adequately explained by either the metabolic or myogenic hypothesis. Other experimental designs have provided more definitive evidence to implicate metabolically linked chemicals in blood flow autoregulation.

Some support for the view that metabolically linked chemicals play an important role in muscle blood flow autoregulation comes from studies of the vasoactivity of venous blood. Folkow (34) has shown that intra-arterial injection of venous blood elicits vasodilation in the cat hindlimb. Similar results have been obtained by Haddy and Scott (46) in the dog forelimb. Scott *et al.* (105) found that perfusion of the dog forelimb with the venous effluent from the hindlimb produced an almost immediate fall in vascular

resistance in the assay forelimb. They employed this same bioassay preparation to demonstrate that a reduction in hindlimb blood flow, produced by compressing the femoral artery, led to vasodilation not only in the hindlimb, but also in the forelimb. Opposite responses were observed when active vasoconstriction was induced in the hindlimb by elevating flow rate. Although these bioassay studies provide evidence to support the metabolic hypothesis of skeletal muscle blood flow autoregulation, they do not identify a specific chemical mediator. As described below, the metabolically linked chemicals that are potential mediators of skeletal muscle blood flow control include oxygen, hydrogen, potassium, and adenosine and its nucleotides.

A. Metabolically Linked Vasoactive Chemicals

1. Oxygen

Several different lines of experimental evidence indicate that oxygen may participate in autoregulation of skeletal muscle blood flow. These include studies of: a) changes in venous blood oxygen content associated with skeletal muscle blood flow autoregulation; and b) the effects of alterations in arterial blood oxygen content on skeletal muscle vascular resistance, blood flow autoregulation, arteriole diameter, and small artery conductance.

A fall in the oxygen content or partial pressure of venous blood draining skeletal muscle has been shown to

accompany autoregulatory responses to decreased arterial perfusion pressure (14, 51, 73-75, 118). Jones and Berne (73, 75), using a pump perfused canine hindlimb preparation, reported that resistance responses to step changes in flow were accompanied by qualitatively similar changes in venous oxygen content. These investigators also observed that blood flow autoregulation was present in dog hindlimbs when venous blood oxygen content was low but not when oxygen content of the venous effluent was high (74). Using the canine gastrocnemius-plantaris muscle group, Stainsby and Otis (118) have shown that muscle venous blood oxygen content decreases with reductions in perfusion pressure which elicit blood flow autoregulation. Similar results have been obtained by Bond et al. (14) and Hanson and Johnson (51) in canine hindlimb preparations. From such observations it has been suggested that autoregulatory changes in vessel caliber result from changes in tissue oxygen tension. Such a hypothesis is tenable only if it can be shown that relatively small changes in tissue fluid pO_2 elicit significant changes in vascular resistance.

Several investigations indicate that a fall in oxygen delivery will elicit vasodilation (19, 24, 57, 96, 110). When the hemoglobin saturation of blood perfusing the dog hindlimb was reduced by 10%, Ross et al. (96) observed a 25% increase in flow. Further reduction of arterial blood hemoglobin saturation to 0% elicited a 3-4 fold increase in

flow. Similar results were obtained by Skinner and Powell (110) in canine gracilis preparations where 70% reductions in arterial blood oxygen content elicited 60% decreases in vascular resistance. Hutchins et al. (57) reduced arterial pO_2 by 15% and observed a 2 to 18% increase in the diameter of small precapillary vessels in the rat cremaster muscle. Carrier et al. (19) reported an inverse relationship between vascular diameter and pO_2 in small arteries (0.5-1.0 mm O.D.) from the dog hindlimb. The smallest vessels examined in this study displayed a 15% decrease in resistance when pO_2 was reduced from 100 to 90 mm Hg, whereas with the larger vessels it was necessary to reduce pO_2 from 100 to 50 mm Hg in order to obtain an equivalent change in resistance. Detar and Bohr (24) found that the active tension developed by aortic strips exposed to epinephrine was diminished when pO_2 in the bathing fluid was decreased from 100 to 70 mm Hg. The reports cited above suggest that vasomotor tone in skeletal muscle is modulated by oxygen so that a given fall in O_2 delivery elicits a corresponding fall in vascular resistance. There is also evidence that supernormal oxygen levels in arterial blood cause vasoconstriction. Bachofen et al. (4), using a hyperbaric chamber, observed an 18% increase in canine hindlimb vascular resistance when arterial blood pO_2 was elevated from 92 to 1660 mm Hg.

Not all available data support the view that small changes in oxygen delivery elicit vasomotor responses.

Daugherty et al. (21) failed to observe a change in dog forelimb vascular resistance when brachial artery pO_2 was reduced from 120 to 30 mm Hg. Only when arterial pO_2 was reduced to very low levels (from 30 to 8 mm Hg) did these investigators observe significant vasodilation in the forelimb. Chalmers et al. (20) were unable to show dilation in the hindlegs of rabbits perfused with hypoxic arterial blood until the oxygen tension was reduced below 30 mm Hg. These studies suggest a threshold for hypoxia-induced vasodilation which might reflect a shift to anaerobic metabolism in either the vascular smooth muscle or the parenchymal cells at low pO_2. While there is agreement that oxygen can effect skeletal muscle vascular tone, the question of whether or not the oxygen effect has a threshold is difficult to resolve since evidence has been presented to support each view.

There are two investigations which specifically address the effects of altered blood oxygen content and pO_2 on skeletal muscle blood flow autoregulation. Using the canine hindlimb, Walker and Guyton (125) found that flow was autoregulated at progressively higher levels when the arterial blood oxygen saturation was progressively decreased from 98 to 30%. It is interesting to note that even though vascular resistance fell with decreasing arterial blood oxygen saturation, autoregulatory behavior was essentially unchanged; that is, perfusion pressure reductions from 150 to 70 mm Hg

elicited similar percent reductions in blood flow and vascular resistance regardless of the arterial blood oxygen content. Similar results have been reported by Bond et al. (14) in the dog hindlimb when blood pO_2 was elevated by changing the inspired gas from room air to 2 atmospheres of oxygen. Even though hindlimb venous blood pO_2 was increased from 50 to 240 mm Hg by this maneuver, no alterations were observed in either the resting level of blood flow or blood flow autoregulation. The results from these two studies indicate that although vascular tone is diminished by local hypoxia it is not effected by hyperoxia and neither maneuver appreciably changes the extent to which blood flow is autoregulated in skeletal muscle.

The mechanism by which hypoxia elicits dilation is not known. Many investigators believe that oxygen alters vascular smooth muscle tone directly (19, 24, 96, 110). The work of Detar and Bohr (24) and Carrier et al. (19) on isolated small arteries and aortic strips provide strong support for this view. Honig (56) has reported in vitro evidence to suggest that hypoxia may lower vasomotor tone directly through the inhibition of myosin ATPase by inorganic phosphate and cyclic AMP elaborated within the vascular smooth muscle cells. However, other investigators have proposed that the dilator effect of hypoxia is secondary to an alteration in parenchymal tissue metabolism which releases vasoactive substances (11, 26, 28, 29). Berne (11) reports

that oxygen lack in the myocardium leads to release of the potent vasodilator adenosine, and suggests a similar type of control for skeletal muscle vascular beds. Large amounts of adenosine are produced by skeletal muscle during severe ischemic exercise and lesser amounts may also be present in resting muscle (26).

2. Hydrogen

Although the hydrogen ion is clearly vasoactive (21, 23, 32, 87, 92, 130), experimental evidence suggests that its role in the mediation of skeletal muscle blood flow autoregulation is probably minor. The difficulty in assigning a major role to the hydrogen ion results primarily from two observations: 1) induced changes in the pH of blood perfusing skeletal muscle must be very large to produce measurable changes in resistance (21, 86, 87, 104, 125); and 2) large decreases in vascular resistance associated with skeletal muscle exercise cause only small alterations in venous blood pH (97, 102, 120). For example, Daugherty et al. (21) observed that a severe local hypercapnic acidosis which reduced forelimb blood pH from 7.58 to 7.19 only lowered vascular resistance 24% below control. In addition, periods of skeletal muscle exercise that produce reductions in resistance ranging from 25 to 70% below control levels are associated with only 0.03 to 0.07-unit reductions in effluent blood pH (97, 102, 120). Since tissue fluid pH probably falls less during local hypotension than during exercise,

the contribution of altered hydrogen ion alone to blood flow autoregulation appears to be very small.

The hydrogen ion may however, exert effects in combination with hypoxia. Stowe et al. (120) perfused a canine gracilis muscle (assay gracilis) with the venous effluent from the contralateral, exercising gracilis and studied the effects of restoring assay gracilis blood pO_2 and pH to pre-exercise levels. Correction of either pO_2 alone or pH alone only slightly reduced the vasodilator activity of venous blood from the exercising muscle. However, when both pO_2 and pH were restored to pre-exercise levels, dilation in the assay gracilis was abolished. These findings indicate that the steady state vasodilator activity of venous blood from exercising skeletal muscle appears to be mainly the combined result of decreased pO_2 and pH. Although the mechanisms for maintenance of a constant flow to metabolism ratio during exercise may be different than those which mediate autoregulation, these results suggest that hydrogen ion could be important in skeletal muscle blood flow autoregulation through a combined effect with the hypoxemia which accompanies reductions in perfusion pressure.

3. Potassium

Although the potassium ion is vasoactive (31, 42, 45, 77, 110) and is probably involved in the initiation of exercise dilation (1, 93, 106, 107), it is not believed to be important in skeletal muscle blood flow autoregulation.

This conclusion is based on the observation that the marked reactive hyperemia which follows periods of circulatory arrest in skeletal muscle occurs without a measurable change in venous potassium concentration (102, 106, 107). However, Skinner and Powell (110) have shown that the hyperemia accompanying reduction of gracilis arterial blood oxygen saturation is enhanced when the arterial potassium ion concentration is elevated above normal.

4. Adenosine and the Adenine Nucleotides

Adenosine and its nucleotides appear to be important in the regulation of coronary blood flow (9, 76, 95, 101) and may be involved in exercise hyperemia (26, 37, 105, 108). However, evidence that these agents are involved in skeletal muscle blood flow autoregulation is lacking because they do not appear to be present in the venous effluent from muscles after release from long periods of flow reduction. Scott et al. (108) reported that femoral venous blood contains adenosine and/or adenosine monophosphate during hindlimb exercise but not during reactive hyperemia.

II. Myogenic Regulation of Vascular Tone

The myogenic hypothesis of blood flow autoregulation states that vascular smooth muscle cells have an intrinsic ability to respond to changes in transmural pressure with contraction or relaxation. Support for this hypothesis has

been derived from studies of: a) the electrophysiological properties of isolated vascular smooth muscle; b) the electrical and mechanical responses of isolated vascular smooth muscle to stretch; c) the responses of intact microvascular networks to change in transmural pressure; and d) the resistance responses of whole vascular networks to changes in transmural pressure.

A. Electrical and Mechanical Properties of Vascular Smooth Muscle

There appear to be two distinct types of smooth muscle, "visceral" or single unit and multiunit smooth muscle (15). In the single unit variety, the cells act as a functional syncytium so that current spreads from cell to cell via low resistance pathways. In the multiunit type current does not pass from cell to cell and conduction is dependent upon nerves. Some cells of the single unit type appear to act as pacemakers since they are able to spontaneously depolarize to threshold. These cells are characterized as having a low and unstable membrane potential which slowly rises through some intrinsic pacemaker mechanism initiating complete depolarizations when threshold is reached. Periods of spike activity are followed by quiescent periods giving rise to phasic contractures. Cells of the multiunit variety show no tendency for spontaneous electrical activity and are thought to be driven by neural influences.

Prior to 1960 it was generally believed that vascular smooth muscle was of the multiunit type, incapable of myogenic activity, since large arteries and veins failed to conduct action potentials in response to direct electrical stimulation (18, 38). It has recently been established that smooth muscle cells of small arteries and veins possess myogenic activity (3, 21, 39, 40, 60, 82, 114, 119). Funaki (39, 40) was the first to obtain intracellular electrical recordings from small blood vessels. He inserted microelectrodes into smooth muscle cells of small pre- and postcapillary vessels of the frog tongue and observed a low, phasic, resting membrane potential. Local potentials were observed to gradually build until a depolarization occurred which was propagated to neighboring cells. Axelsson et al. (3) reported similar behavior in smooth muscle cells of the rat portal vein. During the quiescent period between bursts of action potentials he described a gradual rise in membrane potential suggestive of pacemaker activity. Reports have also appeared documenting the spread of induced activity in isolated blood vessels. In response to electrical or chemical stimulation of rat portal vein strips, Johansson (60), Ljung and Stage (82), and Bevan and Ljung (12) observed a longitudinal spread of electrical and mechanical activity. Bevan and Ljung (12) obtained similar results in rabbit arteries, with propagation being especially pronounced in small arteries. These studies indicate that vascular smooth

muscle cells possess myogenic automaticity and behave as a functional syncytium capable of conducting electrical activity.

B. Vascular Smooth Muscle Responses to Stretch

Several studies have shown that vascular smooth muscle, like other single unit smooth muscles responds to passive stretch with contraction (7, 8, 22, 59, 61, 112, 113, 124). Using an isolated segment of carotid artery, Bayliss (8) was the first to observe contraction in response to elevation of internal pressure. From this and other less conclusive experiments on reactive hyperemia, he originated the myogenic hypothesis of blood flow control. The conclusions of Bayliss were criticized by Anrep (2) who could find no evidence of large vessel contraction with elevation of internal pressure. In support of Bayliss' observation, Wachholder (124) reported rhythmic contraction of isolated carotid segments after elevation of internal pressure. Similar results were obtained twenty years later by Burgi (17) in his experiments on isolated segments of mesenteric arteries. More recently, Davignon et al. (22) perfused norepinephrine free human umbilical arteries and observed constriction when transmural pressure was elevated by either increasing perfusion pressure or applying vacuum outside the arteries.

Similar results have been obtained with helically cut strips of isolated arteries and veins. Using strips from canine paw arteries (200-500 u O.D.), Johansson and Bohr (59) observed slow spontaneous rhythmic contractions which increased in frequency with passive stretch. Sparks (112) found that the active tension development of human umbilical artery strips in response to passive stretch was related to the rate and increment of stretch, and to resting tension of the strip. Similar results were obtained by Sparks and Bohr (113) using helical strips from small branches of the canine superior mesenteric artery. In a recent study, Johansson and Mellander (61) observed that the electrical and contractile activity of portal vein strips depended on muscle length and were strongly influenced by variation in the rate of change in length. Active force and spike frequency showed graded increases with increasing rates of stretch. These studies on whole arterial segments and venous and arterial strips provide support for the view that vascular smooth muscle is myogenically active, responding to increased stretch with contraction.

C. Microvascular Responses to Transmural Pressure Changes

A number of studies on intact microvascular networks provide support for the myogenic hypothesis (5, 33, 71, 91, 127-129). Fog (33) observed dilation of pial arteries with arterial pressure reduction and contraction with pressure

elevation. Similar results were obtained by Nicoll and Webb (91) in bat wing arterioles. Weideman (127, 129) studied the bat wing microvasculature and observed that venous pressure elevation elicited an increased frequency of rhythmic motion in venules. When pressure was elevated in the bat wing microvasculature by injection of saline, Weideman (128) found that the frequency of arteriole vasomotion was increased. There is also evidence that precapillary sphincters respond to changes in transmural pressure. Johnson and Wayland (71) observed a periodic flow pattern in individual capillaries of cat mesentery that appeared to be caused by contraction and relaxation of the precapillary sphincters. Reduction of arterial pressure removed the periodic flow pattern but, when intravascular pressure was restored by increasing venous pressure, vasomotion was also restored.

The responses described above do not appear to result from neural reflexes since bat wing vasomotion is still present after denervation and the periodic flow patterns in mesenteric capillaries were observed in a surgically isolated loop of intestine. However, in each of these studies the induced changes in transmural pressure were accompanied by simultaneous changes in flow which might account for the responses via metabolic mechanisms. Evidence against this explanation is provided by Baez's (5) studies on rat mesoappendix arteriole diameter under conditions of no flow.

He found that intravascular pressure elevation caused some arterioles to constrict, with vessel radius actually becoming smaller at elevated intravascular pressure.

D. Transmural Pressure Effects on Vascular Resistance

There is a substantial amount of less direct evidence to suggest that the resistance vessels respond actively to changes in transmural pressure. The rise in precapillary resistance observed in many organs upon venous pressure elevation (venous-arteriolar response) is presently believed to be myogenically mediated (36, 49, 53, 62, 74, 88). Johnson (62) observed a strong venous arteriolar response in the intestine which was not dependent upon neural mechanisms nor due to purely physical factors. Hanson and Johnson (53) observed increases in hepatic arterial resistance in response to hepatic venous pressure elevation. Since the metabolic hypothesis predicts that these maneuvers would decrease arterial resistance, these results suggest myogenic mechanisms are operative in the intestine and liver.

The venous-arteriolar response has not been consistently observed in skeletal muscle vascular beds. Nagle et al. (88) and Folkow and Oberg (36) were able to consistently observe vasoconstriction with venous pressure elevation in denervated canine gracilis muscles and in the hindlegs of reserpinized cats. However, Jones and Berne (74) and Hanson (49) only observed a venous-arteriolar response in a small

portion of their isolated canine hindlimb preparations.

It has been shown that the capillary filtration coefficient (CFC) of limbs is sensitive to intravascular pressure, an increase in pressure causing the CFC to decrease. In the hindquarters of cats, Mellander et al. (84) observed that simultaneous 50 mm Hg elevations of both venous and arterial pressure elicits an 18% increase in vascular resistance and a 59% reduction in CFC. Since both these responses were abolished by the smooth muscle poison chloral hydrate, they were attributed to a myogenic constriction of precapillary sphincters triggered by elevated intraluminal pressure.

There is also evidence to suggest that skeletal muscle vessels respond to the transmural pressure changes associated with pulsatile perfusion. Rovick and Robertson (99) examined the effects of pulse pressure distension upon vascular resistance in the isolated dog tongue. At mean arterial perfusion pressures of from 80 to 160 mm Hg, vascular resistance increased when pulse amplitude was increased from 0 to 60 mm Hg, with the effect being most pronounced at pulse pressures of 30-40 mm Hg. Mellander and Arvidsson (85) have performed similar experiments in the sympathectomized lower leg muscles of the cat. A sudden shift from nonpulsatile to pulsatile perfusion (mean perfusion pressure kept constant) elicited a 6% increase in muscle vascular resistance. When the vasculature was poisoned by papaverine, this response was abolished and replaced by a 10% decrease

in vascular resistance. These data indicate that the pulse pressure induced stretch of the vascular smooth muscle initiates a myogenic constrictor response which overrides the effect of passive distension. Mellander and Arvidsson also examined the contribution of pulse pressure distension to the increased vascular resistance observed with elevated transmural pressure. Simultaneous 20 mm Hg elevations of venous and arterial pressure elicited a 7% increase in vascular resistance during non-pulsatile and a 14% increase during pulsatile perfusion. In the papaverine treated, passive vasculature, this same elevation of transmural pressure decreased resistance by 20%. Thus, the distending effect of increased transmural pressure observed in the passive vasculature was abolished and replaced by an active, presumably myogenic, constrictor response in the normal vascular bed, but more effectively so during pulsatile than during non-pulsatile perfusion.

METHODS

Adult mongrel dogs of either sex, weighing 27-32 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and a cuffed endotracheal tube was inserted to provide a patent airway. Supplements of sodium pentobarbital were given as necessary during the surgical and experimental procedures.

The right gracilis muscle was exposed via a cutaneous incision and the overlying fascia was stripped away. All branches of the gracilis artery and vein which did not go directly to or come directly from the muscle were ligated, as were other vascular connections to the muscle not arising from the gracilis artery and vein. The tendons of origin and insertion were tied to prevent collateral blood flow.

After surgery was completed, sodium heparin (Wolins Pharmacal Corp., Mellville, New York) was administered in an initial dose of 700 USP units/kg body weight with hourly supplements of 300 USP units/kg body weight. Gracilis artery and vein pressures were measured from small bore (outside diameter (O.D.) 0.038"-0.048") polyethylene (P.E.) cannulae (Intramedic Tubing, Clay Adams, Parsippany, New Jersey) inserted into side branches of the gracilis artery

and vein. Gracilis muscle blood flow was determined by a different method for each experimental series (see below), and gracilis muscle vascular resistance was calculated by dividing the pressure drop across the vasculature by gracilis muscle blood flow.

In all experiments mean systemic arterial pressure was measured from a P.E. 240 catheter (O.D. - 0.095") inserted into the thoracic aorta via the left carotid artery. All cannulae used for pressure measurements were filled with saline and connected to low volume displacement transducers (No. P23 Gb, Statham Laboratories, Hato Rey, Puerto Rico) coupled to a direct writing oscillograph (No. 7784A, Hewlett Packard Co., Waltham, Massachusetts).

The animals were warmed with a heating pad (Walker Co., Middleboro, Massachusetts) and with radiant heat supplied from a lamp (No. 1755, Burton Manufacturing Co., Van Nuys, California) directed at the legs and abdomen, so that rectal and muscle surface temperatures were maintained between 36.5 and 38° C. Rectal and gracilis muscle surface temperatures were monitored via thermister probes coupled to a direct reading tele-thermometer (No. 44TD, Yellow Springs Instrument Co., Yellow Springs, Ohio). The gracilis muscle was covered with saline saturated gauze which was coated with an inert silicone spray (Antifoam A, Dow Corning, Midland, Michigan) to prevent drying.

Series I: Naturally perfused, innervated gracilis muscles exposed to local hypotension during normovolemic and hypovolemic periods.

In 10 spontaneously breathing dogs, the right gracilis muscle was prepared as described above with the gracilis nerve left intact. The gracilis vein was cannulated with a 6-8" section of P.E. 240 tubing downstream from the site of venous pressure measurement. Flow from the vein was directed into an open reservoir maintained at a constant volume by a variable speed, roller pump (Lange Model RE 161, Extracorporeal Medical Specialties, Inc., Mt. Laurel Township, New Jersey) which returned blood to the animal via a large vein. Gracilis muscle blood flow was determined by timed collection of venous outflow.

An illustration of the experimental protocol is provided in Figure 1. After a 30-40 minute control period during which intravascular pressures and muscle blood flow were allowed to stabilize, mean gracilis artery pressure was reduced approximately 20 mm Hg (minute zero on abscissa) by compression of the gracilis artery with a screw clamp. Intravascular pressures were recorded continuously and flow was determined 2 and 4 minutes after the reduction in gracilis artery pressure. This procedure for pressure reduction and data collection was repeated 3 more times, with mean gracilis artery pressure being lowered an additional 10-25 mm Hg each time. The clamp was then released so that

Figure 1. Changes in mean systemic arterial pressure (mm Hg), gracilis artery pressure (mm Hg), and gracilis vein flow (cc/min) in response to local hypotension during normovolemia and during two levels of systemic arterial hypotension. Data are from a selected natural flow experiment. (See text for detailed description.)

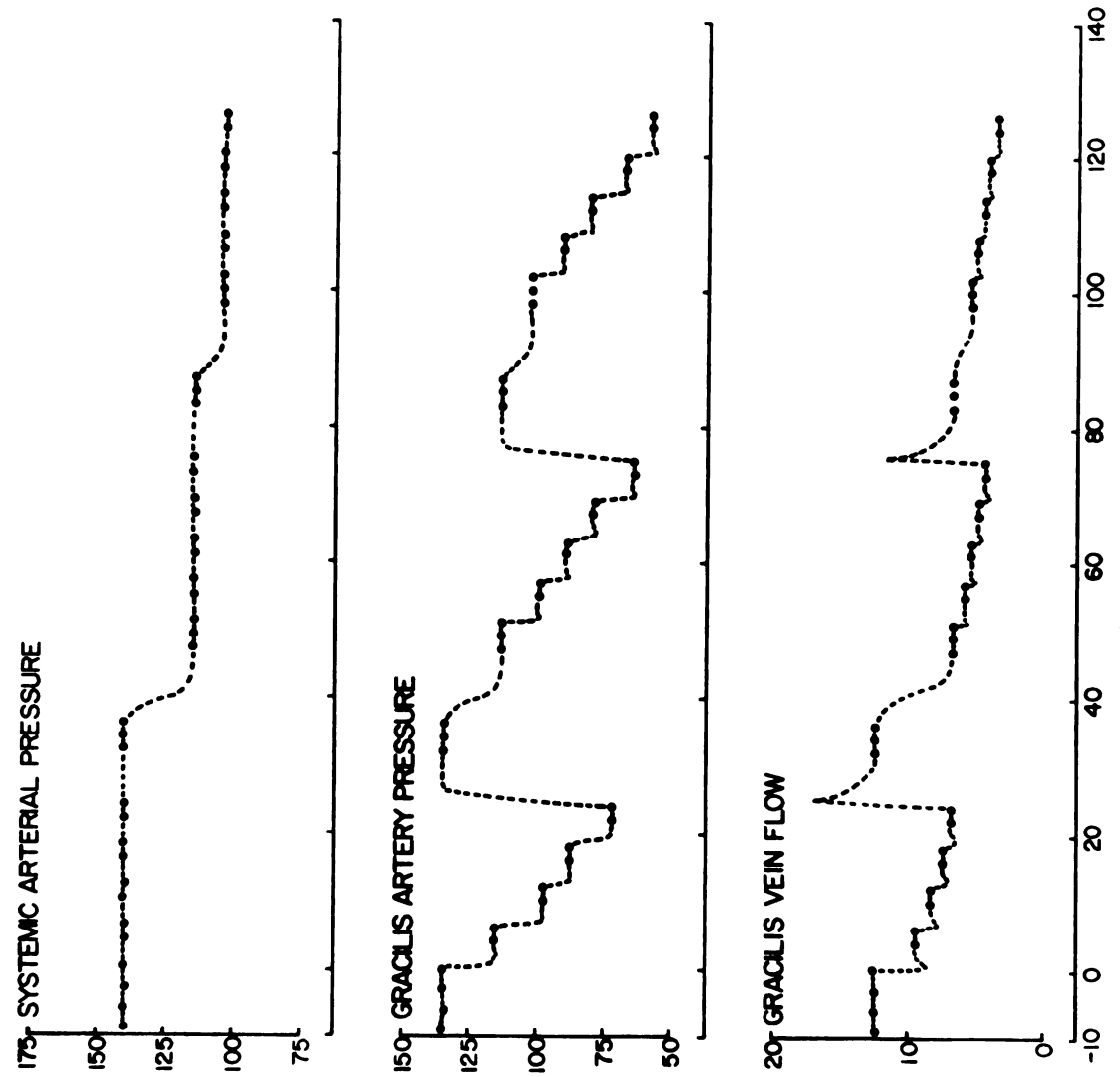
SYSTEMIC ARTERIAL PRESSURE
175
150
125
100
75
GRACILIS ARTERY PRESSURE
150
125
100
75
50
GRACILIS VEIN FLOW
20
10
0
-10
0
20
40
60
80
100
120
140
MINUTES

Figure 1

perfusion pressure and blood flow returned to pre-clamp control levels.

After a recovery period which was terminated when pressures and flow had stabilized (minute 37 on abscissa), the animals were rapidly hemorrhaged from a carotid artery into a pressurized reservoir until mean systemic arterial pressure was reduced by approximately 10%. When the hemorrhage induced constriction had developed, the procedure described above for graded gracilis artery pressure reduction and data collection was repeated while the animals were maintained hypovolemic and hypotensive. After the last flow determination, the clamp was released to allow pressures and flow to return to pre-clamp control levels. In 4 of the 10 experiments, the animals were rapidly hemorrhaged again (minute 89 on abscissa) to a systemic arterial pressure approximately 20% below normal. When pressures and flow had stabilized following the second hemorrhage, the procedure described above for local, graded hypotension and data collection was again repeated exactly as before.

Series II: Pump perfused, innervated gracilis muscles exposed to alterations in mean arterial pressure during normovolemic and hypovolemic periods.

The right gracilis muscles of 8 dogs were prepared as described in Series I and a variable speed, roller pump (Lange Model RE 161, Extracorporeal Medical Specialties, Inc., Mt. Laurel Township, New Jersey) was interposed

between the femoral and gracilis arteries. The pump was fitted with a 4-5" section of silicone treated (Siliclad, Clay Adams, Parsippany, New Jersey) latex rubber rubing so that pump flow rate was independent of outflow resistance. Latex rubber tubing with an internal diameter (i.d.) of 0.094" was used so that pulse frequency was approximately 9/min/cc of flow delivered. A t-tube connected to an air-filled 12" section of P.E. 360 tubing was inserted into the gracilis arterial perfusion line to serve as a compliance chamber for damping the pulse delivered by the pump. Pulse pressure was maintained approximately 1/3 of mean gracilis artery pressure by adjusting the volume of air within the compliance chamber. The d.c. output of the pump was coupled to a direct writing oscillograph (No. 7784A, Hewlett Packard Co., Waltham, Massachusetts) and pump flow was calibrated before each experiment so that each 1 cc increment of flow produced a 4 mm pen deflection. The relationship between pump flow and galvanometer pen deflection was linear throughout the flow ranges studied and the calibration was checked periodically during each experiment by comparing a timed collection of gracilis vein flow with the oscillographic pen deflection. A heat exchanger was placed around the arterial inflow tubing between the pump and the muscle. Gracilis arterial blood temperature was measured downstream from the heat exchanger by a thermister probe (No. PC-130, Gormann-Rupp Industries, Belleville, Ohio) coupled to a direct

reading temperature controller (No. EC-250, Gormann-Rupp Industries, Belleville, Ohio) which maintained gracilis arterial blood temperatures between 37 and 38° C by continuously adjusting fluid temperature in the heat exchanger.

A servosystem (Figure 2) continuously adjusted the flow rate of the perfusion pump to maintain mean gracilis artery pressure constant at 60, 80, 100, 120, 140, 180, or 200 mm Hg. After the muscles had been prepared and pump perfusion established, 10-20 minutes were allowed to: A) determine 7 optimal combinations of the servosystem's setpoint, proportional gain, and integral control action required to maintain mean gracilis artery pressure constant at 60, 80, 100, 120, 140, 180, and 200 mm Hg; and B) determine the 6 optimal combinations of the servosystem's proportional gain and integral control action required to change mean gracilis artery pressure from 140 to 60, 80, 100, 120, 180, and 200 mm Hg in 1-2 seconds with little or no overshoot (step pressure change). The servosystem parameters determined in B were used to return gracilus artery pressure from the pressures mentioned in B back to 140 mm Hg.

After a 30 minute control period in which the muscle was perfused at a mean gracilis artery pressure of 140 mm Hg, the servosystem control parameters were adjusted to that mean gracilis artery pressure was rapidly reduced to and maintained at 80 mm Hg for 4-5 minutes. The servosystem control parameters were then adjusted so that mean gracilis

Figure 2. Schematic of the servosystem used to control gracilis artery pressure.

1. Set point circuit--establishes a reference voltage which is compared to the voltage from the AC amplifier. When the controlled variable deviates from set point, an error voltage is developed.

2. Control panel adjustments--adds adjustable proportional gain, reset and rate, or lag functions to the error and feedback voltages. The feedback voltage is derived from the output current of the controller.

3. Control amplifier--compares the modified error and feedback voltages and provides an output voltage proportional to the difference voltage.

4. C.A.T. (Current Adjusting Type) output control--provides and maintains a current output at a constant operating level in the absence of a voltage from the amplifier; varies the output current according to polarity and amplitude of the amplifier output signal.

5. Automatic-Manual (A-M) switching circuits--transfers the control system bumplessly between automatic and manual modes, and permits manual adjustment of the final control element position in the manual mode. With automatic mode, perfusion pump speed is controlled to maintain perfusion pressure constant; with manual mode, constant flow perfusion is maintained.

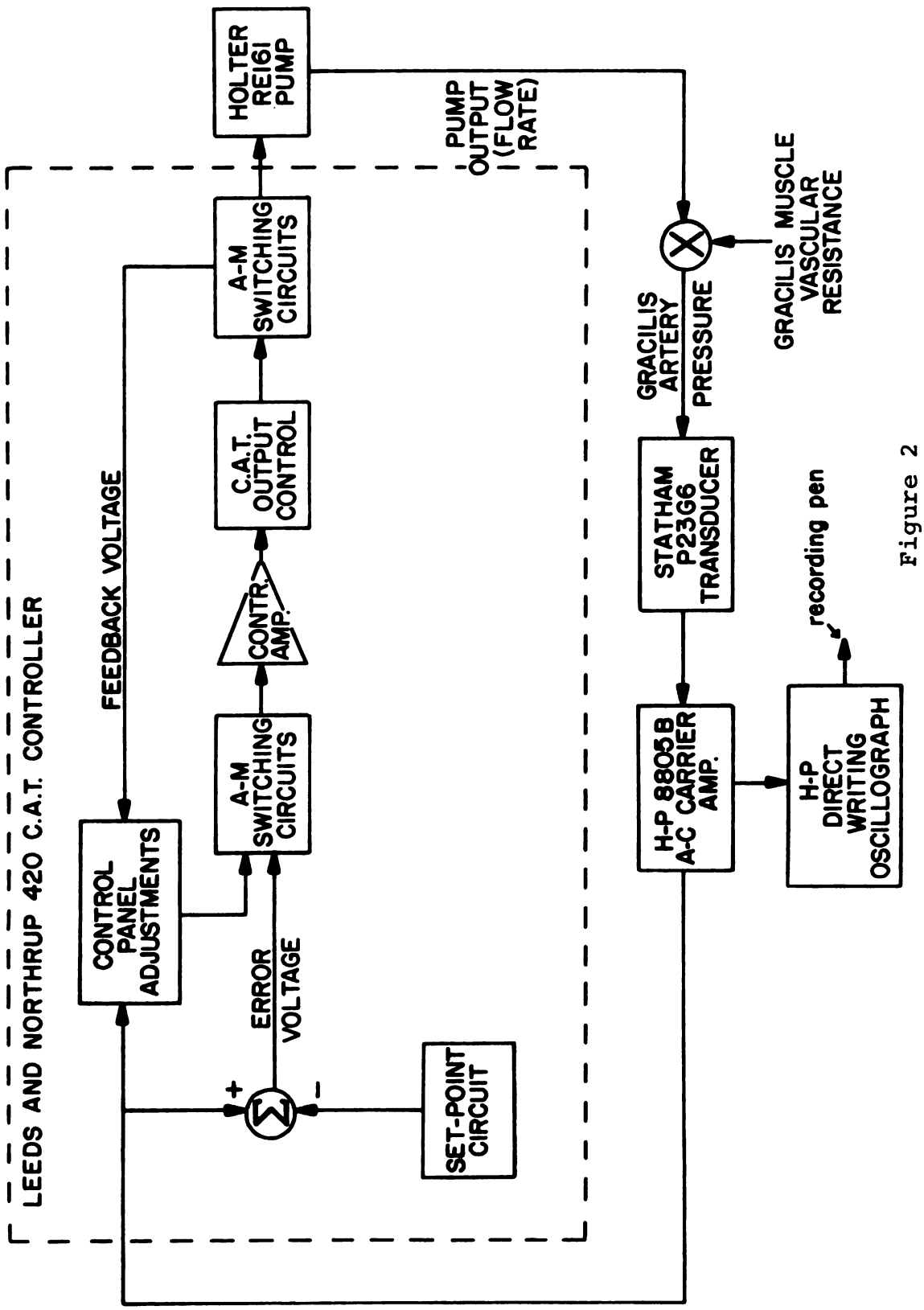
LEEDS AND NORTHRUP 420 C.A.T. CONTROLLER
HOLTER RE161 PUMP
PUMP OUTPUT (FLOW RATE)
A-M SWITCHING CIRCUITS
C.A.T. OUTPUT CONTROL
CONTR. AMP.
FEEDBACK VOLTAGE
CONTROL PANEL ADJUSTMENTS
A-M SWITCHING CIRCUITS
ERROR VOLTAGE
SET-POINT CIRCUIT
GRACILIS ARTERY PRESSURE
GRACILIS MUSCLE VASCULAR RESISTANCE
STATHAM P23G6 TRANSDUCER
recording pen
H-P 8803B A-C CARRIER AMP.
H-P DIRECT WRITING OSCILLOGRAPH

Figure 2

artery pressure was rapidly restored to and maintained at 140 mm Hg for 4 minutes. The procedure just described for a square wave change in mean gracilis artery pressure from 140 to 80 and back to 140 mm Hg was then repeated for mean gracilis artery pressures of 60, 100, 120, 180, and 200 mm Hg in a random sequence. Finally, the protocol described above for pressure reduction to 80 mm Hg and pressure restoration to 140 mm Hg was repeated.

While mean gracilis artery pressure was maintained constant at 140 mm Hg by the action of the servosystem on pump flow rate, the animals were rapidly hemorrhaged from a carotid artery into a pressurized reservoir until mean systemic arterial pressure fell to 100 mm Hg. After a control period of 20-40 minutes during which the hemorrhage induced constriction was allowed to develop fully, the protocol described for alterations in mean gracilis artery pressure was repeated while the animals were maintained hypovolemic and hypotensive. During hypovolemia the sequence of gracilis artery pressure changes and the times at a given pressure were matched to those obtained during normovolemia. After the last gracilis artery pressure manipulation, the gracilis nerve was coated with a local anesthetic (Cetacaine, Cetylite Industries, Long Island City, New York), severed, and the responses to denervation were followed for 30-40 minutes.

As described above, mean gracilis artery pressure was altered throughout each experiment by changing the servosystem's control parameters so as to alter pressure within 1-2 seconds (Figure 3). The most immediate blood flow responses to sudden changes in perfusion pressure are flow minimums or maximums occurring within 5-10 seconds after the pressure change. Similar transient responses have also been described by other investigators (116, 117), and are thought to result from passive collapse or expansion of the vasculature subsequent to the change in distending pressure (117) (see Discussion, Section I-B). Since the initial, rapid changes in pressure reported here are the result of sudden alterations in pump flow rate, it is possible that a significant portion of the recorded changes in blood flow represent artifacts of the servocontrolled pump flow rate and do not accurately reflect passively mediated changes in vascular resistance. To resolve this question, a manually operated pump (Lange Model RE 161, Extracorporeal Medical Specialties, Inc., Mt. Laurel Township, New Jersey) fitted with latex rubber tubing, was connected in series with the servocontrolled pump to provide a downstream resistance. When flow rate in the manually operated, downstream pump was halved or doubled within 0.2 seconds, the servocontrolled pressure and flow transients lasted approximately 1.0 seconds (Figure 4, panel B). Since all recorded blood flow transients to square wave changes in gracilis artery

Figure 3. Gracilis artery blood flow responses to step changes in perfusion pressure. Mean gracilis and systemic arterial pressures are expressed in mm Hg; gracilis artery flow is represented in ml/min. T_1 indicates time to peak minimum or maximum flow transients, while T_2 depicts time to steady state response. Data are from a selected pump perfused gracilis muscle weighing 93.5 gms.

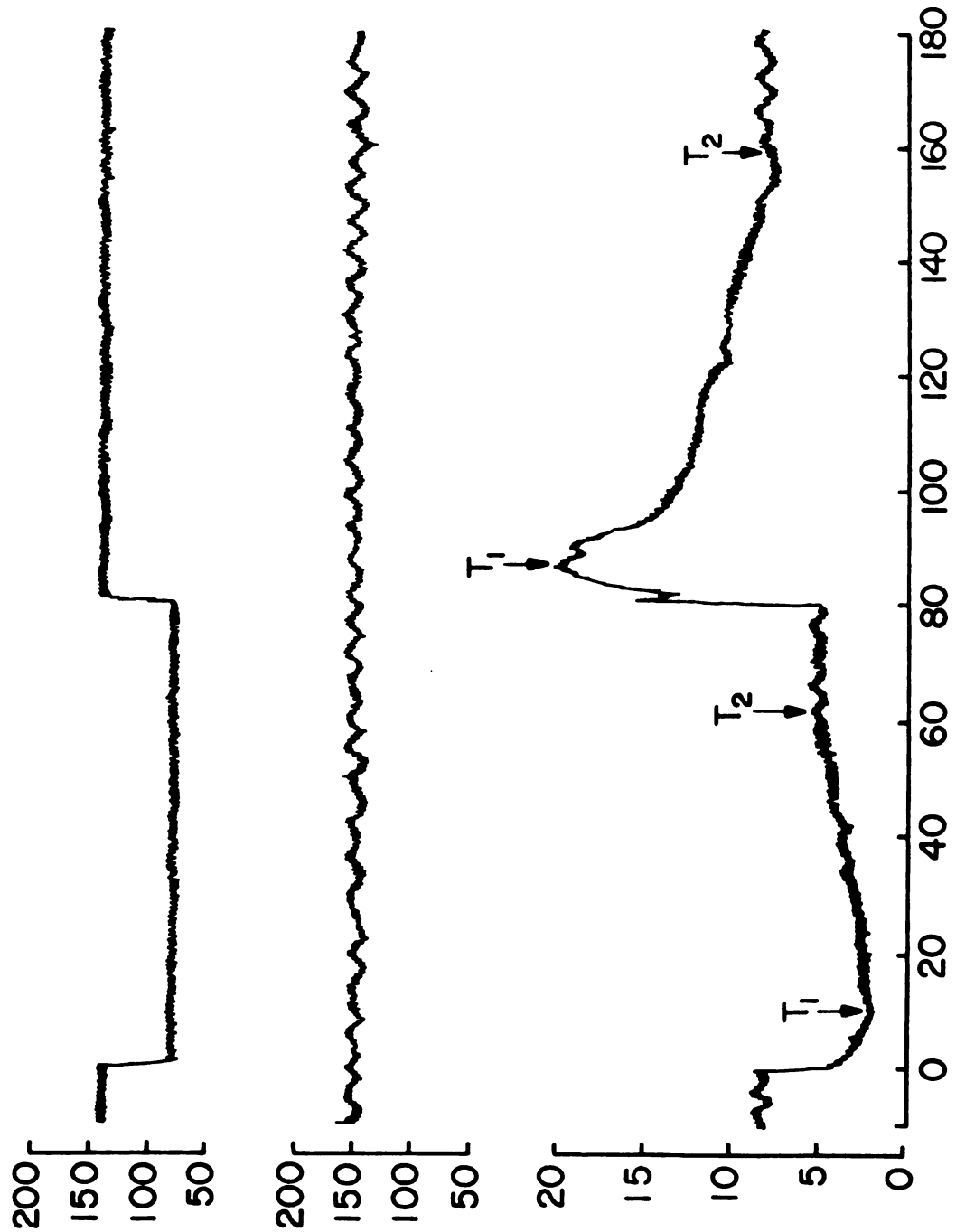
GRACILIS ARTERY PRESSURE
200
150
100
50
SYSTEMIC ARTERIAL PRESSURE
200
150
100
50
GRACILIS ARTERY FLOW
20
15
10
5
0
T1
T2
T1
T2
0
20
40
60
80
100
120
140
160
180
SECONDS

Figure 3

Figure 4. Responses of servocontrolled pressure (mm Hg) and pump flow rate (cc/min) to step changes in load. Panel A, manually operated pump connected in series upstream to the servocontrolled pump so that the manually operated pump provided an upstream flow source to the servocontrolled pump. Servosystem is controlling pressure between the pumps. Panel B, manually operated pump connected in series downstream from the servocontrolled pump so that the manually operated pump provided a downstream resistance to the servocontrolled pump. Servosystem is controlling pressure between the pumps.

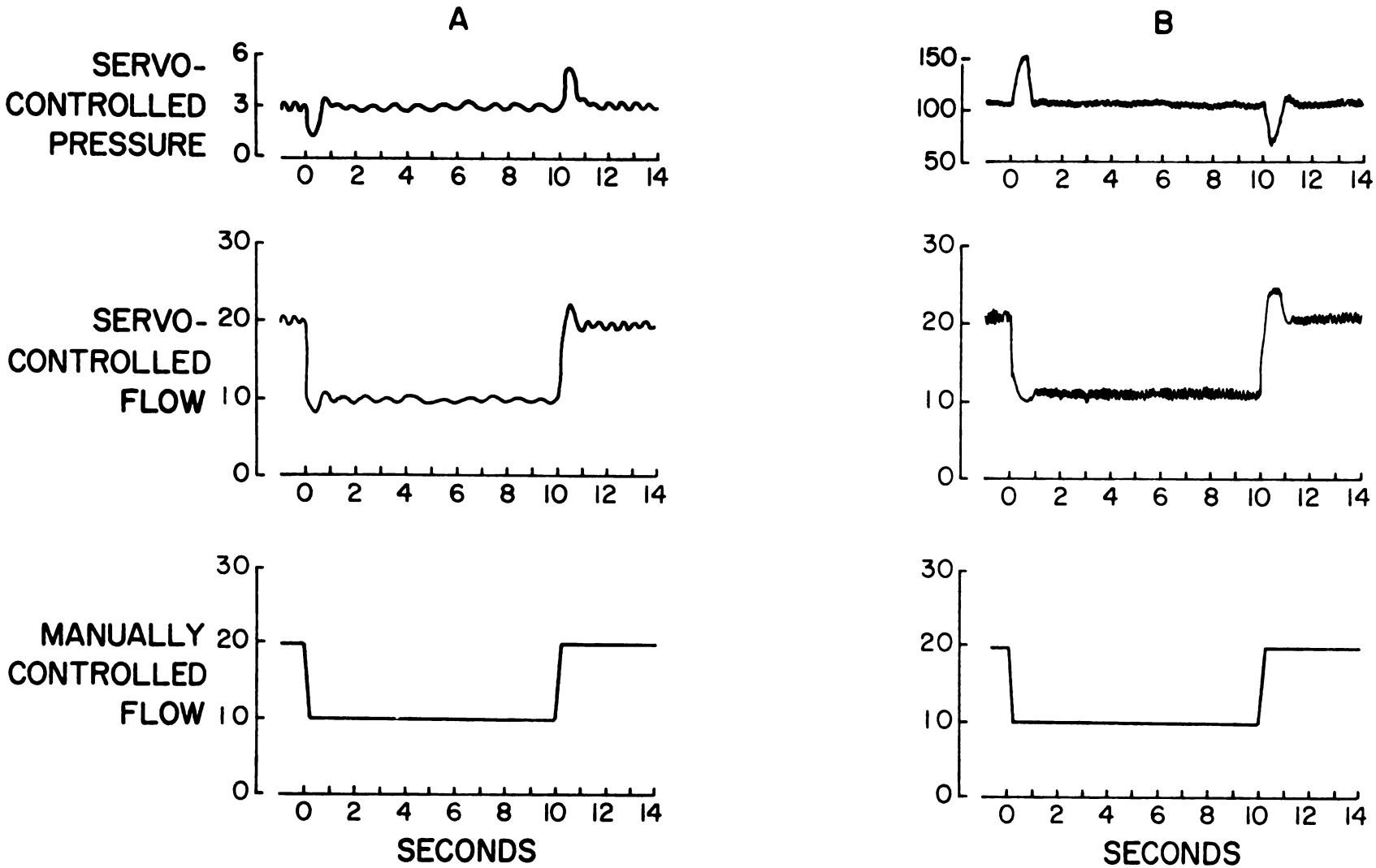
A
B
SERVO-
CONTROLLED
PRESSURE
6
3
0
150
100
50
0 2 4 6 8 10 12 14
SERVO-
CONTROLLED
FLOW
30
20
10
0
MANUALLY
CONTROLLED
FLOW
SECONDS

Figure 4

pressure took at least 6 seconds to develop (see Results, section II-A), the oscillographic blood flow recordings were assumed to reflect actual changes in vascular resistance.

After each step change in gracilis artery pressure, transient muscle blood flow responses similar to those depicted in Figure 3 were observed in all 8 experiments. Data collected for subsequent analysis included: a) pressure and flow values prior to a change in perfusion pressure; b) intravascular pressures and peak minimum or maximum flow transient values at time T_1 following a step change in perfusion pressure; c) the time to reach these initial flow transients (T_1); d) the time to reach the new steady-state level of flow (T_2); e) intravascular pressures and muscle blood flow at time T_2; f) the total time at each pressure; and g) the planimetered area of the oscillographic flow recording above or below a horizontal line projected to the left from the steady state flow at time T_2.

Series III: Naturally perfused, denervated gracilis muscles exposed to elevation of mean vascular distending pressure and to local hypotension during pulsatile or non-pulsatile perfusion.

The right gracilis muscles of 10 dogs were prepared as described above and acutely denervated. In all experiments, the animals were ventilated with room air through a cuffed endotracheal tube with a positive pressure respirator (No. 607, Harvard Apparatus Co., Dover, Massachusetts).

The muscle was perfused through a 6-8" section of silicone treated (Siliclad, Clay Adams, Parsippany, New Jersey) latex rubber tubing (i.d. = 0.25") inserted between the femoral and gracilis arteries. Within the length of rubber tubing a t-tube was inserted to which was attached a 25" section of P.E. 360 tubing. The distal end of the P.E. 360 tubing was fitted with a rubber bulb containing an air inlet valve to control pressure within the air-filled length of P.E. 360 tubing (compliance chamber). When pressure within the compliance chamber was equal to mean gracilis artery pressure, perfusion could be suddenly converted from pulsatile to nearly non-pulsatile without changing mean pressure by removing a clamp placed at the blood-air interface in the compliance chamber. In addition to mean gracilis artery and venous outflow pressures, gracilis artery pulse pressure was also measured from a side branch of the gracilis artery.

The gracilis vein was cannulated with P.E. 240 tubing downstream from the site of pressure measurement and the venous outflow was directed into the right femoral vein via a servocontrolled pump (Lange Model RE 161, Extracorporeal Medical Specialties, Inc., Mt. Laurel Township, New Jersey) which maintained a constant gracilis vein pressure. The servosystem depicted in Figure 2 was used except that the direction of control was reversed so that, for example, elevations in gracilis vein pressure caused by an increased muscle blood flow resulted in an increased pump flow.

That is, the pump functioned as part of a closed-loop, negative feedback, system which operated to keep gracilis vein pressure nearly constant. As described in Series II, the pump was fitted with latex rubber tubing so that pump flow rate was independent of outflow resistance. An air-filled compliance chamber was inserted on the inflow side of the pump to remove pulse pressure oscillations originating from the pump. The d.c. output of the pump was coupled to a direct writing oscillograph (No. 778A, Hewlett Packard Co., Waltham, Massachusetts) and pump flow rate was calibrated before each experiment so that a 1 cc increment of flow corresponded to a 4 mm pen deflection. The relationship between pump flow and galvanometer pen deflection was linear throughout the flow ranges studied and the calibration was checked several times during each experiment by comparing timed collections of pump flow with oscillographic pen deflections.

To determine that the oscillographic recording of pump flow accurately reflected changes in gracilis vein flow, a manually operated pump (Lange Model RE 161, Extracorporeal Medical Specialties, Inc., Mt. Laurel Township, New Jersey) fitted with latex rubber rubing, was connected in series upstream to the servocontrolled pump, thereby providing a flow source to the servocontrolled pump. When the manually operated, upstream pump flow was halved or doubled within 0.2 seconds, the servocontrolled pressure and flow transients

lasted approximately 0.8 seconds (Figure 4, Panel A). Since all recorded flow transients in this series of experiments took at least 4 seconds to develop (see Results, section III-A) the oscillographic blood flow recordings were assumed to accurately reflect gracilis muscle blood flow.

After the muscles had been prepared and perfusion established, the servosystem parameters required to maintain gracilis vein pressure constant at 4 or 24 mm Hg were determined and the preparation was allowed to stabilize for a 30-40 minute period during non-pulsatile perfusion with gracilis vein pressure held constant at 4 mm Hg. As illustrated in Figure 5, when intravascular pressures and muscle blood flow had stabilized, a screw clamp on the gracilis artery was tightened so that mean gracilis artery pressure fell 20 mm Hg. After the transient flow responses had subsided, the arterial screw clamp and the setpoint of the outflow pump servocontroller were adjusted so that mean gracilis arterial and venous pressures were simultaneously increased 20 mm Hg, producing an elevation in mean vascular distending pressure with no change in effective pressure gradient. After the transient flow responses had subsided, vein pressure was reduced to the control level by adjusting the outflow pump servocontroller parameters and pulsatile perfusion was restored by closing off the arterial compliance chamber. Control pressures and flow during pulsatile perfusion were determined and the procedure just described

Figure 5. Gracilis arterial and venous pressures (mm Hg) and gracilis vein flow (cc/min/100 gms) as functions of time from the initial portion of a selected Series III experiment. Mean vascular distending pressure was first elevated 20 mm Hg during non-pulsatile and then during pulsatile perfusion. Next mean gracilis artery pressure was reduced in steps to 120, 100, 80, and 60 mm Hg during pulsatile perfusion.

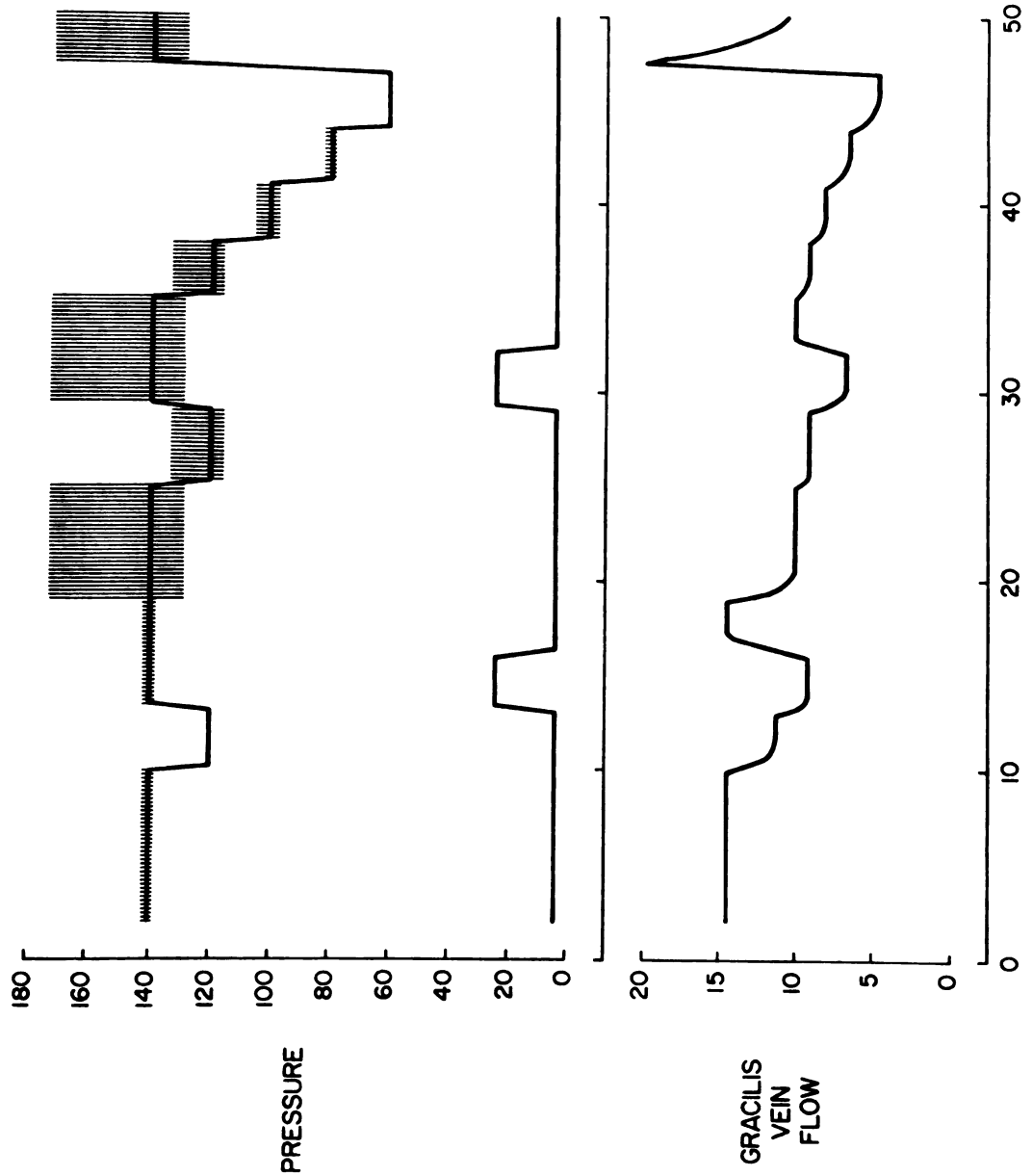
180
160
140
120
100
80
60
40
20
0
PRESSURE
20
15
10
5
0
GRACILIS
VEIN
FLOW
0
10
20
30
40
50
MINUTES

Figure 5

for elevation of mean vascular distending pressure was repeated while the perfusion was pulsatile. When pressures and flow had stabilized after the manuever to increase mean vascular distending pressure, the screw clamp on the gracilis artery was tightened so that mean gracilis artery pressure was reduced in steps to 120, 100, 80, and 60 mm Hg. After each pressure reduction, time was allowed for the transient responses to subside. The clamp was then released so that pressures and flow returned to control levels and the entire protocol sequence depicted in Figure 5 (minutes 0-50) was repeated except that the pulsatile mode of perfusion was reversed.

To determine that the observed resistance responses resulted from active changes in vascular radius, a constant infusion of papaverine hydrochloride (a smooth muscle poison) was administered into the gracilis arterial supply. In all experiments a papaverine concentration was used that increased muscle blood flow 5-8 fold without reducing mean gracilis artery pressure more than 5-10 mm Hg. In 4 separate preparations the concentration range of papaverine used in these experiments was found to be effective in blocking the normal gracilis vasculature responses to test doses of norepinephrine and acetylcholine (Table 1).

Because it was not possible to produce step changes in mean gracilis artery pressure by adjustment of the arterial screw clamp, an analysis of blood flow transients

Table 1. Effects of papaverine infusions on gracilis muscle vascular responses to graded infusions of norepinephrine and acetylcholine. Values are means from 4 experiments.

	Gracilis Artery Pressure[a]	Gracilis Vein Flow[b]	Gracilis Vascular Resistance[c]	% of Control Flow	% of Control Resistance
		Before Papaverine Infusion			
Control	142.2	13.4	10.6	100	100
Norepinephrine					
0.1 µg/min	142.3	7.9	17.9	58.9	169.2
0.5 µg/min	141.7	3.2	44.0	23.7	416.1
1.0 µg/min	141.9	1.4	101.2	10.6	955.2
Acetylcholine					
0.1 µg/min	141.9	34.3	4.2	261.5	38.7
0.5 µg/min	142.3	52.1	2.7	400.2	24.9
1.0 µg/min	141.8	65.1	2.2	493.1	21.0
		Constant Papaverine Infusion 1.9 mg/min			
Control	138.1	64.9	2.16	100	100
Norepinephrine					
0.1 µg/min	138.1	61.8	2.21	95.4	104.8
0.5 µg/min	138.3	59.4	2.34	92.5	109.4
1.0 µg/min	138.2	57.3	2.42	86.9	114.2
Acetylcholine					
0.1 µg/min	138.1	65.1	2.12	100.4	99.8
0.5 µg/min	138.1	65.5	2.10	101.1	96.4
1.0 µg/min	138.3	66.6	2.07	103.3	95.2
		Constant Papaverine Infusion 3.8 mg/min			
Control	133.2	93.9	1.41	100	100
Norepinephrine					
0.1 µg/min	133.4	91.2	1.45	97.2	102.8
0.5 µg/min	133.4	90.4	1.47	96.2	104.3
1.0 µg/min	133.3	89.6	1.49	95.4	105.7
Acetylcholine					
0.1 µg/min	133.1	94.2	1.41	100.3	99.8
0.5 µg/min	133.2	95.0	1.40	101.2	99.4
1.0 µg/min	133.1	95.3	1.39	101.5	99.2

[a] mm Hg

[b] cc/min/100 gms

[c] mm Hg/cc/min/100 gms

subsequent to reductions in perfusion pressure or elevations of mean vascular distending pressure was not performed. However, since it was possible to instantaneously switch the mode of perfusion from pulsatile to non-pulsatile or vice-versa, analysis of the transient blood flow patterns resulting from a change in the mode of perfusion was accomplished. After each mode change of gracilis artery perfusion, muscle blood flow responses similar to those depicted in Figure 6 were observed throughout all 8 experiments. Data collected from subsequent analysis were: a) mean and pulsatile intravascular pressures and venous flow prior to a change in the mode of perfusion; b) intravascular pressures and peak maximum of minimum transient flow values resulting from a change in the mode of perfusion; c) the time required to reach these initial flow transients; d) the time required to reach the new steady-state level of flow; and e) intravascular pressures and muscle blood flow at the new steady state.

Figure 6. Gracilis arterial and venous pressure (mm Hg) and venous flow (cc/min) responses to changing the mode of perfusion from non-pulsatile to pulsatile and vice versa. Data are from a selected naturally perfused gracilis muscle weighing 103.5 gms.

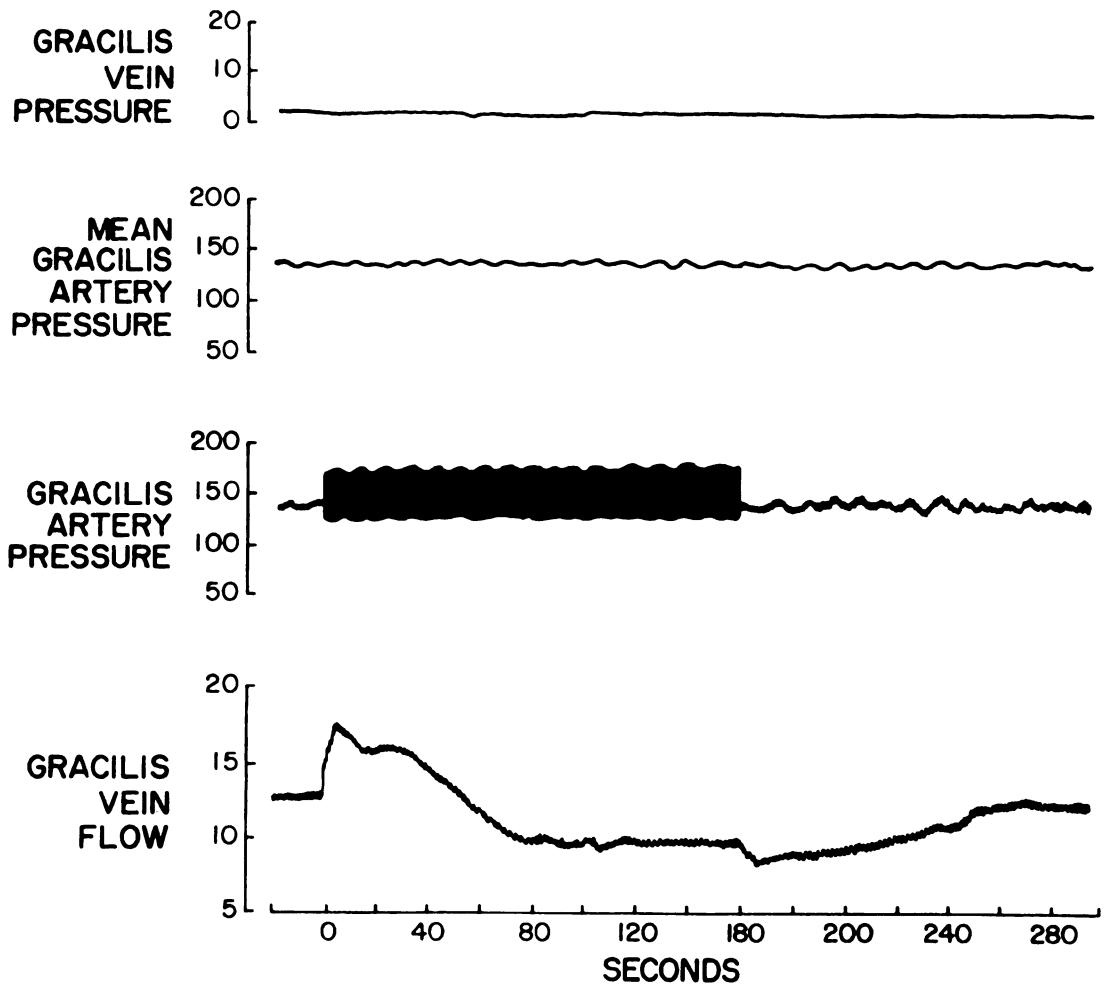
GRACILIS VEIN PRESSURE
20
10
0
MEAN GRACILIS ARTERY PRESSURE
200
150
100
50
GRACILIS ARTERY PRESSURE
200
150
100
50
GRACILIS VEIN FLOW
20
15
10
5
0
40
80
120
180
200
240
280
SECONDS

Figure 6

DATA ANALYSIS

Differences among means (muscle blood flow and vascular resistance) at the different levels of local hypotension obtained during normovolemia, hypovolemia, pulsatile perfusion, or non-pulsatile perfusion were compared by using the Student-Newman-Kuels procedure, a stepwise method in which the range is the test statistic. A Students t test for paired observations was used to analyze whether hemorrhage (Series II) or non-pulsatile perfusion (Series III) produced significantly different values from their respective controls with regard to a given parameter. A least square linear regression analysis was performed on each set of % of control flow and resistance data obtained over the autoregulatory range of perfusion pressures. The slopes obtained from the linear regression of control data were compared with the slopes of experimental data by using an analysis of covariance. In all three series of experiments, blood flow autoregulation was determined to be present if a given percent change in local perfusion pressure elicited a smaller percent change in flow and a directionally similar change in vascular resistance. For all comparisons, differences between means or regression coefficients were considered significant only if the

probability of making a type I error (α) was less than 0.05. A more detailed description of the statistical methods used is presented in Appendix B.

RESULTS

I. Series I: Naturally Perfused, Innervated Gracilis Muscle; Effects of Local Hypotension During Normovolemic and Hypovolemic Periods

A. Muscle Blood Flow

Figure 7 reports gracilis vein flow as a function of gracilis artery pressure during graded, local hypotension in normovolemic and hypovolemic dogs. When the animals were normovolemic, muscle blood flow was significantly reduced from control with each step reduction in perfusion pressure. The gracilis vasculature of these normovolemic dogs displayed blood flow autoregulation in response to graded local hypotension as evidenced by the fact that a 45 $\pm$ 3.1% decrease in arterial pressure from 130 $\pm$ 4.2 to 72 $\pm$ 2.6 mm Hg elicited only a 39 $\pm$ 2.6% decrease in flow from 12.6 $\pm$ 2.0 to 7.7 $\pm$ 1.1 cc/min/100 gms.

When gracilis artery pressure was reduced from the normovolemic control value of 130 $\pm$ 4.2 to 115 $\pm$ 4.1 mm Hg by hemorrhage, muscle blood flow decreased significantly from 12.6 $\pm$ 2.0 to 6.8 $\pm$ 0.7 cc/min/100 gms. This hemorrhage induced reduction of gracilis artery pressure is quantitatively similar to the first local reduction of perfusion pressure obtained during normovolemia. The fall in blood flow accompanying hemorrhage is however, significantly

Figure 7. Effects of local hypotension during normovolemic (solid dots) and hypovolemic (circles) periods on gracilis vein flow. Ordinate reports gracilis vein flow in cc/min/100 gms and abscissa reports gracilis artery pressure in mm Hg. Data represent means ± standard errors from 10 experiments.

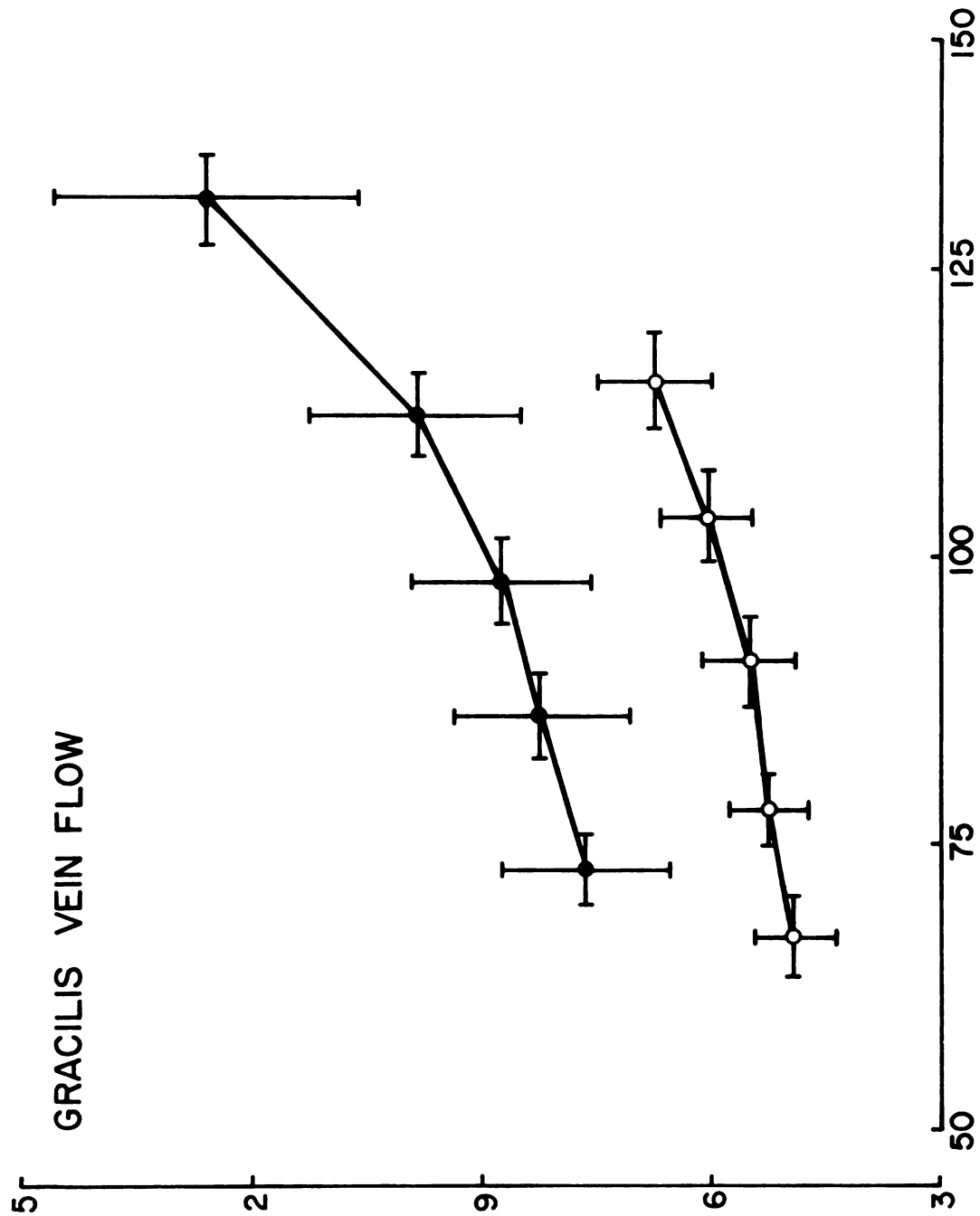
GRACILIS VEIN FLOW
15
12
9
6
3
50
75
100
125
150
GRACILIS ARTERY PRESSURE

Figure 7

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greater than that accompanying equivalent local hypotension.

When the animals were maintained hypovolemic and hypotensive, muscle blood flow was significantly reduced from control with each step reduction in perfusion pressure. During hypovolemia, the responses to graded local hypotension elicited blood flow autoregulation as evidenced by the fact that a 43 ± 2.9% decrease in gracilis artery pressure from 115 ± 4.1 to 66 ± 3.0 mm Hg produced only a 28 ± 2.4% decrease in flow from 6.8 ± 0.8 to 4.9 ± 0.5 cc/min/100 gms.

Figure 8 reports gracilis vein flow as a function of gracilis artery pressure for the four experiments in which the animals were hemorrhaged a second time. During normovolemia, step reductions of gracilis artery pressure from a control value of 137.8 ± 5.0 to 101 ± 5.1 mm Hg and below significantly decreased gracilis muscle blood flow. Autoregulation of blood flow occurred while the animals were normovolemic since a 46 ± 3.0% decrease in gracilis artery pressure from 138 ± 5.0 to 74 ± 2.5 mm Hg elicited only a 33 ± 2.7% decrease in flow from 11.5 ± 1.7 to 7.7 ± 1.4 cc/min/100 gms.

When gracilis artery pressure was reduced from the normovolemic control of 138 ± 5.0 to 121 ± 5.6 and 103 ± 4.8 mm Hg by two successive arterial hemorrhages, muscle blood flow decreased significantly from 11.5 ± 1.7 to 7.9 ± 1.4 and 5.1 ± 1.3 cc/min/100 gms. These flow reductions elicited by hemorrhage were significantly greater than

Figure 8. Effects of local hypotension during normovolemic (dots) and two hypovolemic (circles and squares) periods. Ordinate represents gracilis vein flow in cc/min/100 gms and abscissa depicts gracilis artery pressure in mm Hg. Data are means ± standard errors from four experiments.

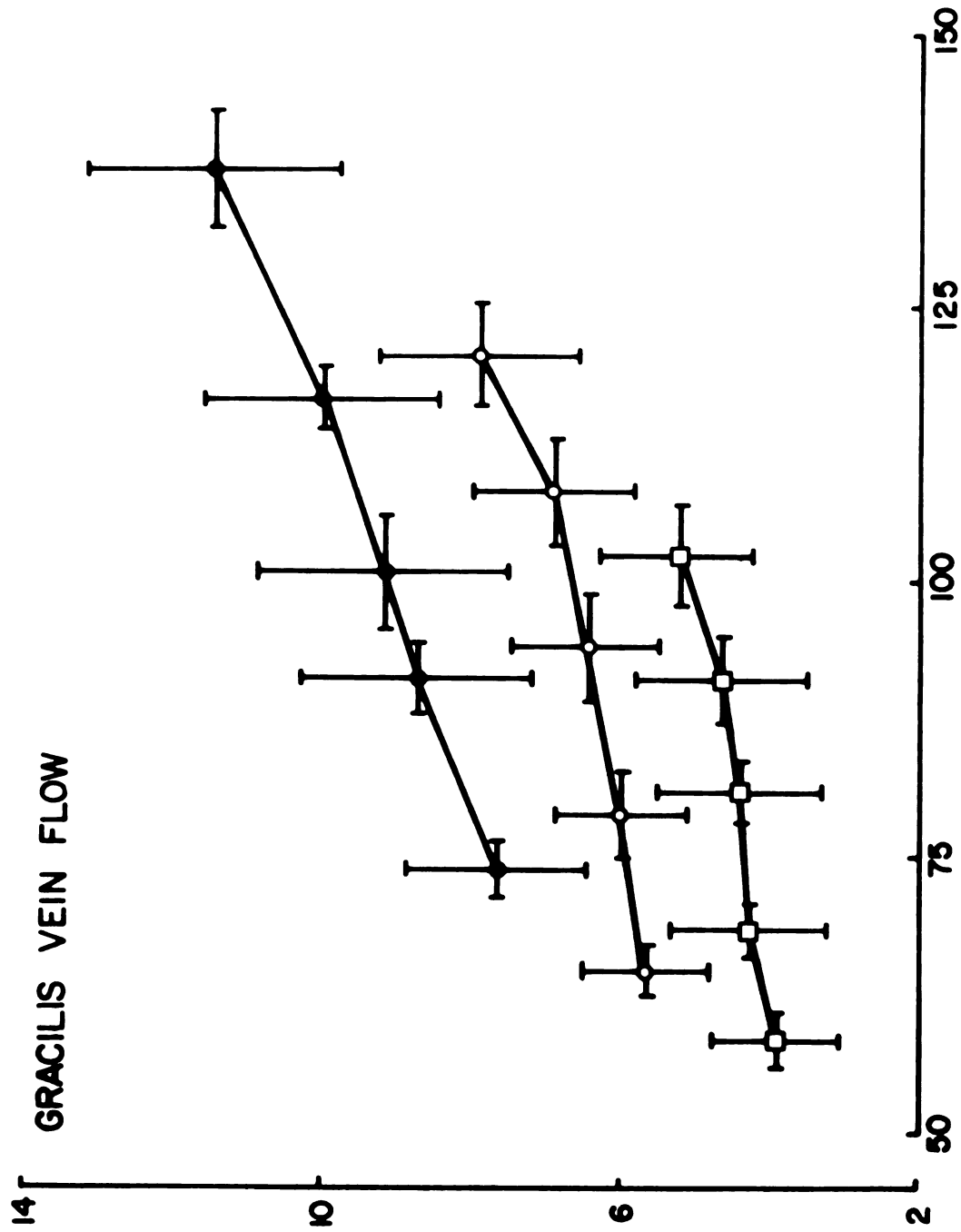
GRACILIS VEIN FLOW
14
10
6
2
50
75
100
125
150
GRACILIS ARTERY PRESSURE

Figure 8

those accompanying equivalent local pressure reductions produced during normovolemia.

During moderate hypovolemia (line connecting circles), step reductions of gracilis artery pressure from a control value of 120.8 ± 5.6 to 94.3 ± 7.2 mm Hg and below significantly decreased gracilis muscle blood flow. The ability to autoregulate blood flow was not compromised during the first hemorrhage, since a 46 ± 2.5% local reduction in gracilis artery pressure from 120 ± 5.6 to 65 ± 2.4 mm Hg produced only a 28 ± 2.3% fall in muscle blood flow from 7.9 ± 1.4 to 5.7 ± 0.9 cc/min/100 gms. During the second, more severe hemorrhage (line connecting squares), step reductions of gracilis artery pressure from a control value of 102.5 ± 4.8 to 81 ± 2.9 mm Hg and below significantly decreased gracilis muscle blood flow. The ability to autoregulate blood flow was again maintained since a 43 ± 3.1% reduction in gracilis artery pressure from 103 ± 4.8 to 59 ± 2.5 mm Hg elicited only a 24 ± 2.4% fall in muscle blood flow from 5.1 ± 1.6 to 3.9 ± 0.5 cc/min/100 gms.

To more accurately describe and compare the relationships between muscle blood flow and perfusion pressure during normovolemia and hypovolemia, the data reported in Figure 7 over the gracilis artery pressure range of 70-120 mm Hg were normalized to percent of control and subjected to linear regression analysis (Figure 9). The data obtained during normovolemia demonstrated a significant regression

Figure 9. Effects of local hypotension during normovolemic (dots and continuous lines) and hypovolemic (circles and dashed lines) periods on gracilis vein flow (upper panel) and gracilis muscle vascular resistance (lower panel). Ordinates represent % of control gracilis vein flow and % of control gracilis muscle vascular resistance; abscissae represent % of control gracilis artery pressure. Data are individual means from 10 experiments over the gracilis artery pressure range of 70-120 mm Hg. For a given experiment, only those data obtained within the same pressure range for both normovolemic and hypovolemic periods are reported. The control values used to normalize the data were always those obtained at the highest gracilis artery pressure within the range described for each experiment. Lines through each set of data are the lines of best fit obtained by least square linear regression analysis.

$$\%\ \text{of C - Flow}_{\text{Normov.}} = 0.623 \pm 0.052\ (\%\ \text{of C - Press.}) + 38.73 \pm 3.18;\ R^2 = 0.689$$

$$\%\ \text{of C - Flow}_{\text{Hypov.}} = 0.701 \pm 0.054\ (\%\ \text{of C - Press.}) + 28.77 \pm 3.23;\ R^2 = 0.713$$

$$\%\ \text{of C - Resist.}_{\text{Normov.}} = 0.712 \pm 0.079\ (\%\ \text{of C - Press.}) + 54.47 \pm 4.32;\ \bar{R}^2 = 0.593$$

$$\%\ \text{of C - Resist.}_{\text{Hypov.}} = 0.679 \pm 0.083\ (\%\ \text{of C - Press.}) + 58.71 \pm 4.56;\ \bar{R}^2 = 0.617$$

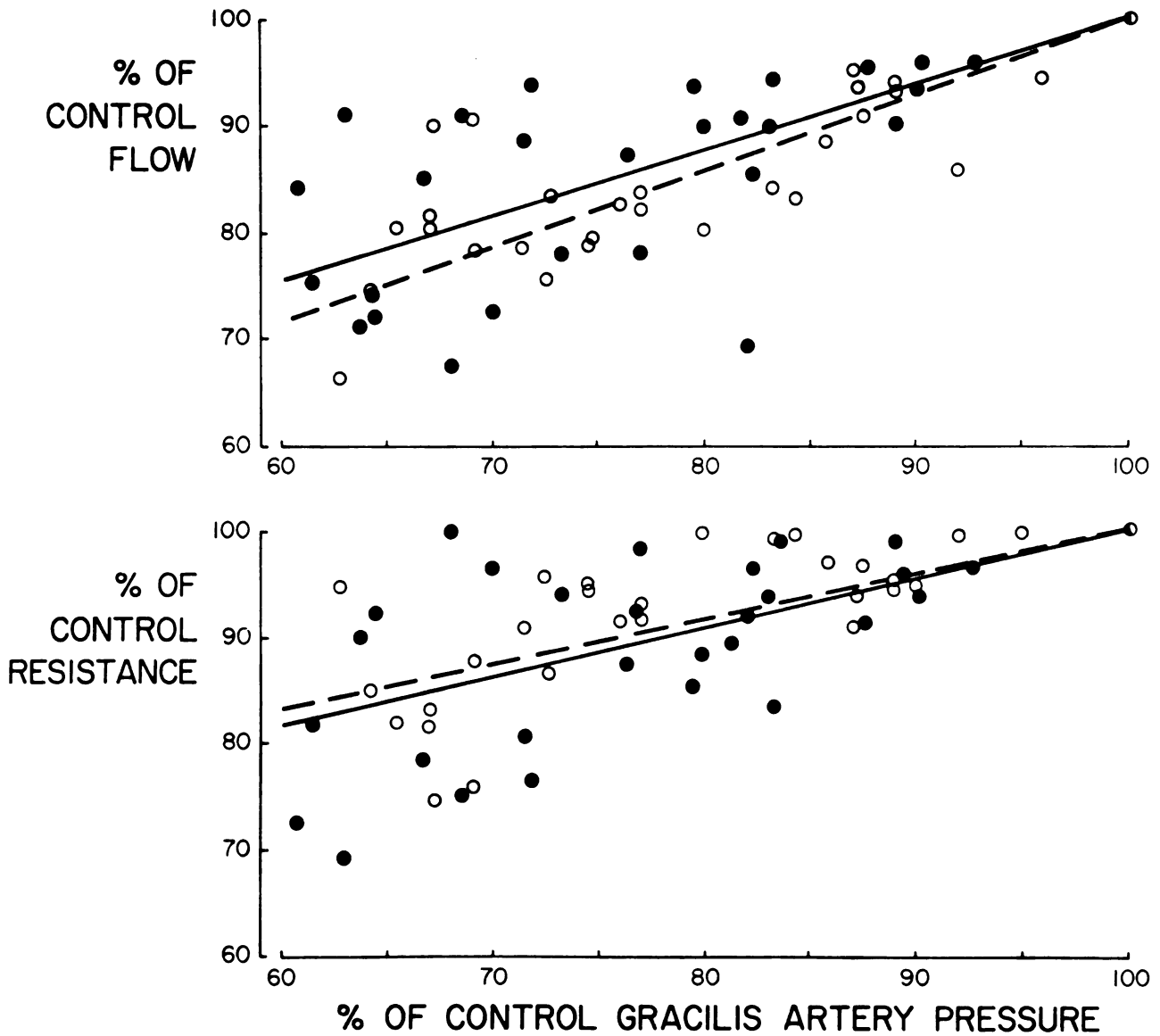
% OF CONTROL FLOW
100
90
80
70
60
60
70
80
90
100
% OF CONTROL RESISTANCE
100
90
80
70
60
60
70
80
90
100
% OF CONTROL GRACILIS ARTERY PRESSURE

Figure 9

of flow as a function of perfusion pressure with a slope of 0.62, indicating that the gracilis vasculature autoregulated its blood flow. The data obtained during hypovolemia also showed a significant regression of flow as a function of perfusion pressure with a slope of 0.70. Because this regression coefficient is not significantly different from that observed during normovolemia, the hemorrhage induced vasoconstriction did not impair the ability of the gracilis vasculature to autoregulate flow in response to local hypotension.

B. Muscle Vascular Resistance

Figure 10 reports gracilis muscle vascular resistance as a function of gracilis artery pressure during graded, local hypotension in normovolemic and hypovolemic dogs. When the animals were normovolemic, reduction of gracilis artery pressure from 112.1 $\pm$ 3.6 to 72.4 $\pm$ 2.6 mm Hg elicited a significant reduction in muscle vascular resistance from 12.8 $\pm$ 1.9 to 10.4 $\pm$ 1.6 mm Hg/cc/min/100 gms. When gracilis artery pressure was reduced below the normovolemic control of 130 $\pm$ 4.2 to 114 $\pm$ 4.1 mm Hg by hemorrhage, muscle vascular resistance increased significantly from 11.7 $\pm$ 1.8 to 17.8 $\pm$ 1.9 mm Hg/cc/min/100 gms. During hypovolemia, local step reductions of gracilis artery pressure from a control value of 115 $\pm$ 4.1 to 77.9 $\pm$ 3.0 and 66.4 $\pm$ 3.0 mm Hg significantly reduced gracilis muscle vascular

Figure 10. Effects of local hypotension during normovolemic (dots) and hypovolemic (circles) periods on gracilis muscle vascular resistance. Ordinate represents gracilis muscle vascular resistance in mm Hg/cc/min/100 gms and abscissa represents gracilis artery pressure in mm Hg. Data are means ± standard errors from 10 experiments.

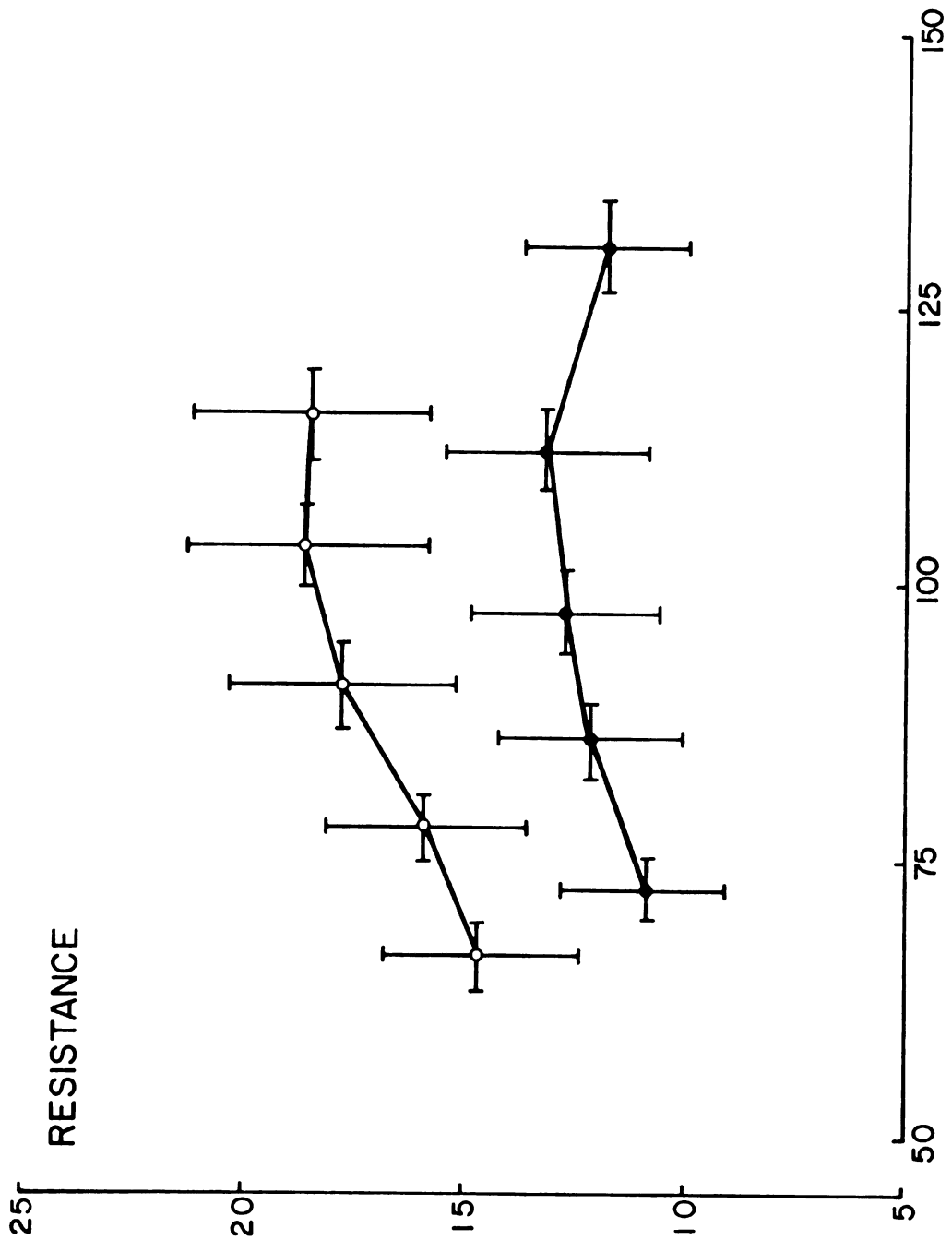
RESISTANCE
25
20
15
10
5
50
75
100
125
150
GRACILIS ARTERY PRESSURE

Figure 10

resistance from a control value of 17.8 ± 1.9 to 15.2 ± 1.8 and 13.9 ± 1.7 mm Hg/cc/min/100 gms.

To better describe and compare the relationships between muscle vascular resistance and perfusion pressure during normovolemia and hypovolemia, the data reported in Figure 10 over the gracilis artery pressure range of 70-120 mm Hg were normalized to percent of control and subjected to linear regression analyses (Figure 9, lower panel). The data obtained during normovolemia showed a significant regression of resistance as a function of perfusion pressure with a slope of 0.71. The data obtained during hypovolemia also showed a significant regression of resistance as a function of perfusion pressure with a slope of 0.68, which was not significantly different from that obtained for the normovolemic data.

Table 2 presents control pressure, flow and resistance data obtained before and after local graded hypotension during both normovolemic and hypovolemic periods. At all systemic arterial pressures, muscle blood flows, intravascular pressure, and vascular resistances were the same before and after each period of local hypotension indicating that the preparations were stable throughout each experimental period.

Table 2. Comparison of intravascular pressures (mm Hg), muscle blood flows (cc/min/100 gms), and muscle vascular resistances (mm Hg/cc/min/100 gms) observed prior to (control) and after (post-control) local, graded hypotension during both normovolemia and hypovolemia. Data are mean values and standard errors for mean systemic arterial pressure (P_S), mean gracilis artery pressure (P_G), gracilis vein flow (F_G), and gracilis muscle vascular resistance (R_G).

	P_S	P_G	F_G	R_G
Normovolemia (n = 10)				
Control	137.1 ± 4.3	130.6 ± 4.2	12.6 ± 2.0	11.8 ± 2.0
Post-control	136.9 ± 4.2	131.9 ± 4.3	12.7 ± 2.0	11.5 ± 1.5
Hypovolemia (n = 10)				
Control	118.1 ± 3.9	115.3 ± 4.1	6.8 ± 0.8	17.8 ± 1.9
Post-control	118.6 ± 4.3	115.5 ± 4.4	7.0 ± 0.8	17.4 ± 2.2
Normovolemia (n = 4)				
Control	142.0 ± 5.3	137.8 ± 5.0	11.5 ± 1.7	12.3 ± 3.0
Post-control	139.8 ± 4.3	136.5 ± 5.0	11.3 ± 1.7	12.3 ± 3.0
Mild hypovolemia (n = 4)				
Control	122.3 ± 4.7	120.8 ± 5.6	7.9 ± 1.4	15.9 ± 4.0
Post-control	122.5 ± 4.9	120.3 ± 5.5	8.0 ± 1.3	15.7 ± 4.0
Moderate hypovolemia (n = 4)				
Control	103.5 ± 4.1	102.5 ± 4.8	5.1 ± 1.3	20.8 ± 3.9
Post-control	103.5 ± 4.4	102.0 ± 4.5	5.3 ± 1.3	20.2 ± 3.9

II. Series II. Pump Perfused, Innervated Gracilis Muscle; Effects of Alterations in Mean Arterial Pressure During Normovolemic and Hypovolemic Periods

A. Muscle Blood Flow

Figure 11 reports changes in gracilis muscle blood flow from a selected experiment in which perfusion pressure was altered locally during both normovolemia and hypovolemia. During normovolemia, reducing gracilis artery pressure from 140 to 80 mm Hg by adjustment of the perfusion pump servocontroller parameters caused an initial rapid decrease in flow to a minimum value at time T_1. Flow then rose slowly to a new steady state value at time T_2. During hypovolemia, a step change in gracilis artery pressure from 140 to 80 mm Hg elicited a similar pattern of blood flow responses. When the animals were normovolemic, restoration of gracilis artery pressure to 140 mm Hg resulted in an initial, rapid increase in flow to a maximum value at time T_1, which was followed by a slower return of blood flow to a new steady state at time T_2. During hypovolemia, restoration of gracilis artery pressure to 140 mm Hg elicited muscle blood flow responses similar in form to those observed during normovolemia. The time to reach the minimum or maximum transient flow values (T_1) and the time to reach the new steady state levels of flow (T_2) were always longer during hypovolemic than during a corresponding normovolemic period.

Figure 11. Gracilis artery blood flow responses to step changes in perfusion pressure during normovolemic and hypovolemic periods. Mean gracilis and systemic arterial pressures are expressed in mm Hg; gracilis artery flow is expressed in ml/min. T_1 indicates time to peak minimum or maximum flow transient values, while T_2 depicts time to steady state responses. Data are from a selected, pump perfused gracilis muscle weighing 93.5 gms.

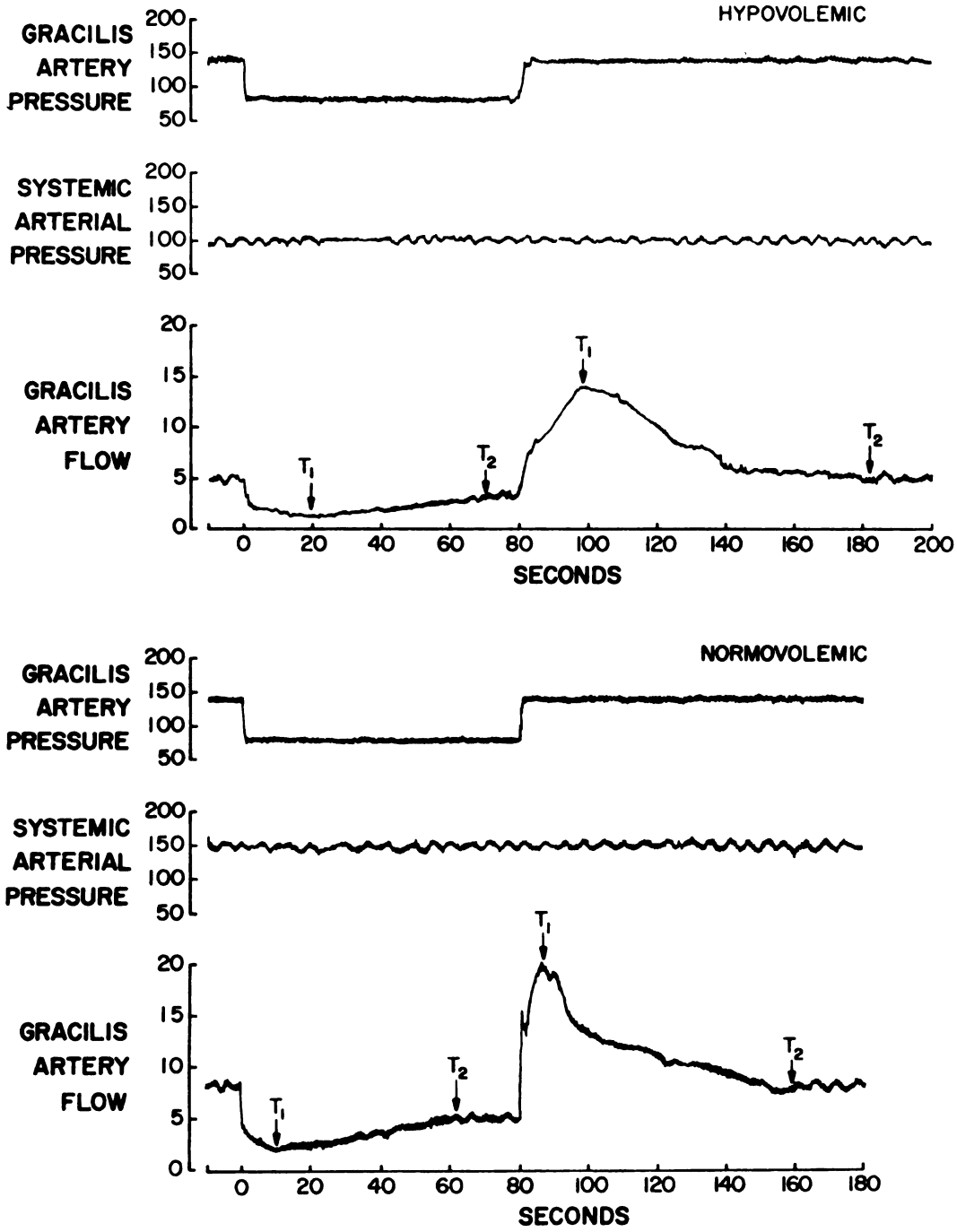
HYPOVOLEMIC
GRACILIS ARTERY PRESSURE
200
150
100
50
SYSTEMIC ARTERIAL PRESSURE
200
150
100
50
GRACILIS ARTERY FLOW
20
15
10
5
0
T1
T2
T1
T2
0
20
40
60
80
100
120
140
160
180
200
SECONDS
NORMOVOLEMIC
GRACILIS ARTERY PRESSURE
200
150
100
50
SYSTEMIC ARTERIAL PRESSURE
200
150
100
50
GRACILIS ARTERY FLOW
20
15
10
5
0
T1
T2
T1
T2
0
20
40
60
80
100
120
140
160
180
SECONDS

Figure 11

Figure 12 reports gracilis muscle blood flow responses to step changes in gracilis artery pressure from 140 to 80 and back to 140 mm Hg. Data are mean values and standard errors for responses obtained during normovolemia (upper panels) and during hypovolemia (lower panels). When the animals were normovolemic (upper left panel), reducing gracilis artery pressure from 140.1 $\pm$ 0.7 to 80.1 $\pm$ 0.6 mm Hg in 1.7 $\pm$ 0.3 seconds elicited a fall in muscle blood flow from 10.0 $\pm$ 1.2 cc/min/100 gms to 2.6 $\pm$ 0.3 cc/min/100 gms. This minimum flow was reached in 13.8 $\pm$ 0.5 seconds and corresponded to a 74.5% reduction from the control value of 10.0 cc/min/100 gms observed at a perfusion pressure of 140 mm Hg. The initial period of declining flow was brief and while gracilis artery pressure remained at 80 mm Hg, muscle blood flow rose to a steady state value of 6.6 $\pm$ 1.0 cc/min/100 gms. This steady state level of flow was attained at 40.6 $\pm$ 1.1 seconds and was 36.3% less than the control flow rate of 10.0 cc/min/100 gms. The gracilis vasculature of these normovolemic dogs displayed blood flow autoregulation, since steady state flow fell only 36.3% when perfusion pressure was reduced 42.9% (from 140 to 80 mm Hg). The area enclosed within the oscillographic flow recordings below horizontal lines projected to the left from the steady state flows averaged 63.6 $\pm$ 7.7 ml x seconds.

Figure 12. Muscle blood flow responses to sequential gracilis artery pressure alterations from 140 to 80 to 140 mm Hg obtained during both normovolemic (upper two panels) and hypovolemic (lower two panels) periods. Panels on the left depict flow responses to perfusion pressure reductions from 140 to 80 mm Hg, panels on the right show flow responses to perfusion pressure elevations from 80 to 140 mm Hg. Ordinates represent gracilis vein flow in cc/min/100 gms and abscissae represent time in seconds. The shaded areas above and below lines connecting data points represent standard errors for flow and each vertical and horizontal bar through the data points represents 2 standard errors for flow and time. The percent values below the peak minimum flows in the left hand panels and above the peak maximum flows in the right hand panels represent the percent of control flow obtained at the peak minimum or maximum flow. The other number within each panel represents the average planimetered area of the oscillographic blood flow recordings below (left panels) or above (right panels) horizontal lines projected to the left from the steady state flows. These areas are expressed in cc x seconds and all data are mean values and standard errors from 8 experiments.

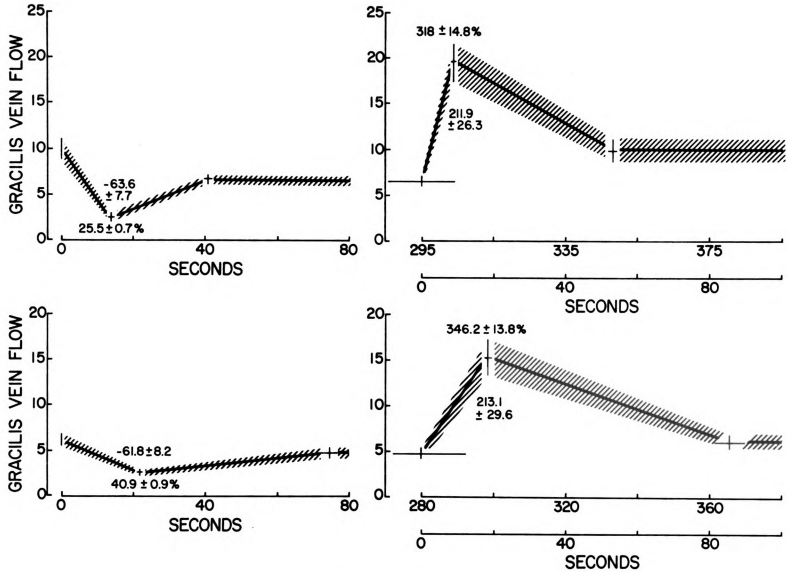
GRACILIS VEIN FLOW
25
20
15
10
5
0
-63.6
± 7.7
25.5 ± 0.7%
0
40
80
SECONDS
318 ± 14.8%
211.9
± 26.3
295
335
375
0
40
80
SECONDS
GRACILIS VEIN FLOW
-61.8 ± 8.2
40.9 ± 0.9%
SECONDS
346.2 ± 13.8%
213.1
± 29.6
280
320
360
SECONDS

Figure 12

When the animals were hypovolemic, reducing gracilis artery pressure from 140.2 ± 0.6 to 79.9 ± 0.5 mm Hg in 1.7 ± 0.4 seconds elicited a fall in muscle blood flow from 6.1 ± 0.7 cc/min/100 gms to 2.5 ± 0.3 cc/min/100 gms (Figure 12, lower left panel). This minimum flow level was reached in 19.8 ± 1.1 seconds and represented a 59.1% reduction from the control flow rate. This 59.1 ± 1.0% fall in blood flow observed in response to perfusion pressure reduction from 140 to 80 mm Hg during hypovolemia was significantly less than the 74.5 ± 0.9% reduction observed in response to a similar maneuver performed during normovolemia. In addition, the time required to reach this peak minimum flow was significantly longer during hypovolemia than during normovolemia.

As observed during normovolemia, the initial period of declining flow associated with a decrease in perfusion pressure was brief and flow subsequently rose to a steady state value of 4.3 ± 0.6 cc/min/100 gms. This new steady state rate was 31.1 ± 1.3% below the control rate of 6.1 cc/min/100 gms. The percent decrease in steady state flow accompanying a perfusion pressure reduction from 140 to 80 mm Hg was significantly less during hypovolemia (31.1 ± 1.3% versus 36.3 ± 1.5% during normovolemia), indicating that blood flow autoregulation may actually be enhanced by hemorrhage. However, the 74.4 ± 3.0 seconds required to reach steady state flow during hypovolemia was significantly

greater than the 40.6 ± 1.1 seconds required during normovolemia, indicating that the autoregulatory response to a step reduction in perfusion pressure develops more slowly during systemic arterial hypotension.

The area enclosed within the oscillographic blood flow recordings below horizontal lines projected to the left from the steady state flows averaged 61.8 ± 8.2 ml x seconds during hypovolemia and was not significantly different from that observed during normovolemia. These areas were not significantly different even though the amplitude of the transient and steady state flow responses to perfusion pressure reduction during hypovolemia were significantly smaller than those observed during normovolemia. This discrepancy results from the longer time required to reach steady state flow responses during hypovolemia.

After gracilis artery pressure had been maintained at 80 mm Hg for 295.3 ± 9.4 seconds during normovolemia (Figure 12, upper right panel), elevation of perfusion pressure to 140 ± 0.8 mm Hg in 1.8 ± 0.5 seconds elicited an increase in muscle blood flow from 6.6 ± 1.0 cc/min/100 gms to a maximum of 19.7 ± 2.2 cc/min/100 gms. This peak flow took 8.8 ± 0.5 seconds to develop and represented an elevation to 318 ± 14.8% of the control value of 6.6 cc/min/100 gms observed at a perfusion pressure of 80 mm Hg. This initial period of rising flow was brief and muscle blood flow subsequently fell to a steady state value of

10.0 ± 1.3 cc/min/100 gms. This steady state level of flow required 53 ± 1.6 seconds to develop and corresponded to a 150.1 ± 2.7% increase from the control rate observed at 80 mm Hg. The gracilis vasculature displays blood flow autoregulation in response to perfusion pressure elevation from 80 to 140 mm Hg since the 175% elevation in perfusion pressure elicited only a 150% increase in flow. The area enclosed within the oscillographic blood flow recordings above horizontal lines projected to the left from the steady state flows averaged 211.9 ± 26.3 ml x seconds.

After perfusion pressure had been maintained at 80 mm Hg for 281.1 ± 13.6 seconds during hypovolemia (Figure 12, lower right panel), elevation of gracilis artery pressure to 140.1 ± 0.5 mm Hg in 1.8 ± 0.3 seconds elicited an increase in muscle blood flow from 4.3 ± 0.6 cc/min/100 gms to a maximum of 14.4 ± 1.9 cc/min/100 gms. This peak flow took 22.5 ± 1.8 seconds to develop and represented a flow elevation to 346.2 ± 13.8% of the control rate observed at a perfusion pressure of 80 mm Hg. This 346% increase in blood flow observed in response to perfusion pressure elevation from 80 to 140 mm Hg during hypovolemia was significantly greater than the 318% increase in flow observed in response to a similar maneuver performed during normovolemia. Furthermore, time required to reach peak flow was significantly greater during hypovolemia than during normovolemia.

As observed during normovolemia, the initial rising phase of blood flow accompanying increased perfusion pressure was brief and flow subsequently fell to a steady state level of 6.1 ± 0.8 cc/min/100 gms which corresponded to a flow elevation to 145.2 ± 1.8% of the control level of 4.3 cc/min/100 gms observed at 80 mm Hg. Since this 145% flow increase observed in response to a 175% elevation of perfusion pressure from 80 to 140 mm Hg during hypovolemia was significantly less than the 150% elevation of flow accompanying a similar maneuver performed during normovolemia, blood flow autoregulation again appeared to be more pronounced during systemic arterial hypotension. However, the 85.8 ± 4.1 seconds required to reach this steady state level of flow during hypovolemia was significantly greater than the 53.0 ± 1.6 seconds required during normovolemia, indicating that the autoregulatory response to a step elevation of perfusion pressure develops more slowly during systemic arterial hypotension.

The area enclosed within the oscillographic blood flow recordings above horizontal lines projected to the left from the steady state flows averaged 213.1 ± 29.6 ml x seconds during hypovolemia and was not significantly different from that observed during normovolemia. These areas were not significantly different in normovolemic versus hypovolemic periods even though the amplitude of the transient and steady state flow responses to perfusion pressure

elevation during hypovolemia were significantly smaller than those observed during normovolemia. This discrepancy results from the longer time required to reach the steady state flow responses during hypovolemia.

During both normovolemic and hypovolemic periods, the time required to reach peak minimum flow when perfusion pressure was reduced to 80 mm Hg was significantly greater than that time required to reach peak maximum flow when perfusion pressure was restored to 140 mm Hg. In addition, the time required to reach a new steady state flow after perfusion pressure reduction to 80 mm Hg was always significantly less than that time required to reach a new steady state flow after perfusion pressure was restored to 140 mm Hg.

Table 3 reports all data described above in Figure 12 as well as the flow responses to step changes in perfusion pressure to and from 200, 180, 120, 100, and 60 mm Hg. The data depicted in Figure 12 are contained in the first four rows of Table 3 and represent responses to the first set of gracilis artery pressure alterations performed in each normovolemic and hypovolemic period. The last four rows of Table 3 report the flow responses to this same set of gracilis artery pressure manipulations performed at the end of each normovolemic and hypovolemic period. Because all responses to step changes in gracilis artery pressure from 140 to 80 and back to 140 mm Hg were statistically

Table 3. Gracilis muscle blood flow responses to step changes in perfusion pressure. P_S=mean systemic arterial pressure; P_G=mean gracilis artery pressure; F_G=gracilis vein flow; T_C=duration of control period; T_P=time required to reach new pressure; T_F=time required to reach flow value; F_G%C=percent of control gracilis vein flow; A=area above or below transient flow recordings. Pressures are expressed in mm Hg; flows in cc/min/100 gms; time in seconds; and area in cc x seconds. Values are means and standard errors from 8 experiments.

CONTROL				TRANSIENT RESPONSES						STEADY STATE RESPONSES			
P_S	P_G	F_G	T_C	P_G	T_P	T_F	F_G	F_G%C	A	P_G	T_F	F_G	F_G%C
141.9	140.1	10.0	243.4	80.1	1.7	13.8	2.6	25.5	-63.6	80.1	40.6	6.6	63.7
±5.8	±0.7	±1.2	±8.9	±0.6	±0.3	±0.5	±0.3	±0.7	±7.1	±0.5	±1.1	±1.0	±2.7
141.9	80.1	6.6	295.3	140.0	1.8	8.8	19.7	318.0	211.9	140.1	53.0	10.0	150.1
±5.8	±0.5	±1.0	±8.2	±0.8	±0.5	±0.5	±2.2	±14.8	±26.3	±0.6	±1.6	±1.3	±2.7
100.1	140.2	6.1	238.7	79.9	1.7	19.8	2.5	40.9	-61.8	80.0	74.4	4.3	68.9
±1.1	±0.6	±0.7	±6.9	±0.5	±0.4	±1.1	±0.3	±0.9	±8.2	±0.5	±3.0	±0.6	±2.1
100.1	80.0	4.3	281.1	140.1	1.8	22.5	14.4	346.2	213.1	140.0	85.8	6.1	145.2
±1.2	±0.5	±0.6	±13.6	±0.5	±0.3	±1.8	±1.9	±13.8	±29.6	±0.6	±4.1	±0.8	±1.8

continued

Table 3--continued

CONTROL				TRANSIENT RESPONSES						STEADY STATE RESPONSES			
P_S	P_G	F_G	T_C	P_G	T_P	T_F	F_G	F_G%C	A	P_G	T_F	F_G	F_G%C
142.7	140.1	10.1	239.4	201.2	2.1	16.5	29.0	289.1	261.6	201.2	95.2	20.1	192.3
±6.2	±0.6	±1.3	±9.1	±1.3	±0.5	±0.6	±3.5	±7.4	±29.6	±0.4	±2.3	±2.1	±9.0
142.7	201.2	20.1	232.0	140.1	2.0	13.3	9.3	50.3	-10.1	140.1	59.0	10.0	48.3
±6.1	±1.3	±3.1	±8.9	±0.5	±0.6	±0.6	±1.3	±3.1	±6.6	±0.5	±3.0	±1.2	±2.2
100.4	140.0	6.1	241.8	201.1	2.0	27.0	17.4	285.7	265.8	201.1	141.9	11.8	189.5
±2.1	±0.6	±0.8	±7.6	±1.4	±0.6	±1.5	±2.3	±4.5	±31.1	±1.4	±6.6	±1.7	±9.5
100.4	201.1	11.8	221.6	140.1	2.1	23.8	7.0	65.9	12.9	140.1	93.8	6.1	50.3
±2.1	±1.4	±1.7	±10.1	±0.6	±0.5	±1.6	±0.9	±7.1	±2.9	±0.6	±3.2	±0.7	±1.9
142.6	140.0	10.1	244.2	179.8	1.9	11.6	19.7	198.2	84.3	179.8	85.4	14.4	145.4
±6.2	±0.4	±1.4	±8.3	±0.9	±0.4	±0.5	±2.4	±4.6	±0.3	±0.9	±1.4	±1.7	±5.1
142.6	179.8	14.4	212.6	140.0	1.8	10.9	7.8	54.1	-21.1	140.0	54.1	10.1	70.3
±6.2	±0.9	±1.7	±3.0	±0.4	±0.4	±0.3	±0.9	±2.1	±4.4	±0.4	±1.1	±1.3	±2.8
100.4	140.0	6.1	242.1	179.9	1.9	21.6	12.6	201.4	82.3	179.9	109.4	8.8	138.5
±2.0	±0.5	±0.8	±7.6	±0.9	±0.6	±0.6	±1.7	±4.0	±10.5	±0.9	±3.8	±1.2	±5.4
100.4	179.9	8.8	206.9	140.1	1.8	20.3	6.7	80.3	9.7	140.0	87.9	6.0	68.2
±2.1	±0.9	±1.2	±3.6	±3.6	±0.6	±0.9	±0.9	±4.9	±2.4	±0.4	±2.6	±0.9	±2.5

continued

Table 3--continued

CONTROL				TRANSIENT RESPONSES						STEADY STATE RESPONSES			
P_S	P_G	F_G	T_C	P_G	T_P	T_F	F_G	F_G%C	A	P_G	T_F	F_G	F_G%C
142.6	140.0	10.0	239.6	120.6	0.9	11.4	6.8	68.2	-18.9	120.6	38.8	8.7	85.8
±6.1	±0.4	±1.3	±4.8	±0.5	±0.2	±0.4	±0.8	±1.6	±2.2	±0.5	±1.2	±1.2	±1.9
142.6	120.6	8.7	215.6	140.0	0.9	10.9	13.6	159.9	61.7	140.0	56.9	10.0	114.3
±6.1	±0.5	±1.2	±8.9	±0.4	±0.2	±0.5	±1.7	±3.5	±6.4	±0.4	±1.1	±1.1	±2.2
100.4	140.0	6.1	242.1	120.6	0.9	20.3	4.7	77.9	-15.9	120.6	80.6	5.7	91.2
±2.2	±0.5	±0.7	±6.7	±0.5	±0.3	±2.2	±0.5	±1.0	±5.8	±0.5	±2.2	±0.7	±1.3
100.4	120.6	5.7	220.1	140.0	0.9	21.0	9.9	175.3	65.3	140.0	85.8	6.1	108.1
±2.1	±0.4	±0.7	±9.4	±0.5	±0.3	±1.9	±1.3	±2.7	±8.2	±0.5	±3.7	±0.8	±2.1
142.6	140.1	10.1	239.1	100.8	1.2	11.0	4.5	44.7	-30.3	100.8	45.8	7.6	71.8
±6.2	±0.5	±1.2	±6.5	±0.7	±0.3	±0.3	±0.5	±1.0	±4.4	±0.7	±1.1	±1.1	±3.6
142.7	100.8	7.6	240.3	140.1	1.2	9.4	18.1	247.8	65.3	140.1	63.5	10.0	130.7
±6.2	±0.7	±1.1	±9.2	±0.7	±0.3	±0.3	±2.1	±8.9	±6.7	±0.7	±1.3	±1.2	±2.7
100.3	140.1	6.1	243.8	100.8	1.2	19.8	3.4	56.2	-29.4	100.8	75.8	4.9	79.2
±2.1	±0.4	±0.9	±6.1	±0.8	±0.4	±1.1	±0.5	±1.0	±3.7	±0.8	±2.8	±0.7	±2.7
100.3	100.8	4.9	232.8	140.0	1.2	21.5	11.5	238.6	73.7	140.0	92.3	6.1	123.7
±2.1	±0.7	±0.7	±8.4	±0.4	±0.4	±1.4	±1.5	±8.4	±9.1	±0.4	±2.1	±0.7	±1.9

continued

Table 3--continued

CONTROL				TRANSIENT RESPONSES						STEADY STATE RESPONSES			
P_S	P_G	F_G	T_C	P_G	T_P	T_F	F_G	F_G%C	A	P_G	T_F	F_G	F_G%C
142.6	140.1	10.1	245.4	60.3	1.9	12.5	1.6	15.9	-64.9	60.3	77.9	4.9	48.9
±6.2	±0.5	±1.2	±5.3	±0.9	±0.3	±0.5	±0.2	±0.9	±7.9	±0.9	±1.1	±0.6	±1.0
142.6	60.3	4.9	240.3	140.1	1.8	11.3	26.6	543.9	312.8	140.1	72.1	10.0	203.8
±6.2	±0.9	±0.6	±8.2	±0.5	±0.3	±0.3	±3.2	±8.3	±38.6	±0.5	±1.8	±1.3	±4.2
100.3	140.2	6.1	241.2	60.4	1.9	22.6	1.0	16.9	-54.9	60.4	104.1	3.2	52.7
±2.1	±0.5	±0.8	±6.4	±0.8	±0.4	±1.6	±0.1	±0.8	±8.6	±0.8	±3.6	±0.4	±1.2
100.3	60.4	3.2	235.1	140.3	1.8	22.5	17.6	540.7	304.8	140.3	109.6	6.1	191.3
±2.1	±0.9	±0.4	±5.1	±0.5	±0.4	±1.8	±2.3	±11.5	±36.4	±0.5	±4.7	±0.8	±2.8
143.4	140.2	10.1	242.7	80.0	1.7	13.6	2.5	25.4	-62.4	80.0	42.8	6.6	61.8
±5.6	±0.7	±1.4	±8.1	±0.5	±0.4	±0.5	±0.3	±0.6	±7.9	±0.4	±1.7	±1.3	±2.3
143.4	80.0	6.6	291.4	140.0	1.8	8.9	19.9	323.4	223.4	140.0	54.9	10.1	153.4
±5.6	±0.5	±1.3	±8.2	±0.6	±0.5	±0.6	±2.1	±13.8	±28.7	±0.7	±1.9	±1.4	±4.0
100.7	140.2	6.0	244.3	80.0	1.7	20.0	2.5	41.4	-65.7	80.0	77.3	4.3	64.8
±2.9	±0.7	±0.8	±8.8	±0.6	±0.4	±1.2	±0.2	±1.0	±7.8	±0.5	±2.9	±0.8	±3.1
100.7	80.0	4.3	283.5	140.1	1.8	21.9	14.5	338.1	219.4	140.0	86.4	6.0	144.8
±2.9	±0.5	±0.8	±9.9	±0.6	±0.5	±1.7	±1.8	±12.6	±23.5	±0.7	±5.0	±0.7	±3.2

similar before and after each experimental period, the preparations were assumed to have been stable throughout the normovolemic and hypovolemic periods.

Transient and steady state flow responses to gracilis artery pressure alterations to and from 120, 100 and 60 mm Hg during normovolemia and hypovolemia are similar to those responses described above for perfusion pressure alterations to and from 80 mm Hg. For example, after each change in perfusion pressure to and from 120, 100, 80, and 60 mm Hg, more time was required to reach the peak transient and steady state flows during hypovolemia than during normovolemia. In addition, the areas above the minimum transient flows and below the maximum transient flows were the same in normovolemic versus hypovolemic periods. As observed for pressure alterations from 80 to 140 mm Hg, changes in gracilis artery pressure from 120, 100, or 60 to 140 mm Hg elicited greater percent changes in initial transient flow in hypovolemic versus normovolemic periods. Furthermore, a given change in gracilis artery pressure always produced a proportionately smaller change in steady state flow, indicating that the vasculature autoregulated its blood flow in response to each of the step changes in perfusion pressure.

Changing gracilis artery pressure to 180 or 200 mm Hg and back to 140 mm Hg elicited a somewhat different set of flow responses than those described above for perfusion

pressure alterations below 140 mm Hg. As described previously, more time was required to reach transient and steady state flows when the dogs were hypovolemic. However, when gracilis artery pressure was elevated from 140 to 180 or 200 mm Hg, the percent increases in flow occurring at T_1 and T_2 were not significantly different during normovolemic versus hypovolemic periods. Further, the gracilis vasculature appeared less able to autoregulate its blood flow in response to elevations in perfusion pressure above 140 mm Hg. This conclusion is based on the observation that 29 and 43% increases in gracilis artery pressure (140 to 180 and 140 to 200 mm Hg, respectively) elicited 45 and 92% increases in steady state flow.

Figure 13 reports steady state gracilis vein flow as a function of gracilis artery pressure when perfusion pressure was altered from 140 mm Hg by changing the perfusion pump servosystem parameters during normovolemic (dots) and hypovolemic (circles) periods. During normovolemia, reductions of gracilis artery pressure from 140 to 100 mm Hg and below, or elevations of perfusion pressure from 140 to 180 and 200 mm Hg significantly altered muscle blood flow. During hypovolemia, muscle blood flow was significantly reduced from the normovolemic control values observed at each level of perfusion pressure. Except for the local pressure reduction from 140 to 120 mm Hg, all changes of gracilis artery pressure from 140 mm Hg performed during

Figure 13. Effects of gracilis artery pressure alterations during normovolemic (solid dots) and hypovolemic (circles) periods on gracilis vein flow. Ordinate reports gracilis vein flow in cc/min/100 gms and abscissa reports gracilis artery pressure in mm Hg. Data represent mean values ± standard errors from 8 experiments.

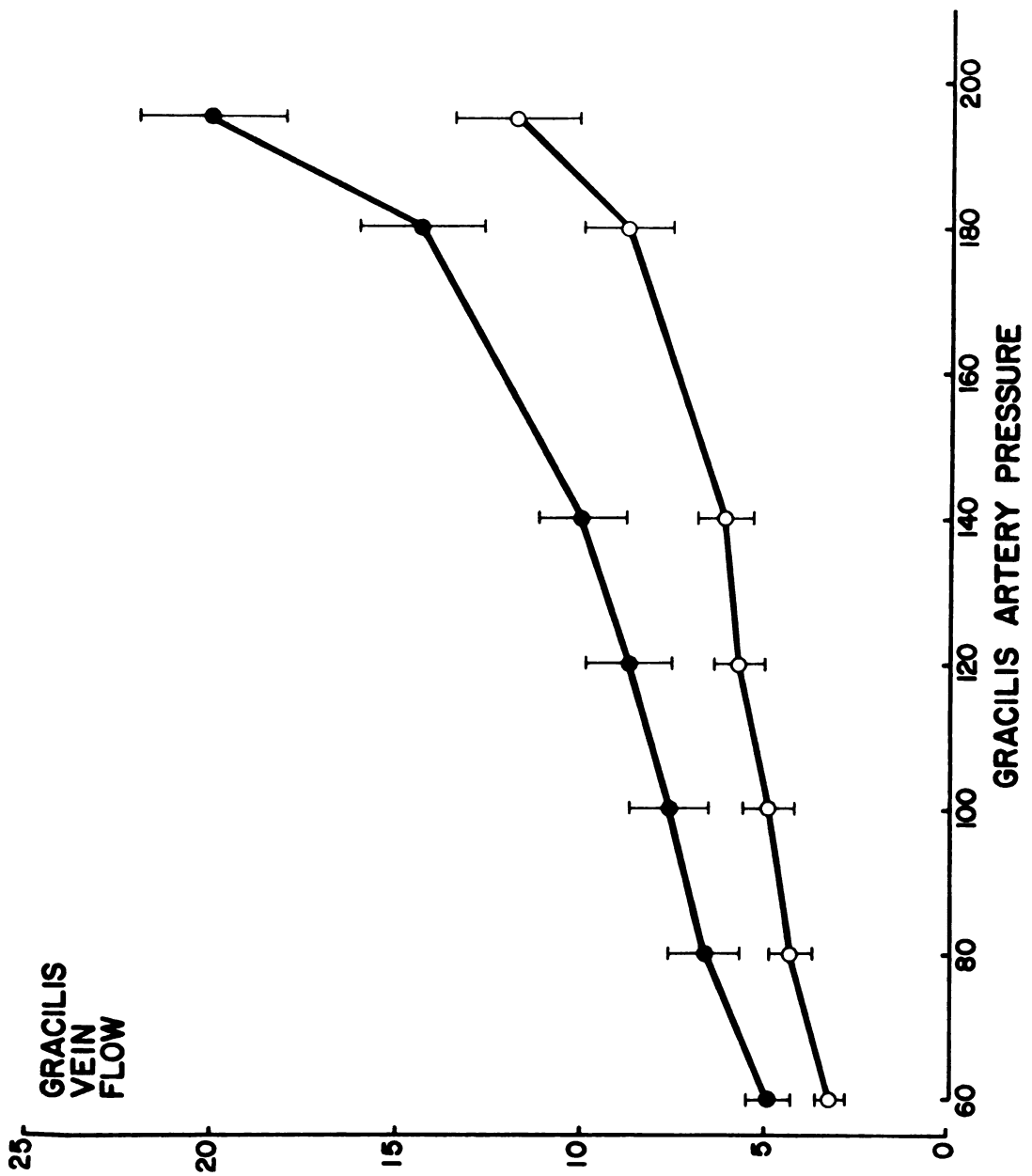
GRACILIS VEIN FLOW
25
20
15
10
5
0
60
80
100
120
140
160
180
200
GRACILIS ARTERY PRESSURE

Figure 13

hypovolemia significantly altered gracilis muscle blood flow.

To more accurately compare the relationships between muscle blood flow and perfusion pressure during normovolemia and hypovolemia, the flow data reported in Figure 13 were normalized to percent of control (Figure 14). During normovolemia the vasculature autoregulated its flow at perfusion pressures of 140 mm Hg and below since a 57% local reduction in gracilis artery pressure from 140 to 60 mm Hg elicited only a 51.1 $\pm$1.0% decrease in blood flow. However, when pressure was altered from 140 to 180 or 200 mm Hg (29 and 43% increases respectively), blood flow increased 45.4 $\pm$ 5.1 and 92.3 $\pm$ 9.0%. The fact that steady state flow increased proportionately more than pressure indicates that the gracilis vasculature is less able to autoregulate flow at high perfusion pressures. The ability to autoregulate flow is not completely lost at these higher pressures since flow always declined after the initial passive dilation associated with a given pressure elevation (Table 3).

The ability to autoregulate flow in response to perfusion pressure alterations below 140 mm Hg was evident during systemic hypotension since a 57% local reduction in perfusion pressure from 140 to 60 mm Hg elicited only a 47.3 $\pm$ 1.2% decrease in steady state blood flow. In fact, hemorrhage appeared to potentiate autoregulation slightly since step reductions in gracilis artery pressure from 140

Figure 14. Effects of gracilis artery pressure alterations during normovolemic (solid dots) and hypovolemic (circles) periods on gracilis vein flow normalized to % of control. Ordinate reports % of control gracilis vein flow and abscissa reports gracilis artery pressure in mm Hg. Data obtained at gracilis artery pressures of 140 mm Hg were used to normalize the data from each experiment. Data represent mean values ± standard errors from 8 experiments.

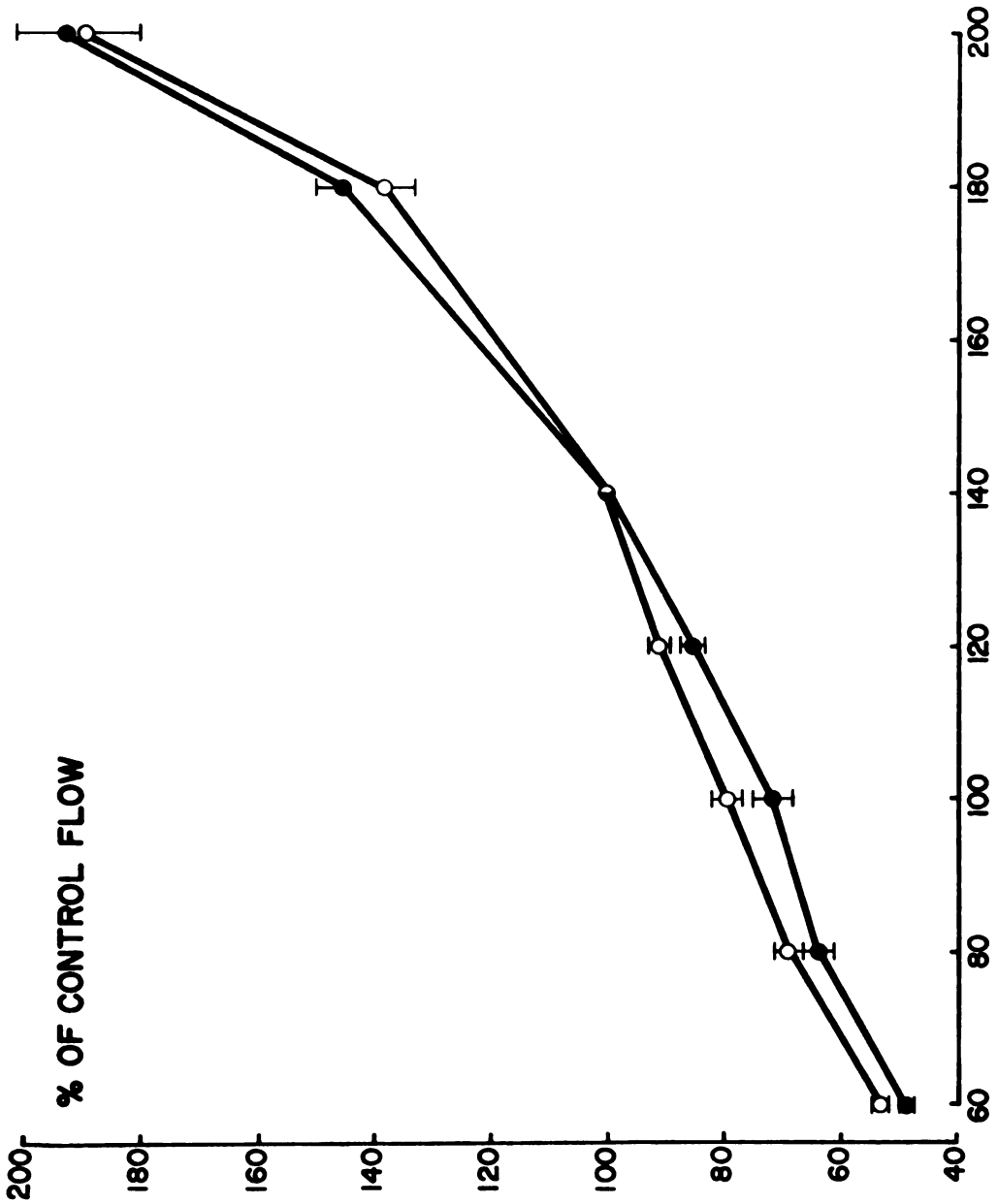
% OF CONTROL FLOW
200
180
160
140
120
100
80
60
40
60
80
100
120
140
160
180
200
GRACILIS ARTERY PRESSURE

Figure 14

to 120, 100, 80, and 60 mm Hg each elicited a significantly smaller percent change in flow during hypovolemia. As during normovolemia, blood flow autoregulation during hypovolemia was diminished at pressures above 140 mm Hg since 29 and 43% elevations of perfusion pressure from 140 to 180 and 200 mm Hg elicited 38.5 $\pm$ 5.4 and 89.5 $\pm$9.5 percent increases in blood flow. These flow changes in response to increasing perfusion pressure from 140 to 180 and 200 mm Hg during hypovolemia were not significantly different from those observed in response to identical pressure manipulations performed during normovolemia.

B. Muscle Vascular Resistance

Figure 15 reports steady state gracilis muscle vascular resistance as a function of gracilis artery pressure when perfusion pressure was altered from 140 mm Hg by changing the perfusion pump servosystem parameters during normovolemic (dots) and hypovolemic (circles) periods. During normovolemia, elevation of gracilis artery pressure from 140 to 180 or 200 mm Hg significantly reduced muscle vascular resistance from the control value observed at 140 mm Hg; all other perfusion pressure manipulations failed to significantly alter vascular resistance.

During hypovolemia, muscle vascular resistance was significantly elevated from the normovolemic control values observed at each level of perfusion pressure. As was

Figure 15. Effects of gracilis artery pressure alterations during normovolemic (solid dots) and hypovolemic (circles) periods on gracilis muscle vascular resistance. Ordinate reports gracilis muscle vascular resistance in mm Hg/cc/min/100 gms and abscissa reports gracilis artery pressure in mm Hg. Data represent mean values ± standard errors from 8 experiments.

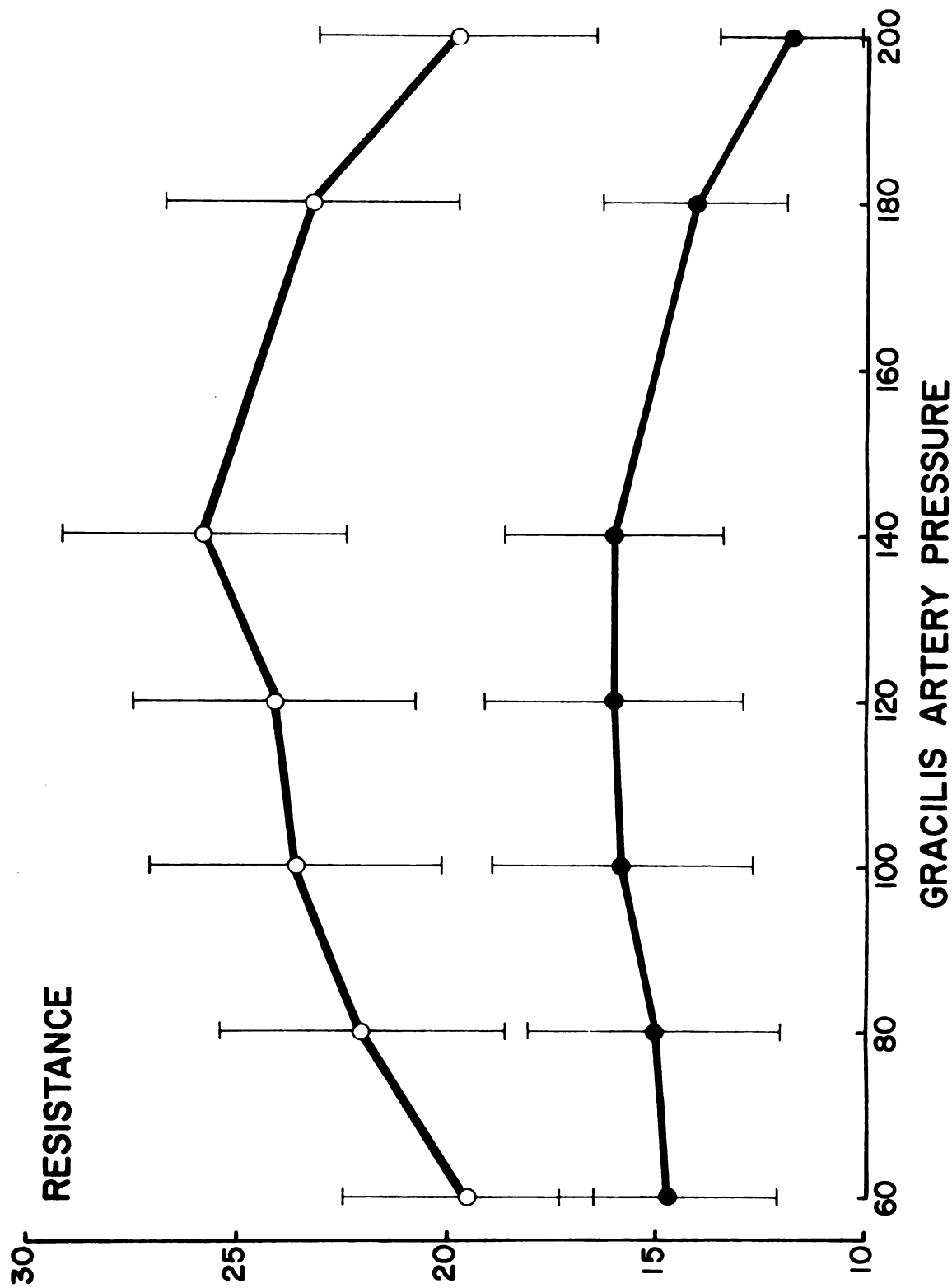
RESISTANCE
30
25
20
15
10
60
80
100
120
140
160
180
200
GRACILIS ARTERY PRESSURE

Figure 15

observed during normovolemia, elevation of gracilis artery pressure from 140 to 180 or 200 mm Hg significantly reduced muscle vascular resistance. However, decreasing gracilis artery pressure from 140 to 80 and 60 mm Hg significantly reduced muscle vascular resistance below the control value observed at 140 mm Hg.

To more accurately compare the relationships between muscle vascular resistance and perfusion pressure during normovolemia and hypovolemia, the resistance data reported in Figure 15 were normalized to percent of control (Figure 16). During normovolemia, the vasculature autoregulated its flow at perfusion pressures below 140 mm Hg since significant reductions in resistance were observed when gracilis artery pressure was reduced from 140 to 100 mm Hg and below. When perfusion pressure was increased from 140 to 180 and 200 mm Hg resistance fell significantly, indicating that the gracilis vasculature is less able to autoregulate flow over this high pressure range. The vasculature's ability to autoregulate is not completely lost at these higher pressures since resistance always increased after the initial, passive dilation associated with a given pressure elevation.

The ability of the vasculature to autoregulate flow in response to perfusion pressure reductions below 140 mm Hg was enhanced during hypovolemia, since gracilis artery pressure reductions from 140 to 120, 100, 80, and 60 mm Hg

Figure 16. Effects of gracilis artery pressure alterations during normovolemic (solid dots) and hypovolemic (circles) periods on gracilis muscle vascular resistance normalized to percent of control. Ordinate reports percent of control gracilis muscle vascular resistance and abscissa reports gracilis artery pressure in mm Hg. Resistances obtained at gracilis artery pressure of 140 mm Hg were used to normalize the data from each experiment. Data represent mean values ± standard errors from 8 experiments.

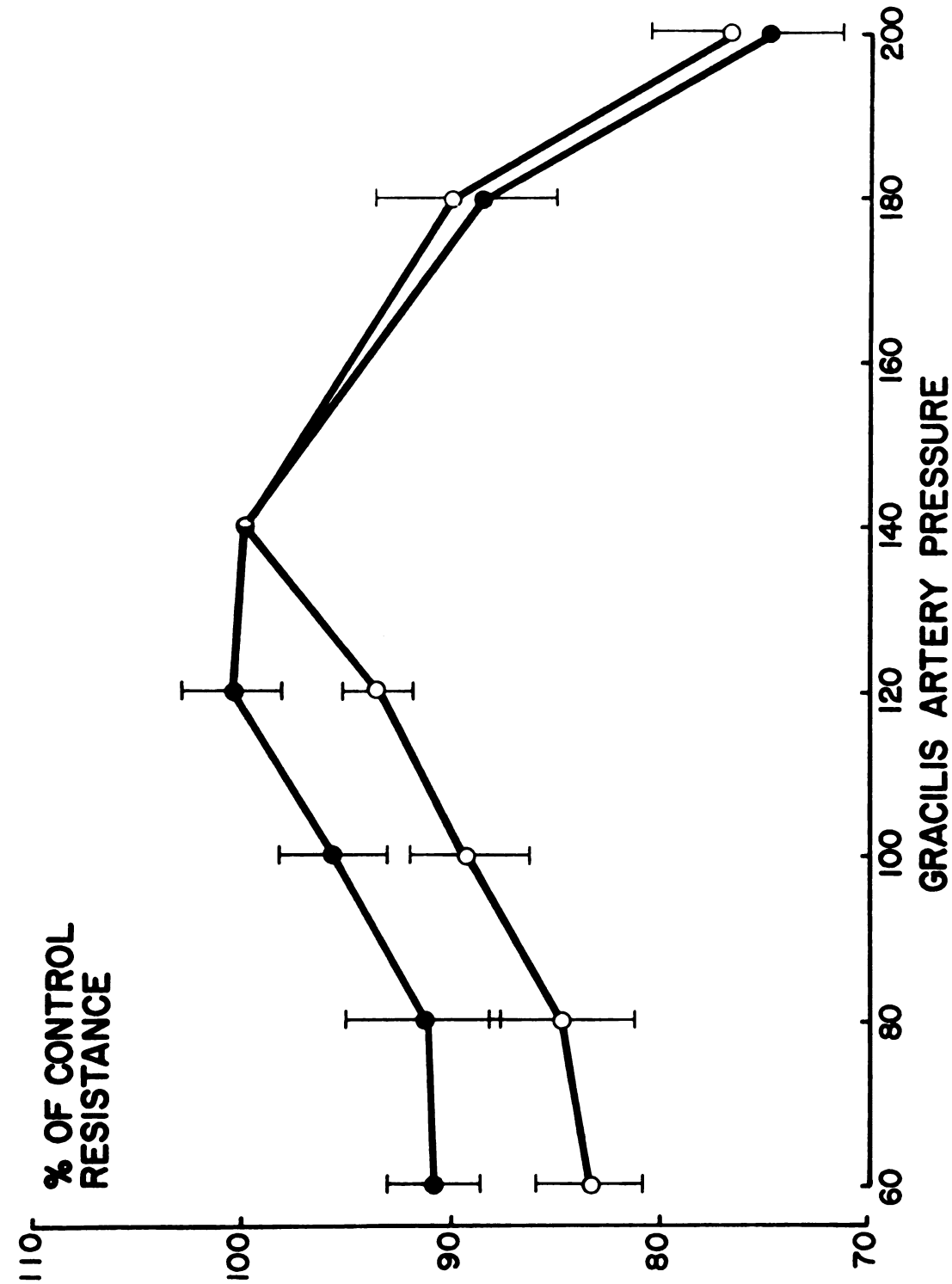
% OF CONTROL RESISTANCE
110
100
90
80
70
60
80
100
120
140
160
180
200
GRACILIS ARTERY PRESSURE

Figure 16

each elicited percent decreases in resistance which were significantly larger than those observed during normovolemia. During hypovolemia, blood flow autoregulation was diminished at perfusion pressures above 140 mm Hg since elevations of gracilis artery pressure from 140 to 180 and 200 mm Hg elicited significant reductions in muscle vascular resistance. Those resistance changes in response to increasing perfusion pressure from 140 to 180 and 200 mm Hg during hypovolemia were not significantly different from those observed in response to identical pressure elevations performed during normovolemia.

C. Vascular Responses to Denervation During Hypovolemia

Table 4 reports the effects of denervation during hemorrhage on gracilis muscle blood flow and muscle vascular resistance. With reduction of systemic arterial pressure from 143.4 ± 5.6 to 100.7 ± 2.9 mm Hg by arterial hemorrhage, muscle blood flow fell from 10.1 ± 1.4 to 6.0 ± 0.7 cc/min/100 gms as a result of an increase in muscle vascular resistance from 15.9 ± 2.6 to 25.8 ± 3.4 mm Hg/cc/min/100 gms. This flow change accompanying hemorrhage represented a 41.6% decrease from the normobolemic control value. The resistance response to hemorrhage represented a 63.2% increase from the normovolemic control value.

During hypovolemia, acute denervation caused gracilis muscle blood flow and vascular resistance to return toward

Table 4. **Effects of denervation during hemorrhage on gracilis muscle blood flow (cc/min/100 gms) and vascular resistance (mm Hg/cc/min/100 gms). Time is expressed in minutes while pressures are expressed in mm Hg. Data are mean values and standard errors from 8 experiments.**

	Time of Experiment	Mean Systemic Arterial Pressure	Mean Gracilis Artery Pressure	Gracilis Vein Flow	Gracilis Muscle Vascular Resistance	% Of Control Flow	% Of Control Resistance
Control Innervated	63.1 ±4.8	143.4 ±5.6	140.0 ±0.7	10.1 ±1.4	15.9 ±2.6	100	100
Hypovolemia Innervated	162.4 ±5.4	100.7 ±2.9	140.1 ±0.7	6.0 ±0.7	25.8 ±3.4	58.4 ±2.1	163.2 ±3.1
Hypovolemia Denervated	197.2 ±6.8	100.4 ±2.8	140.2 ±0.7	7.8 ±0.9	19.3 ±3.2	78.2 ±2.5	122.5 ±2.9

their normovolemic control values. Vascular resistance decreased from 163.2 ± 3.1 to 122.5 ± 2.9% of control resistance when the muscles were acutely denervating during hypovolemia, suggesting that approximately 60% of the resistance elevation observed with arterial hemorrhage to 100 mm Hg could be attributed to neurogenically mediated vasoconstriction. However, the interpretation of these data is complicated by at least two factors which make it difficult to assess what portion of the hemorrhage induced vasoconstriction is neurally mediated: 1) a portion of the decrease in resistance observed in response to acute denervation during hypovolemia could be due to a removal of the neural component of basal vascular tone that is present during normovolemia since acute denervation during normovolemia also increases muscle blood flow; 2) a portion of the decrease in resistance observed in response to acute denervation during hypovolemia could be due to increased sympathetic vasodilator fiber activity secondary to the trauma the nerve receives with section.

III. Series III: Naturally Perfused, Denervated Gracilis Muscles; Vascular Responses to Increased Venous and Mean Distending Pressures and to Local Hypotension During Pulsatile or Non-Pulsatile Perfusion

A. Vascular Response to Alterations in Perfusion Mode

Figure 17 reports data from a selected experiment in which the mode of perfusion was altered from non-pulsatile to pulsatile and back to non-pulsatile. Panels on the left show responses to perfusion mode alterations in the normal vasculature, those on the right depict vascular responses to similar alterations performed during papaverine infusion. In the normal vasculature, switching from non-pulsatile to pulsatile perfusion (second 0 on abscissa) elicited a rapid increase in muscle blood flow followed by a slower decline to a new steady state flow rate lower than that observed during non-pulsatile perfusion. When non-pulsatile perfusion was restored (second 160 on abscissa), there occurred an initial, rapid decrease in flow followed by a gradual return to a new steady state value higher than that observed with pulsatile perfusion.

The effect of papaverine infusion on the blood flow responses to changes in perfusion mode are reported in the right hand panels of Figure 17. During papaverine infusion, switching from non-pulsatile perfusion (second 0 on abscissa) resulted in a rapid, sustained increase in muscle blood

Figure 17. Gracilis muscle vascular responses to changing the mode of perfusion from non-pulsatile to pulsatile and back to non-pulsatile. Panels on the left depict responses to perfusion mode alterations in the normal vasculature, those on the right show responses during a local infusion of papaverine. Ordinates depict gracilis vein pressure, mean (electronically integrated) gracilis artery pressure and non-integrated gracilis artery pressure in mm Hg; and gracilis vein flow in cc/min. Abscissae depict time in seconds. Data are from a selected experiment with a naturally perfused gracilis muscle weighing 103.5 gms.

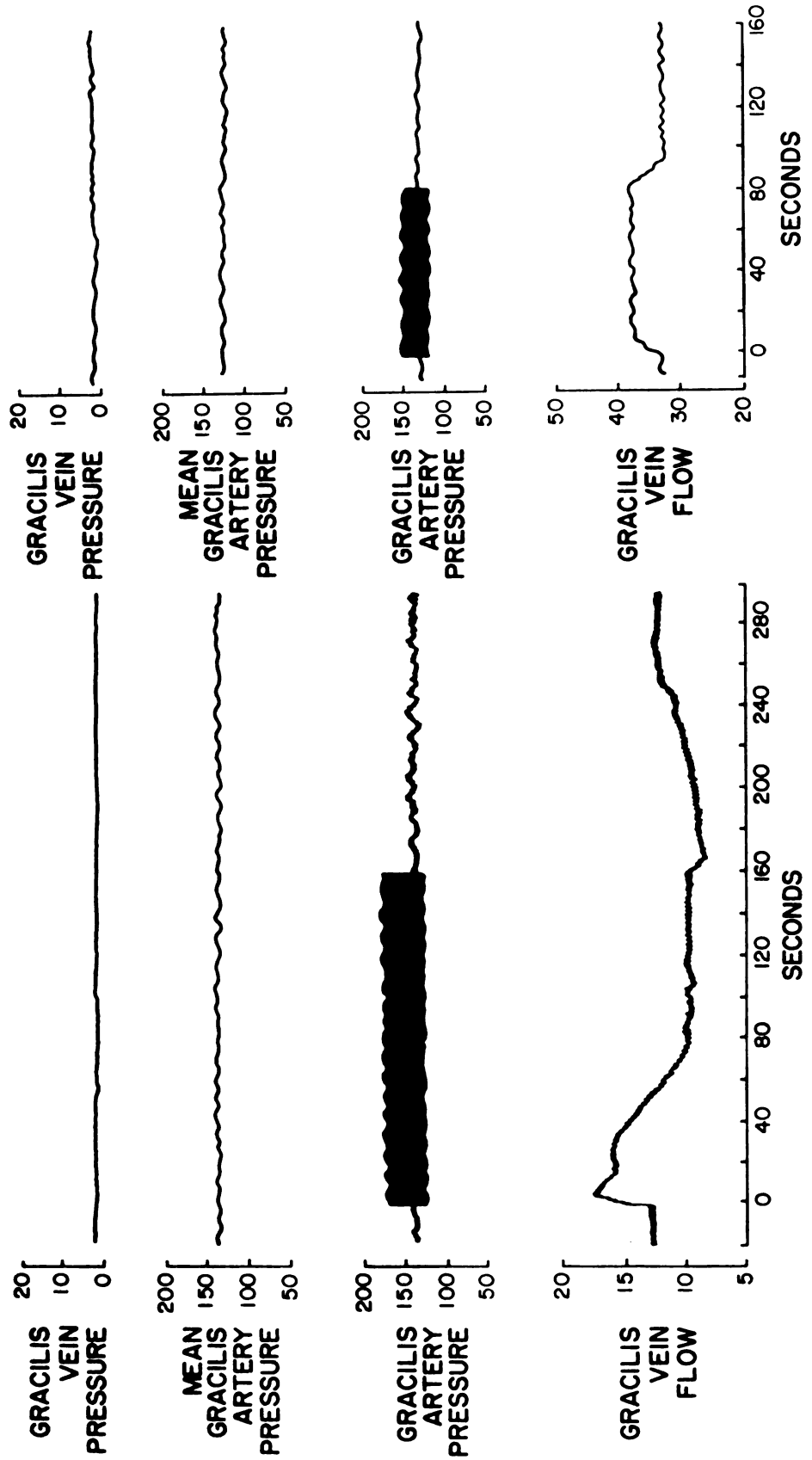
GRACILIS VEIN PRESSURE
20
10
0
MEAN GRACILIS ARTERY PRESSURE
200
150
100
50
GRACILIS ARTERY PRESSURE
200
150
100
50
GRACILIS VEIN FLOW
50
40
30
20
0
40
80
120
160
SECONDS
GRACILIS VEIN FLOW
20
15
10
5
0
40
80
120
160
200
240
280
SECONDS

Figure 17

flow. Restoration of non-pulsatile perfusion (second 80 on abscissa) resulted in a rapid, sustained decrease of blood flow.

Table 5 reports mean values and standard errors from 10 experiments for gracilis vascular responses to perfusion mode changes in normal and papverine treated muscles. In the normal vasculature, rapid elevation of gracilis artery pulse pressure from 2.6 $\pm$ 0.8 to 40.9 $\pm$ 5.5 mm Hg elicited a 43.2 $\pm$ 3.7% increase in blood flow from 14.7 $\pm$ 2.1 cc/min/100 gms to 21.8 $\pm$ 2.7 cc/min/100 gms. This peak maximum flow was reached in 5.3 $\pm$ 0.4 seconds and occurred because vascular resistance fell 22% from 11.5 $\pm$ 1.8 to 8.4 $\pm$ 1.7 mm Hg/cc/min/100 gms. Following the initial, rapid elevation, muscle blood flow declined to a steady state value of 10.2 $\pm$ 1.3 cc/min/100 gms. This steady state level of flow required 78.5 $\pm$ 4.3 seconds to develop and represented a 25.7% reduction from the flow rate observed during non-pulsatile perfusion. Thus, switching from non-pulsatile to pulsatile perfusion elicited an initial, rapid fall in muscle vascular resistance followed by a slower rise in resistance to a new steady state value 30.6 $\pm$ 3.2% above the level observed during non-pulsatile perfusion.

During papverine infusion, the secondary phase of increasing resistance was not observed; switching from non-pulsatile to pulsatile perfusion elicited only a rapid

Table 5. Gracilis muscle vascular responses to perfusion mode changes in normal and papaverine treated muscles. Pressures are expressed in mm Hg; flow in cc/min/100 gms; resistance in mm Hg/cc/min/100 gms; and time in seconds. Data are mean values ± standard errors from 10 experiments.

	Control	Transient Responses	Steady State Responses
	Normal Vasculature		
Mean Perfusion Pressure	141.4 ± 2.8	141.5 ± 1.8	141.5 ± 1.9
Pulse Pressure	2.6 ± 0.8	40.9 ± 5.5	40.9 ± 5.4
Muscle Blood Flow	14.7 ± 2.1	21.8 ± 2.7	10.2 ± 1.3
Muscle Vascular Resistance	11.5 ± 1.8	8.4 ± 1.7	15.6 ± 2.0
Time to Response	-	5.3 ± 0.4	78.5 ± 4.3
% of Control Flow	100	143.2 ± 3.7	74.2 ± 2.2
% of Control Resistance	100	78.0 ± 1.9	130.6 ± 3.2
Mean Perfusion Pressure	142.2 ± 2.4	142.2 ± 2.3	142.2 ± 2.3
Pulse Pressure	40.4 ± 5.1	2.7 ± 0.8	2.7 ± 0.8
Muscle Blood Flow	10.1 ± 1.2	8.7 ± 1.0	14.6 ± 2.6
Muscle Vascular Resistance	15.7 ± 2.1	17.9 ± 2.6	11.6 ± 1.7
Time to Response	-	5.7 ± 0.3	81.5 ± 4.6
% of Control Flow	100	88.1 ± 2.1	137.4 ± 3.4
% or Control Resistance	100	113.2 ± 2.4	77.8 ± 1.9
	Papaverine Treated Vasculature		
Mean Perfusion Pressure	133.9 ± 2.3	-	133.8 ± 2.2
Pulse Pressure	1.7 ± 0.6	-	32.8 ± 3.4
Muscle Blood Flow	64.3 ± 7.4	-	78.7 ± 8.8
Muscle Vascular Resistance	2.0 ± 0.6	-	1.7 ± 0.5
Time to Response	-	-	5.8 ± 0.5
% of Control Flow	100	-	125.5 ± 4.1
% of Control Resistance	100	-	83.7 ± 2.1
Mean Perfusion Pressure	132.6 ± 2.4	-	132.7 ± 2.4
Pulse Pressure	32.4 ± 3.1	-	1.8 ± 0.6
Muscle Blood Flow	78.9 ± 8.6	-	63.7 ± 7.2
Muscle Vascular Resistance	1.7 ± 0.5	-	2.0 ± 0.6
Time to Response	-	-	6.3 ± 0.3
% of Control Flow	100	-	81.2 ± 1.9
% of Control Resistance	100	-	127.1 ± 4.7

16.3% reduction in resistance from 2.0 ± 0.6 mm Hg/cc/min/100 gms to a steady state value of 1.7 ± 0.5 mm Hg/cc/min/100 gms. This response was very similar to the initial transient response observed prior to papaverine treatment, suggesting that the normal vasculature's transient responses to switching the mode of perfusion from non-pulsatile to pulsatile probably results from a passive expansion of vascular radius due to pulse pressure distension. Since papaverine removed the secondary slow phase of increasing resistance, this response probably represents an active, myogenic reduction in vascular radius triggered by the increase in pulse pressure.

Restoration of non-pulsatile perfusion in the normal vasculature elicited a pattern of responses that were an approximate mirror image of those described above to switching from non-pulsatile to pulsatile perfusion. Blood flow fell rapidly and then slowly rose to a new steady state value 37.4% above the level observed during pulsatile perfusion. During papaverine infusion, switching to non-pulsatile perfusion resulted in a rapid fall in flow to a new steady state value 18.8% below the level observed with pulsatile perfusion. As was observed for the opposite perfusion mode change, papaverine removed only the slow phase of changing resistance, suggesting that the initial, rapid responses to perfusion mode changes are passive, while the more slowly developing responses represent

actively mediated alterations in vascular radius.

B. Vascular Responses to Increasing Mean Distending Pressure

Table 6 and Figure 18 report gracilis muscle vascular responses to 20 mm Hg elevations in mean vascular distending pressure performed during pulsatile and non-pulsatile perfusion in normal and papaverine treated muscles. When gracilis arterial and venous pressures were simultaneously elevated 20 mm Hg during non-pulsatile perfusion, muscle blood flow was reduced 25.3% below control as the result of a 15.6 $\pm$ 1.7% elevation of vascular resistance from 12.2 $\pm$ 1.6 to 14.0 $\pm$ 1.8 mm Hg/cc/min/100 gms. However, during pulsatile perfusion, increasing mean vascular distending pressure 20 mm Hg elicited a significantly greater percent reduction in muscle blood flow as the result of a 26.6 $\pm$ 1.8% elevation of vascular resistance from 14.9 $\pm$ 1.9 to 20.1 $\pm$ 2.2 mm Hg/cc/min/100 gms.

When the vasculature was treated with papaverine, increasing mean distending pressure produced significant elevations in muscle blood flow due to an 18.2 $\pm$ 1.5% reduction in resistance during pulsatile perfusion and an 18.8 $\pm$ 1.6% resistance decrease during non-pulsatile perfusion. Therefore, elevations of mean vascular distending pressure in the face of a constant pressure head results in a passive dilation when the vascular smooth muscle cells are paralyzed with papaverine. In the normal vasculature

Table 6. Gracilis muscle vascular responses to elevations in mean vascular distending pressure during pulsatile and non-pulsatile perfusion in normal and papaverine treated muscles. Pressures are represented in mm Hg; flow in cc/min/100 gms; resistance in mm Hg/cc/min/100 gms. C_P = pulsatile control; C_{N-P} = non-pulsatile control; and ↑MDP = 20 mm Hg elevation in mean distending pressure. Data are mean values ± standard errors from 10 experiments.

	Gracilis Artery Pulse Pressure	Mean Gracilis Artery Pressure	Gracilis Vein Pressure	Gracilis Vein Flow	Gracilis Vascular Resistance	% Of Control Flow	% Of Control Resistance
			Normal Vasculature				
First Trial							
C_{N-P}	0.2 ± 0.1	119.8 ± 1.8	3.9 ± 0.3	11.3 ± 1.6	12.2 ± 1.6	100	100
↑MDP	2.5 ± 0.8	140.1 ± 2.0	23.9 ± 0.2	8.3 ± 1.1	14.0 ± 1.8	74.7 ± 2.6	115.6 ± 1.7
C_P	18.5 ± 2.4	120.1 ± 1.8	3.9 ± 0.4	9.1 ± 1.1	14.9 ± 1.9	100	100
↑MDP	41.0 ± 5.3	140.6 ± 1.9	23.8 ± 0.3	5.8 ± 0.8	20.1 ± 2.2	69.9 ± 2.4	126.2 ± 1.8
Second Trial							
C_P	17.9 ± 2.5	119.7 ± 1.9	3.9 ± 0.3	9.0 ± 1.1	14.9 ± 1.8	100	100
↑MDP	41.2 ± 5.1	140.4 ± 2.1	23.9 ± 0.3	5.9 ± 0.8	19.7 ± 2.3	68.4 ± 2.4	127.8 ± 1.9
C_{N-P}	0.3 ± 0.2	119.9 ± 1.9	3.9 ± 0.3	11.1 ± 1.7	12.1 ± 1.8	100	100
↑MDP	2.7 ± 0.6	139.7 ± 2.0	23.9 ± 0.3	8.4 ± 1.3	13.8 ± 1.9	75.9 ± 2.8	116.7 ± 1.7
			Papaverine Treated Vasculature				
C_P	14.6 ± 2.1	113.4 ± 2.2	3.9 ± 0.6	52.2 ± 5.3	2.09 ± 0.4	100	100
↑MDP	32.3 ± 3.2	133.6 ± 2.4	23.9 ± 0.7	66.5 ± 6.9	1.67 ± 0.3	125.5 ± 2.0	81.8 ± 2.3
C_{N-P}	0.2 ± 0.2	113.6 ± 2.3	3.9 ± 0.7	42.9 ± 4.5	2.58 ± 0.8	100	100
↑MDP	1.7 ± 0.6	133.3 ± 2.7	23.9 ± 0.7	54.3 ± 6.3	2.07 ± 0.5	129.5 ± 2.1	80.6 ± 2.5

Figure 18. Gracilis muscle resistance responses to elevations in mean vascular distending pressure during pulsatile or non-pulsatile perfusion in normal (left hand bars) and papaverine treated (right hand bars) muscles. Ordinate depicts the percent change in gracilis muscle vascular resistance. Data are means ± standard errors from 10 experiments.

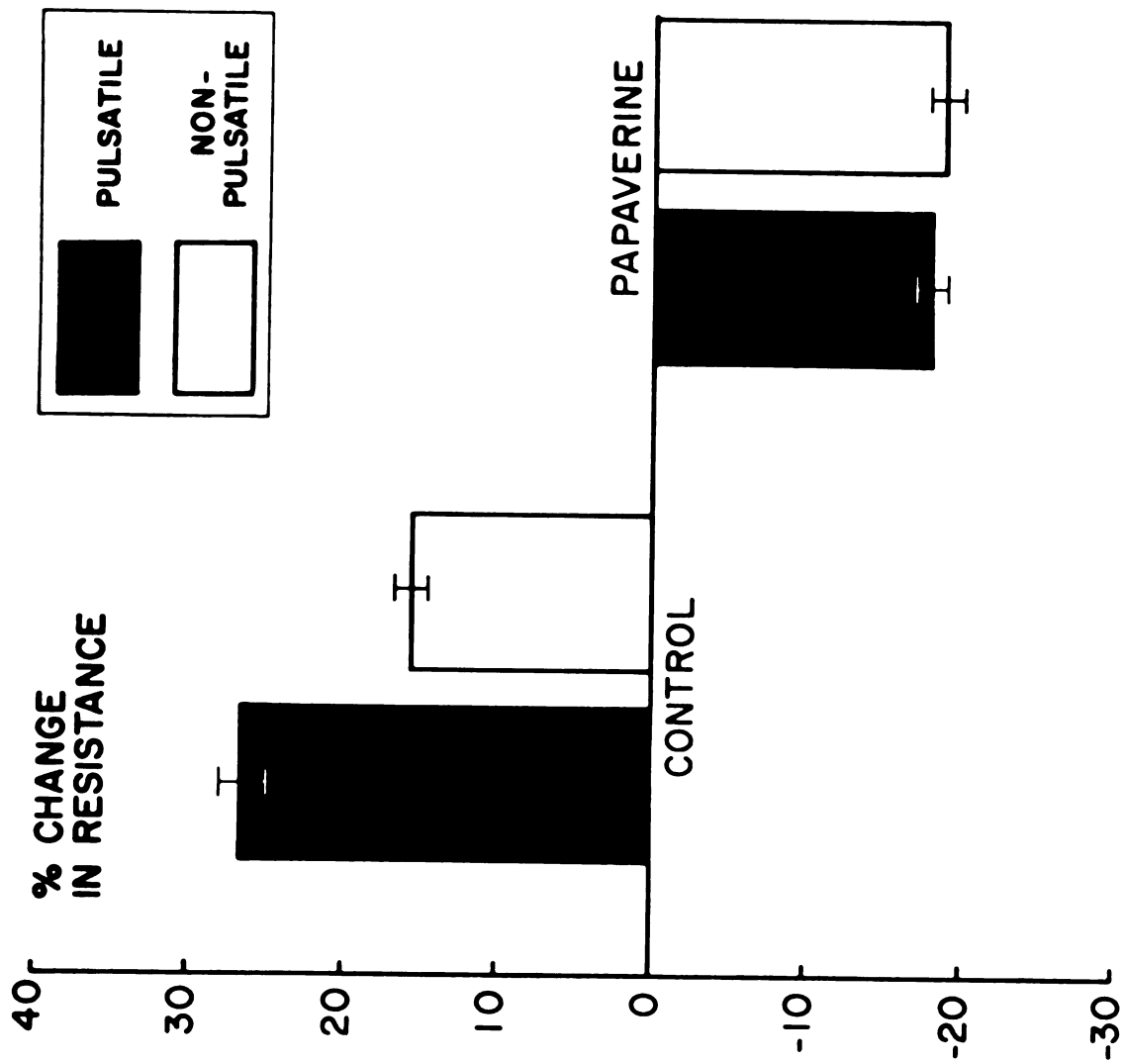
PULSATILE
NON-
PULSATILE
% CHANGE
IN RESISTANCE
40
30
20
10
0
-10
-20
-30
CONTROL
PAPAVERINE

Figure 18

however, the passive dilation is abolished and replaced by an active constriction which is more pronounced during pulsatile than during non-pulsatile perfusion.

The data reported in the first four rows of Table 6 represent the vascular responses to the first set of experimental maneuvers performed in these dogs. The second four rows of Table 6 report the vascular responses to this same set of experimental maneuvers performed just prior to papaverine infusion, after the vasculature had been exposed to two periods of local, graded hypotension. Because all responses to elevations of mean vascular distending pressure were statistically similar before and after periods of local hypotension, the preparations were assumed to have been stable throughout the course of the experiments.

C. Vascular Responses to Increased Venous Pressure

Table 7 and Figure 19 report gracilis muscle vascular responses to increasing gracilis vein pressure 20 mm Hg during pulsatile and non-pulsatile perfusion in normal and papaverine treated muscles. When gracilis vein pressure was elevated 20 mm Hg during non-pulsatile perfusion, muscle blood flow was reduced 38.7% below control, partly because of a decreased pressure gradient but also because of a 21.5 $\pm$ 2.1% elevation of vascular resistance from 11.5 $\pm$ 1.8 to 14.1 $\pm$ 2.0 mm Hg/cc/min/100 gms. However, during pulsatile perfusion, increasing vein pressure

Table 7. Gracilis muscle vascular responses to 20 mm Hg elevations of venous pressure during pulsatile and non-pulsatile perfusion in normal and papaverine treated muscles. Pressures are represented in mm Hg; flow in cc/min/100 gms; and resistance in mm Hg/cc/min/100 gms. C_{N-P} = non-pulsatile control data; C_P = pulsatile control data; and $\uparrow V_P$ = 20 mm Hg elevation of vein pressure. Data are mean values ± standard errors from 10 experiments.

	Gracilis Artery Pulse Pressure	Mean Gracilis Artery Pressure	Gracilis Vein Pressure	Gracilis Vein Flow	Gracilis Vascular Resistance	% Of Control Flow	% Of Control Resistance
				Normal Vasculature			
First Trial							
C_{N-P}	2.7 ± 0.8	141.5 ± 1.8	3.9 ± 0.3	14.7 ± 2.1	11.5 ± 1.8	100	100
$\uparrow V_P$	2.7 ± 0.8	141.5 ± 1.8	23.9 ± 0.2	8.3 ± 1.1	14.1 ± 2.0	61.3 ± 2.1	121.5 + 2.1
C_P	41.0 ± 5.3	140.6 ± 1.8	23.8 ± 0.3	10.2 ± 1.3	15.6 ± 2.0	100	100
$\uparrow V_P$	41.0 ± 5.3	140.6 ± 1.8	23.8 ± 0.3	5.8 ± 0.9	20.1 ± 2.2	56.9 ± 1.9	129.3 ± 2.2
Second Trial							
C_P	41.2 ± 5.1	140.1 ± 2.1	3.9 ± 0.3	10.3 ± 1.3	15.4 ± 2.1	100	100
$\uparrow V_P$	41.2 ± 5.1	140.4 ± 2.1	23.9 ± 0.3	5.9 ± 0.8	19.7 ± 2.3	57.2 ± 1.8	128.4 ± 2.1
C_{N-P}	2.7 ± 0.6	139.7 ± 2.0	3.9 ± 0.3	14.9 ± 2.2	11.5 ± 1.7	100	100
$\uparrow V_P$	2.7 ± 0.6	139.7 ± 2.0	23.9 ± 0.3	8.4 ± 1.3	13.8 ± 1.9	62.6 ± 2.2	119.9 ± 2.0
				Papaverine Treated Vasculature			
C_P	32.3 ± 3.2	133.6 ± 2.4	3.9 ± 0.6	78.6 ± 8.3	1.63 ± 0.3	100	100
$\uparrow V_P$	32.3 ± 3.2	133.6 ± 2.4	23.9 ± 0.7	66.5 ± 6.9	1.65 ± 0.3	85.3 ± 1.9	102.3 ± 0.7
C_{N-P}	1.7 ± 0.6	133.3 ± 2.7	3.7 ± 0.7	64.1 ± 6.8	2.05 ± 0.4	100	100
$\uparrow V_P$	1.7 ± 0.6	133.3 ± 2.7	23.9 ± 0.7	54.3 ± 6.3	2.08 ± 0.4	85.7 ± 1.9	101.9 ± 0.6

Figure 19. Gracilis muscle resistance responses to 20 mm Hg elevations of venous pressure during pulsatile or non-pulsatile perfusion in normal (left hand bars) and papaverins treated (right hand bars) muscles. Ordinate depicts the percent change in gracilis muscle vascular resistance. Data are means ± standard errors from 10 experiments.

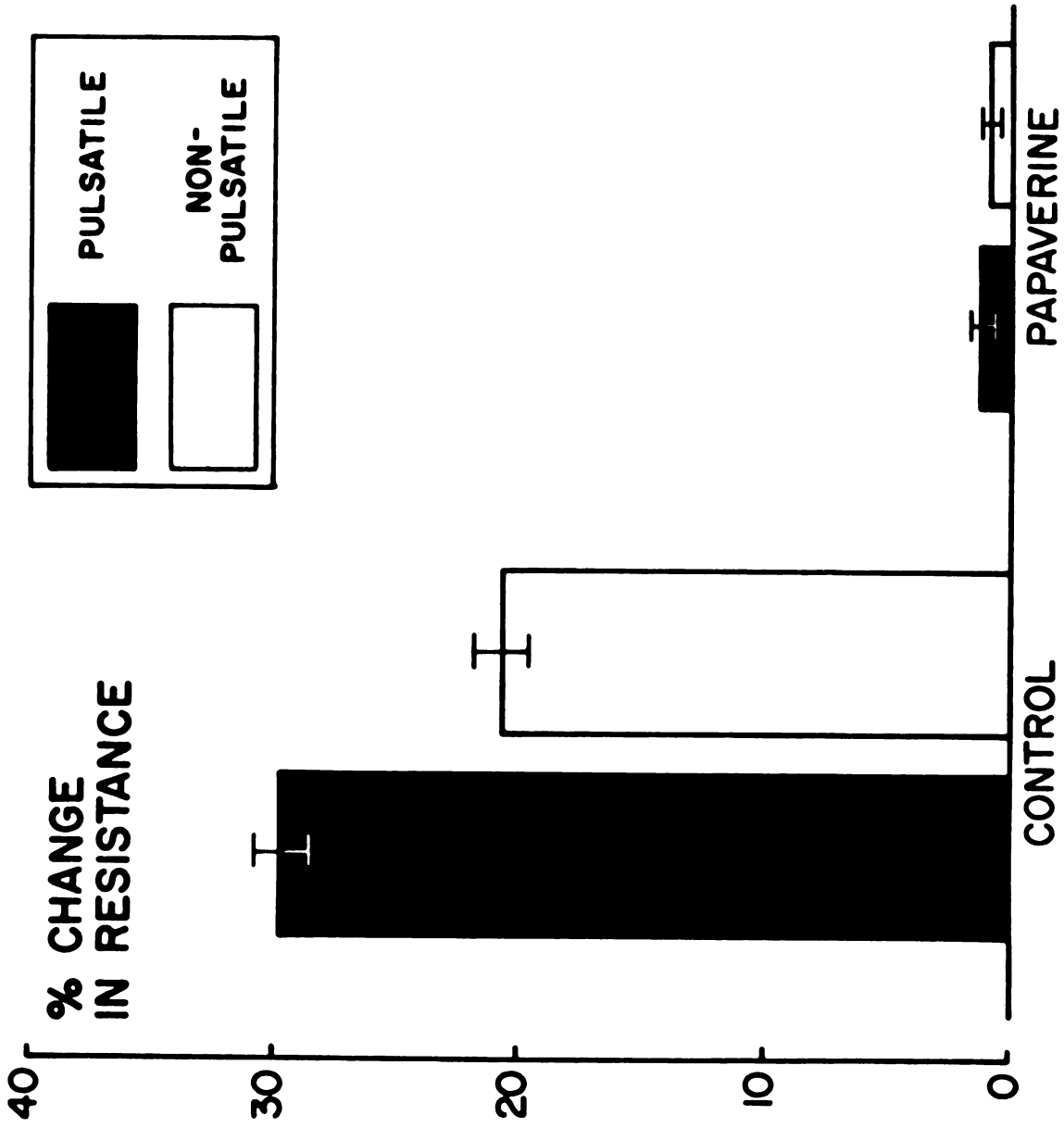
% CHANGE
IN RESISTANCE
40
30
20
10
0
PULSATILE
NON-
PULSATILE
CONTROL
PAPAVERINE

Figure 19

20 mm Hg elicited a significantly greater percent reduction in muscle blood flow because vascular resistance was elevated 29.3 ± 2.2% from 15.6 ± 2.0 to 20.1 ± 2.2 mm Hg/cc/min/100 gms.

When the vasculature was treated with papaverine, a 20 mm Hg increase in vein pressure elicited only a 2.3 ± 0.7% increase in vascular resistance during pulsatile perfusion and only a 1.9 ± 0.6% increase in vascular resistance during non-pulsatile perfusion. Thus, in the normal vasculature, the constriction elicited by venous pressure elevation was more pronounced during pulsatile perfusion and was almost abolished by papaverine. The latter observation suggests that this response represents an active smooth muscle contraction triggered by increased vascular distending pressure and potentiated by arterial pulse pressure distension.

The data reported in the first four rows of Table 7 represent the mean responses to the first set of experimental maneuvers performed in these dogs. The second four rows of Table 7 report the vascular responses to this same set of venous pressure elevations performed just prior to papaverine infusion, after the vasculature had been exposed to two periods of local, graded hypotension. Because all responses to elevations in venous pressure were statistically similar before and after periods of local

hypotension, the preparations were assumed to have been stable throughout the experiments.

D. Vascular Responses to Graded, Local Hypotension

Figure 20 reports gracilis muscle blood flow and vascular resistance as functions of gracilis artery pressure during pulsatile (dots) and non-pulsatile (circles) perfusion. During pulsatile perfusion, artery pulse pressure and muscle blood flow were significantly reduced from control with each local step reduction in perfusion pressure produced by compression of the gracilis artery. During pulsatile perfusion, the gracilis vasculature displayed blood flow autoregulation in response to graded local hypotension, as evidenced by the fact that a 29.6 $\pm$ 1.3% decrease in gracilis artery pressure from 141.5 $\pm$ 1.8 to 99.6 $\pm$ 1.5 mm Hg elicited only a 20.3 $\pm$ 1.1% reduction in flow from 10.2 $\pm$ 1.3 to 8.1 $\pm$ 1.0 cc/min/100 gms.

As described previously, reducing pulse pressure from 40.4 $\pm$ 5.1 to 2.6 $\pm$ 0.8 mm Hg without changing the pressure head, caused gracilis muscle blood flow to increase significantly from 10.1 $\pm$ 1.2 to 14.6 $\pm$ 2.6 cc/min/100 gms. During non-pulsatile perfusion, muscle blood flow was also significantly reduced from control with each local step reduction in perfusion pressure produced by compression of the gracilis artery. However, blood flow autoregulation was less pronounced than during pulsatile perfusion;

Figure 20. Gracilis muscle vascular responses to graded, local hypotension during pulsatile (dots) and non-pulsatile (circles) perfusion. Ordinates represent gracilis vein flow in cc/min/100 gms and gracilis muscle vascular resistance in mm Hg/cc/min/100 gms; abscissae represent gracilis artery pressure in mm Hg. Shaded areas above and below the lines indicate 1 standard error for the mean values and the numbers near the points indicate the average pulse pressure at each data value. Data are mean values from 10 natural flow experiments.

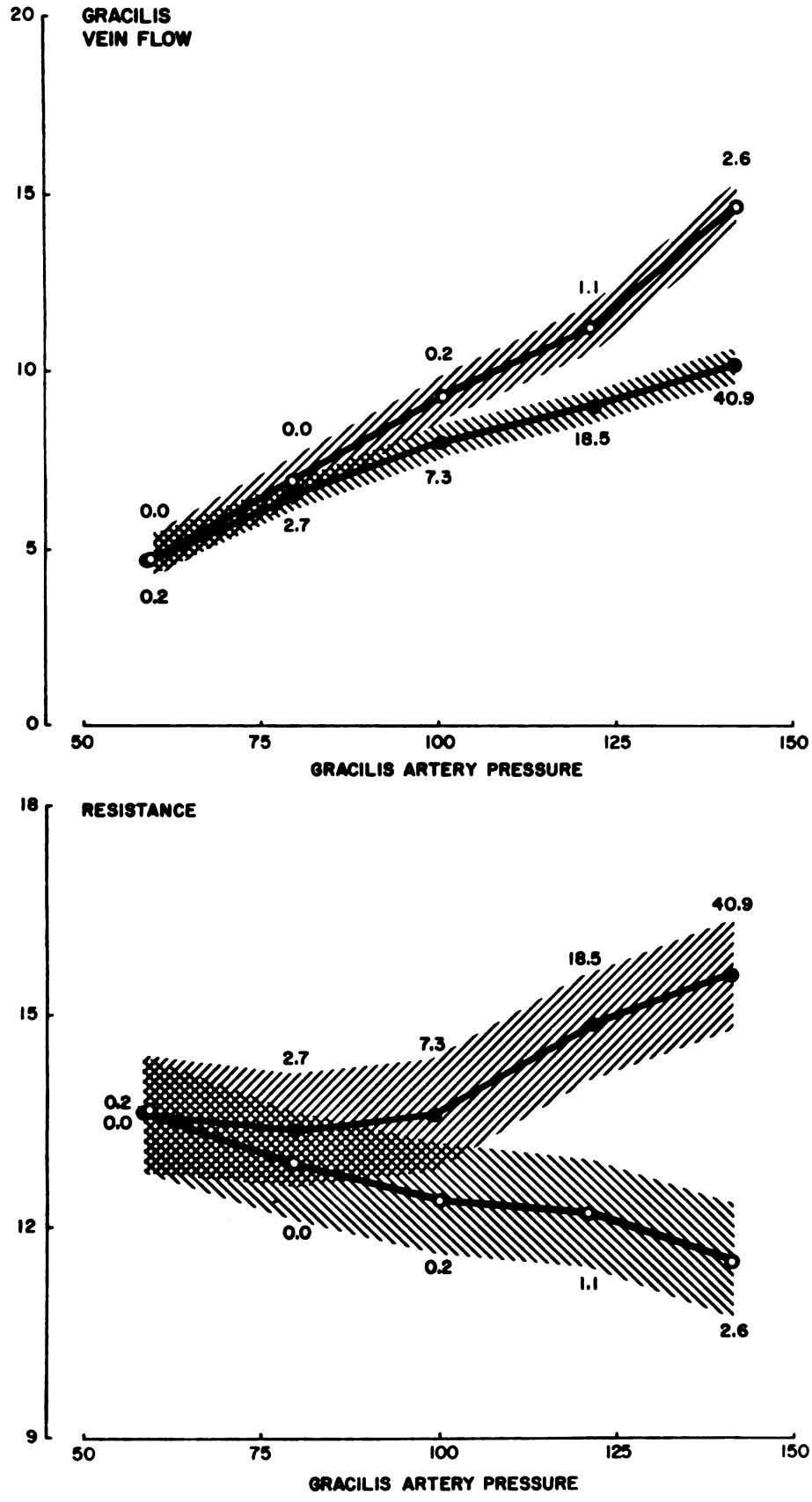
GRACILIS
VEIN FLOW
20
15
10
5
0
2.6
1.1
0.2
40.9
0.0
18.5
7.3
0.0
2.7
0.2
50
75
100
125
150
GRACILIS ARTERY PRESSURE
RESISTANCE
18
15
12
9
40.9
18.5
7.3
2.7
0.2
0.0
0.0
0.2
1.1
2.6
50
75
100
125
150
GRACILIS ARTERY PRESSURE

Figure 20

for example, a 29.7 ± 0.7% reduction in gracilis artery pressure from 141.4 ± 2.3 to 100.3 ± 0.4 mm Hg elicited a 35.2 ± 1.1% reduction in flow from 14.6 ± 2.6 to 9.3 ± 1.4 cc/min/100 gms.

The lower panel of Figure 20 reports the resistance responses to graded local hypotension during pulsatile and non-pulsatile perfusion. During pulsatile perfusion, gracilis muscle vascular resistance was significantly reduced from control when mean gracilis artery pressure was lowered from 140 to 100 mm Hg and below. During non-pulsatile perfusion however, muscle vascular resistance increased significantly as mean gracilis artery pressure was reduced from 140 to 60 mm Hg.

To better describe and compare the relationships between muscle vascular resistance and gracilis artery pressure during pulsatile and non-pulsatile perfusion, the resistance data in Figure 20 over the gracilis artery pressure range of 80-140 mm Hg were normalized to % of control and subjected to linear regression analysis (Figure 21). The data obtained during pulsatile perfusion demonstrated a significant regression of resistance as a function of perfusion pressure with a slope of 0.349 ± 0.056, indicating that the gracilis vasculature autoregulated its blood flow during pulsatile perfusion. The data obtained during non-pulsatile perfusion also demonstrated a significant regression of resistance as a function of perfusion

Figure 21. Effects of graded, local hypotension during pulsatile (dots) and non-pulsatile (circles) perfusion on gracilis muscle vascular resistance. Ordinate represents percent of control gracilis muscle vascular resistance; abscissa represents percent of control gracilis artery pressure. Data are individual values from 10 experiments over the gracilis artery pressure range of 80-140 mm Hg. The control values used to normalize the data were those obtained at the highest gracilis artery pressure within the range described for each experiment. Lines through each set of data are the lines of best fit obtained by least square linear regression analyses:

$$\%\ \text{of C - Resist}_{\text{Pulsatile}} = 0.349 \pm 0.056\ (\%\ \text{of C - Press.}) + 65.09 \pm 4.49;\ \bar{R}^2 = 0.711$$

$$\%\ \text{of C - Resist}_{\text{Non-puls.}} = -0.381 \pm 0.089\ (\%\ \text{of C - Press.}) + 138.4 \pm 7.16;\ R^2 = 0.569$$

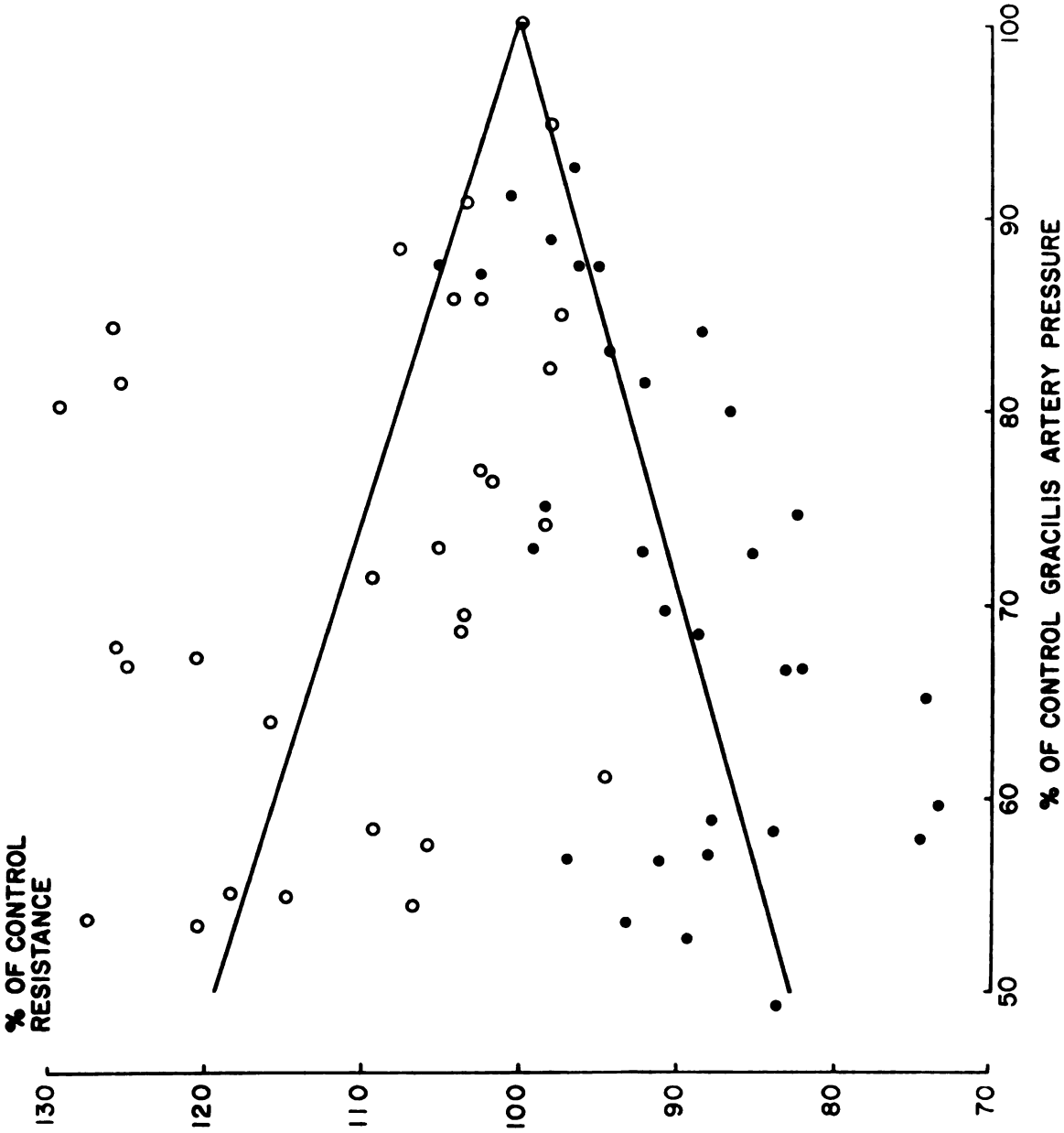
% OF CONTROL RESISTANCE
130
120
110
100
90
80
70
50
60
70
80
90
100
% OF CONTROL GRACILIS ARTERY PRESSURE

Figure 21

pressure. However, the slope for the resistance data obtained during non-pulsatile perfusion was -0.381 ± 0.089; a value significantly different from that observed during pulsatile perfusion. Thus, blood flow autoregulation was significantly attenuated during non-pulsatile perfusion.

Table 8 reports the vascular responses to 20 mm Hg reductions in gracilis artery pressure during non-pulsatile perfusion in normal and papaverine treated muscles. When gracilis artery pressure was reduced 14.2% below the control value of 139.7 ± 2.0 to 119.9 ± 1.9 mm Hg in the normal vasculature, gracilis muscle blood flow fell 21.7% below the control level of 14.9 ± 2.2 to 11.1 ± 1.7 cc/min/100 gms due to the fall in perfusion pressure and to a 9.3 ± 3.9% increase in muscle vascular resistance. However, during papaverine infusion, a 14.8% reduction in gracilis artery pressure from 133.3 ± 2.7 to 113.6 ± 2.3 mm Hg elicited a significantly greater percent reduction in flow as a result of a 23.7 ± 4.3% increase in gracilis muscle vascular resistance. These data indicate that although muscle blood flow autoregulation was attenuated with non-pulsatile perfusion, the vasculature was still capable of some degree of flow autoregulation since the resistance increase observed in response to gracilis artery pressure reduction was greater in the papaverine treated versus the normal vasculature.

Table 8. Gracilis muscle vascular responses to 20 mm Hg reductions of gracilis artery pressure during non-pulsatile perfusion in normal and papaverine treated muscles. Pressures are represented in mm Hg; flow in cc/min/100 gms; and resistance in mm Hg/cc/min/100 gms. C_{N-P} = non-pulsatile control data; and $\downarrow P_G$ = 20 mm Hg reduction of gracilis artery pressure. Data are mean values ± standard errors from 10 experiments.

	Gracilis Artery Pulse Pressure	Mean Gracilis Artery Pressure	Gracilis Vein Pressure	Gracilis Vein Flow	Gracilis Vascular Resistance	% Of Control Flow	% Of Control Resistance
				Normal Vasculature			
C_{N-P}	2.7 ± 0.6	139.7 ± 2.0	3.9 ± 0.6	14.9 ± 2.2	11.5 ± 1.7	100	100
$\downarrow P_G$	0.3 ± 0.2	119.9 ± 1.9	3.9 ± 0.3	11.1 ± 1.7	12.1 ± 1.8	78.3 ± 3.4	109.3 ± 3.9
				Papaverine Treated Vasculature			
C_{N-P}	1.7 ± 0.6	133.3 ± 2.7	3.9 ± 0.7	64.1 ± 6.8	2.05 ± 0.4	100	100
$\downarrow P_G$	0.2 ± 0.2	113.6 ± 2.3	3.9 ± 0.7	42.9 ± 4.5	2.58 ± 0.8	68.3 ± 3.7	123.7 ± 4.3

DISCUSSION

I. Series I and II: Naturally Perfused and Pump Perfused, Innervated Gracilis Muscles; Gracilis Artery Pressure Alterations During Normovolemic and Hypovolemic Periods

A. Steady State Autoregulatory Responses to Local Alterations in Perfusion Pressure

For naturally perfused muscles, the autoregulatory adjustments in vascular tone accompanying step reductions in gracilis artery pressure were not significantly altered by hypovolemia (Figure 9). However, when gracilis artery pressure was lowered from or increased to 140 mm Hg in pump perfused muscles, a small improvement of blood flow autoregulation was detected when vascular tone was elevated by hemorrhage (i.e., a given percent change in pressure produced a smaller percent change in flow during hypovolemia) (Table 3; Figures 14, 16). Bond and Green (13, 43) also reported improved autoregulatory adjustment of canine hindlimb blood flow in response to local perfusion pressure alterations performed during hypovolemia.

Both metabolic and myogenic mechanisms may contribute to the maintenance or improvement of blood flow autoregulation when vascular tone is elevated by hemorrhage.

The reduced blood flow levels accompanying hemorrhage could lower the flow to metabolism ratio and increase the tissue concentration of vasodilator metabolites thereby enhancing blood flow autoregulation via metabolic mechanisms. Some support for this view is provided by the studies of Stainsby (115) in which blood flow autoregulation was found to be more effective when the flow to metabolism ratio was lowered by skeletal muscle exercise. Furthermore, Jones and Berne (70) report that muscle blood flow autoregulation is more pronounced in preparations displaying low venous blood oxygen saturation. It is also possible that myogenic mechanisms, augmented by a reflex increase in vascular smooth muscle tension, account for the improved autoregulation seen during hemorrhage. It has been shown for example that isolated arterial strips exhibit increased myogenic responsiveness to passive stretch when resting tension is high (112, 113).

B. Transient Vascular Responses to Alterations in Perfusion Pressure

In the pump perfused muscles, each elevation of arterial pressure elicited a rapid, disproportionate increase in flow which was followed by a slower decline of flow toward control levels (Table 3; Figures 11, 12). Other workers have suggested that this initial, brief period of declining resistance is due to a passive vascular distention (43, 116, 117). Since the vascular wall should be less compliant

during elevated levels of vasomotor tone accompanying hemorrhage, it might be expected that the peak transient flow would be proportionately less during hemorrhage if this initial period of rising flow is due to purely passive vascular behavior. However, just the opposite occurred: when perfusion pressure was elevated to 140 mm Hg, the initial peak flows were proportionately greater during hypovolemia than during normovolemia (Table 3). Assuming that the vasculature is less compliant during hemorrhage, these results suggest that the initial period of declining resistance observed in response to perfusion pressure elevation is not due solely to a passive distension of the resistance vessels.

The metabolic hypothesis provides one explanation for the relatively greater increase in peak transient flow observed during hemorrhage. Since the initial level of tissue vasodilator metabolites should be higher at the low flow rates accompanying hemorrhage, elevation of perfusion pressure could via metabolic mechanisms, elicit a proportionately greater initial decrease in resistance during hemorrhage. Some support for this interpretation is obtained from an analysis of Jones' and Bernes' (73) data, where perfusion pressure elevations in muscles having low flows and low venous oxygen content elicited initial vasodilations which were relatively greater than those

observed in preparations having higher resting flows and venous oxygen contents.

When perfusion pressure was lowered during normovolemia, muscle blood flow at first rapidly decreased to minimum values and then slowly rose toward control levels (Table 3; Figures 11, 12). When vascular tone was elevated during hypovolemia, perfusion pressure reductions elicited proportionately smaller reductions in initial transient flows (Table 3; Figure 12). For example, a 57% reduction in gracilis artery pressure from 140 to 80 mm Hg elicited a transient 75% reduction in flow during normovolemia, whereas flow fell only 59% below control when local pressure was similarly reduced in hypovolemia. Other investigators have proposed that this initial rise in resistance accompanying perfusion pressure reduction is due to passive vascular collapse (116, 117). If it is assumed that the vasculature is less distensible when vascular smooth muscle tone is elevated by hemorrhage, the observation that perfusion pressure reductions elicit proportionately smaller initial reductions in flow during hemorrhage supports this hypothesis.

When perfusion pressure was altered to or from 140 mm Hg, more time was required to reach the minimum and maximum transient flow values when vascular tone was elevated by hemorrhage. These data possibly reflect a decreased

distensibility of the vascular smooth muscle cells when active tension is elevated by hemorrhage.

During hemorrhage, more time was also required to reach the steady state flows after gracilis artery pressure alterations (Table 3), indicating that the autoregulatory responses to step changes in perfusion pressure develop more slowly during systemic arterial hypotension. This central, vasoconstrictor influence on blood flow autoregulation could be mediated by several different mechanisms. Folkow (35) has proposed that the smooth muscle of resistance vessels is arranged in two functionally different sheaths; one inner, myogenically active layer surrounded by an outer sheath where the smooth muscle cells essentially lack myogenic activity but are subordinated to adrenergic vasoconstrictor fiber control. According to this hypothesis, the myogenically active inner cells would become unloaded by contraction of an outer, well innervated layer during hemorrhage, possibly leading to a prolonged development of autoregulatory adjustments in vascular caliber during hemorrhage. Of course, this hypothesis could also be extended to encompass delayed local metabolic influences on the inner smooth muscle cells.

However, it is not necessary to propose a differential neuroeffector organization within the vascular wall to explain the prolonged development of autoregulatory adjustments in vascular tone during hemorrhage. Vasodilator

metabolites could exert their effects more slowly on the same population of smooth muscle cells which are driven by neurally released or blood borne vasoconstrictors during hemorrhage. Some support for this view is provided from the studies of Chalmers et al. (20) who observed that the rate of development of gracilis exercise hyperemia is progressively slower at increasing levels of vasomotor tone elicited by systemic hypoxemia. Alternatively, during hemorrhage the myogenically active cells may be driven to such a large extent by neurally released or blood-borne vasoconstrictors that they are unable to respond quickly to changes in transmural pressure.

When gracilis artery pressure was altered from or increased to 140 mm Hg, the areas under or above the transient flow peaks were the same in normovolemic versus hypovolemic periods (Table 3). The fact that these areas were the same in normovolemic and hypovolemic periods provide further indication that the overall autoregulatory response is not appreciably altered by hemorrhage induced elevations in vascular tone.

II. Series III: Naturally Perfused, Denervated Gracilis Muscles; Vascular Responses During Pulsatile and Non-pulsatile Perfusion

A. Vascular Responses to Alterations in Perfusion Mode

Switching the mode of perfusion from non-pulsatile to pulsatile elicited a rapid increase in blood flow followed

by a slower decline to a new steady state flow rate lower than that observed during non-pulsatile perfusion (Figure 17; Table 5). Conversely, restoration of non-pulsatile perfusion elicited an initial, rapid decrease in flow followed by a gradual return to a new steady state level higher than that observed with pulsatile perfusion (Figure 17; Table 5). The initial, rapid changes in resistance seen with perfusion mode alterations are attributed to the addition or removal of pulse pressure induced vascular distension since qualitatively similar passive responses were observed when perfusion mode alterations were performed in vascular networks poisoned with papaverine (Figure 17, Table 5). The secondary phases of increasing resistance observed after switching to pulsatile perfusion or decreasing resistance after switching to non-pulsatile perfusion probably represent active responses since papaverine abolished these changes in vascular resistance (Figure 17; Table 5).

When pulse pressure was increased from 2.6 to 41 mm Hg in these experiments, steady state gracilis vascular resistance increased 31% above control levels (Table 5). Rovick and Robertson (99) reported quantitatively similar increases in dog tongue vascular resistance in response to similar increases in pulse pressure. These investigators observed a progressive increase in muscle vascular resistance in response to step elevations in pulse pressure,

with a maximum 37% increase in resistance occurring when pulse pressure was increased to 40 mm Hg. With further elevations in pulse pressure to 64 mm Hg, tongue vascular resistance was observed to return to control levels. However, when Mellander and Arvidsson (85) elevated arterial pulse pressure from 8 to 56 mm Hg in sympathectomized cat hindlimbs, they observed only a 6% increase in muscle vascular resistance. It is possible that these relatively small resistance responses observed by Mellander and Arvidsson are due to species and/or preparation differences. However, an analysis of Rovick and Robertson's data suggests that only small increases in resistance would be expected with pulse pressure elevation from 8 to 56 mm Hg, since passive vascular distension apparently overrides the active vasoconstriction when pulse pressure is increased beyond 40 mm Hg.

The increased vascular tone accompanying pulse pressure distension could be either metabolically or myogenically mediated. When perfusion is altered from non-pulsatile to pulsatile, it is conceivable that flow could be redistributed within the muscle microvasculature in such a manner as to produce transvascular exchange conditions that would result in a lower tissue concentration of vasodilator metabolites leading to an increased total muscle vascular resistance via metabolic mechanisms. However, because the venous-arteriolar response is more pronounced during

pulsatile versus non-pulsatile perfusion (Figure 19; Table 7), it is more probable that the increased resistance seen with pulsatile perfusion represents an active myogenic reduction in vascular radius triggered by the increase in pulse pressure.

Transmural pressure changes associated with pulse pressure distension could elicit increased myogenic activity by at least two mechanisms. The increased vascular resistance seen with pulsatile perfusion could result from an increased myogenic activity elicited by the higher peak systolic distending pressures. Alternatively, myogenically active pacemaker cells could also be sensitive to the dynamic stretch stimulus afforded by repetitive pulsatile distensions. Both mechanisms seem plausible since active tension development and spike frequency in isolated artery and vein strips is directly related to the rate and increment of stretch as well as to resting tension (61, 112, 113).

B. Vascular Responses to Increased Venous Pressure

A 20 mm Hg increase in vein pressure elicited a 21.5% increase in gracilis vascular resistance during non-pulsatile perfusion and a 29.3% increase in resistance during pulsatile perfusion (Table 7; Figure 19). These responses were almost completely abolished when the vasculature was poisoned with papaverine (Table 7; Figure 19). Since the muscles were acutely denervated, these venous-arteriolar responses do not

appear to result from neural reflexes. Because the metabolic hypothesis predicts a decreased resistance with venous pressure elevation and because the venous-arteriolar response should persist during papaverine administration if tissue pressure effects mediate the response, these data indicate that myogenic mechanisms of local blood flow control are present within the gracilis muscle vasculature. While it is possible that local neural networks mediate these venous-arteriolar responses, this seems unlikely since the response is present in the hindlimbs of reserpinized cats (36). Also, Johnson (62) has demonstrated that procaine administration does not effect the venous-arteriolar response in the intestine.

The venous-arteriolar response has not been consistently observed in skeletal muscle vascular beds. Jones and Berne (73, 74) and Hanson (49) observed small venous-arteriolar responses in only a few of their isolated canine hindlimb preparations. Since in these experiments, the muscles being studied were sectioned, it is possible that the preparations were less myogenically active. When venous pressure was elevated in muscle preparations that required little surgical disruption (canine gracilis and cat hindquarters), Nagle et al. (88) and Folkow and Oberg (36) consistently observed large venous-arteriolar responses.

The presence and magnitude of the venous-arteriolar response also appears to depend on the resting blood flow

level. In experiments reported here, gracilis muscle blood flow averaged 10 cc/min/100 gms and all preparations displayed prominent venous-arteriolar responses. The gracilis muscles used by Nagle et al. (88) and the cat hindlimbs studied by Folkow and Oberg (36) displayed similar resting blood flows per unit tissue weight and quantitatively similar venous-arteriolar responses. Resting blood flow averaged only 3.4 cc/min/100 gms in the canine hindlimb preparations used by Jones and Berne (73, 74), and these preparations usually did not exhibit venous-arteriolar responses. In the few preparations which displayed relatively high control blood flows and venous oxygen content, Jones and Berne did observe an appreciable increase in vascular resistance with elevated venous pressure. These results suggest that at low resting blood flow rates, increased tissue concentrations of vasodilator metabolites may override myogenic mechanisms so that the venous-arteriolar response is abolished.

In the experiments reported in this thesis, venous pressure elevation produced larger elevations in gracilis vascular resistance during pulsatile compared to non-pulsatile perfusion (Table 7, Figure 19). This potentiation of the venous-arteriolar response by arterial pulse pressure distension implies that the gain of the myogenically active smooth muscle cells is increased with repetitive

pulse pressure distension. Pulsatile changes in transmural pressure could elicit increased myogenic activity in response to elevation of venous pressure by at least two mechanisms. Myogenic pacemaker cells could be more active at the higher peak systolic distending pressures since it is known that isolated artery strips develop more active tension in response to a given increment of passive stretch when resting tension is high (112, 113). Alternately, myogenic pacemaker cells could become more sensitive to a given increment of stretch when they are exposed to the dynamic changes in length associated with repetitive pulse pressure distension, since active tension development and spike frequency in isolated artery and vein strips are increased with elevated rates of stretch (61, 112, 113).

C. Vascular Responses to Increasing Mean Distending Pressure

When mean vascular distending pressure was elevated by increasing mean gracilis arterial and venous pressure 20 mm Hg, muscle vascular resistance increased 15.6% during non-pulsatile and 26.6% during pulsatile perfusion (Table 6, Figure 18). When the vasculature was treated with papaverine, this same increase in mean distending pressure elicited an 18.2% reduction in resistance during pulsatile perfusion and an 18.8% resistance decrease during non-pulsatile perfusion (Table 6, Figure 18). Mellander and Arvidsson (85) have obtained similar results in the

sympathectomized lower leg muscles of the cat. When these investigators increased mean distending pressure 20 mm Hg in the normal vasculature, they observed a 7% increase in vascular resistance during non-pulsatile and a 14% increase during pulsatile perfusion, whereas the same elevation in transmural pressure in the papaverine treated vasculature decreased resistance by 20%.

These data indicate that the distending effect of increased transmural pressure observed in the passive muscle vasculature is abolished and replaced by an active, presumably myogenic, constrictor response in the normal vascular bed. Further, since the constriction is more pronounced with pulsatile perfusion, it would appear that myogenically active smooth muscle cells are also sensitive to the transmusal pressure changes accompanying pulse pressure distension. These data do not conclusively demonstrate a vascular sensitivity to pulse pressure distension because, in the same experiments, simultaneous elevations of mean arterial and venous pressures are accompanied by greater increases in peak systolic pressures during pulsatile compared to non-pulsatile perfusion (Table 6). It could be argued that the greater constriction observed in response to elevation of mean distending pressure during pulsatile perfusion is instead related to the larger increase in systolic pressure during pulsatile compared to non-pulsatile perfusion. However, this seems unlikely since, when venous

pressure alone is elevated, the venous-arteriolar response is also more pronounced during pulsatile perfusion (Table 7, Figure 19).

Thus, it appears that transmural pressure changes associated with pulse pressure distension potentiate the myogenically mediated vasoconstriction observed in response to elevations of mean distending pressure. Since it has been shown that isolated artery strips develop more active tension to a given increment of stretch when resting tension is high (112, 113), myogenically active pacemaker cells could be operating more efficiently at the higher systolic distending pressures thereby eliciting more pronounced constriction in response to mean distending pressure elevation during pulsatile perfusion. Because isolated vessels do show more active tension development and elevated spike frequency at increasing rates of stretch (61, 112, 113), these pacemaker cells could also be sensitive to the rate of transmural pressure change and operate at higher gain during pulsatile perfusion.

D. Vascular Responses to Graded, Local Hypotension

When mean gracilis artery pressure was progressively lowered from 140 to 60 mm Hg during pulsatile perfusion, both pulse pressure and muscle vascular resistance decreased significantly (Figures 20, 21). However, during non-pulsatile perfusion, the same maneuver elicited a progressive

rise in muscle vascular resistance (Figures 20, 21). Even though resistance increased in response to graded pressure reductions during non-pulsatile perfusion, some degree of blood flow autoregulation remained because the papaverine treated, passive vasculature displayed significantly larger increases in resistance when perfusion pressure was reduced from 140 to 120 mm Hg (Table 8).

Vascular resistance was observed to decrease only when both mean arterial and pulse pressure were reduced, indicating that muscle blood flow autoregulation is mediated to a large extent by alterations in pulse pressure rather than simply by changes in mean transmural pressure. The fact that the venous-arteriolar response and the constriction in response to mean distending pressure elevation are both larger during pulsatile perfusion suggests that alterations in pulse pressure mediate blood flow autoregulation by myogenic rather than metabolic mechanisms. Myogenically active pacemaker cells could elicit autoregulatory decreases in resistance when pulse pressure is reduced by relaxing in response to lower peak systolic pressures. This interpretation is consistent with the reports of other investigators that isolated artery strips develop less active tension to a given increment of stretch when resting tension is low (112, 113).

SUMMARY AND CONCLUSIONS

1. A given percent change in gracilis artery pressure elicited either similarly less proportionate (naturally perfused muscles) or slightly smaller (pump perfused muscles) percent changes in flow during hypovolemia, indicating that steady state blood flow autoregulation was either maintained or slightly improved when vascular tone was elevated by hemorrhage. Both metabolic and myogenic mechanisms may have contributed to the maintenance or improvement of steady state autoregulatory adjustments in vascular tone.

2. The rate of development of autoregulatory responses to perfusion pressure alterations were prolonged during hemorrhage induced elevation of vascular tone due to some interaction or competition between local metabolic and/or myogenic mechanisms and remote vasoconstrictor influences.

3. Altering the mode of perfusion from non-pulsatile to pulsatile or vice versa elicited initial passive changes in muscle resistance due to the addition or removal of pulse pressure induced vascular distension. These brief, passive alterations in resistance were followed by the addition or removal of pulse pressure induced myogenic vascular tone.

4. Increased venous or mean vascular distending pressures produced more vasoconstriction during pulsatile versus non-pulsatile perfusion, indicating that myogenic mechanisms are present within the muscle vasculature and are sensitive to pulse pressure induced changes in transmural pressure.

5. When mean gracilis artery pressure was progressively lowered from 140 to 60 mm Hg during pulsatile perfusion both pulse pressure and muscle vascular resistance decreased, whereas during non-pulsatile perfusion the same maneuver elicited a progressive rise in vascular resistance. These data indicate that muscle blood flow autoregulation is mediated to a large extent by alterations in pulse pressure induced myogenic activity rather than simply by changes in mean transmural pressure.

APPENDICES

APPENDIX A

PRESSURE, FLOW, AND RESISTANCE DATA

Table A-1. Effects of local hypotension during normovolemic and hypovolemic periods on mean systemic arterial and gracilis artery and vein pressures (mm Hg), gracilis vein flow (cc/min/100 gms), and vascular resistance (mm Hg/cc/min/100 gms). Values are means ± standard errors from 10 experiments.

	Systemic Arterial Pressure	Gracilis Artery Pressure	Gracilis Vein Pressure	Gracilis Vein Flow	Muscle Vascular Resistance
			Normovolemia		
Control	137±4.3	130±4.2	4.8±1.6	12.6±2.0	11.8±2.0
Clamp	137±4.0	112±3.6	3.4±1.4	9.9±1.4	12.8±1.9
	136±3.9	98±3.6	2.7±1.3	8.8±1.2	12.1±1.8
	137±4.1	86±3.4	2.4±1.2	8.3±1.2	11.5±1.6
	137±4.2	72±2.6	2.1±1.3	7.7±1.1	10.4±1.6
Control	137±4.2	132±4.3	4.8±1.5	12.7±2.0	11.6±1.5
			Hypovolemia		
Control	118±3.9	115±4.1	1.8±0.8	6.8±0.8	17.8±1.9
Clamp	118±4.3	103±3.6	1.6±0.8	6.1±0.7	17.8±1.9
	119±4.3	91±4.0	1.4±0.7	5.5±0.6	17.1±1.8
	118±4.3	78±3.0	1.3±0.6	5.3±0.5	15.2±1.8
	118±4.2	66±3.0	1.1±0.6	4.9±0.5	13.9±1.7
Control	119±4.3	115±4.4	1.9±0.8	7.0±0.8	17.4±2.2

Table A-2. Effects of local hypotension during normovolemic and two hypovolemic periods on mean systemic arterial and gracilis artery and vein pressures (mm Hg), gracilis vein flow (cc/min/100 gms), and vascular resistance (mm Hg/cc/min/100 gms). Values are means ± standard errors from 4 experiments.

	Systemic Arterial Pressure	Gracilis Artery Pressure	Gracilis Vein Pressure	Gracilis Vein Flow	Muscle Vascular Resistance
			Normovolemia		
Control	142±5.3	138±5.0	4.5±1.5	11.5±1.7	12.3±3.0
Clamp	141±4.0	117±2.6	3.8±1.4	10.0±1.9	13.1±4.3
	142±2.9	101±5.1	3.0±1.4	9.2±1.7	12.1±3.9
	140±2.5	91±3.0	2.8±1.3	8.8±1.6	11.4±3.6
	141±3.6	74±2.5	2.4±1.3	7.7±1.4	10.3±2.9
Control	140±4.3	137±5.0	4.4±1.5	11.3±1.7	12.3±3.0
			Mild Hypovolemia		
Control	122±4.7	120±5.6	2.8±1.2	7.9±1.4	15.9±4.0
Clamp	121±5.1	108±4.9	2.1±1.1	6.9±1.1	16.5±4.4
	122±5.5	94±7.2	1.8±0.9	6.5±1.0	15.0±3.9
	121±5.1	79±3.4	1.7±0.9	6.0±0.9	13.1±3.0
	122±4.9	65±2.4	1.6±0.8	5.7±0.9	11.3±2.3
Control	123±4.9	120±5.5	2.9±1.2	8.0±1.3	15.7±4.0
			Moderate Hypovolemia		
Control	104±4.1	103±4.8	1.4±0.9	5.1±0.6	20.8±3.9
Clamp	104±4.2	91±3.9	1.3±0.9	4.6±0.7	20.5±3.9
	103±4.4	81±2.9	1.2±0.8	4.4±0.6	19.2±3.8
	103±4.3	69±2.5	1.2±0.8	4.3±0.5	16.8±3.6
	103±4.3	59±2.5	1.0±0.7	3.9±0.5	16.1±3.8
Control	104±4.4	102±4.5	1.4±1.0	5.3±0.7	20.2±3.9

Table A-3. Effects of local gracilis artery pressure alterations during normovolemic and hypovolemic periods on gracilis muscle vascular resistance. Pressures are expressed in mm Hg; resistance in mm Hg/cc/min/100 gms. Values are means ± standard errors from 8 experiments.

	Systemic Arterial Pressure	Gracilis Artery Pressure	Gracilis Vascular Resistance	% of Control Resistance
		Normovolemic		
Control	143±6.0	140±0.7	15.9±2.6	100
Local pressure change	143±6.1	201±1.3	11.7±1.8	74.8±3.5
	143±6.2	180±0.9	14.1±2.2	88.4±3.5
	143±6.1	121±0.5	16.5±3.1	100.4±2.4
	143±6.2	101±0.7	15.8±3.1	95.7±2.5
	143±5.8	80±0.4	15.0±2.9	91.2±3.9
	143±6.2	60±0.9	14.7±2.6	90.8±2.1
		Hypovolemic		
Control	100±2.1	140±0.7	25.8±3.4	100
Local pressure change	100±2.1	201±1.4	19.7±3.3	76.2±4.1
	100±2.0	180±0.9	23.2±3.5	89.9±3.8
	100±2.2	121±0.5	24.1±3.4	93.9±1.7
	100±2.1	101±0.8	23.6±3.5	89.4±1.7
	100±2.9	80±0.5	22.1±3.4	84.7±3.5
	100±2.1	60±0.8	19.6±3.0	83.2±2.2

Table A-4. Gracilis muscle vascular responses to graded, local hypotension during pulsatile and non-pulsatile perfusion. Pressures are represented in mm Hg; flow in cc/min/100 gms; and resistance in mm Hg/cc/min/100 gms. Values are means ± standard errors from 10 experiments.

	Gracilis Artery Pulse Pressure	Mean Gracilis Artery Pressure	Gracilis Vein Pressure	Gracilis Vein Flow	Gracilis Vascular Resistance	% of Control Flow	% of Control Resistance
				Non-pulsatile Perfusion			
Control	2.7±0.8	142±1.8	3.9±0.3	14.7±2.1	11.5±1.7	100	100
Clamp	1.1±0.6	121±0.8	3.9±0.3	11.3±1.6	12.2±1.6	78.3±3.4	109.3±3.9
	0.2±0.2	100±0.4	3.9±0.4	9.3±1.4	12.4±1.7	64.8±1.1	109.2±1.2
	0.0±0.0	79±0.7	3.9±0.3	6.9±1.0	12.9±1.7	48.0±1.6	114.5±1.9
	0.0±0.0	59±0.8	3.9±0.3	4.7±0.6	13.7±1.7	33.3±2.1	123.0±3.1
				Pulsatile Perfusion			
Control	41.5±5.3	141±1.8	3.9±0.3	10.2±1.3	15.6±2.0	100	100
Clamp	18.5±2.4	122±0.9	3.9±0.3	9.1±1.1	14.9±1.9	89.7±1.3	96.0±1.9
	7.3±1.0	100±1.5	3.9±0.3	8.1±0.9	13.6±1.7	79.7±1.0	89.0±1.6
	2.7±0.5	79±1.0	3.9±0.3	6.5±0.8	13.3±1.6	65.3±1.7	87.6±1.4
	0.2±0.2	59±0.6	3.9±0.3	4.6±0.6	13.6±1.7	46.9±2.1	89.2±1.7

APPENDIX B

STATISTICAL METHODS

APPENDIX B

STATISTICAL METHODS

I. Series I

Gracilis vein flow and muscle vascular resistance were determined for control periods and during four experimental periods of local hypotension obtained during normovolemia or hypovolemia. For each period individual means ($\bar{x}_i$) were calculated for each parameter from two values obtained 2 and 4 minutes after gracilis artery pressure stabilized. The individual means were used to calculate a grand mean ($\bar{X}$), variance (S^2), and standard error of the mean ($SE_{\bar{X}}$) for each period during normovolemia or hypovolemia as follows:

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

$$S^2 = \frac{\sum_{i=1}^{n} \bar{X}_i^2 - \frac{\left(\sum_{i=1} \bar{X}_i\right)^2}{n}}{n - 1}$$

$$SE_{\bar{X}} = \sqrt{S^2} / \sqrt{n}$$

A. Comparison of Control Means with Four Experimental Means Produced by Local Hypotension During Normovolemia or Hypovolemia

An analysis of variance was performed to determine if the population means for each parameter at the five different

perfusion pressures were identical. Prior to the analysis of variance, the sample mean variances among periods (S^2) were determined to be homogeneous (a prerequisite for analysis of variance) by using the F max test. In this procedure the maximum variance ratio (S^2_{max}/S^2_{min}) is computed from the greatest (S^2_{max}) and the smallest (S^2_{min}) sample variances. This test statistic is compared with critical values (F_{max}, 0.05, k, υ) from a tabled cumulative probability distribution of maximum F for 2 sided comparisons based on k experimental periods and υ degrees of freedom (n - 1). In all vases the test statistic was smaller than the tabled F max value, so the null hypothesis ($S^2_{max} = S^2_{min}$) was accepted, indicating that the sample mean variances among periods were homogeneous.

An analysis of variance partitions the total variation of items into two distinct sources of variation: 1) that due to variation among groups (treatment effects); 2) that variation within groups (inter-dog variation). The variation among groups (mean square error among groups, MM_{among}) is expressed as the sum of squares among groups divided by the n - 1 degrees of freedom ($SS_{among}/n - 1$). The sum of squares among groups is computed from the following expression:

$$SS_{among} = \frac{1}{n}\sum_{i=1}^{a}\left(\sum_{i=1}^{n} Y_i\right)^2 - \frac{1}{an}\left(\sum_{i=1}^{a}\sum_{i=1}^{n} Y_i\right)^2$$

where a is the number of treatments, n the number of

experiments, and Y_i are the individual parameter values. The mean square error among groups (MS_{among}) is based on the variance of group means, which describes the dispersion of the group means around the grand mean. The variation within groups (mean square error within groups, MS_{within}) is expressed as the sum of squares within groups divided by $a(n - 1)$ degrees of freedom ($SS_{within}/a(n - 1)$. The sum of squares within is computed from the following expression:

$$SS_{within} = -\frac{1}{n}\sum_{i=1}^{a}\left(\sum_{i=1}^{n} Y_i\right)^2 + \sum_{i=1}^{a}\sum_{i=1}^{n} Y^2 \quad \text{where:}$$

a is the number of treatments, n is the number of experiments, and Y_i are the individual parameter values. The mean square error within groups (MS_{within}) gives the average dispersion of the n items in each group around the group means.

The test statistic for the analysis of variance is: MS_{among}/MS_{within}. The test statistic was compared with critical values ($F, 0.05, \upsilon_1, \upsilon_2$) from an F distribution table for υ_1 degrees of freedom of numerator mean square and υ_2 degrees of freedom of denominator mean square. If the test statistic exceeded the critical value, the null hypothesis of no added effect of treatments was rejected and the alternative hypothesis of a significant effect of local hypotension on vascular resistance or muscle blood flow was accepted.

If the analysis of variance demonstrated that local hypotension produced a significant change in a parameter value within the range of treatments, it was of interest to determine which treatments were significantly different from each other. If c independent comparisons among means are made, the probability of finding at least one significant comparison by chance is $1 - (1 - \alpha)^c$ (where α = error rate for each comparison) (78). Therefore, as the number of comparisons increases, the probability of finding at least one spuriously significant result also increases. The Student-Newman-Kuels procedure is a method in which comparisons are made in a stepwise fashion so that the probability of making a type I error (α) for the collection of comparisons can be set at a desired level. The Student-Newman-Kuels procedure was used in the present study to determine what levels of local hypotension produced significant changes in parameter values. The procedure requires the calculation of the standard error of a group ($S_{\bar{y}}$) using the error mean square (MS_{within}) from the analysis of variance as a pooled estimate of the variance among items within a group:

$$S_{\bar{y}} = \sqrt{\frac{MS_{within}}{a(n - 1)}}$$

The least significant ranges (LSR) for 2, 3, 4, and 5 means were then calculated using a table of studentized ranges (Q) at a significance level of 0.05 for groups 5, 4, 3, and

2 means with a(n - 1) degrees of freedom:

$$\text{LSR (for k groups)} = [Q, 0.05, k, a(n - 1)] \times S_{\bar{y}}$$

The means were then arranged in order of magnitude and the total range from the largest to the smallest mean ($\bar{y}_5 - \bar{y}_1$) was compared with the LSR for k = 5. If this range was significant, the range from the smallest to the next to largest mean ($\bar{y}_4 - \bar{y}_1$) was compared with the LSR for k = 4. The testing was continued in a similar manner until a range was encountered that was not significant and the testing was then discontinued. If the range enclosed by any two means was greater than the critical LSR the null hypothesis ($\mu_a = \mu_b$) was rejected and the alternative hypothesis ($\mu_a \neq \mu_b$) was accepted.

B. Comparison of Gracilis Muscle Vascular Responses to Local Hypotension Produced During Normovolemia with Responses Obtained During Hypovolemia

Gracilis artery pressures, muscle blood flows and vascular resistances obtained at the animals' prevailing systemic arterial pressure either during normovolemia or hypovolemia were used as control values to normalize the data obtained in response to graded local hypotension to percent of control. To determine whether hemorrhage influenced gracilis muscle vascular responses to local hypotension, separate least square linear regressions were performed on the normovolemic and hypovolemic percent of

control flow and resistance data, as functions of percent of control gracilis artery pressure. Only data obtained over the autoregulatory range of perfusion pressures (70-120 mm Hg) were used for the regressions and the analysis was performed on the Control Data Corporation 6500 Digital Computer using the MSU STAT LS program. The slopes of the regression equations for flow and resistance obtained during normovolemia or hypovolemia were compared by an analysis of covariance. In an analysis of covariance the features of regression and analysis variance are combined to provide a test statistic. Briefly, the procedure requires that a regression of pooled normovolemic and hypovolemic data be performed and the slope of this pooled regression compared to the normovolemic or hypovolemic regression line slope using the F statistic. If the calculated F value exceeds the critical F value ($F, 0.05, \upsilon_1, \upsilon_2$) from an F distribution table, the null hypothesis ($Slope_{normovolemic} = Slope_{hypovolemic}$) was rejected and the slopes were considered to be significantly different.

II. Series II and III

The statistical methods described for Series I (F max test, analysis of variance, Student-Newman-Kuels procedure) were used to determine whether alterations in gracilis artery perfusion pressure during either normovolemia,

hypovolemia (Series II), pulsatile or non-pulsatile perfusion (Series III) produced significant changes in gracilis muscle blood flow and vascular resistance. As described for Series I experiments, separate least square linear regressions were performed on the percent of control flow and resistance data obtained in response to graded local hypotension as functions of percent of control gracilis artery pressure during either normovolemia, hypovolemia, pulsatile, or non-pulsatile perfusion. Only data obtained below 140 mm Hg gracilis artery pressure were used for the regressions and as described for Series I, analysis of covariance was used to determine whether hypovolemia or non-pulsatile perfusion significantly altered gracilis muscle vascular responses to local hypotension compared to their respective controls.

A standard paired difference test was used to determine whether blood flows and vascular resistances during local hypotension were significantly different in normovolemic vs. hypovolemic periods (Series II) and in pulsatile vs. non-pulsatile perfusion (Series III) at corresponding gracilis artery pressures. The test statistic (t_s) for each comparison was:

$$t_s = \frac{\bar{d}}{s_d/\sqrt{n}}$$

where:

$\bar{d}$ = mean difference between control and experimentsl values (i.e., mean pulsatile control resistance minus mean non-pulsatile resistance at gracilis artery pressure = 100 mm Hg).

S_d = standard deviation of the difference between the control and experimental mean.

n = number of observations.

The test statistic was compared with critical values ($t_{0.05}$, υ) obtained from a Students t distribution table. If t_s exceeded $t_{0.05}$, υ, the null hypothesis ($\mu_{\bar{d}} = 0$) was rejected and the alternative hypothesis ($\mu_{\bar{d}} \neq 0$) was accepted. The paired difference test was also used to evaluate: 1) whether hemorrhage produced significantly different transient vascular responses to a step change in perfusion pressure compared to control (Series II); and 2) whether elevation of mean vascular distending pressure produced significantly different vascular responses during pulsatile vs. non-pulsatile perfusion (Series III). In these studies a significance level of 0.05 was used for all comparisons.

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