LOCAL CONTROL OF BLOOD FLOW IN CANINE SKELETAL MUSCLE:

I. VASOACTIVE METABOLITES IN LYMPH FROM EXERCISING MUSCLE

II. EFFECTS OF VASCULAR TRANSMURAL PRESSURE CHANGES IN AN ISOLATED MUSCLE

By

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ABSTRACT

LOCAL CONTROL OF BLOOD FLOW IN CANINE SKELETAL MUSCLE:

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Contraction of skeletal muscle is associated with a decrease in vascular resistance which is seen in the intact or naturally perfused organ as an increase in blood flow. This increase in blood flow is termed active (functional, exercise or contraction) hyperemia and can be quite large (up to 8-10 times resting flow) depending on the severity of the work load. Bioassay and chemical analysis of the venous effluent from contracting muscles have provided strong evidence that metabolically linked chemicals play a role in active hyperemia. It is thought that these vasodilator agents accumulate in the interstitium where they cause relaxation of the resistance vessels. Among the agents proposed are inorganic phosphate, potassium and hyperosmolality. If these agents are involved in active hyperemia, their interstitial fluid concentrations would help to define their importance. One purpose of the present studies was

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to attempt to measure the interstitial fluid concentrations of inorganic phosphate, potassium and osmolality.

Intramuscular pressure increases during muscle contraction. This may compress blood vessels and trigger a relaxation of vascular smooth muscle. Thus, the myogenic response could also play a role in active hyperemia. This hypothesis is attractive since it is known that smooth muscle responds to a decrease in stretch with relaxation, and since vasoactive metabolites singly or in combination do not produce the full sequence of events seen in active hyperemia.
A second purpose of the present studies was to attempt to isolate the contribution (if any) of the myogenic response to active hyperemia.

Since it is not possible to collect interstitial fluid, it was decided to analyze muscle lymph obtained at rest and during contraction. The dog hindlimb was used and lymph was collected from the femoral lymphatic. Lymph from the paw and skin was excluded by an occlusive clamp on the limb immediately central to the popliteal lymph node. Lymph obtained during exercise showed a small increase in potassium and inorganic phosphate, but no change in osmolality. Venous blood samples showed a rise in potassium and osmolality, but not in inorganic phosphate, with muscle contraction. Exogenous inorganic phosphate proved to be quite vasoinactive. These studies show that inorganic phosphate

does not play a role in active hyperemia of canine skeletal muscle. The results do not give additional support to nor do they detract from the proposal that potassium and osmolality are involved in active hyperemia.

In an attempt to evaluate the effects of vascular transmural pressure changes, per se, on vascular reactivity, an in vitro muscle perfusion system was designed. This allowed uniform changes in extravascular pressure, both pulsatile and non-pulsatile, to be made. The excised gracilis muscle suspended at its in situ length within the muscle chamber was used. Intramuscular pressure measured electronically increased during muscle contraction. Passive increases in intramuscular pressure within the range seen with contraction did not appear to elicit myogenically induced vasodilations. A decrease in blood flow during the passive extravascular pressure increase was followed by a reactive hyperemia upon termination of the pressure change. These results indicate only a very minimal myogenic component to active hyperemia.

The data obtained in these studies indicate that (i) inorganic phosphate is not one of the metabolites that play a role in active hyperemia of canine skeletal muscle, and (ii) myogenic relaxation of vascular smooth muscle due to a decrease in vascular transmural pressure is not an important mechanism in active hyperemia in canine skeletal muscle.

DEDICATION

To those whose love and loyal support

made this endeavor worthwhile.

My wife, Carol,

My children, Michael, David and Annette

and

My parents, Mr. and Mrs. Gerald Bacchus.

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I. INTRODUCTION

The local control of blood flow refers to those mechanisms which operate within a specified vascular bed to regulate its blood supply. This excludes the influence of extrinsic nerve activity, and vasoactive substances in the inflowing blood. The investigations in this area have been directed at discovering what is controlled, how the control mechanisms operate, and how local regulation of blood flow contributes to the physiologic adaptations of the intact animal.

The arterioles, metarterioles and precapillary sphincters are the sites in the microcirculation where local control is effected (83,104,134). The smooth muscle component of these vascular structures, then, is the target of any factors that would play a role in regulating blood flow to an organ or tissue. It is currently believed that by adjustments of the contractile state of the smooth muscle of arterioles, metarterioles and precapillary sphincters, the caliber of these vessels are controlled, the vascular resistance of the organ or tissue is changed, and the supply of blood is regulated.

It is also believed that although all three elements of the micro-circulation contribute to the control of resistance

to flow, arterioles may function more in the control of flow to the whole vascular bed, metarterioles may be more responsible for local pressure and volume flow, while precapillary sphincters may control surface area for exchange with the interstitium (173).

In the intact animal, the minute to minute regulation of blood supply to an organ is the result of the interaction of the local control mechanisms, with remote control mechanisms such as nerve fibers and blood-borne vasoactive agents. These two systems influence the same effector unit (the vascular smooth muscle), and sometimes when they become opposed the local mechanisms over-ride the remote (37,118).

At least four distinct types of local control phenomenon have been observed in various organs or vascular beds (81,104). These are, autoregulation, active hyperemia, reactive hyperemia and the venous-arteriolar response. Autoregulation is defined as the ability to maintain a constant blood flow following a change in perfusion pressure over a certain limited range. Active hyperemia (also referred to as functional hyperemia) refers to the increase in blood flow accompanying increased metabolic activity. Reactive hyperemia is the increase in blood flow above control that is seen on relief of ischemia. The venous-arteriolar response refers to the resistance increase seen upstream, following an elevation of venous pressure.

Autoregulation has been observed in the kidney, heart, cerebral, intestine and skeletal muscle vascular beds. In the kidney the autoregulatory response is prominent and consistently observed between 70-180 mmHg. Skeletal muscle exhibits a weak and sometimes inconsistently autoregulatory response. Active hyperemia is seen in all organs in which metabolic activity can be appreciably increased (heart and skeletal muscle contraction, secretory activity by glands, transport activity in organs such as the kidney and GI tract). Blood flow seems to be regulated in order to maintain a constant flow to metabolism ratio (79). Reactive hyperemia is seen in most systemic vascular beds being most prominent in the cardiac muscle, skeletal muscle, cerebral, intestinal and renal vasculature. The circulation of the skin and lungs do not show reactive hyperemia to any great extent. The venous-arteriolar response is seen in the vasculature of the intestines, and also skeletal muscle.

Over the years, several theories have been proposed to explain local regulatory phenomena. These hypotheses have tried to take into account all the mechanical, neural, and chemical factors that may influence the local vasculature. It now appears unlikely that the same mechanism can be responsible for all types of local regulation in all organs, or even that the same mechanism is responsible for a particular type of local regulation in different systemic organs.

Consequently, it is beyond the scope of this literature review to cover all the theories that have evolved to explain local regulation of blood flow. Rather, only those hypotheses that are relevant to local regulation of blood flow in skeletal muscle will be discussed. Emphasis will be given to control mechanisms in active hyperemia.

Perhaps the oldest and most attractive theory to explain local regulatory phenomenon is the so-called "metabolic hypotheses" (21,79,81,82) which proposes that when tissue metabolism or blood flow changes, the concentration of certain vasoactive chemicals is altered and the vascular smooth muscle tone is adjusted appropriately to meet the new requirements of the tissue. This hypothesis has been used to explain autoregulation, reactive hyperemia and active hyperemia. For example, when perfusion pressure is suddenly increased to an organ or vascular bed there is a transient increase in blood flow which acts to lower the interstitial concentration of vasodilator metabolites (wash-out), and hence vascular resistance increases (autoregulation). A fall in perfusion pressure would produce a reverse effect. By the same token an increase in tissue metabolism would also lead to an increased interstitial concentration of dilator metabolites (via increased production), and vasodilation (active hyperemia).

The myogenic hypothesis holds that since smooth muscle reacts to stretch by contraction, and to reduction of

stretch by relaxation, any change that would affect the vascular transmural pressure would elicit a related myogenic response (18,54,107,126). Thus, it has been proposed that the myogenic or Bayliss response (so named because it was first described by Bayliss) plays a role in autoregulation, reactive hyperemia, the venous-arteriolar response and active hyperemia (11,18,32,53,107,126). In autoregulation, an increase or a decrease in the vascular transmural pressure would elicit a myogenically induced resistance change in the same direction. In arterial occlusion the fall in the intravascular pressure, and hence in vascular transmural pressure, would trigger a myogenic relaxation of the smooth muscle of the resistance vessels. Also, during contraction of skeletal muscle intramuscular pressure is elevated, thereby causing a fall in transmural pressure which would elicit vascular smooth muscle relaxation.

The tissue pressure hypothesis based on a so-called capillaron model (19,154,155) holds that changes in tissue pressure affect capillary and venous diameter. According to this theory, capillaries and veins are compressed and vascular conductance is decreased when tissue pressure increases. When tissue pressure falls, capillary and venous diameter increase and vascular conductance increases. In this model, the control of tissue perfusion is by strictly passive factors. For example, the contraction of muscle during

exercise supposedly expresses volume from the extravascular compartment, thereby decreasing tissue pressure and increasing vascular conductance. On termination of exercise, extravascular volume and pressure return to their precontraction levels as does vascular conductance. A similar explanation would apply to reactive hyperemia. During autoregulation, elevation of perfusion pressure causes a net transfer of intravascular volume to the extravascular space. This would increase tissue pressure with a resultant compression of capillaries and small veins. Consequently, vascular conductance would be decreased out of proportion to the new pressure gradient for flow. Although the capillaron model shows changes resembling an autoregulatory response (19), other investigators have shown that the resistance changes in autoregulation occur at the precapillary resistance vessels rather than at the capillary and postcapillary vessels as is called for by the tissue pressure hypothesis (76,76, 84,134,143). Consequently, this theory has fallen out of vogue. Moreover, in exercise tissue volume increases (83).

Endogenous free histamine has been suggested as an intrinsic regulator of blood flow (160). The evidence is based on the recovery from vascular tissue of an inducible form of histidine decarboxylase. A wide variety of nontraumatic stimuli that trigger blood flow changes has been shown to increase the activity of this enzyme. However,

since antihistamines do not attenuate the blood flow changes seen during active and reactive hyperemia (79), and since capillary permeability does not change during exercise while injected histamine increases capillary permeability (6,83), it is believed that histamine is not involved in local control of blood flow in skeletal muscle.

A more recent hypothesis to explain active hyperemia in skeletal muscle has been advanced by Honig and colleagues (94-96). With the use of histochemical staining techniques and various pharmacologic agents, they have provided evidence for the existence of neurons in the walls of the arterioles of skeletal muscle. They postulate that these ganglion cells are sensitive to mechanical stimuli, and are responsible for post-contraction as well as sympathetic vasodilation. With the use of graded work load, bioassay techniques and local anesthetics, they have shown that these intrinsic neurons could be responsible for the entire post-contraction hyperemia at mild work loads (1 twitch or brief trains of phasic contractions), and could account for the initiation of the hyperemia at greater work load. In this schema it seems that little importance is given to the myogenic response. Metabolites are considered to contribute to the hyperemia of moderate and severe work (twitches at 2/sec. and greater for more than 1 minute) by helping to maintain the hyperemia during work and during the recovery from work.

The chemical mediator of these neurons is not an already known neurotransmitter (96). The presence of these arteriolar neurons needs confirmation by other techniques (such as electrophysiologic recordings).

The bulk of the current experimental evidence lend support to the metabolic and myogenic theories of local regulation of blood flow (21,77,79,80,82,103,105,134), especially in skeletal muscle. However, the degree of participation of these two mechanisms to the various local regulatory responses is unknown. In regards to the metabolic hypothesis, many metabolically linked chemicals have been suggested for roles in local control of blood flow (21,79,82,87,153,161, 163-166). Interest in the various candidates or mediators of the responses have waxed and waned as experimental evidence for or against their candidacy has been uncovered. For example, there has been no experimental support for important roles for the catecholamines, acetycholine, histamine, serotonin or bradykinin (79,161). Likewise, to date the Krebs cycle intermediates have not been shown to play prominent roles in local vascular control although some have been shown to affect vascular resistance, or to increase in the muscle tissue during some types of local control phenomenon (58,68,140). In fact, the products of intermediary metabolism are only considered as they may contribute to the osmolality of the interstitium (79).

The vasoactive agents that are currently studied in respect to their roles in local regulation of skeletal muscle blood flow are: the potassium ion, oxygen, the hydrogen ion, adenosine and adenine nucleotides, osmolality, inorganic phosphate, and the prostoglandins of the E series (79,82,112). A brief overview of the experimental evidence for or against the candidacy of these vasoactive substances will be presented here. A more detailed examination of the experimental data in the case of potassium, inorganic phosphate and osmolality (along with a detailed review of the myogenic hypothesis) will be presented in the literature survey.

The Metabolic Hypothesis

Bioassay studies, chemical analysis of venous blood, and estimation of the interstitial fluid concentration in a few instances, have provided strong evidence for the participation of metabolites in local blood flow control (21,77, 79-82,108,109,161-166). This is especially true in the case of exercise hyperemia in skeletal muscle. In a bioassay system where the venous blood from one muscle (the donor or regulatory organ) is diverted into the arterial inflow of a recipient or bioassay organ it can be shown that: (i) venous blood from resting muscle is vasodilatory with respect to arterial blood and (ii) venous blood from the exercising donor muscle has increased vasodilatory properties as

compared to venous blood from the donor at rest (80,176). Also, during exercise venous PO_2 decreases markedly. Such evidence strongly indicates that some substance(s) produced in the active organ is produced in sufficient quantities to affect resistance within the donor organ, and is stable enough to enter the interstitium of the recipient organ and affect resistance there. Recently, additional support has been given to this hypothesis by the finding that the recovery times from exercise and arterial occlusion in skeletal muscle are longer at constant flow than at natural flow (149). This strengthens the conclusion that there is a build-up of vasodilator substances within the active tissue, and prevention of their dispersion prolongs their action.

For a chemical to be considered as an important causal factor in local blood flow control, it must satisfy all of the following criteria (2,79,134). (i) It should be a substance naturally present within the particular tissue and it should be possible to detect it or its breakdown products in the tissue or venous effluent. Its concentration should relate to its vasodilator activity. (ii) When the substance is introduced intra-arterially into a resting organ in amounts similar to that which may be produced within a given vascular bed, it should produce a response similar to its proposed action. (iii) It should not disrupt any normal functions of the organ to which it is administered.

(iv) Its mode of action should be compatible with the established electrical and mechanical properties of the tissue component upon which it acts. (v) The time course of its appearance and or disappearance should correlate with the time course of the local regulatory response.

Vasoactive Agents

Oxygen

Decreases in the venous blood oxygen content, oxygen saturation and oxygen tension have been shown to occur during active hyperemia, reactive hyperemia and autoregulation (seen with reduced perfusion pressure) in skeletal muscle (16,46, 73,80,109,120,132,156,157,164,167). Local hypoxemia was shown to have a vasodilatory effect on the skin and muscle vasculature of the dog forelimb (39,162). But the hypoxemia has to be severe and exercise dilation can occur even if the change in oxygen tension is prevented (164). More recently, it has been shown that correction of the oxygen tension alone will not abolish the vasodilator effect of venous blood from an exercising muscle (176). Both pH and PO_2 must be corrected.

The mechanism by which oxygen lack produces its effect is a matter of controversy. Some investigators advocate a direct effect of oxygen on the vascular smooth muscle tone (33,43,73,156). Others believe that the decrease in oxygen supply prior to reactive and active hyperemia, for example,

causes a change in tissue metabolism, and some subsequent metabolite elicits the vasodilator effect on the vascular smooth muscle (21,45,80,164).

Hydrogen

Hydrogen ion concentration in the tissue fluid and venous blood increases during active and reactive hyperemia in skeletal muscle (25,67,120,164). That the hydrogen ion is locally vasoactive is shown by studies where intra-arterial infusion or topical application of acid, causes a fall in vascular resistance (42,50,140). The vasodilator effect of increased CO_2 tension to a local vascular bed is probably mediated by an increased intracellular hydrogen ion concentration (123). Although changes in hydrogen ion concentration do occur during active and reactive hyperemia, its role may be minor because (i) the changes do not correlate with the magnitude of the hyperemia (38,170), (ii) the hyperemia of exercise is established before an increased hydrogen ion concentration can be detected (64), (iii) exercise hyperemia can occur in cases where no decrease in pH is possible (67, 79) and (iv) correction of the pH of venous blood from exercising muscle does not abolish its vasodilator effect--PO_2 must also be corrected (176).

Adenine Nucleotides and Adenosine

Adenosine, AMP, ADP and ATP are vasodilators in most vascular beds (22,82). In 1963, Berne produced evidence

that adenosine is involved in the local control of coronary blood flow (20). Since then, many studies have established a role for adenosine in both reactive and active hyperemia in the coronary vascular bed (22,158), and more recently in active hyperemia of skeletal muscle (26,27,34,44). Also, the enzyme for adenosine production has been shown to be present in skeletal muscle (158).

The venous blood from contracting skeletal muscle has been reported to show increased concentrations of ATP (56), ATP and AMP (34) and adenosine (44). Bioassay studies of the femoral venous blood in dogs have shown evidence for the presence of AMP and/or adenosine during exercise hyperemia (166). Recent studies measuring adenosine levels of muscle tissue samples have demonstrated increased adenosine content in muscle from the dog hindlimb muscles after 10 minutes of contractions (27). Thus adenosine may contribute to active hyperemia in skeletal muscle, but the exercise has to be severe before it is measurable in the venous blood (27,44,177). Its role as a vasodilator is subsequent to a decrease in oxygen tension within the tissue (22). A possible mechanism of action on the smooth muscle cell is by blocking the cellular uptake of calcium, thus causing relaxation of the smooth muscle (23).

Potassium

The potassium ion has been shown to increase in the venous effluent from skeletal muscle during exercise

(9,27,116,138,153,164,178), but not during reactive hyperemia (160,164). When potassium salts are infused into the arterial supply of resting muscles to give a final venous plasma concentration of less than 10 mEq/L, vascular resistance falls (41,57,66,116,125,163,164,167). However, the decrease in resistance is much less than that seen during exercise, for an equivalent increase in venous plasma potassium. The source of the increased potassium during exercise appears to be the skeletal muscle cell as a consequence of depolarization (11,113,164). Increased potassium ions in the interstitium can stimulate the sodium-potassium pump in the membrane of the vascular smooth muscle cell causing a hyperpolarization and subsequent relaxation (3,4,24,35,145). Because the peak of the increase in venous plasma potassium concentration occurs at approximately 10 seconds after the start of muscle contraction (164,165), and the potassium concentration wanes while the increase in blood flow does not decrease during maintained muscle stimulation (153), it has been suggested that the potassium ion may play an important role in the initiation but not in the maintenance of active hyperemia.

Osmolality

The osmolality of the venous plasma from exercising skeletal muscle increases shortly after the initiation of muscle contraction (127-129,135,142,164,165). However, when

the osmolality of the arterial inflow is raised by the same amount as the increase seen with exercise, the fall in resistance is considerably less (164,165). Also, the rise in osmolality reaches its peak in about one minute while the hyperemia is established in 10-20 seconds, and osmolality may return to control levels while the hyperemia is maintained (142,153,164,165).

From these observation, hyperosmolality in the interstitium is thought to add to the hyperemia of exercise shortly after the hyperemia is established, but not to play a major role in either the initiation or the maintenance of the hyperemia (79,81,82).

Inorganic Phosphate

The major support for inorganic phosphate as a mediator of exercise hyperemia comes from Dr. Hilton's laboratory where comparisons of fast and slow muscles of the cat hindlimb show that inorganic phosphate is increased in fast but not slow muscles during exercise (88-92). This observation is considered to be of major importance since the slow muscle studied (the soleus) shows only a small increase in flow during nerve stimulation (89,91). Other investigators have observed small increases in venous plasma inorganic phosphate from contracting muscle (15,26,27,44,178), but none have confirmed the vasodilatory action of phosphate claimed by Hilton and colleagues. Thus the role of inorganic phosphate in exercise hyperemia is still questioned.

Prostoglandins

The prostoglandins (PG) of the A and E series have been shown to be vasodilatory in the canine forelimb and hindlimb (38,70). Endogenous prostoglandins have been proposed to play a role in active and reactive hyperemia in skeletal muscle (112,137). This hypothesis is based on the use of the prostoglandin synthesis inhibitors, indomethacin and eicosatetraynoic acid, and on bioassay of a venous plasma fraction obtained by column separation and extraction procedures.

Messina et al. (137) have shown that the increase in arteriolar diameter (measured by in vivo microscopy of rat cremaster muscle) following 15-60 seconds of occlusion was attenuated by infusion of PG inhibitors. Also, the duration of the response was less. PG inhibitors did not affect arteriolar diameter in the resting muscle preparation.

Kilbom and Wennmalm (112) have shown that indomethacin reduces the hyperemia following arterial occlusion or contraction of human forearm. There was no effect on the resting muscle. Also, a venous plasma fraction was extracted by Amberlite column separation and ethanol elution. This plasma fraction had PGE-like action and was assayed by its effect on isolated rat stomach strips. There was greater PGE-like activity from plasma obtained during reactive hyperemia than from plasma obtained during exercise hyperemia.

These studies indicate that PG's do not contribute to the basal tone of the resistance vessels in skeletal muscle but may be involved in ischemic dilation and to a small extent in exercise hyperemia. However, the production of PG's in reactive and exercise dilation may not be related to the accompanying hypoxia or hypercapnea, as Kientz et al. (111) have shown that indomethacin does not affect the dilation due to locally produced hypoxia and hypercapnea.

The Myogenic Hypothesis

The myogenic response (Bayliss effect) refers to the property of smooth muscle that causes it to contract in response to stretch, and relax in response to a decrease in stretch. The smooth muscle of the resistance vessels has been shown to possess basal electrical activity capable of cell to cell propagation (28,53,59). Variations in the vascular transmural pressure can serve as a stimulus for this myogenic activity, and the myogenic response can participate in local regulation of blood flow.

In skeletal muscle, the myogenic response may contribute to autoregulation, reactive hyperemia and active hyperemia. An increase or a decrease in perfusion pressure can trigger a constriction or a relaxation, respectively, of vascular smooth muscle, giving rise to the observed autoregulatory changes in vascular resistance. With arterial occlusion,

intravascular pressure falls. This can trigger a myogenic relaxation of the vascular smooth muscle which would contribute to the subsequent reactive hyperemia. During muscle contraction, intramuscular pressure can compress the blood vessels. This can reduce the vascular transmural pressure, and cause a myogenic relaxation of the vascular smooth muscle.

A general survey of the literature reveals that no one mechanism can account for local control of blood flow in skeletal muscle. In reference to the metabolic hypothesis, no known metabolite can produce the effects seen during the various types of local control, neither can any one metabolite account for the complete sequence of events seen in one type of local control. Also, quantitative measurements of the interstitial fluid concentration of the various metabolites are lacking. In regards to the myogenic hypothesis, it is difficult to separate the effects of transmural pressure changes from other effects-transient changes in flow or metabolite concentration, for example.

The aims of the present work were two-fold. Firstly, we wanted to see whether prenodal lymph from muscle may reflect any changes in metabolite concentration as may occur in the interstitium during muscle contraction. Since the quantity of lymph which can be collected from muscle is small, we decided to focus this study on potassium, osmolality and inorganic phosphate.

Secondly, we attempted to isolate the contribution of the myogenic response to the hyperemia of moderate exercise. To do this, we designed a special muscle chamber (described in detail in Methods section) which enabled us to change pressure around an in vitro muscle.

II. LITERATURE SURVEY

The first suggestion that metabolites produced within a vascular bed could play a role in the regulation of its blood flow, was made by Gaskell in 1880 (62). At that time, the accepted explanation for all circulatory changes was nervous control via chemical mediators. In fact, when Gaskell in 1877 made one of the first quantitative descriptions of the changes in flow accompanying muscle contraction, he explained his findings by proposing that vasodilator nerves were stimulated along with the motor nerves (61). His metabolic theory was proposed after he observed, in frog heart and frog muscle preparations, that topically applied acids dilate, and alkali constrict blood vessels (62).

Bayliss laid the foundation for the myogenic theory when, in 1902, he observed that a brief (8-10 sec.) reduction in the perfusion pressure of the hindlimb of cats, rabbits and dogs resulted in a subsequent volume increase above control. A brief elevation in perfusion pressure resulted in a subsequent decrease in volume below the control level (18). From these studies, and from his observations on excised carotid arteries, Bayliss concluded that the muscular coat of the arterial vessels react like other smooth muscle by contracting in response to a stretching

force, and relaxing in response to a decreased tension. Thus, he concluded that the local vascular response observed in an intact organ to alterations in perfusion pressure, was due to a myogenic response. Anrep (5) challenged the conclusions of Bayliss and pointed out that the time course of the observed changes was sufficient for local accumulation or washout of vasodilator metabolites, and for an adrenal medullary response.

Since the postulation of these two theories, much work has been done to determine the relative importance of each in the various types of local regulation, and to more clearly define the mechanisms by which metabolites and myogenic factors adjust the tone of the smooth muscle of the resistance vessels. The purpose of this literature review is to critically examine the experimental evidence for the participation of inorganic phosphate, hyperosmolality, the potassium ion and the myogenic response in local regulation of blood flow in skeletal muscle, with particular emphasis on the role of these factors in active hyperemia.

Inorganic Phosphate

Evidence for the Involvement of Inorganic Phosphate (Pi) as a Vasodilator Metabolite in Active Hyperemia

In 1969, Hilton and Vrbova (88) proposed that inorganic phosphate is the chief mediator of functional vasodilation

in skeletal muscle. This conclusion came from comparisons of blood flow and venous plasma inorganic phosphate in the soleus (a slow muscle) and the gastrocnemius (a fast muscle) of the cat. They found that the soleus exhibited little or no exercise hyperemia and showed no increase in venous plasma Pi. On the other hand, the gastrocnemius showed excellent exercise hyperemia (4-5 fold increase inflow), and an increase in venous plasma Pi that was proportional to the contraction frequency. They reasoned that in a muscle with a high oxidative metabolism (slow muscle = red muscle = tonic muscle), Pi does not accumulate because the oxygen supply (from high blood flow and large myoglobin stores) is sufficient to meet the demand of aerobic metabolism (55,88,89). In the muscle with low oxidative metabolism (fast muscle = white muscle = phasic muscle), Pi does accumulate because of anaerobic metabolism. Thus it seemed that Pi was associated with active hyperemia, and lack of Pi with lack of active hyperemia.

Several other investigators have superfically looked at a possible role for inorganic phosphate in active hyperemia, but the evidence has been mostly negative. Barcroft et al. (15) observed an increase of 20% in the inorganic phosphate concentration of the venous plasma from the exercising human forearm. Chou et al. (36) reported that venous plasma Pi concentration was increased after 20 minutes of ischemia,

and also after 15 minutes of tetany but not after 5 minutes of moderate (6/sec) exercise. Dobson et al. (44) have shown that Pi in the bath containing a frog sartorius muscle increased by the same amount when the muscle was at rest, as it did when contracting. More recently, Bockman et al. (27) showed in the dog calf muscle that although venous plasma Pi increased by 0.2 millimoles/L after 2 minutes of contraction (4/sec), the arterial plasma Pi had also increased, so that the arterio-venous difference after contraction was the same as control.

Vasoactivity of Exogenous Inorganic Phosphate

When Hilton infused solutions of inorganic phosphate into the arterial supply of both slow and fast muscles, they showed vasodilation in proportion to the concentration of Pi (91). The monobasic phosphate was found to be a more potent vasodilator than the dibasic. In another study, it was reported that inorganic phosphate solutions topically applied to the rat spinotrapezius muscle preparation caused an increase in arteriolar diameter (130).

This vasodilatory action of Pi has not been confirmed by other investigators. Barcroft et al. increased venous plasma Pi concentration to 400% of normal and saw no effects on muscle resistance (15). Similar results were reported by Overbeck et al. (147) and Dobson et al. (44).

Mechanism of Action of Inorganic Phosphate

There are as yet no definitive studies of the mechanisms by which Pi may cause relaxation of vascular smooth muscle. However, Hilton has suggested that a possible mechanism could be by removal of calcium at the cell membrane binding sites (91).

Hilton postulated that inorganic phosphate does not cross the capillary membrane very readily (92). In this case, the interstitial fluid levels of Pi would remain increased over a long period during muscle contraction, and be much higher than the venous plasma levels. Thus we decided to collect lymph from contracting muscle to test Hilton's hypothesis.

The marked vasodilatory effect of Pi infusions reported by Hilton could be related to simultaneous changes in pH and osmolality. Venous blood pH fell to below 7.0 in some instances, and venous plasma osmolality was increased by 10-15 mOsm/kg. In fact, it has been reported that equivalent changes in pH alone can cause vasodilation. Kontos et al. (122) used sodium monobasic phosphate as the source of H^+ in a study on the effects of acidosis on the human forearm vascular bed. They found that the magnitude and time course of the vasodilation was the same for a given change in pH, whether phosphate or CO_2 was the cause of the acidosis. These considerations led us to test the

vasoactivity of inorganic phosphate (the monobasic phosphate) in both unbuffered (pH 4.5) and buffered (with sodium bicarbonate at pH 6.8) solutions.

Interstitial Hyperosmolality

Evidence for the Involvement of Tissue Hyperosmolality in Exercise Hyperemia in Skeletal Muscle

In 1967, Mellander et al. (133) reported that the osmolality of the venous plasma from exercising leg muscles of cats, increased by up to 40 mOsm/kg in proportion to the rate of stimulation. There was no concomitant increase in arterial plasma osmolality. In these studies, the increase in effluent plasma osmolality seemed to be maintained for up to 15 minutes of exercise, although maxium values were reached in 2-5 minutes. Other laboratories have confirmed the rise in osmolality of the venous plasma from exercising muscle, but the magnitude and time course of the changes are different. Scott et al. (164) observed a 9 mOsm/kg increase in the venous plasma from the exercising dog gracilis muscle at constant pressure perfusion. There was no increase by the 10th second of exercise and the rise which was seen after 1 minute had disappeared by the 5th minute. At constant flow perfusion, the magnitude of the increase was greater (as would be expected) but the tendency of the increase to wane towards the control value was still apparent.

These studies also showed that there were no osmolality changes associated with reactive hyperemia. In a similar preparation, Radawski et al. (153) found that the arterio-venous difference in plasma osmolality is not maintained during prolonged exercise. Murray et al. (142) observed an increase of 17 mOsm/kg in the osmolality of the venous plasma from the contracting calf muscles of the dog. This increase occurred by the 2nd minute of exercise and disappeared completely by the 55th minute while resistance was still decreased. In fact, venous plasma osmolality during the recovery period was below the control value, yet resistance was still decreased.

These findings, and similar results in other laboratories using dog skeletal muscle (27,178), and forearm and leg muscles of man (127-129), indicate that hyperosmolality of the interstitium may play only a minor role in the hyperemia seen in contracting skeletal muscle. This role may be to add to the hyperemia shortly after its initiation. Other agents may initiate and maintain the hyperemia (77,79-82, 161-165). However, there may be species and muscle-type differences (135,165). In cats, the magnitude of the osmolality increase and the time course of its appearance are different from that found in dogs (133,164). In predominantly fast (white, phasic) muscle the increase in osmolality can be detected by 25-30 seconds after initiation of

contraction (129), whereas in a mixed muscle (predominantly slow fibers) the increase is not seen until 1 minute after contraction begins (164).

Effects of Locally Produced Hyperosmolality

It has been amply illustrated that local changes in plasma osmolality affect vascular resistance. These studies include intra-arterial infusions of hyperosmolal and hypo-osmolal solutions of many different agents while recording changes in vascular response (63,127,129,133,147,164,172). In other studies, arteriolar diameter changes have been measured by microscopy during topical application to, or suffusion of a vascular bed with hyperosmotic solutions (48, 69,130). In vitro vascular smooth muscle preparations also contribute data on this subject (101,133).

Overbeck et al. (147) demonstrated that hyperosmolality dilates and hypoosmolality constricts the small vessels of the dog forelimb. Mellander et al. (133), and Lundvall (129) have shown that hyperosmolality is vasodilatory in the cat leg muscles and in human forearm and leg muscles. In these studies, the resistance changes (in time course and magnitude) were similar during exercise and arterial infusions of hyperosmotic agents. This only holds, though, if the results are interpreted in the light of the fact that venous plasma osmolality would underestimate interstitial space osmolality during exercise, but over-estimate it during

arterial infusions. Scott et al. (164) and Scott and Radawski (165) have shown in the dog gracilis muscle, that exercise which produced a venous plasma osmolality increase of only 9 mOsm/kg was associated with about an 80% fall in resistance, whereas infusion of hyperosmotic NaCl to produce a venous plasma osmolality increase of 20 mOsm/kg was associated with only a 25% fall in resistance. Also, the vasodilatory effect of the hyperosmolality produced by intra-arterial infusion waned by the 6th minute of infusion.

Gray (69) and Marshall (130) have observed increases in arteriolar diameter when hyperosmotic solutions were topically applied to a rat spinotrapezius muscle preparation. The vasodilation seen with osmolality increases similar to that seen with exercise was not well maintained, and maximum dilation occurred only with increases of 50-400 mOsm/kg. In both these studies the observed effects were predominantly on the arterioles and not the venules. These observations, together with the data from exercise and hyperosmotic infusion studies, would support only a minor role for interstitial hyperosmolality in exercise hypereima. Hyperosmolality may add to the hyperemia shortly after its initiation, then the effect wears off.

Mechanism of Action of Interstitial Hyperosmolality

While a passive effect on blood vessels due to cellular dehydration of the vessel wall, increased transcapillary

reabsorption of water, shrinkage of blood cells, or release of a vasodilatory agent has not been ruled out as the mode of action for hyperosmolality, it has been shown that the response has an active component (63,133,135). Gazitua et al. (63) showed that local hyperosmolality produced with cell-free solutions still caused vasodilation. A maximally dilated (papaverine-induced) organ did not show any further vasodilation in response to hyperosmolality (63,135). In in vitro vascular strips, hyperosmolality caused a relaxation of mechanical tension and a decrease in electrical activity (133,135). From these observations, it has been proposed that hyperosmolality acts by hyperpolarizing the smooth muscle cell by affecting ionic gradients (63,129,133,135). It seems reasonable that hyperosmolality may contribute to the establishment of the hyperemia of exercise within the 1st minute, but that it has only a minor role in the maintenance of the response.

In summary, it seems that interstitial hyperosmolality may play only a very minor role in active hyperemia, because the venous plasma osmolality changes are small and of short duration. However, it is possible that the changes in interstitial osmolality may be much greater than the changes in venous plasma osmolality. This would be the case if osmotic particles in the interstitium cannot readily cross the capillary membrane as has been postulated for inorganic phosphate (92). Thus, measuring the osmolality of interstitial fluid

during muscle contraction could possibly extend the role of tissue hyperosmolality as a factor in active hyperemia.

The Potassium Ion

Evidence for the Involvement of Potassium in Active Hyperemia

The bulk of the experimental evidence to date suggest that the potassium ion is involved in exercise hyperemia, but not in reactive hyperemia nor autoregulation in skeletal muscle. The evidence comes mainly from analysis of venous plasma obtained during local regulatory maneuvers. Baetjer (10) reported a 21% increase in potassium levels in the femoral venous blood of the cat hindlimb following clamping of the abdominal aorta for 5-23 minutes. In this study, there was no evidence that the potassium came from skeletal muscle. In more relevant studies, Rudko and Haddy (159), and Scott et al. (164), showed that in the dog hindlimb there was no increase in venous plasma potassium following complete occlusion of the femoral artery for up to 5 minutes. Although there are no data on venous plasma potassium levels during autoregulation in skeletal muscle, it seems quite unlikely that potassium is involved (161).

There is abundant evidence for the involvement of the potassium ion in exercise hyperemia in skeletal muscle (77, 79,81,82,161,163,164). Baetjer (9) was the first to demonstrate that venous plasma from contracting muscle contained

increased amounts of potassium. Kjellmer (116) found that increasing the frequency of contraction in the cat calf muscles increased the amounts of potassium recovered in the venous plasma, and at maximum exercise dilation (constant flow) potassium concentration was doubled. The fall in resistance correlated well with the rise in plasma potassium levels. Also, when KCl was infused to produce venous plasma potassium levels similar to those seen during exercise, 25 to 65% of the change in resistance seen with exercise was obtained.

Scott et al. (164), Scott and Radawski (165), and Radawski et al. (153) followed the time course of the potassium increase in the venous plasma from exercising skeletal muscle. The isolated gracilis muscle and isolated hindlimb of dogs were used. These studies showed that venous plasma potassium rose significantly after 10 seconds of electrical stimulation of the nerve to the isolated organ, and then gradually returned towards control levels. However, the arterio-venous difference at the end of 120 minutes of contraction was still significantly different from control. Since the rise in venous potassium levels preceded the fall in resistance, the potassium ion is thought to be importantly involved in the initiation of exercise hyperemia. Studies by Tominaga et al. (178) also support this view. They measured PO_2, PCO_2, potassium, osmolality, pH, inorganic

phosphate, and adenosine changes in the venous plasma from the exercising canine hindlimb and found that the resistance changes correlated best with the potassium changes. Mohrman and Sparks (138) have shown that increased amounts of potassium can be found in venous plasma from skeletal muscle following a 1 second tetany (32 pulses per second), and have calculated that the release of potassium into the interstitium is rapid and large enough to account for the resistance changes.

The skeletal muscle cells have been shown to be the source of the potassium. Scott et al. (164) perfused an isolated muscle with homologons plasma or a salts-supplemented cell-free dextran solution and still observed the rise in venous effluent potassium associated with exercise. Fenn (51), earlier, had shown that muscle tissue lost potassium in exchange for sodium during muscle contraction, while there were no changes in the blood potassium levels. In the human, Kilburn (113) demonstrated that treadmill exercise, which caused a 1.2 mEq/L increase in venous plasma potassium, did not affect plasma water volume or calculated RBC potassium levels. Release of potassium by the skeletal muscle fiber upon depolarization is accepted as the source of the increase seen with muscle contraction.

Evidence that the Potassium Ion is Vasoactive in Skeletal Muscle

Katz and Linder (110) first showed that local intra-arterial injections of potassium into a vascular bed produced a dual response. An increase of up to 50% above the control plasma potassium levels produced vasodilation, whereas greater increases produced vasoconstriction. In 1941, Dawes (41) demonstrated a similar response to injections of KCl. Since then, numerous studies have confirmed the vasodilatory action of the potassium ion with increases within the physiological range.

In 1959, Emanuel et al. (49) using the canine forelimb preparation showed a differential effect of KCl infusion on the large and small vessels of this vascular bed. Resistance across the small vessels fell in proportion to the increase in potassium, whereas resistance across the large vessels only increased at the high potassium levels. Total resistance fell at first and then increased at the higher potassium levels. This study also showed that increased potassium caused a decreased response to injected norepinephrine, and the effect was the same whether KCl or K_2HPO_4 was used as the source of potassium. Thus the effect of infusions of potassium salts seemed to be specifically the effect of the potassium ion, and its action on the vasculature seemed to be direct.

Other studies which demonstrated the vascular effects of the potassium ion, include those of Overbeck et al. (147) who recorded a 22% fall in resistance across the forelimb vascular bed when isotonic KCl was infused intra-arterially to raise the blood concentration of potassium by 1.4 mEq/L. Haddy et al. (74) then saw that reducing the potassium concentration in the arterial inflow causes vasoconstriction. Lowe and Thompson (125) infused KCl in the human brachial artery and measured the increase in blood flow by venous occlusion plethysmography. Flow increased in proportion to the increased potassium levels. Similar results were obtained by Glover et al. (66). Kjellmer (115,116) has shown that intra-arterial infusions of KCl or KNO_3 decrease resistance in the cat calf-muscles with maximum dilation reached with potassium levels of 15-20 mEq/L. Above this level vasoconstriction occurred. Chen et al. (35) lowered and raised potassium levels in the arterial blood of the canine gracilis muscle by dialyzing the blood against a modified Ringer solution. Hypokalemia produced a significant rise, and hyperkalemia produced a significant decrease in perfusion pressure at constant flow perfusion.

In vitro vascular strips, was well as in vivo microscopy, have been used to study the action of the potassium ion on vascular smooth muscle. Gellai and Detar (65) have shown that increasing the potassium concentration by 2-6 mM

in the bath containing a strip of small artery from the leg of the rabbit caused a decrease in tension of about 40%. But complete recovery occurred in 5 minutes, even though the increase in potassium was maintained. In a similar preparation, Johansson and Bohr (99) used small subsutaneous arteries from the dog's feet to show that increasing the potassium concentration in the bath caused a decrease in frequency of the phasic contractions of the smooth muscle. Marshall (130), using in vivo microscopy of the rat spinotrapezuis muscle, has shown that topically applied potassium solutions caused dilation of the arterial but not the venous side of the vascular bed. Maximal response was obtained with 9.5 mEq/L of potassium.

Since the potassium increase in the venous plasma during exercise wanes within a few minutes (164), since exogenous potassium is not a very good vasodilator and the vascular effects of hypokalemia and hyperkalemia are transient (29,65), and since ouabain pretreatment only delays the onset of exercise hyperemia without affecting the magnitude of the hyperemia (3,29,35), it is believed that the potassium ion is involved only in the initiation of active hyperemia in skeletal muscle.

Mechanism of Action of the Potassium Ion

An increase in the potassium concentration on the outside of the vascular smooth muscle cell, should result in

depolarization (and contraction), caused by a decreased passive outflux of potassium in the presence of an electroneutral sodium-potassium pump. This, however, does not agree with the observations that increased potassium causes vasodilation. If an electrogenic pump that is stimulated by an increase in extracellular potassium is assumed, then the observed effect of the changes in potassium concentration can be accounted for (3,29). The experimental evidence supports this concept, and it is presently accepted that an increase in potassium concentration in the interstitium causes hyperpolarization of the smooth muscle cell (relaxation of tension and hence vasodilation), while a decrease causes depolarization (contraction of smooth muscle and hence vasoconstriction). Gellai and Detar (65) have shown that the ratio of the potassium concentrations across the cell membrane is more important than the absolute concentration.

The major evidence for the above explanation comes from studies on the action of ouabain on the vascular response to hypokalemia and hyperkalemia. Chen et al. (35) observed that ouabain, a cardiac glycoside and well-known sodium-potassium ATP-ase inhibitor, reversed or eliminated the effects of hyper- and hypokalemia on the isolated dog gracilis muscle. Thus during ouabain block the membrane potential is controlled by passive ion fluxes and the vascular response

to potassium is opposite to that of an unblocked preparation. It is interesting to note that the effect of ouabain pretreatment on exercise dilation in skeletal muscle is to prolong both the onset of the dilation and the time to reach maximum response (35). This observation has been used to strengthen the proposal that potassium is involved mainly in the initiation of exercise hyperemia. Cell modeling experiments have demonstrated that data obtained from in vivo experiments are better explained by an electrogenic rather than an electroneutral sodium-potassium pump (3,29). In vitro arterial preparations have supported this concept. Nguyen-Duong et al. (145) showed that a reduction in the internal sodium concentration abolished the response to changes in external potassium. Also, they found that cesium and rubidium, which are known to activate the sodium-potassium pump, mimicked the effects of potassium.

Other mechanisms such as adrenergic receptor stimulation by cyclic-AMP accumulation, increased potassium conductance, and adrenergic antagonisms have been suggested but there are no data for their support (4,57,125,145).

In summary, there is quite a bit of experimental evidence to suggest a role for the potassium ion in active hyperemia of skeletal muscle. It is evident that contracting skeletal muscle immediately releases potassium into the interstitium. Judging from the rise in potassium concentration in effluent blood leaving exercising muscle, the

interstitial plasma concentration reaches vasodilatory levels. Thus, one must conclude that potassium is partly responsible for the response. On the other hand, quite a lot of data suggests that its role is not one of major importance, especially in sustained exercise hyperemia. First, potassium is not a potent vasodilator under any circumstance. During heavy muscular exercise, muscle flow has been reported to increase by as much as 800%. The maximum response that can be produced by K^+ infused intra-arterially is a doubling of flow. Moreover, the vasodilator response to K^+ is not maintained for more than a few minutes during intra-arterial administration. In addition, the release of measurable amount of K^+ from exercising skeletal muscle is transient while the hyperemia is sustained for as long as the exercise continues. Finally, rendering the vasculature insensitive to potassium only delays the onset and not the magnitude of exercise hyperemia. Based on the information at hand, it seems reasonable to postulate that potassium is only involved in the initiation of exercise hyperemia, and even here to only a minimal extent. It is possible that the postulated role of potassium could be extended if one could accurately measure interstitial potassium levels during exercise.

Myogenic Regulation of Blood Flow in Skeletal Muscle

Myogenic Phenomena in Vascular Smooth Muscle

It was once held that all vascular smooth muscle was of the multiunit type with no cell to cell propagation of electrical activity (11,31). It has been established, however, that in contrast to the smooth muscle of the large arteries and veins, the smooth muscle of the small arteries and veins is of the single unit type in which there is cell to cell propagation of electrical activity (28,54,59,60,99, 100,103,175,180,181). Thus the resistance vessels in the skeletal muscle vasculature are capable of myogenic activity, and this activity is said to contribute greatly to the basal tone (53,105,107).

Direct evidence for the existence of intrinsic basal electrical activity in smooth muscle cells was first introduced by Funaki (59,60). With the use of intracellular microelectrodes inserted in smooth muscle cells of arterioles and venules, Funaki showed that vascular smooth muscle exhibited a phasic resting membrane potential and spontaneous spike discharge. The spike discharges followed slow potentials (similar to pre-potentials in some other tissues) and were propagated from cell to cell. Other investigators have confirmed the presence in vascular smooth muscle of rhythmic electrical activity, the longitudinal spread of induced

activity, and slow rising potentials suggestive of pacemaker activity (28,31,99,100,175,181).

Studies with in vitro vascular strips show that vascular smooth muscle constrict in response to passive stretch. Johansson and Bohr (99), using helical strips from canine paw arteries, observed slow spontaneous rhythmic contractions that increased in frequency with passive stretch. Sparks (169) saw active tension development in the human unbilical artery in response to passive stretch. He showed that the response was directly related to the resting tension, the rate and the increment of stretch. Recently, Johansson and Mellander (102) have shown that both the electrical and mechanical activity of portal vein strips increased in direct proportion to increasing rates of stretch.

These observations make it more probable that an active response by the vascular smooth muscle could function in local blood flow regulation. Thus, an increase or decrease in perfusion pressure could cause, respectively, a constriction or relaxation of the vascular smooth muscle. This could play a role in autoregulation. The reduction in internal pressure of the vasculature subsequent to arterial occlusion could trigger a myogenic relaxation of the vascular smooth muscle that could be a factor in causing the reactive hyperemia. Theoretically, the rhythmic contractions of an exercising muscle could passively decrease the stretch

on the vascular smooth muscle whose active relaxation could contribute to the exercise hyperemia.

The Myogenic Response in Local Control of Skeletal Muscle Blood Flow

Bayliss (18) was the first to suggest that an active myogenic relaxation of vascular smooth muscle could be the cause of the hyperemia seen following brief occlusion of the arterial supply to the dog hindlimb muscles. One criticism of the conclusions of Bayliss is that the periods of occlusion (8-15 secs) were long enough to allow for metabolite accumulation. Folkow (53) attempted to overcome this objection by only partially reducing the intravascular pressure. He suggested that since flow was not drastically compromised, the resulting vasodilation was due to myogenic relaxation of the vascular smooth muscle.

In a further attempt to isolate possible myogenic factors from metabolic factors, some investigators have increased the intravascular pressure of an organ by raising the venous pressure. This would put the myogenic and metabolic mechanisms in opposition to each other. Thus the net change in vascular resistance following elevation of venous pressure could indicate which control factor was dominant. However, the results of these studies have been inconsistent. Nagle *et al.* (143), using the denervated canine gracilis muscle reported that a 20 mmHg increase in venous pressure caused a 40% increase in vascular resistance. Since venous

resistance did not change or decrease, it was concluded that the resistance increase was due to precapillary constriction. Jones and Berne (108,109) found that when venous pressure was raised there was a transient increase in vascular resistance, but in the subsequent steady-state the resistance was decreased.

Other techniques have been used to evaluate the myogenic response of the vascular bed of skeletal muscle. These studies have tried to change the distending or transmural pressure of a vascular bed without affecting flow. Lundvall et al. (126) increased the transmural pressure in the lower leg muscles of cats by increasing both the arterial and venous pressure by the same amount. Changes in precapillary resistance were reflected by changes in the capillary filtration coefficient (CFC). At rest, an increase in the transmural pressure caused a reduction in CFC of 35% with no change in flow. This was interpreted as constriction of the precapillary resistance vessels just sufficient to balance the passive distension. During exercise, elevation of the transmural pressure decreased CFC by 50% and increased flow by 38%. This was interpreted as showing that, while the precapillary vessels could still respond to myogenic stimuli, the metabolic control of blood flow did over-ride the myogenic response causing the resistance vessels to respond passively.

Smiesko (168) used a different approach in the isolated dog gracilis muscle. The muscle was perfused with blood from a reservoir, while the inflow was monitored. With this non-pulsatile pressure perfusion system, 1 second square-wave changes in pressure were produced. A transient increase or decrease in perfusion pressure resulted in a vasodilation with the same magnitude and time-course. This myogenic vasodilation seemed to be different from the classical Bayliss effect, and also to be in response to the ascending limb of the pressure change.

Microscopic observation of several vascular beds have provided strong evidence for a myogenic contribution to reactive hyperemia (32,106,144,179). Johnson et al. (106) measured red cell velocity in individual capillaries of the cat sartorius muscle and found that peak velocity, following release of upstream occlusion, was the same for occlusions from 5-30 seconds. With occlusions of longer duration (60-120 secs.) peak velocity increased. This indicates that a myogenic relaxation of precapillary vessels may be involved in the first few seconds of reactive hyperemia, and other factors such as vasodilator metabolites come into play subsequently. In a similar preparation, Tuma et al. (179) showed that the duration but not the peak of the hyperemia was increased by low PO_2. This, together with the studies of Owen et al. (149) would support the idea that both

myogenic and metabolic factors are responsible for reactive hyperemia.

Mohrman and Sparks (139) have provided more direct evidence that the myogenic response could play a role in the hyperemia following muscle contraction. They measured intramuscular pressure by a small saline-filled balloon during 1 second periods of tetany (16/sec). With the muscle at rest, they increased extravascular pressure by the same amount (65-80 mmHg), by rapidly inflating a rubber bladder wrapped around the isolated muscle. This maneuver was followed by a vasodilation with similar time course but only one-third the magnitude of the vasodilation which followed tetany. These investigators concluded that these findings could be explained by the classical Bayliss effect (decreased vascular transmural pressure triggering relaxation of smooth muscle), or by the phenomenon described by Smiesko (a vasodilation in response to the ascending phase of a transient pressure change).

The myogenic response may thus be involved in the hyperemia associated with rhythmic muscle contraction. This suggestion has been made by several authors (81,82,104,134,153) but the evidence is less direct. With contraction frequencies of greater than 4-6 pulses per second, blood flow has been observed to decrease during each contraction (61,174). Intramuscular pressure increases with muscle contraction

depending on the frequency of contraction (139,184). These observations make it reasonable to hypothesize that compression of the vasculature during muscle contraction could trigger a myogenic relaxation of the vascular smooth muscle. In this respect, Radawski et al. (153) have suggested that since mild exercise (less than 2/sec) does not cause any dilation in the recipient muscle in a bioassay system, the myogenic response could be involved. However, they also pointed out that labile metabolites may be responsible.

III. STATEMENT OF OBJECTIVES

The venous effluent from exercising muscle has provided considerable evidence that locally produced metabolites are involved in the production of exercise hyperemia. However, the interstitial fluid concentration of these metabolites is the more relevant measure of their participation in this type of blood flow regulation. Since it is not possible to obtain interstitial fluid, we decided to analyze lymph from skeletal muscle, as the changes in interstitial fluid may be reflected there. The potassium concentration, the osmolality and the inorganic phosphate concentration of lymph from resting and from exercising muscle, together with these same values in corresponding venous plasma samples, should add new and useful data on the involvement of these metabolites in exercise hyperemia.

In an effort to isolate the proposed contribution of the myogenic response to the hyperemia accompanying muscle contraction, an in vitro system was designed so that changes in the vascular transmural pressure of an excised muscle could be made. With this apparatus, pulsatile and non-pulsatile decreases in transmural pressure, and non-pulsatile increases in transmural pressure were possible. Thus, by

simulating in the resting muscle, the changes in vascular transmural pressure observed in the exercising muscle, the vascular resistance changes attributable to purely myogenic factors could be determined.

IV. METHODS

Adult mongrel dogs of either sex weighing 12-25 kg were used in all experiments. The animals were anesthetized with sodium pentobarbital (30 mg/kg I.V.) and were artificially ventilated with room air by a positive pressure respirator (Harvard Apparatus Co., Model 613, Millis, Mass.). Supplemental doses of sodium pentobarbital were given as required. Following the surgical preparation, sodium heparin (The Upjohn Co., Kalamazoo, Mi.) was administered in an initial dose of 500 USP units/kg with hourly supplements of 250 USP units/kg.

Blood pressures were continuously recorded via heparinized-saline-filled polyethylene (PE) tubing (Intramedic Tubing, Clay Adams, Parsippany, N.J.) connected to low volume displacement pressure transducers (Statham Laboratories, Model P23Gb, Hato Rey, Puerto Rico) with inputs into a direct-writing oscillograph (Hewlett-Packard, Model 7796A, Boston, Mass.). Blood flow was measured with an electromagnetic flow meter (Biotronex Laboratory, Model BL-610, BLC-2024-F10 probe, Silver Springs, MD.), and/or with stopwatch and graduated cylinder. All chemicals used were American Chemical Society Certified reagent grade and

supplied by Mallinckrodt Co., St. Louis, Mo., Fisher Scientific Co., Fair Lawn, N.J. or J. T. Baker Chemical Co., Phillipsburg, N. J.

The experiments described in this report fall into two categories. In the first group of experiments lymph from resting and activated skeletal muscle was analyzed for altered concentrations of various vasodilator metabolites that have been postulated to play a major role in exercise hyperemia. In the second group of experiments the role of alterations in vascular transmural pressure in the genesis of exercise hyperemia was examined in an isolated skeletal muscle.

Experimental Design

These experiments were designed for paired comparisons. Steady-state control conditions were reached before experimental maneuvers were performed. Where possible the order of experimental maneuvers was randomized and post-experimental control data were obtained.

Studies on Lymph

Series 1: Composition of Lymph from the Exercising Hindlimb at Natural Flow

At the beginning of surgery, the animals were hydrated by IV infusion of saline (50 ml/kg body weight) in an

attempt to encourage lymph flow. The preparation used is shown in Figure 1. The femoral, obturator, and sciatic nerves were isolated and cut for electrical stimulation of their distal ends to produce muscle contraction. The femoral artery was ligated at the level of the inguinal region and cannulated distally with an in-line electromagnetic flow probe through which blood flowed from the contralateral femoral artery. Blood flow was recorded continuously by feeding the output from the electromagnetic flow meter into the DC amplifier of the direct-writing oscillograph. The flow meter was calibrated with saline immediately before each experiment, and rechecked with whole blood at the end of most experiments. A femoral lymphatic in the inguinal region was isolated, ligated and cannulated distal to the lymph nodes for collection of lymph. Lymph was collected via PE-10 tubing in miniature graduated cylinders made from plastic pipettes. To minimize evaporation the collection tubes were narrow and covered by parafilm through which the PE tubing entered. Injections of a 2% aqueous solution of Patent-Blue Violet (Sigma Chemical Co., St. Louis, Mo.) into the Gracilis, Semi-membranosus, Semi-tendonosus, Sartorius and Vastus medialis muscles was used to visualize the lymphatics. In experiments where the lymphatic was cannulated without previous dye injection, dye was injected during the course of the experiment to show that muscle lymph was

Figure 1. Schematic of the intact canine hindlimb preparation.

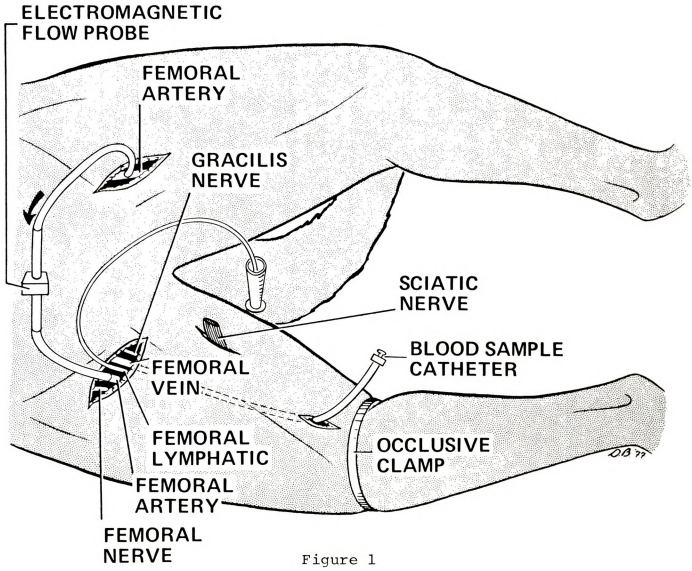

ELECTROMAGNETIC
FLOW PROBE
FEMORAL
ARTERY
GRACILIS
NERVE
SCIATIC
NERVE
BLOOD SAMPLE
CATHETER
FEMORAL
VEIN
FEMORAL
LYMPHATIC
OCCLUSIVE
CLAMP
FEMORAL
ARTERY
FEMORAL
NERVE

Figure 1

collected during exercise and that lymph from the lower leg was not collected.

Venous blood samples were collected from a catheter inserted into the femoral vein via a side branch and advanced to the level of the femoral lymphatic cannula. A radiator hose clamp was tightly positioned at the knee, central to the popliteal lymph node. This was done in order to exclude the possibility of collecting post-nodal lymph from the lower leg.

There were three periods of lymph collection: (1) A control period with the limb at rest. During this period gentle massage and elevation of the limb was often necessary to encourage lymph flow. (2) A period of exercise produced by simultaneous electrical stimulation (S5 Stimulator, Grass Instruments, Model S5E, Quincy, Mass.) of the Femoral, Gracilis and Sciatic nerves at a stimulus strength of 6 volts, frequency of 6 pulses per second and a pulse duration of 1.6 msec. (3) A post-exercise control period. The length of these periods (40-90 mins in the control periods and 20-50 mins during exercise) varied with the lymph flow rate as a minimum lymph sample of 0.4 ml was required for analysis. A venous blood sample was drawn at the beginning and end of each lymph collection period. In addition, a blood sample was taken 2 minutes after the start of exercise. All blood samples were immediately centrifuged, the plasma refrigerated and analyzed as soon as possible.

Lymph and plasma samples were analyzed for potassium, osmolality, inorganic phosphate, and total protein. Potassium concentration was determined by Flame photometry (Beckman Instruments, Model 105, Fullerton, Ca.), osmolality by freezing point depression (Advanced instruments, Model 67-3lLAS, Newton Highland, Mass.), and inorganic phosphate by the Fiske-SubbaRow manual method (50) as modified by the Sigma Chemical Company. Total protein content was determined by the ultraviolet spectrophotometric method of Waddell (186).

Series 2: Intramuscular Injection of a Hyperosmotic Solution Containing Elevated Levels of Inorganic Phosphate and Potassium

The purpose of this series of experiments was to show that if osmolality, potassium and/or inorganic phosphate rose in muscle interstitial fluid, the increase would be reflected in the collected lymph. The hindlimb preparation (Figure 1) used in the first series was used here also. Lymph was collected during two periods: (1) a control and (2) a period immediately following intramuscular injection of a 1500 milliosmolar solution of sodium chloride containing 15 meq/L potassium (the chloride salt) and 6 mM/L inorganic phosphate (the sodium monobasic phosphate). The total injection volume was 30 ml and was injected in 3 ml doses into 10 different areas of the muscle mass.

Injections were delivered slowly through a 27 gauge 3-inch hypodermic needle so as to avoid major blood vessels and to distribute the solution into as large a muscle mass as possible.

Blood samples were obtained at the beginning and end of each lymph collection period. In addition, a blood sample was taken 2 minutes after the injection. All samples were handled as in Series 1, and analyzed for osmolality, potassium and inorganic phosphate.

Series 3: Close Arterial Infusion of Inorganic Phosphate into the Hindlimb and into the Isolated, Denervated, in situ Gracilis Muscle

The purpose of this series of experiments was to test the response of the vascular bed of canine skeletal muscle to increased phosphate concentration and to ascertain whether an increase in intravascular phosphate concentration could be detected in lymph.

A 198 mM solution of inorganic phosphate of the sodium monobasic phosphate ($NaH_2PO_4H_2O$) dissolved in water and buffered with sodium bicarbonate at pH 6.9, was infused into the arterial inflow in the hindlimb preparation of Figure 1. The osmolality of the solution was 680 mOsm/kg. The infusion was at three different rates selected to increase the venous blood concentration of phosphate between 3 to 12 times normal. Infusions were made with an infusion pump

(Harvard Apparatus, Model 940, Millis, Md.), and maintained for 10 minutes at each rate. A sodium chloride solution was infused in the same manner to serve as a control. Since the hindlimb preparation used was at natural flow conditions any changes in vascular resistance would be seen as a directionally opposite change in flow.

One lymph sample was collected during the sodium chloride infusion and another during the phosphate infusion. Venous blood samples were obtained during the infusion periods at each infusion rate. Lymph and venous plasma were analyzed for inorganic phosphate. Also, determinations of pH (PHM 72 MK2 Digital Acid-base Analyzer, Radiometer Copenhagen, The London Co., Westlake, Ohio), hematocrit (Adams Micro-Hematocrit Centrifuge, Clay Adams Inc., N.Y.), and osmolality were done on all venous blood samples. Arterial inflow and arterial perfusion pressure were continuously monitored.

In order to determine the effect of inorganic phosphate on a pure muscle preparation, a 198 mM solution of inorganic phosphate of the sodium monobasic phosphate buffered with sodium bicarbonate (pH 6.9, 680 mOsm/kg) was infused into the arterial inflow of the isolated, denervated, in situ Gracilis muscle (Figure 2). The Gracilis muscle was surgically isolated and tightly ligated at its origin and insertion. All collateral vessels were ligated and blood flowed

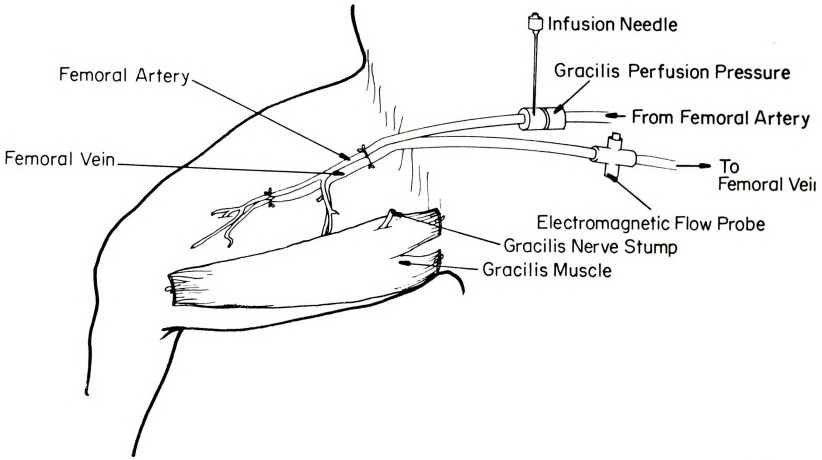


Figure 2. Schematic of the isolated denervated in situ Gracilis muscle preparation.

to and from the muscle only by the main gracilis artery and vein. For convenience, the arterial inflow was from the contralateral femoral artery and the cannula rested in the ipsilateral femoral artery. The venous blood cannula rested in the femoral vein central to the junction of the gracilis vein. The femoral artery and vein were ligated distal to the junction of the gracilis vessels and all collaterals were tied off. An electromagnetic flow probe was inserted in the venous cannula to continuously record venous outflow. Changes in vascular resistance were calculated from changes in blood flow, and the arterial perfusion pressure. Infusions were done in the same manner as in the hindlimb above. Venous blood samples were collected during each infusion period and analyzed for inorganic phosphate, osmolality, pH and hematocrit. In one-half of the animals used, the phosphate solution was unbuffered (pH 4.5, 340 mOsm/kg).

Studies on the Myogenic Response

To accomplish this group of studies it was necessary to design and build an in vitro muscle perfusion chamber which allowed production of near square wave pulsatile and non-pulsatile changes in pressure within the chamber. The apparatus is shown in Figure 3 and consisted of a muscle chamber separated from a gas chamber by a flexible, impermeable diaphragm, and a variable voltage controller connected to an

Figure 3. Photograph of the extracorporeal apparatus used to change vascular transmural pressure.

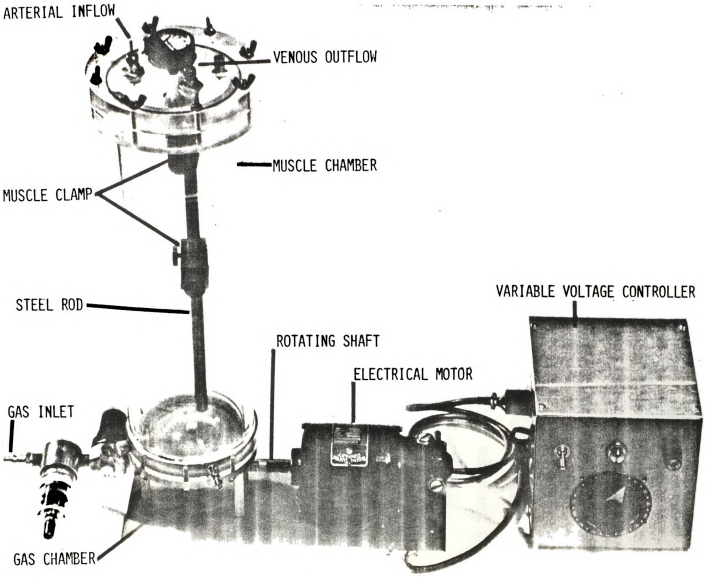

ARTERIAL INFLOW
VENOUS OUTFLOW
MUSCLE CHAMBER
MUSCLE CLAMP
STEEL ROD
VARIABLE VOLTAGE CONTROLLER
ROTATING SHAFT
ELECTRICAL MOTOR
GAS INLET
GAS CHAMBER

Figure 3

electrical motor. The motor turned a shaft to open and close the gas chamber. The rate of rotation of the shaft determined the frequency of pulsing the muscle chamber, and the volume of gas admitted to the gas chamber determined the pulse pressure. The muscle chamber had inflow and outflow ports at the top for arterial and venous blood flow, wire electrodes for nerve stimulation, a steel rod for hanging the muscle, and inflow and outflow ports for circulation of Ringers solution which bathed the muscle at a constant temperature between 35°C and 38°C. The top of the muscle chamber with the steel rod attached was removable. This system permitted us to determine in skeletal muscle the vascular effects of alterations in transmural pressure produced by changes in extravascular pressure. By pulsing the chamber at various rates and pulse pressures it was possible to simulate the changes in transmural pressure that occur during exercise in a quiescent muscle. In addition, the vascular response to transient and sustained square-wave increases or decreases in extravascular pressures could be examined.

Series 1: Constant Pressure Studies

The right Gracilis muscle was surgically isolated with the tendons securely tied and attached to rubber rings. The Gracilis nerve was isolated and cut leaving a length of about 3 cm attached to the muscle. The femoral artery and

vein were dissected free from the surrounding tissue for about 6 cm above and below the junction of the main Gracilis vessels. All branches of the gracilis vessels and any other collateral vessels to the muscle were tied off. Blood cannulas were connected to the inflow and outflow ports of the top of the muscle chamber to bring arterial blood from the left femoral artery and conduct the venous outflow to the venous blood reservoir. With these cannulas as well as the connections on the inner side of the muscle chamber top in place, the top with the attached steel rod was placed close to the in situ isolated muscle. By cannulating the muscle venous outflow, then the arterial inflow, the length of time that the muscle blood supply was interrupted was minimized. The blood cannulas were placed in the upper portion and the arterial and venous pressure cannulas in the lower portion of the femoral vessels. The muscle was then excised, attached to the steel rod with adjustable clamps that held it at its in situ length, and cut free from all other structures. All severed structures were tightly ligated to prevent bleeding. The Gracillis nerve stump was then attached to the wire electrodes. The muscle chamber was assembled, filled with normal Ringers solution at a temperature of 36°C to 38°C and sealed (Figure 4). The muscle chamber pressure was adjusted to a steady resting pressure which varied between -2 and 6 mmHg.

Figure 4. Schematic of the excised gracilis muscle in the muscle chamber.

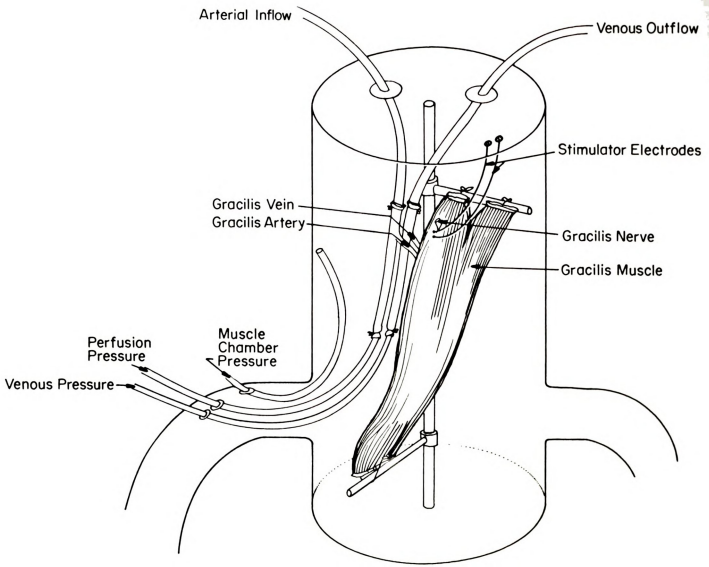

Arterial Inflow
Venous Outflow
Stimulator Electrodes
Gracilis Vein
Gracilis Artery
Gracilis Nerve
Gracilis Muscle
Perfusion Pressure
Muscle Chamber Pressure
Venous Pressure

Figure 4

The venous outflow from the muscle chamber passed to a constant volume reservoir for return to the dog. Flow measurements were made periodically with stop-watch and graduated cylinder at the venous reservoir and were recorded continuously from the inflow pump speed. Normal Ringers solution was circulated through the muscle chamber to maintain a constant muscle chamber temperature. This circulation of fluid was stopped during the experimental maneuvers. Figure 5 illustrates the experimental preparation.

The muscle was pump perfused (Holter Pump Model RE 161, Extracorporeal Med. Spec. Inc., King of Prussia, Pa.) with perfusion pressure maintained constant at the level of the systemic arterial pressure by a servosystem (Figure 6) using the Leeds and Northrup 420 Current Adjusting Tape Controller. The feedback signal was the change in muscle perfusion pressure and the controlled variable was the speed of the perfusion pump. Thus, when the pump is calibrated for flow rate output versus speed, the pump rpms can be fed into a DC Amplifier and oscillograph for continuous recording of the muscle inflow rate. In this system changes in vascular resistance would be seen as directionally opposite changes in flow. The muscle preparation was considered functional and acceptable if (1) pressures and flow remained stable at control for at least 15 minutes after surgery and (2) the muscle reacted with dilation to arterial occlusion and

Figure 5. Overall schematic view of the experimental preparation used to study the effects of transmural pressure changes in the in vitro gracilis muscle.

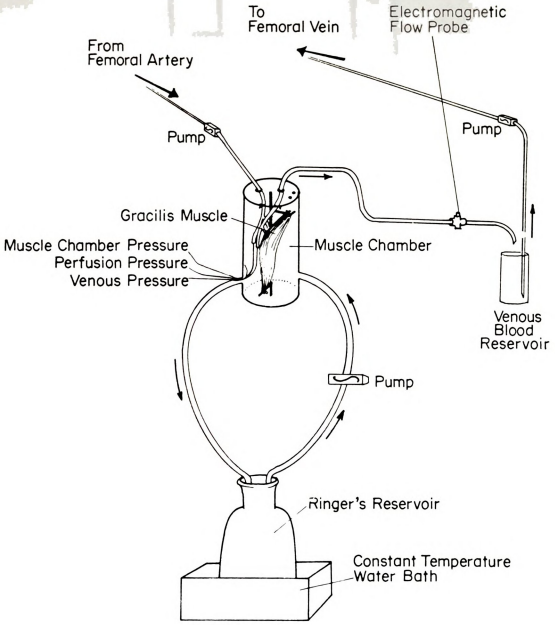

To
Femoral Vein
Electromagnetic
Flow Probe
From
Femoral Artery
Pump
Pump
Gracilis Muscle
Muscle Chamber Pressure
Perfusion Pressure
Venous Pressure
Muscle Chamber
Venous
Blood
Reservoir
Pump
Ringer's Reservoir
Constant Temperature
Water Bath

Figure 5

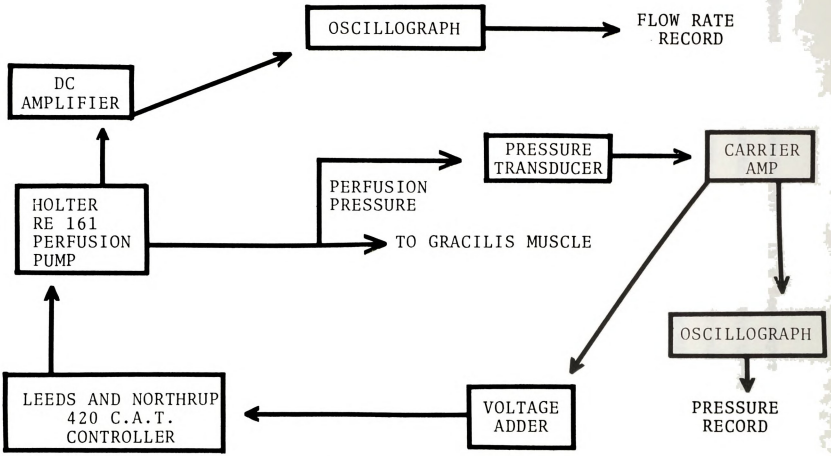


Figure 6. Schematic of the use of a servosystem to maintain muscle perfusion pressure constant by feedback control of the perfusion pump.

30 second nerve stimulation. In a few experiments pressure-flow curves were run to demonstrate that these muscles could autoregulate. In some experiments intramuscular pressure was measured electronically by use of a Mikro-tip pressure transducer (Model #PC-340, Millar Instruments, Houston, Texas) introduced into the thickest portion of the muscle via a 15 gauge bevelled steel needle.

Using this experimental preparation several series of experiments were performed. In 10 muscles the vascular response to one minute of exercise by nerve stimulation at 5 pulses per second (pps), 5 volts and 1.6 millisecond duration were compared with the response to one minute of simulated exercise produced by pulsing the muscle chamber at 5 pps with a pulse pressure of 10, 25, and 50 mmHg. In 10 additional muscles the vascular response to 1 min of exercise produced by nerve stimulation at 2 pps, 5 volts and 1.6 msec. duration were compared with the response to simulated exercise produced by pulsing the muscle chamber at 2 pps with pulse pressures of 10, 25, and 50 mmHg.

Several other experimental maneuvers that could contribute information on myogenic phenomenon in the vascular bed of skeletal muscle were done. In 5 muscles the vascular effects of a transient increase or decrease in muscle chamber pressure was studied. The transient pressure changes were accomplished by making a single excursion in muscle chamber

pressure to +50 mmHg and -50 mmHg by adding and withdrawing volume from the chamber. In 5 muscles the effects of a sustained increase or decrease in muscle chamber pressure was examined. This was done in order to see if the Bayliss phenomenon could be elicited by extravascular pressure changes. The muscle chamber pressure was increased to 10, 25, and 50 mmHg for 1 minute at each level by adding volume to the chamber. By withdrawing volume from the chamber, the inside pressure was made negative to -10, -25, and -50 mmHg and held at each level for 2 minutes.

Series 2: Natural Flow Studies

The right gracilis muscle was surgically isolated, excised and transferred to the muscle chamber as described above. To obtain natural flow conditions the muscle was perfused directly with arterial blood through a catheter from the abdominal aorta. The experimental preparation was the same as illustrated in Figure 5 with the exception that the arterial perfusion pump was eliminated in order to show that the pump did not interfere with the vascular response. Venous outflow was continuously recorded from the electromagnetic flow meter, and changes in vascular resistance were seen as directionally opposite changes in venous outflow.

In 7 muscles the vascular effects of one minute of exercise produced by nerve stimulation at 5 pps, 5 volts, 1.6 msec. duration were compared with (a) 1 minute of

simulated exercise produced by pulsing the muscle chamber at 5 pps with a pulse pressure of 50 mmHg, and (b) 1 minute of simultaneous nerve stimulation exercise (5 pps, 5 volts, and 1.6 msec.) and chamber pulsing simulated exercise (5 pps, 50 mmHg).

In 5 muscles the vascular response to 1 minute of muscle exercise produced by nerve stimulation at 2 pps, 5 volts, 1.6 msec. was compared to (a) the response to 1 minute of simulated exercise produced by pulsing the muscle chamber at 5 pps with a pulse pressure of 50 mmHg, and (b) the response to 1 minute of simultaneous nerve stimulation exercise (2 pps, 5 volts, 1.6 msec.) and chamber pulsing simulated exercise (2 pps, 50 mmHg)

Series 3: Constant Flow Studies

The gracilis muscle was surgically isolated, excised and transferred to the muscle chamber as previously described. Constant flow conditions were obtained by interposing a constant speed pump in the arterial perfusion line. Blood flow was set at a level such that the muscle perfusion pressure was close to the systemic arterial pressure. The venous outflow was continuously recorded from the electromagnetic flow meter. The preparation was identical to that illustrated in Figure 5. Under these conditions, changes in vascular resistance were seen as directionally similar changes in muscle perfusion pressure.

In 12 muscles the vascular effects of 1 minute of exercise produced by nerve stimulation at 5 pps, 5 volts, and 1.6 msec. were compared with (a) the effects of 1 minute of simulated exercise produced by pulsing the muscle chamber at 5 pps with a pulse pressure of 50 mmHg and (b) the effects of 1 minute of simultaneous nerve stimulation exercise (5 pps, 5 volts, and 1.6 msec.) and chamber pulsing simulated exercise (5 pps, 50 mmHg).

In 8 muscles the vascular effects of 1 minute of nerve stimulation exercise at 2 pps, 5 volts, 1.6 msec. were compared with (a) 1 minute of simulated exercise produced by pulsing the muscle chamber at 2 pps with a pulse pressure of 50 mmHg, and (b) 1 minute of simultaneous nerve stimulation exercise (2 pps, 5 volts, 1.6 msec.) and chamber pulsing simulated exercise (2 pps, 50 mmHg).

V. DATA COMPILATION AND STATISTICAL ANALYSIS

Blood flows were converted to ml/min/100 gm of tissue except in the studies on the hindlimb (Part I, Series 1-3) where the weight of the tissue could not be accurately determined. Potassium and osmolality were reported in the units measured, mEq/L and mOsm/kg respectively, inorganic phosphate was assayed in mg/100 ml inorganic phosphorus (P) and converted to millimoles/liter inorganic phosphate (PO_4) as follows:

$$(\text{mg}/100\text{ ml P}) \times \frac{95}{31} \times \frac{10}{95} = \text{mmoles/L } PO_4.$$

(Molecular weights : P=31, PO_4 = 95)

Resistances were calculated by dividing the drop in pressure across the vascular bed by the blood flow and reported in mmHg/ml/min/100 gm of tissue (PRU/100 gm).

In all experiments the experimental organ was its own control. For each sample, the arithmetic mean ($\bar{x}$), the sample variance (s^2), and the standard error of the mean ($S\bar{x}$) were calculated. The data from the studies on lymph (Part I) were analyzed for significant changes by the students t test for paired observations. The data from some of the studies on the myogenic response (Part II) were

analyzed by a two-way analysis of variance (after the test for equality of variance was performed) and means compared by the Tukey's procedure. In the others, the paired-t test was used. All statistical evaluation was done at a 0.05 significance level.

VI. RESULTS

Studies on Lymph

Series 1: Composition of Lymph From the Exercising Hindlimb

Figure 7 shows a typical vascular response of the hindlimb to nerve stimulation. Blood flow increased threefold with a concomitant small decrease in perfusion pressure. Note the rapid and sustained increase in blood flow. In this experiment the control blood and lymph flow rate were 30 ml/min and 0.003 ml/min respectively. During steady-state exercise produced by nerve stimulation, blood flow increased to 80 ml/min, and lymph flow to 0.012 ml/min.

Average data from ten such experiments are shown in Table 1. The flow rate, inorganic phosphate concentration [Pi], potassium concentration [K+], osmolality [Osm], and total protein concentration in lymph and venous plasma during a control period with the limb at rest (R), and at the end of a 25 minute period of nerve stimulation exercise (25 min E) are shown. In addition, the corresponding values for venous plasma obtained at 2 minutes after the start of exercise are given. It can be seen that exercise produced a threefold increase in blood and lymph flow. In lymph collected during the control period the concentrations of

Figure 7. Typical vascular response of the intact hindlimb to faradic stimulation (6 pps, 6 volts, 1.6 msec) of the gracilis, sciatic and femoral nerves. Exercise lasted for 28 min (between arrows). Hindlimb lymph was collected before and during exercise, and venous blood sampled immediately before, at 2nd min and on termination of stimulation.

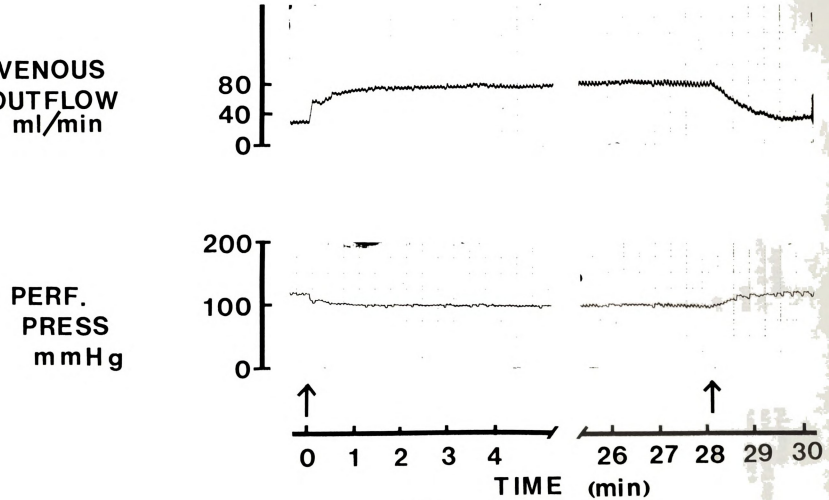

VENOUS
OUTFLOW
ml/min
80
40
0
PERF.
PRESS
mmHg
200
100
0
0 1 2 3 4
26 27 28 29 30
TIME (min)

Figure 7

Table 1. Results of analyses of lymph and venous plasma from the hindlimb collected at rest (R) and during exercise (E) at 6 volts, 6 pps and 1.6 msec duration. Values are mean ± S$\bar{x}$. * denotes a significant change from rest ($P < 0.05$). N = 10.

	Lymph		Venous Plasma		
	R	25 min E	R	2 min E	25 min E
Flow ml/min	.007 ±.002	.025* ±.004	29 ±6	92* ±10	92* ±10
[Pi] mMoles/L	1.2 ±0.008	1.4* ±0.07	1.3 ±0.1	1.3 ±0.1	1.4 ±0.1
[K+] mEq/L	3.2 ±0.06	3.4* ±0.08	3.3 ±0.1	4.0* ±0.1	3.7* ±0.1
[Osm] mOsm/kg	300 ±3	299 ±2	302 ±1.2	306* ±1.4	302 ±1.4
[T. Prot] gm/100ml	1.9 ±0.2	2.0 ±0.2	4.4 ±0.4	4.6 ±0.4	4.5 ±0.3

the solutes measured agree with established values as given in various texts and handbooks. In lymph collected during exercise, there was a small (0.2 mM/L) increase in [Pi] and [K+] (0.2 mEq/L). There was no change in osmolality or total protein. The plasma from venous blood samples taken at 2 and 25 minutes after the start of exercise showed significant increases in [K+] (0.7 mEq/L and 0.4 mEq/L respectively). An increase (4 mOsm/kg) in venous plasma osmolality was seen only in the sample taken at 2 minutes of exercise. There was no change in venous plasma inorganic phosphate or total protein. However, protein transport ([T. Prot] x lymph flow) was elevated during exercise.

Series 2: Intramuscular Injection of a Hyperosmotic Solution Containing Five Times Normal Concentrations of Inorganic Phosphate and Potassium Into the Hindlimb

Table 2 presents the results of analyses of lymph and venous plasma from the hindlimb at rest, and after intramuscular injection of a hyperosmotic (1500 mOsm/kg) solution containing 15 mEq/L of potassium and 6.0 mM/L inorganic phosphate. A control lymph sample (Con) was obtained prior to injection, and another during a 25 minute period after injection (25 min Inj.). Corresponding venous blood samples were obtained with an additional blood sample taken at 2 minutes after injection. Following the injection, lymph flow increased from a control of 0.005 ml/min to 0.016 ml/min

Table 2. Results of analyses of lymph and venous plasma from the hindlimb during a control period (Con) and after intramuscular injection (Inj) of a 1500 mOsm/kg solution of sodium chloride containing 15 mEq/L [K+] and 6.0 mMoles/L inorganic phosphate. Values are means $\pm \bar{x}$. * denotes a significant change from control ($P < 0.05$) N = 5.

	Lymph		Venous Plasma		
	Con	25 min Inf.	Con	2 min Inj.	25 min Inj.
[Pi] mMole/L	1.1 ±0.1	1.8* ±0.2	1.2 ±0.1	1.4 ±0.1	1.5* ±0.1
[K+] mEq/L	2.9 ±0.2	3.6* ±0.2	2.8 ±0.1	3.5* ±0.2	3.4* ±0.4
[Osm] mOsm/kg	283 ±9	321* ±6	294 ±4	317* ±8	299 ±3

within 1-3 minutes after injection. After the injection, lymph [Pi] increased by 0.7 mM/L, [K+] by 0.7 mEq/L and [Osm] by 38 mOsm/kg. The significant changes in the venous plasma were seen in the 2 minute sample in which [K+] increased by 0.7 mEq/L and the osmolality by 23 mOsm/kg, and also in the 25 min sample in which [Pi] and [K+] were increased by 0.3 mM/L and 0.6 mEq/L respectively. These results show that if the concentration of inorganic phosphate, potassium and osmolality increased in the interstitium, the increase should be reflected in the lymph and venous blood.

Series 3: Close Arterial Infusion of Inorganic Phosphate into the Hindlimb and into the Isolated, Denervated, in situ Gracilis Muscle

The vascular response of the hindlimb to a close intra-arterial infusion of inorganic phosphate (198 mM Na H_2PO_4 buffered with sodium bicarbonate) is shown in Table 3. Elevation of [Pi] in the venous blood and lymph from 1.2 to 11.3 mM and 1.3 to 9.7 mM respectively, produced no change in hindlimb blood flow. Equal volume infusion of a sodium chloride solution having the same osmolality as the inorganic phosphate solution was also without effect on hindlimb flow. Neither solution changed lymphatic vessel flow. Venous plasma osmolality was not significantly changed, and lymph samples were insufficient for measurement of osmolality.

Table 3. Effects on blood flow, venous plasma [Pi], venous plasma Osmolality [Osm] and lymph Pi of close arterial infusion of a 198 mM solution of inorganic phosphate buffered with sodium bicarbonate into the hindlimb. Values are means $\pm S_{\bar{x}}$. * denotes a significant change from control ($P < 0.05$) N = 4.

Solution	Infusion Rate ml/min	Blood Flow ml/min	Venous Plasma Pi mM	Venous Plasma Osm	Lymph
NaCl (680 mOsm/kg)	0	38 ± 2	1.2 ± .03	298 ± 7	
	.382	38 ± 3	1.2 ±0.1	300 ± 6	
	.764	39 ± 3	1.2 ±0.1	300 ± 7	
	1.91	39 ± 3	1.2 ±0.1	300 ± 6	1.3 ±0.1
Pi (680 mOsm/kg)	0	35 ± 2	1.2 ±0.1	300 ± 5	1.3 ±0.1
	.382	36 ± 6	3.8* ±0.5	302 ± 6	
	.764	37 ± 5	6.4* ±0.9	302 ± 5	
	1.91	32 ± 1	11.3* ±1.2	307 ± 5	9.7* ±0.6

The hindlimb is a mixture of skeletal muscle, skin and bone and it is possible (and in fact likely) that individual tissues may respond differently to a given vasoactive substance (79). Also, since exercise hyperemia is seen in skeletal muscle and not in skin or bone, another series of experiments was designed to assess the vasoactivity of Pi in pure skeletal muscle. The muscle chosen for these studies was the gracilis because (a) in the dog it is a mixed muscle (containing both red and white fibers), (b) it shows excellent exercise hyperemia and (c) it has a vascular supply that can be easily isolated. It was, however, not possible to collect lymph from this muscle, thus it was not suitable for the initial series of experiments.

The response of the gracilis vasculature to increasing local infusion of Pi is shown in Table 4. It is evident that elevations in the Pi concentration in gracilis venous plasma from 1.2 to 14.9 mM produced no regular effects on gracilis blood flow. Equal volume infusions of a sodium chloride solution of comparable osmolality, also was without effect on gracilis flow indicating that the infusion rates used did not produce dilutional reductions in blood viscosity and/or vasoactive alterations in plasma osmolality.

In a final series of experiments unbuffered inorganic phosphate was infused into the gracilis vasculature to determine if the combination of increased inorganic

Table 4. Effects on blood flow, venous plasma inorganic phosphate, venous plasma osmolality and blood pH of close-arterial infusion of a 198 mM solution of inorganic phosphate (buffered with sodium bicarbonate) into the gracilis muscle. Values are means $\pm S_{\bar{x}}$. * denotes a significant change from control ($P < 0.05$) N = 5.

Solution	Infusion Rate ml/min	Blood Flow ml/min/100gm	Venous Plasma [Pi] mM/L	Venous Plasma [Osm] mOsm/kg	Venous Blood pH
NaCl (680 mOsm/kg)	0	14 ± 2	1.2 ±0.1	294 ± 5	7.46 ± .05
	.123	16 ± 2	1.1 ±0.1	299 ± 3	7.46 ± .05
	.247	17 ± 2	1.1 ±0.1	303 ± 4	7.46 ± .05
	.494	18 ± 2	1.2 ±0.2	312* ± 3	7.45 ± .06
Pi (680 mOsm/kg, pH 6.8)	0	14 ± 1	1.2 ±0.1	304 ± 3	7.43 ± .06
	.123	16 ± 2	5.0* ±1.0	304 ± 3	7.43 ± .05
	.247	17 ± 2	8.5* ±1.2	304 ± 6	7.40 ± .06
	.494	16 ± 1	14.9* ± 1.9	309 ± 3	7.34* ± .06

phosphate and decreased pH would show vasoactivity. The pH of the infusion solution was 4.5 and the osmolality was 340 mOsm/kg. The results of this group of experiments are shown in Table 5. Clearly, the combination showed no more vasoactivity than the phosphate alone even though there was a significant decrease in venous pH at the higher infusion rates of the unbuffered phosphate solution. Other investigations have also reported that the skeletal muscle vasculature is not regularly affected by simple acidosis (48,170).

Studies on the Myogenic Response

Since these studies required that the gracilis muscle be removed from the dog and suspended in a lucite chamber, it was deemed necessary to determine if such a preparation would respond in a normal manner to a variety of physiological challenges. Consequently, in most muscles the vascular response to acute changes in blood flow (autoregulation and reactive hyperemia) and to certain naturally occurring vasoactive agents was determined.

In 10 muscles the arterial inflow was partially occluded to reduce flow to a mean of 66% of the control for 1 minute. Muscle chamber pressure was maintained at the resting control level. Figure 8 is a tracing from one such experiment. The blood flow record shows that when arterial inflow was reduced from 6 to 5 ml/min for 1 minute, the peak

Table 5. Effects on blood flow, venous plasma inorganic phosphate, venous plasma osmolality and venous blood pH of close-arterial infusion of a 198 mM solution of inorganic phosphate (unbuffered) into the gracilis muscle. Values are means $\pm S_{\bar{x}}$. * denotes a significant change from control ($P < 0.05$) N = 5.

Solution	Infusion Rate ml/min	Blood Flow ml/min/100 gm	Venous Plasma [Pi]mM/L	Venous Plasma [Osm] mOsm/kg	Venous Blood pH
NaCl (340 mOsm/kg)	0	19 ± 4	1.1 ±0.2	301 ± 4	7.42 ± .03
	.123	20 ± 4	1.1 ±0.2	300 ± 4	7.43 ± .02
	.247	18 ± 3	1.1 ±0.2	300 ± 4	7.43 ± .02
	.494	19 ± 3	1.1 ±0.2	302 ± 4	7.44 ± .03
Pi (340 mOsm/kg, pH 4.5)	0	17 ± 3	1.1 ±0.2	302 ± 3	7.44 ± .03
	.123	17 ± 3	3.8* ±0.4	299 ± 3	7.38 ± .01
	.247	17 ± 3	6.9* ±0.7	298 ± 3	7.31* ± .02
	.494	17 ± 3	13.0* ± 1.3	298 ± 3	7.13* ± .04

Figure 8. Typical response of the excised gracilis muscle to a short period (indicated by arrows) of reduced blood flow. Flow reduction was accomplished by partial occlusion of the perfusing artery distal to the site of pressure measurement. Chamber pressure was held at per-occlusion level.

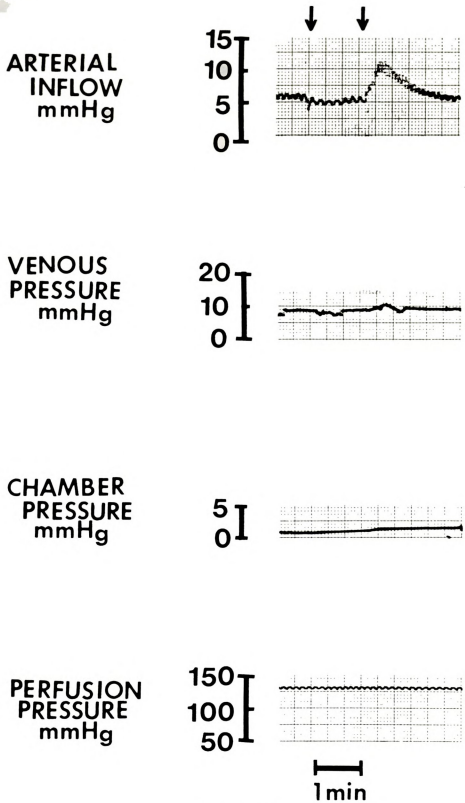

ARTERIAL
INFLOW
mmHg
15
10
5
0
VENOUS
PRESSURE
mmHg
20
10
0
CHAMBER
PRESSURE
mmHg
5
0
PERFUSION
PRESSURE
mmHg
150
100
50
1 min

Figure 8

flow after release of the partial occlusion was 10.0 ml/min. The mean blood flow and resistance from 10 experiments are shown in Figure 9. This shows that when arterial inflow was reduced from 9 ml/min/100 gm to 6 ml/min/100 gm, the peak flow after release of the occlusion was 14 ml/min/100 gm. The vascular resistance at the point of peak post-occlusion hyperemia was 66% of control.

In order to demonstrate that the excised gracilis muscles could autoregulate, pressure-flow curves were run in a few muscles. Figure 10 is a tracing from one such experiment. The muscle was pump perfused at constant flow, thus allowing abrupt increases or decreases in flow while the perfusion pressure was continuously recorded. The example shown illustrates the response to stepwise increases in flow from 5 ml/min to 10, 14, and 19 ml/min. On raising the flow, the perfusion pressure increased, then transiently decreased. The transients represent points of decreased resistance as shown in the pressure-flow curve plotted from the data in this experiment (Figure 11). The resistance then increased to the steady state value, which is indicative of autoregulation.

The vascular responses to an intra-arterial bolus injection of acetylcholine (2 micrograms in 0.1 ml of saline), and to a 20 second period of arterial occlusion are illustrated in Figure 12. The typical dilation in response to

Figure 9. Blood flow and resistance results from 10 experiments in which arterial inflow to the Gracilis muscle was partially occluded for 1 minute as illustrated in Figure 8. Data are control, a point 45 seconds after the beginning of occlusion, and the peak of the hyperemia after release of occlusion. Statistical analysis was done by the paired-t test.

( = control,  = occlusion,  = post-occlusion).

* denotes a significant change from control ($P < 0.05$) n = 10. Values are means + $S_{\bar{x}}$.

ARTERIAL INFLOW
ml/min/100gm
20
15
10
5
0
*
*
RESISTANCE
PRU/100 gm
20
10
0
*

Figure 9

Figure 10. Response of an excised gracilis muscle to step changes in blood flow. The muscle was pump-perfused and flow was increased in a step-wise fashion from 5 ml/min to 10, 14, and 19 ml/min. R = steady-state resistance (mmHg/ml/min) at each flow rate.

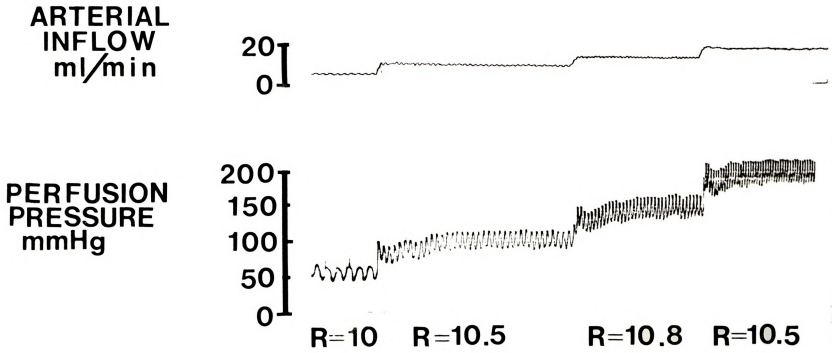

ARTERIAL
INFLOW
ml/min
20
0
PERFUSION
PRESSURE
mmHg
200
150
100
50
0
R=10
R=10.5
R=10.8
R=10.5

Figure 10

Figure 11. Pressure-flow curve and resistances from the experiment described in Figure 10. The unbroken lines show the steady-state values and the broken lines the transients. Autoregulation is demonstrated by the increase in resistance to the steady-state value after a transient decrease.

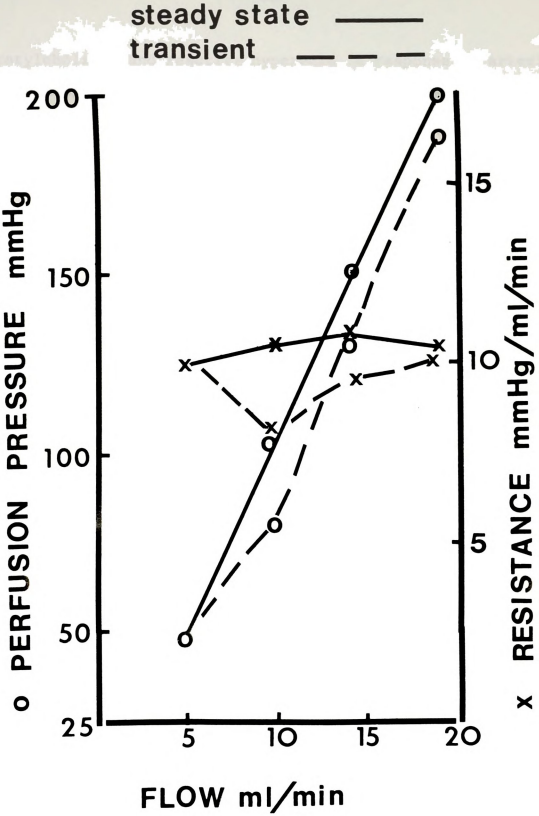

steady state
transient
200
150
100
50
25
o PERFUSION PRESSURE mmHg
15
10
5
x RESISTANCE mmHg/ml/min
5
10
15
20
FLOW ml/min

Figure 11

acetylcholine, and reactive hyperemia in response to arterial occlusion was demonstrated in all experiments. Figure 13 illustrates the vascular response to nerve stimulation (5 volts, 1.6 msec., and 5 pps), and to chamber pulsing. This typical exercise hyperemia (Panel A) was demonstrated in all experiments. Of special importance here is the intramuscular pressure. With each contraction the intramuscular pressure increased by 10 mmHg. The baseline intramuscular pressure changed in the same manner as the chamber pressure which seemed to reflect the changes in volume of the muscle (Figures 12 and 13). In 10 muscles in which intramuscular pressures were recorded, the increase caused by muscle contraction ranged from 10-30 mmHg with an average of 16 mmHg. There was considerable variation between muscles, and pressure within the same muscle was different depending on the location of the pressure transducer. When the muscle chamber pressure was changed, the full amount of the pressure change was transmitted to the intramuscular pressure as seen in Figure 12 (Panel B).

The vascular responses illustrated in Figures 8 to 13 are quite similar to the responses of in situ gracilis muscle preparations used in this laboratory in previous studies (153,164,165). Our measure of intramuscular pressure appeared to be a true measure of the extravascular force that could decrease vascular transmural pressure and trigger a

PERF.
PRESS.
mmHg
150
100
50
0

Figure 12. Response of the in vitro gracilis muscle to an intra-arterial bolus injection of acetylcholine, and to 30 secs of arterial occlusion. Acetylcholine (2 micro-grams in 0.1 ml saline) was injected at first arrow (on left), and the arterial inflow to the muscle was completely occluded for 20 secs (between the pair of arrows on right).

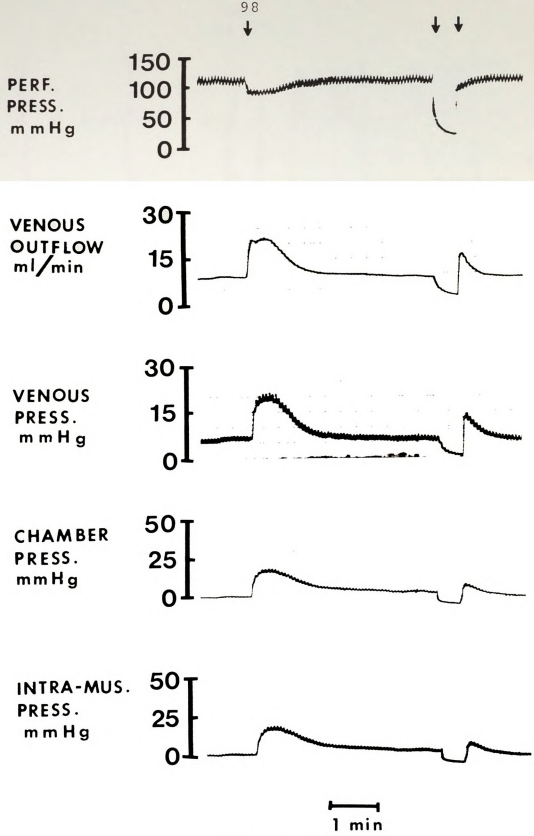

PERF.
PRESS.
mmHg
150
100
50
0
VENOUS
OUTFLOW
ml/min
30
15
0
VENOUS
PRESS.
mmHg
30
15
0
CHAMBER
PRESS.
mmHg
50
25
0
INTRA-MUS.
PRESS.
mmHg
50
25
0
1 min

Figure 12

Figure 13. The vascular response of the in vitro gracilis muscle in 1 min of nerve stimulation (Panel A) and 1 min of chamber pulsing with pulse pressures of 10, 25, and 50 mmHg (Panel B). Experimental maneuvers were carried out between each pair of arrows in each panel.

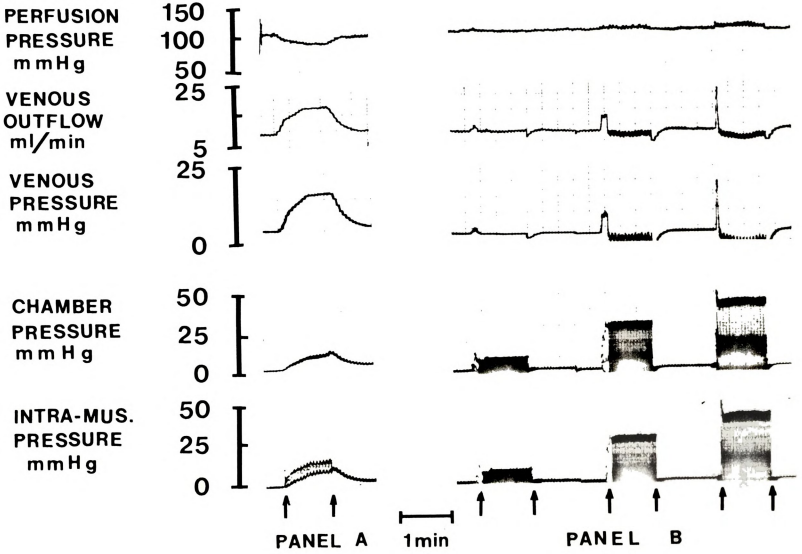

PERFUSION PRESSURE mmHg
150
100
50
VENOUS OUTFLOW ml/min
25
5
VENOUS PRESSURE mmHg
25
0
CHAMBER PRESSURE mmHg
50
25
0
INTRA-MUS. PRESSURE mmHg
50
25
0
PANEL A
1min
PANEL B

Figure 13

myogenic response. Thus we believe that the in vitro gracilis preparation was an adequate model for studying the involvement of the myogenic response in exercise hyperemia.

Series 1: Constant Pressure Studies

In this series of experiments the gracilis muscle was perfused at constant pressure, consequently changes in blood flow are largely the result of changes in vascular resistance. Results obtained from a typical experiment are shown in Figure 14. It is evident that nerve stimulation was immediately associated with a large increase in blood flow (Panel A). After approximately 15 seconds of stimulation gracilis blood flow was threefold greater than resting flow. This increase in flow is comparable to that produced by similar nerve stimulation of an in vivo gracilis muscle (164).

The most important conclusions that can be drawn from this type of experiment are that the hyperemia is independent of extrinsic factors and that it is mediated via alteration in vascular smooth muscle activity rather than alterations in the pressure gradient for flow, blood viscosity and/or passive changes in vessel caliber. However, this type of experiment does not shed light on the mechanism of the smooth muscle relaxation. As stated previously, one major hypothesis indicates that the relaxation is caused by an increased tissue level of vasodilator metabolites, while a second hypothesis states that it is mediated via a myogenic

Figure 14. Tracings obtained from an experiment at constant pressure showing the vascular response to 1 min of nerve stimulation at 5 pps, 5 volts, 1.6 msec (Panel A), 1 minute of chamber pulsing at 5 pps with pulse pressures of 10 mmHg (Panel B), 25 mmHg (Panel C), and 50 mmHg (Panel D). Experimental maneuvers were initiated at the first and terminated at the second arrow in each Panel.

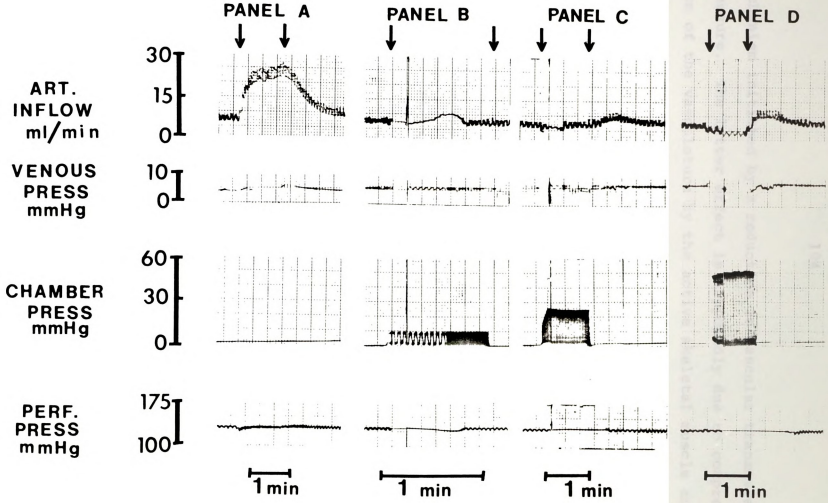

PANEL A
PANEL B
PANEL C
PANEL D
ART. INFLOW ml/min
30
15
0
VENOUS PRESS mmHg
10
0
CHAMBER PRESS mmHg
60
30
0
PERF. PRESS mmHg
175
100
1 min
1 min
1 min
1 min

Figure 14

mechanism initiated by a reduction in vascular transmural pressure. The latter effect is supposedly due to compression of the vasculature by the active skeletal muscle and to an increased interstitial volume.

In order to determine the extent of participation, if any, or a myogenic component to exercise hyperemia, the muscle chamber was pulsed (Figure 14) at 5 pulses per second (pps) with pulse pressures of 10 mmHg (Panel B), 25 mmHg (Panel C), and 50 mmHg (Panel D). It is evident, especially at the highest pulse pressure, that blood flow fell slightly during these pulsatile elevations in extra vascular pressure. The slight rise in flow seen immediately after stopping the chamber pulsations may be due to the previous period of reduced flow. A comparison of Figure 14 Panel D with Figure 8 would support this explanation.

Mean values from ten such experiments are shown in Figure 15. Data are shown for the control period, a point 45 seconds after the start of the experimental maneuver, and 15 secs after the end of chamber pulsing (or the peak of the subsequent hyperemia, if any). On the average, muscle contraction produced by nerve stimulation was associated with a doubling of blood flow and halving of vascular resistance. There were no significant changes in blood flow or resistance during or after muscle chamber pulsing with pulse pressures of 10 and 25 mmHg. However, pulsatile changes in

Figure 15. Comparison of blood flow and resistance results obtained in 10 experiments at constant pressure during 1 minute of muscle chamber pulsing at 5 pps with pulse pressure (PP) of 10, 25, and 50 mmHg with responses obtained during 1 minute of exercise produced by nerve stimulation (5 pps, 5 volts, 1.6 msec). Values are means + $S_{\bar{x}}$ for a control period () a point 45 secs after the start of exercise produced by (NS) nerve stimulation (), a point 45 secs after the start of chamber pulsing (), and a point 15 secs after the end of chamber pulsing (). Statistical evaluation for significant changes was done by the paired-t test.

* significant change from control ($P < 0.05$).

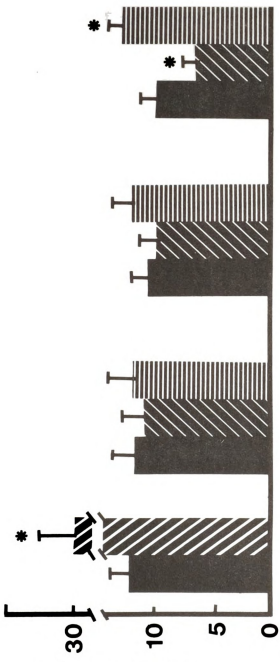

ARTERIAL
INFLOW
ml/min/100gm
30
10
5
0
RESISTANCE
PRU/100gm
20
10
0
NS
PP=10
PP=25
PP=50

Figure 15

external pressure from 0 to 50 mmHg produced an elevation in resistance and a reduction in muscle blood flow to about 60% of control. After pulsing was stopped blood flow increased to a peak at about 30% above control.

An additional group of experiments was done to see whether pulsatile alterations in external pressure at a frequency of 2 pulses per second would elicit a myogenic dilation. The protocol followed is illustrated in Figure 16. Nerve stimulation at 2 pps, 5 volts and 1.6 msec duration, and chamber pulsing at 2 pps with pulse pressures of 10, 25, and 50 mmHg were performed. As seen from these representative tracings, nerve stimulation produced the expected hyperemia. On the other hand, chamber pulsing with pulse pressures of 10 and 25 mmHg had no vascular effect, except that there seems to be a very slight increase in inflow after the pulsing at 25 mmHg was stopped. Again, the decrease in flow during pulsing and the subsequent increase afterwards, is seen with the 50 mmHg pulse pressure.

Average blood flow and resistance data from 10 experiments are shown in Figure 17. Values are presented for a control period, a point 45 secs after the start of the experimental maneuver, and 15 secs after chamber pulsing (or the peak of the resulting hyperemia when present). The results are qualitatively similar to those from the group where the frequency of pulsing was 5 pps. There were no

Figure 16. Tracings obtained from an experiment at constant pressure showing the vascular response to 1 minute of nerve stimulation at 2 pps, 5 volts and 1.6 msec duration (Panel A), and 1 minute of chamber pulsing at 2 pps with pulse pressures of 10 mmHg (Panel B), 25 mmHg (Panel C), and 50 mmHg (Panel D). Experimental maneuvers were carried out between arrows in each Panel.

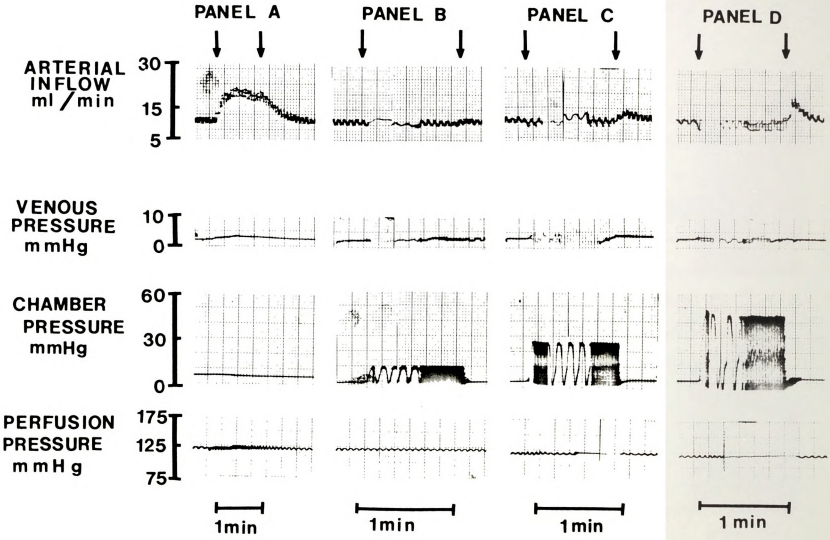

PANEL A
PANEL B
PANEL C
PANEL D
ARTERIAL INFLOW ml/min
30
15
5
VENOUS PRESSURE mmHg
10
0
CHAMBER PRESSURE mmHg
60
30
0
PERFUSION PRESSURE mmHg
175
125
75
1min
1min
1min
1 min

Figure 16

Figure 17. Blood flow and resistance results obtained in 10 experiments during constant pressure perfusion when 1 minute of chamber pulsing at 2 pps with pulse pressures (PP) of 10, 25, and 50 mmHg, and 1 minute of nerve stimulation (2 pps, 5 volts, 1.6 msec) were performed. Values are means + $S_{\bar{x}}$ for a control period ( ), a point 45 secs after the start of exercise produced by (NS) nerve stimulation ( ), a point 45 secs after the start of chamber pulsing ( ), and a point 15 secs after the end of chamber pulsing ( ). Statistical evaluation for significant changes was done by the paired-t test.

* significant change from control ($P < 0.05$).

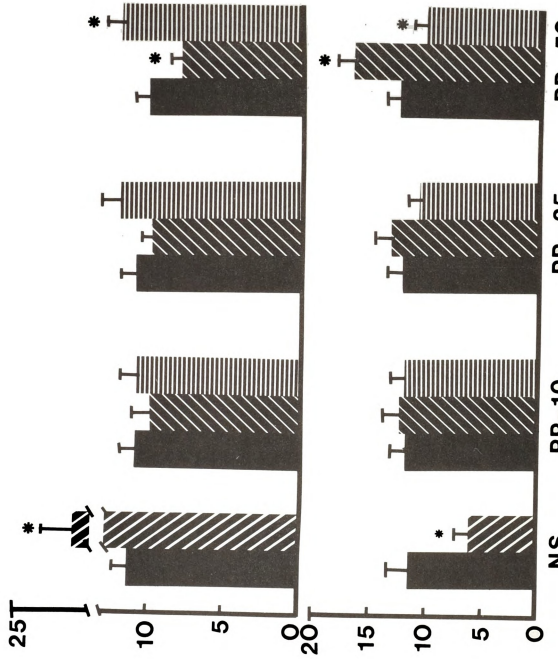

ARTERIAL INFLOW ml/min/100gm
RESISTANCE PRU/100gm
25
10
5
0
20
15
10
5
0
NS
PP=10
PP=25
PP=50

Figure 17

significant changes in flow or resistance during or after pulsing at 10 and 25 mmHg. With the 50 mmHg pulse pressure, there was a significant decrease in flow (by about 25% of control) during pulsing, and a significant increase (peak at about 25% above control) after pulsing ended. The corresponding resistance values show a significant increase during pulsing and a significant fall shortly after pulsing was stopped. Nerve stimulation exercise at 2 pps showed a 50% fall in resistance and a hyperemia which peaked at close to twice the control flow.

The data presented in this section thus far, show that pulsatile decreases in extravascular pressure do not elicit a myogenic dilation. Presence of a myogenic dilation would be manifest as a fall in resistance during chamber pulsing alone, but instead an increase in resistance occurred. The mild hyperemia seen after ending chamber pulsing with 50 mmHg pulse pressure can be explained by the decrease in flow during the pulsing. In additional studies carried out in the constant pressure mode the effects of transient and prolonged increases and decreases in chamber pressure on muscle blood flow were examined.

In one group of experiments the muscle chamber pressure was increased with single pulses of 10, 25, and 50 mmHg, and also decreased with single pulses of -10, -25, and -50 mmHg. The single pulses were obtained by adding or withdrawing

volume from the air-tight chamber by a large syringe through a rubber sleeve at the Ringer's inflow port. Because of the time lag inherent in this procedure, the single pulse excursions lasted for 4-8 secs. A representative tracing of the response to positive single pulse pressures is shown in Figure 18. It can be seen that the 10 mmHg pulse did not affect blood flow, while the 25 mmHg pulse produced a slight flow increase after the pulse. The 50 mmHg pulse produced a noticeable decrease in flow at the time the pressure pulse peaked, which was followed by a marked hyperemia. This hyperemia is most likely a reactive hyperemia due to the brief decrease in flow at the time the pressure peaked (Panel C). Figure 19 shows the blood flows and calculated resistances before, at the point the pressure pulse peaked, and 15 secs after the muscle chamber pressure was returned to zero (or at the time of the peak hyperemia, if any). Only the 50 mmHg pulse produced a significant hyperemia (a 20% fall in resistance and a peak flow increase of about 40% above control) following the pulse. At the time the pressure excursion peaked, the blood flow was significantly reduced (by 15% of control).

Figure 20 is a representative tracing of the vascular response to single negative pulses of -10, -25, and -50 mmHg. There was no significant vascular response induced by these changes. However, during the -50 mmHg pulse there was an

Figure 18. Tracings obtained from an experiment at constant pressure showing the vascular response to single positive pulse changes of 10 mmHg (Panel A), 25 mmHg (Panel B), and 50 mmHg (Panel C) in muscle chamber pressure. Arrows in each panel designate the peak of the pressure change.

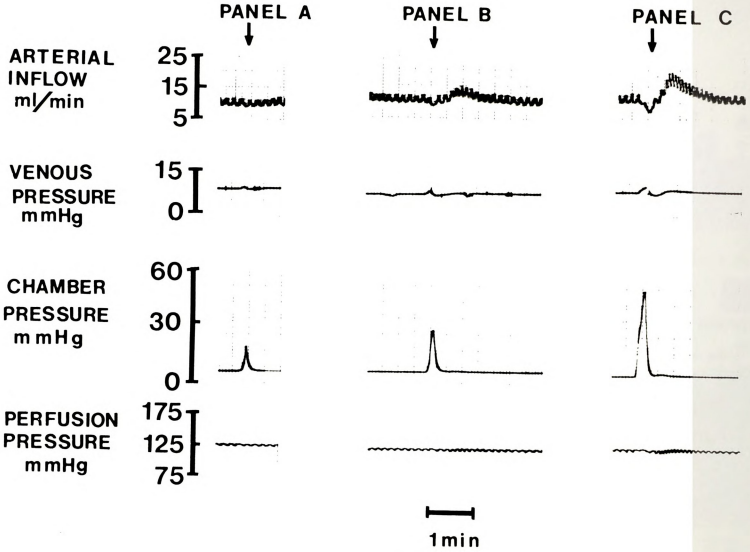

PANEL A
PANEL B
PANEL C
ARTERIAL INFLOW ml/min
25
15
5
VENOUS PRESSURE mmHg
15
0
CHAMBER PRESSURE mmHg
60
30
0
PERFUSION PRESSURE mmHg
175
125
75
1min

Figure 18

Figure 19. Blood flow and resistance results obtained at constant pressure when single pulse pressure (PP) changes of 10, 25, and 50 mmHg were made in muscle chamber pressure. Values are means + $S_{\bar{x}}$ for the control (), at the peak of the pulse (), and 15 seconds after the muscle chamber pressure returned to zero (or the peak of the resulting hyperemia) (). Statistical evaluation was done by the paired-t test. * significant change from control ($P < 0.05$) $n = 5$.

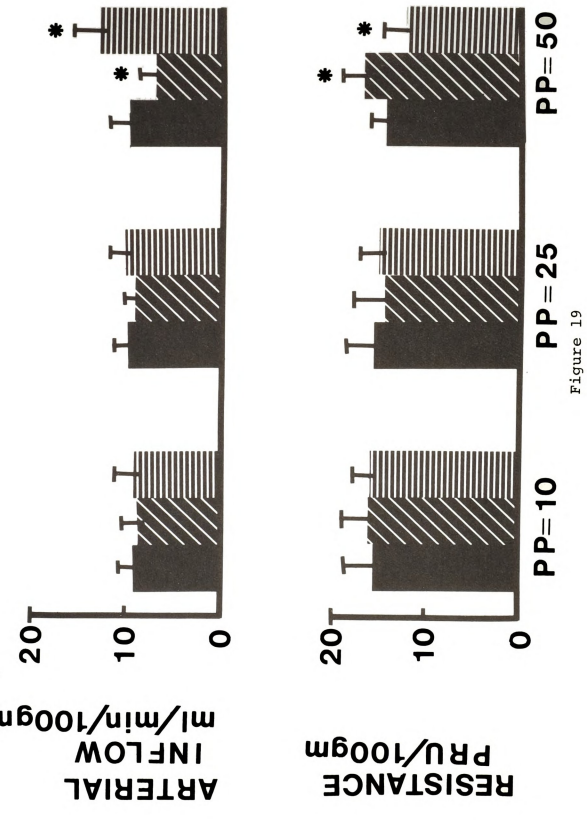

ARTERIAL
INFLOW
ml/min/100gm
20
10
0
*
*
RESISTANCE
PRU/100gm
20
10
0
*
*
PP=10
PP=25
PP=50

Figure 19

Figure 20. Tracings obtained from an experiment at constant pressure showing the vascular response to single negative pulse changes of -10 mmHg (Panel A), -25 mmHg (Panel B), and -50 mmHg (Panel C) in muscle chamber pressure. Arrows in each panel designate the peak of the pressure change.

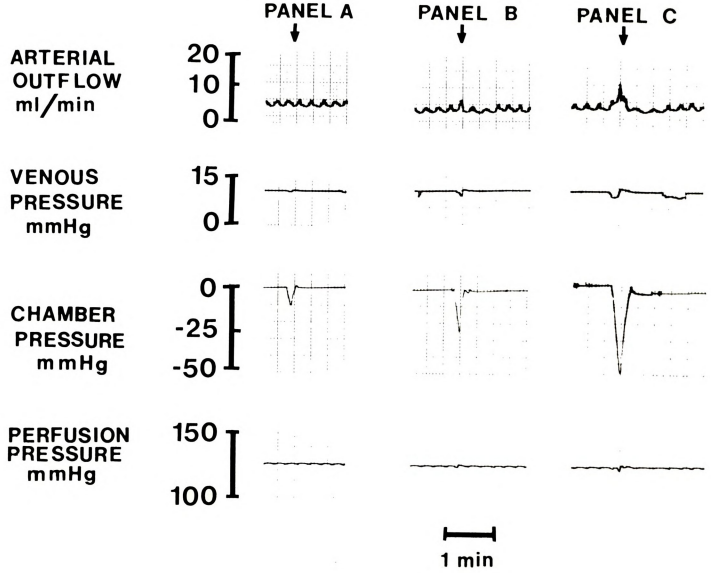

PANEL A
PANEL B
PANEL C
ARTERIAL OUTFLOW ml/min
20
10
0
VENOUS PRESSURE mmHg
15
0
CHAMBER PRESSURE mmHg
0
-25
-50
PERFUSION PRESSURE mmHg
150
100
1 min

Figure 20

increase in flow which can be attributed to a passive increase in vessel diameter. If there had been any myogenic constriction, a decrease in flow below control shortly after the pressure change, should have resulted. The data summary (Figure 21) shows that the only effects of negative single pulse changes in muscle chamber pressure was the passive increase in flow at the peak of the -50 mmHg pulse.

In another group of experiments the vascular effects of sustained positive and sustained negative changes in muscle chamber pressure were examined. Figure 22 shows representative tracings from an experiment where the muscle chamber pressure was increased by 10, 25, and 50 mmHg for periods of 1 minute. It can be seen that the 10 mmHg increase was without effect while the 25 and 50 mmHg increases produced significant hyperemia after the muscle chamber pressure returned to zero. Note that muscle flow fell during the period of increased chamber pressure. Calculated resistances and blood flow were obtained for the control period, a point 45 secs after the pressure increase began, and the peak of the hyperemia after muscle chamber pressure was returned to zero. These data are shown in Figure 23. With the 25 mmHg increase in pressure there was a 10% decrease in flow (approximately 35% increase in resistance) during, and a 50% increase in flow (approximately 35% decrease in resistance) after application of the pressure. The 50 mmHg

Figure 21. Blood flow and resistance results obtained at constant pressure when single pulse pressure (PP) of -10, -25, and -50 mmHg were made in the muscle chamber pressure. Values are means + $S_{\bar{x}}$ for the control period ( ), at the peak of the pulse ( ), and 15 seconds after the muscle chamber pressure returned to zero ( ). Statistical analysis was done by the paired-t test. * significant change ($P < 0.05$) $n = 5$.

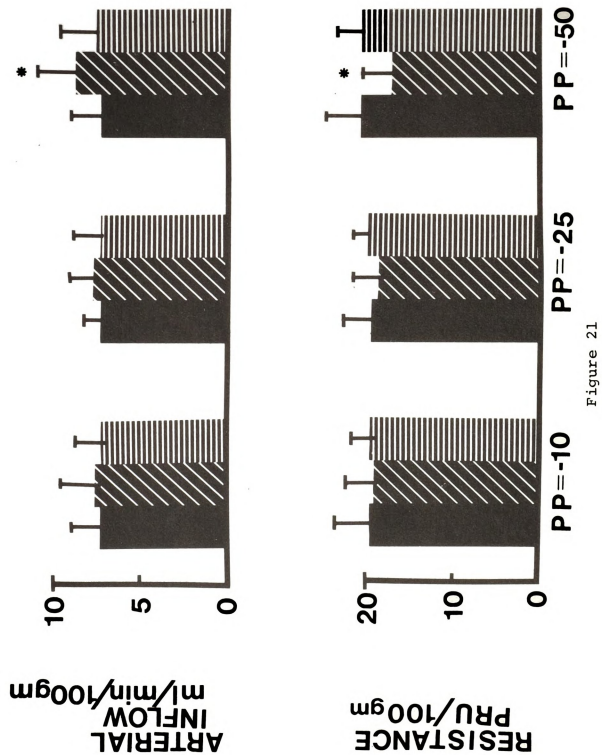

ARTERIAL INFLOW ml/min/100gm
10
5
0
*
RESISTANCE PRU/100gm
20
10
0
*
PP=-10
PP=-25
PP=-50

Figure 21

Figure 22. Representative tracings from an experiment at constant pressure where the muscle chamber pressure was increased for periods of 1 minute to 10 mmHg (Panel A), 25 mmHg (Panel B), and 50 mmHg (Panel C). In each panel, the first arrow marks the beginning and the second arrow the ending to change in chamber pressure.

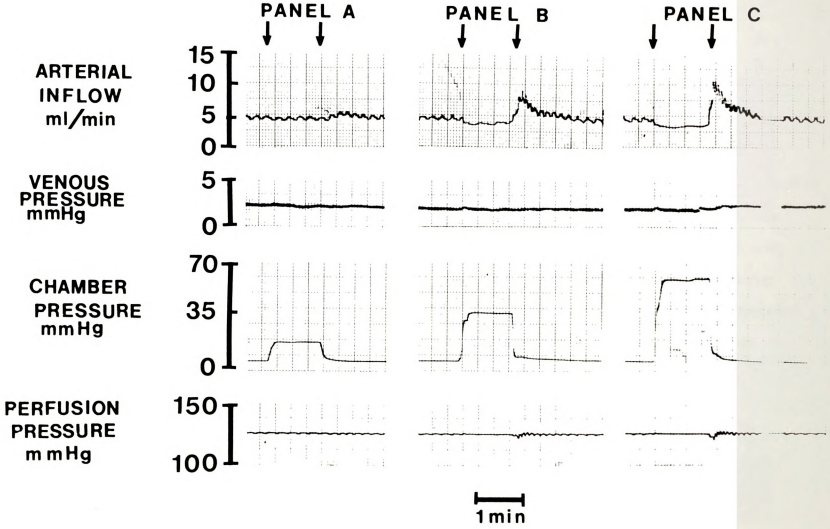

PANEL A
PANEL B
PANEL C
ARTERIAL INFLOW ml/min
15
10
5
0
VENOUS PRESSURE mmHg
5
0
CHAMBER PRESSURE mmHg
70
35
0
PERFUSION PRESSURE mmHg
150
100
1 min

Figure 22

Figure 23. Blood flow and resistance results obtained at constant perfusion pressure when chamber pressure (P) was increased for 1 minute periods to 10, 25, or 50 mmHg. Values are means + $S_{\bar{x}}$ for a control period (), a point 45 secs after the pressure increase was initiated (), and a point 15 secs (or the peak of the hyperemia) after the muscle chamber was returned to zero pressure (). Statistical evaluation was done with the paired-t test. * significant change ($P < 0.05$) n = 5.

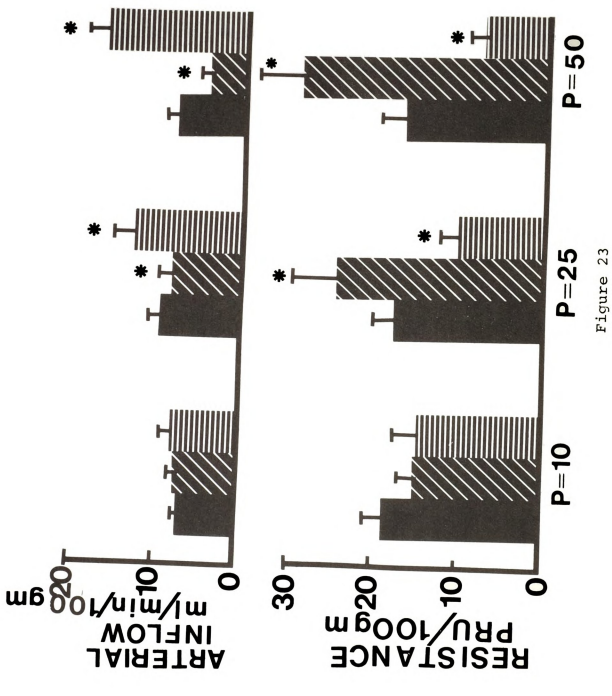

ARTERIAL INFLOW
ml/min/100gm
0
10
20
RESISTANCE
PRU/100gm
0
10
20
30
P=10
P=25
P=50
*

Figure 23

pressure increase showed a 50% decrease in flow during pressure application and a 100% increase afterwards.

Representative tracings from an experiment where the muscle chamber pressure was decreased by 10, 25 and 50 mmHg for 2 minutes are shown in Figure 24. It can be seen that the 10 mmHg decrease had no effect. With the 25 and 50 mmHg decreases, blood flow increased with the application of the pressure, and then gradually returned towards control even though the negative pressure was maintained constant. Blood flow and calculated resistance for a control period, the peak of the flow increase after application of the negative pressure, the points immediately before and after the muscle chamber pressure was returned to zero are shown in Figure 25. The increase inflow seen during the 25 mmHg decrease in pressure was not statistically significant but that seen with the 50 mmHg was. It is important to note that the flow and resistance were back to control values by the end of the 2 minutes of sustained negative pressure. This suggests that active constriction of blood vessels occurred during the increase in transmural pressure. However, the mechanism of the constriction cannot be delineated from these experiments. Both myogenic factors and loss of vasodilator metabolites may be responsible.

Series 2: Natural Flow

This series of experiments was performed to confirm the results of the studies on simulated exercise at constant

Figure 24. Representative tracing from experiments designed to study the vascular effects of sustained increases in transmural pressure. The muscle chamber pressure was decreased by 10 mmHg (Panel A), 25 mmHg (Panel B), and 50 mmHg (Panel C). Each negative pressure was maintained for 2 minutes. Perfusion pressure was maintained constant throughout the experiment. In each panel the pressure change was initiated at the first and terminated at the second arrow.

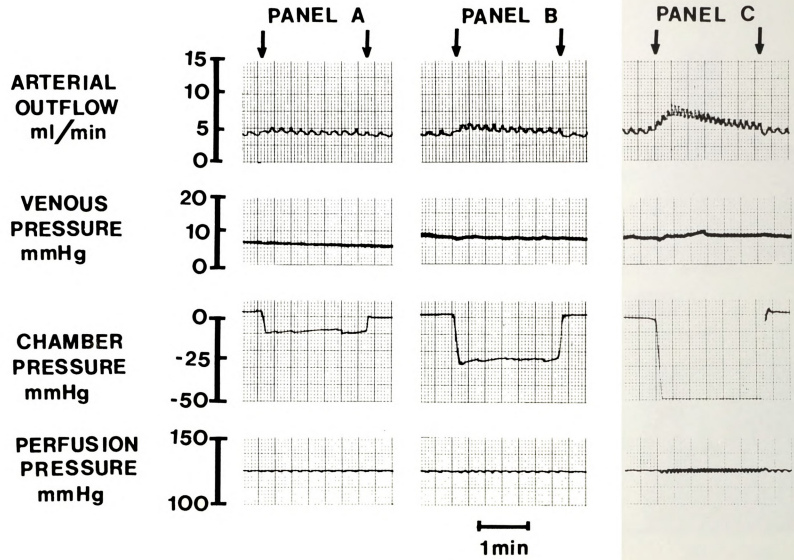

PANEL A
PANEL B
PANEL C
ARTERIAL OUTFLOW ml/min
15
10
5
0
VENOUS PRESSURE mmHg
20
10
0
CHAMBER PRESSURE mmHg
0
-25
-50
PERFUSION PRESSURE mmHg
150
100
1min

Figure 24

Figure 25. Blood flow and resistance results obtained at constant pressure when muscle chamber pressure (P), was decreased to -10, -25, and -50 mmHg for 2 minute periods. Values are means + $S_{\bar{x}}$ for control ( ), peak change after application of pressure ( ), immediately before chamber pressure was returned to zero pressure ( ), and immediately after the chamber pressure was returned to zero ( ). Statistical evaluation was done by the paired-t test. * denotes a significant change from control ($P < 0.05$) n = 5.

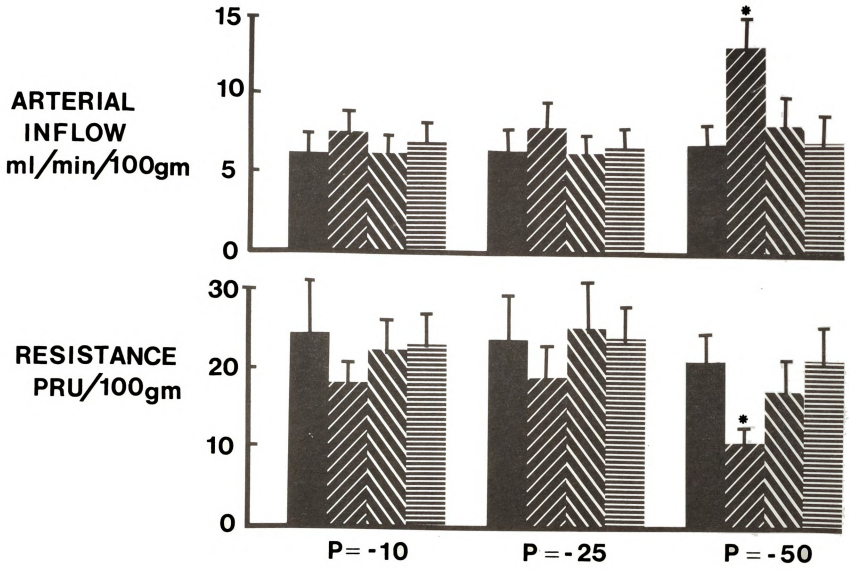
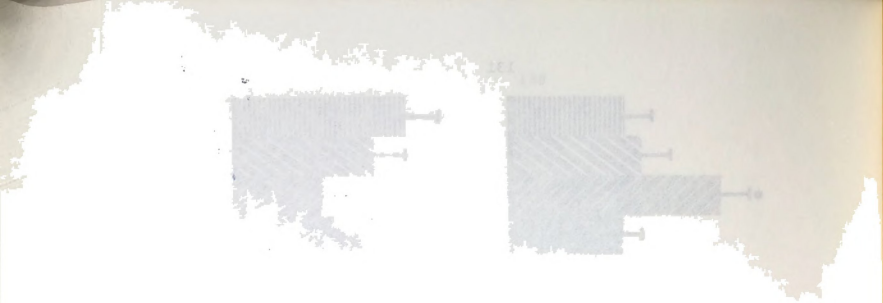

ARTERIAL
INFLOW
ml/min/100gm
15
10
5
0
*
RESISTANCE
PRU/100gm
30
20
10
0
*
P = -10
P = -25
P = -50

Figure 25



pressure by showing that the pump and artificial control system did not affect the results obtained, and to show that the muscle preparation was capable of showing an active hypereia during pulsing of the muscle chamber.

In 7 muscles, the vascular responses to 1 minute of nerve stimulation at 5 pps, 5 volts and 1.6 msec duration were compared to 1 minute of chamber pulsing at 5 pps with a pulse pressure of 50 mmHg, and also to 1 minute of simultaneous nerve stimulation plus chamber pulsing. In this group of experiments, muscle perfusion was directly from the abdominal aorta via a catheter in the femoral artery, and venous outflow was recorded continuously with an electromagnetic flow-meter. The muscle perfusion and venous pressures were measured via catheters resting in the respective femoral vessels at the junction with the gracilis vessels. Figure 26 shows typical responses obtained in one such experiment. With nerve stimulation alone there was an increase in venous outflow from 9 to 18 ml/min. Associated with the rise in blood flow there was a slight decrease in muscle perfusion pressure and increases in venous as well as muscle chamber pressures. With chamber pulsing alone there was a transient increase in venous outflow followed by a sustained decrease; perfusion pressure increased by 10 mmHg and venous pressure decreased. After the pulsing ended there was a transient decrease in venous outflow followed by

Figure 26. Representative tracings from an experiment at natural flow where the vascular response to 1 minute of exercise produced by nerve stimulation at 5 pps, 5 volts, 1.6 msec (Panel A), were compared to 1 minute of chamber pulsing at 5 pps with a pulse pressure of 50 mmHg (Panel B), and 1 minute of simultaneous nerve stimulation plus chamber pulsing (Panel C). In each panel, experimental maneuvers began at first and ended at second arrow.

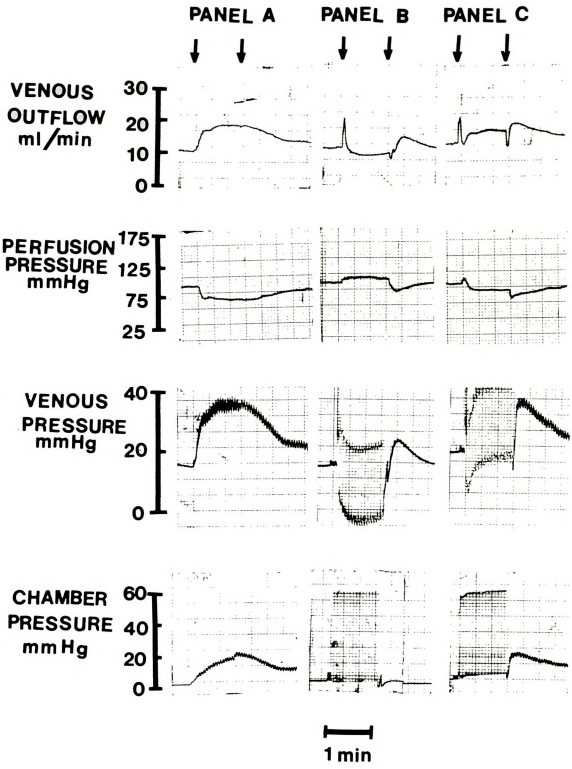

PANEL A
PANEL B
PANEL C
VENOUS OUTFLOW ml/min
30
20
10
0
PERFUSION PRESSURE mmHg
175
125
75
25
VENOUS PRESSURE mmHg
40
20
0
CHAMBER PRESSURE mmHg
60
40
20
0
1 min

Figure 26

a significant hyperemia. The transient increase seen at the beginning of the pulsing most likely resulted from the passive expression of vascular volume, and the transient decrease at the end of pulsing resulted from the refilling of the blood vessels. With simultaneous nerve stimulation plus chamber pulsing, there was a transient increase in outflow followed by a sustained hyperemia (flow increased from 8 to 16 ml/min. Perfusion pressure increased transiently then fell below control. Venous and muscle chamber pressure increased slightly. After the pulsing and nerve stimulation ended there was a further increase in venous outflow, drop in perfusion pressure and increase in venous as well as chamber pressure. Blood flow and calculated resistance for a control period, and a point 45 seconds after the start of each maneuver are shown in Figure 27. It can be seen that nerve stimulation and simultaneous nerve stimulation plus chamber pulsing produced quite similar effects on muscle hemodynamics. Both produced approximately a threefold increase in flow and a 70% decrease in resistance. In contrast chamber pulsing alone produced a significant decrease in flow (by about 40% of control) and a 50% increase in resistance.

In 5 of the muscles used in the above experiments, the same experimental maneuvers were done at 2 pps instead of 5 pps. The results were qualitatively the same.

Figure 27. Blood flow and resistance results obtained at natural flow when the vascular response to 1 minute of nerve stimulation (NS) at 5 pps, 5 volts, 1.6 msec, was compared to 1 minute of chamber pulsing (CP) at 5 pps with pulse pressures of 50 mmHg, and 1 minute of simultaneous nerve stimulation plus chamber pulsing (NS + CP). Values are means + $S_{\bar{x}}$ for control ( ) and 45 seconds after the start of the experimental maneuver ( ). Statistical analysis was done by a two-way analysis of variance with the means compared using the Tukeys procedure. Values designated with * are significantly different from their respective controls ($P < 0.05$). Blood flow values marked with an open circle ( ) and those with a closed circle ( ) are not different within their respective group. N = 7.

VENOUS OUTFLOW ml/min/100gm
0
10
20
30
NS
CP
NS+CP
RESISTANCE PRU/100gm
0
5
10
15
20
25
NS
CP
NS+CP

Figure 27

Figure 28. Representative tracings from an experiment at natural flow where the vascular response to 1 minute of nerve stimulation at 2 pps, 5 volts, 1.6 msec (Panel A), were compared to the vascular response to 1 minute of chamber pulsing at 2 pps with a pulse pressure of 50 mmHg (Panel B), and to 1 minute of simultaneous nerve stimulation plus chamber pulsing (Panel C). The experimental maneuver began at the first and ended at the second arrow.

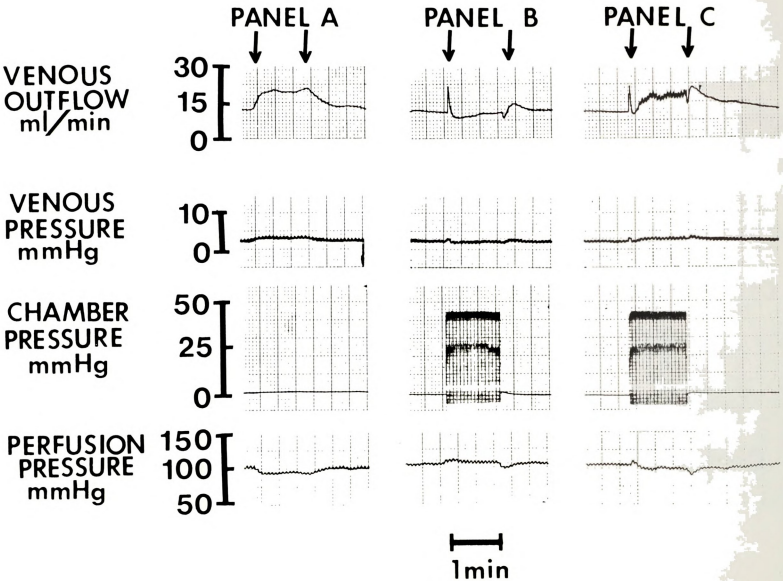

PANEL A
PANEL B
PANEL C
VENOUS OUTFLOW ml/min
30
15
0
VENOUS PRESSURE mmHg
10
0
CHAMBER PRESSURE mmHg
50
25
0
PERFUSION PRESSURE mmHg
150
100
50
1min

Figure 28

Figure 29. Blood flow and resistance results obtained at natural flow when the vascular response to 1 minute of nerve stimulation (NS) at 2 pps, 5 volts, 1.6 msec, was compared to 1 minute of chamber pulsing (CP) at 2 pps with pulse pressures of 50 mmHg, and 1 minute of simultaneous nerve stimulation plus chamber pulsing (NS + CP). Values are means + $S_{\bar{x}}$ for control ( ) and 45 seconds after the start of the experimental maneuver ( ). Statistical analysis was done by a two-way analysis of variance with the means compared using the Tukeys procedure. Values designated with * are significantly different from their respective controls ($P < 0.05$). Blood flow values marked with an open circle ( ) and those with a closed circle ( ) are not different within their respective group. N = 5.

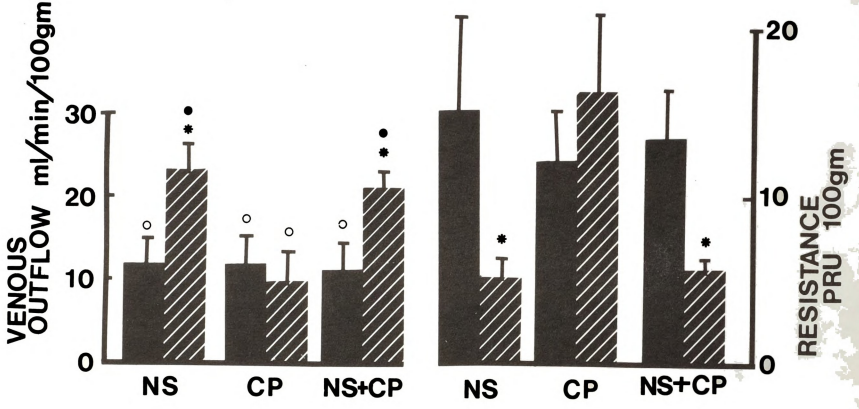

VENOUS OUTFLOW ml/min/100gm
30
20
10
0
NS
CP
NS+CP
NS
CP
NS+CP
20
10
0
RESISTANCE PRU 100gm

Figure 29

Figure 28 shows representative tracings and Figure 29 a summary of the data collected from these experiments. It can be seen that nerve stimulation (NS) and simultaneous nerve stimulation plus chamber pulsing (NS + CP) caused equal increases in flow (about 100% above control) and equivalent decreases in resistance. Chamber pulsing (CP) alone did not affect blood flow or resistance significantly. This strengthens the conclusion that during pulsatile changes in extravascular pressure (decreases in transmural pressure) no active relaxation of the vascular smooth muscle occurred.

Series 3: Constant Flow Studies

The vascular effects of nerve stimulation, chamber pulsing and simultaneous nerve stimulation plus chamber pulsing at 5 pps as well as 2 pps were studied under conditions of constant blood flow. This group of experiments was designed to show whether the hyperemia seen after chamber pulsing was due to a myogenic response or was simply the result of a reduced blood flow during the procedure. If the myogenic response was involved, a dilation evidenced by a fall in perfusion pressure after chamber pulsing would be seen.

A representative experiment from a group of 12 at 5 pps is illustrated in Figure 30. During nerve stimulation, the perfusion pressure showed the expected decrease (to about 30% of control). Upon termination of nerve stimulation, perfusion pressure gradually returned to control.

Figure 30. Tracings obtained from an experiment at constant flow showing the vascular responses to 1 minute of nerve stimulation at 5 pps, 5 volts, 1.6 msec (Panel A), 1 minute of chamber pulsing at 5 pps, 50 mmHg pulse pressure (Panel B) and 1 minute simultaneous nerve stimulation plus chamber pulsing (Panel C). Experimental maneuvers began at first and ended at second arrow in each panel.

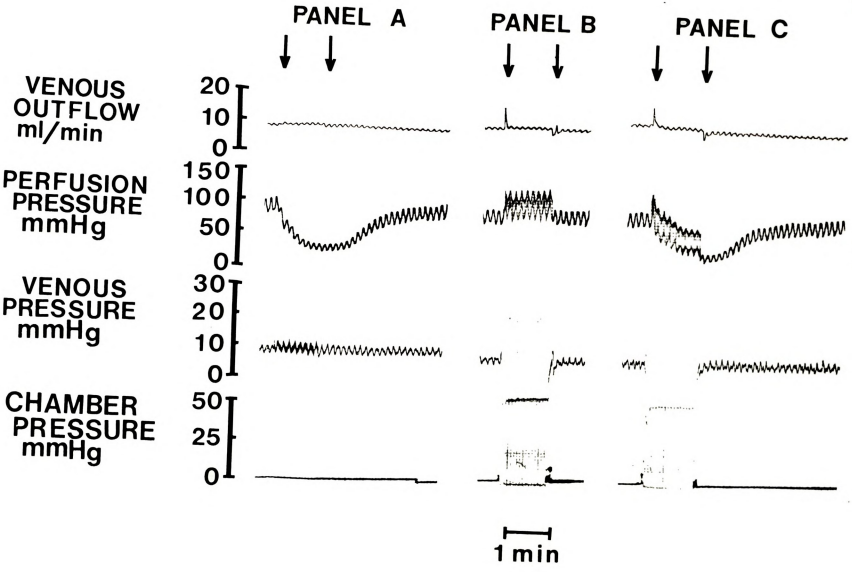

PANEL A
PANEL B
PANEL C
VENOUS OUTFLOW ml/min
20
10
0
PERFUSION PRESSURE mmHg
150
100
50
0
VENOUS PRESSURE mmHg
30
20
10
0
CHAMBER PRESSURE mmHg
50
25
0
1 min

Figure 30

With chamber pulsing alone there was an increase in mean perfusion pressure which amounted to 15 mmHg. It seemed that the full value of the pressure change was transmitted to the arterial pressure as the pulse pressure was 50 mmHg. In this case, the vascular transmural pressure was not being affected. The blood flow tracing shows a transient increase at the beginning and decrease at the end of chamber pulsing. These transients probably represent expression and refilling of blood in the vascular system. Upon termination of chamber pulsing, perfusion pressure returned to control. There was no evidence of active dilation at this point. The response to simultaneous nerve stimulation and chamber pulsing appears quite similar to a superimposition of the separate responses.

Average changes from 12 such experiments are shown in Figure 31. The data shown are for the control period and a point 45 secs after the start of each experimental maneuver. Nerve stimulation (NS) and simultaneous nerve stimulation plus chamber pulsing (NS + CP) produced an approximate 50% fall in perfusion pressure. Chamber pulsing (CP) alone produced a slight increase in perfusion pressure. The corresponding resistance was not significantly different from control. The reason the latter parameter was not increased may well be related to the concomitant increase in venous pressure that occurred during the maneuver.

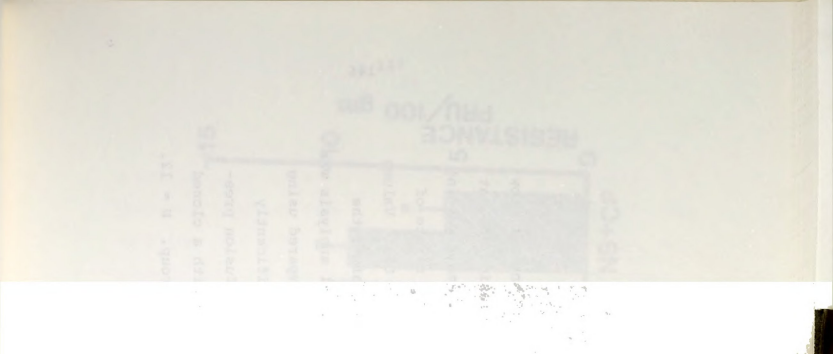


Figure 31. Perfusion pressure and resistance results obtained at constant flow when the vascular response to 1 minute of nerve stimulation (NS) at 5 pps, 5 volts, 1.6 msec was compared to 1 minute of chamber pulsing (CP) at 5 pps with the pulse pressures of 50 mmHg, and 1 minute of simultaneous nerve stimulation and chamber pulsing (NS + CP). Values are means + $S_{\bar{x}}$ for control (▬) and a point 45 secs after the start of the experimental maneuver (▨). Statistical analysis was done by a two-way analysis of variance with the means compared using the Tukeys procedure. Values designated with * are significantly different from their respective controls ($P < 0.05$). Perfusion pressure values marked with an open circle (◎) and those with a closed circle (●) are not different within their respective group. N = 12.

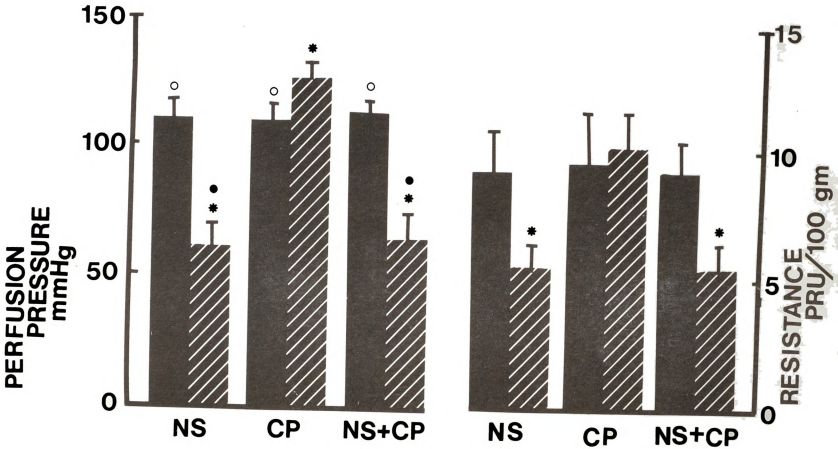

150
100
50
0
PERFUSION PRESSURE mmHg
15
10
5
0
RESISTANCE PRU/100 gm
NS
CP
NS+CP
NS
CP
NS+CP

Figure 31

In 8 muscles the experimental protocol was repeated at a frequency of 2 pps for nerve stimulation and chamber pulsing. Tracings from a representative experiment is shown in Figure 32. The responses obtained are qualitatively the same as those obtained at 5 pps. With nerve stimulation, perfusion pressure decreased from 90 mmHg to about 55 mmHg in 45 secs. With chamber pulsing alone, mean perfusion pressure increased slightly (by about 15 mmHg), mean venous pressure increased by about 10 mmHg and the venous outflow record showed the transient increase at the beginning and decrease at the end of pulsing. As was suggested before, these transients in blood flow resulted from the mechanical compression and relaxation of the muscle vasculature. Again, simultaneous nerve stimulation and chamber pulsing produced a response best described as a superimposition of the two separate responses.

Figure 33 presents average data from this group of experiments. The data shown are for a pre-experimental control and a point 45 secs after the start of the experimental maneuver. It can be seen that nerve stimulation (NS) and simultaneous nerve stimulation plus chamber pulsing (NS + CP) produced an approximate 30% fall in perfusion pressure and a 35% fall in resistance. Chamber pulsing (CP) alone produced a significant increase in perfusion pressure to approximately 15% above control. However, the corresponding

Figure 32. Tracings obtained from an experiment at constant flow showing the vascular responses to 1 minute of nerve stimulation at 2 pps, 5 volts, 1.6 msec (Panel A), 1 minute of chamber pulsing at 2 pps, 50 mmHg pulse pressure (Panel B) and 1 minute simultaneous nerve stimulation plus chamber pulsing (Panel C). Experimental maneuvers began at first and ended at second arrow in each panel.

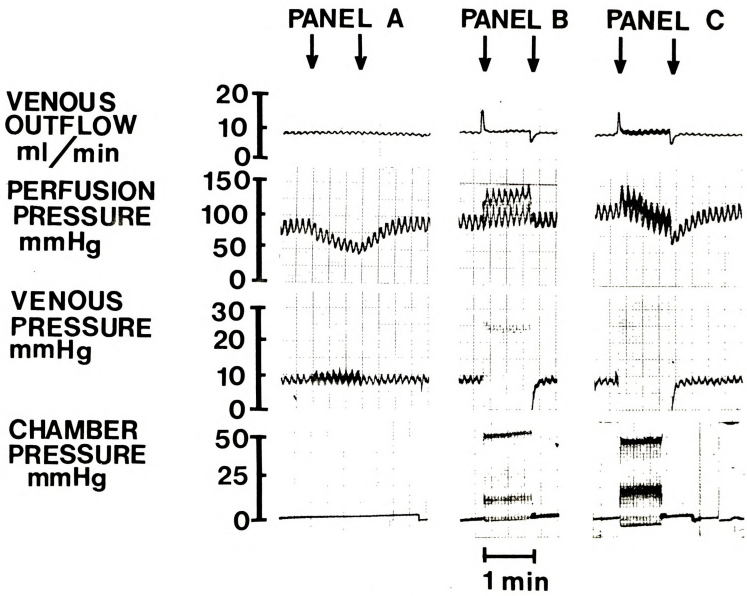

PANEL A
PANEL B
PANEL C
VENOUS OUTFLOW ml/min
20
10
0
PERFUSION PRESSURE mmHg
150
100
50
0
VENOUS PRESSURE mmHg
30
20
10
0
CHAMBER PRESSURE mmHg
50
25
0
1 min

Figure 32

LOWER C

Figure 33. Perfusion pressure and resistance results obtained at constant flow when the vascular response to 1 minute of nerve stimulation (NS) at 2 pps, 5 volts, 1.6 msec was compared to 1 minute of chamber pulsing (CP) at 2 pps with pulse pressures of 50 mmHg and 1 minute of simultaneous nerve stimulation plus chamber pulsing (NS + CP). Values are means + $S_{\bar{x}}$ for control (▬) and a point 45 secs after the start of the experimental maneuver (▨). Statistical analysis was done by a two-way analysis of variance with the means compared using the Tukeys procedure. Values designated with an * are significantly different from their respective controls ($P < 0.05$). Perfusion pressure values marked with an open circle (○) and those with a closed circle (●) are not different within their respective group. N = 12.

PERFUSION PRESSURE mmHg
125
100
75
50
NS
CP
NS+CP
RESISTANCE PRU/100gm
15
10
5
0
NS
CP
NS+CP

Figure 33

resistance value is not significantly different from control since the venous pressure had also increased.

This series of studies show that when pulsatile changes in extravascular pressure were made in an isolated muscle perfused at constant flow, there was no active dilation during nor immediately after the pulsing.

VII. DISCUSSION

Numerous metabolites as well as oxygen have been proposed to mediate active hyperemia. Some investigators believe that several of these factors act in concert to produce the responses (163,164,167). However, in recent years two groups of investigators have proposed two separate metabolites that they maintain, play dominant roles in active hyperemia. In 1967 Mellander (133) reported that local changes in interstitial osmolality during exercise could account for 70-80% of the dilation observed. In 1969, Hilton (88) presented data to show that changes in interstitial inorganic phosphate could be the main cause of the response. Other investigators have not been able to confirm these studies, thus the roles of these two factors remain controversial. It was one purpose of the present studies to see whether lymph from contracting muscle would exhibit any changes in the concentration of these agents, since their concentrations in the interstitium is thought to be greatly increased.

A second objective of these studies was to delineate the contribution, if any, of the myogenic response to active hyperemia. Since intramuscular pressure increases during

muscle contraction (61,139,174,184), it has been suggested that blood vessels would be compressed during exercise, thus decreasing vascular transmural pressure and thereby eliciting a myogenic relaxation of vascular smooth muscle.

Vasoactive Metabolites in Lymph From the Hindlimb

The Anatomy of the Lymphatic System

Three different lymphatic beds have been described in the hindlimb of the dog (152). These are: 1) a lateral superficial system which drains the paw and lower leg (skin and some muscle) into the popliteal lymph node, 2) a medial superficial system which drains the skin from mid-calf to the groin into the inguinal lymph node, and 3) a deep medial system which drains predominantly muscle, does not enter the popliteal lymph node, and runs deep with the femoral artery and vein. This deep lymphatic system is the one from which we collected lymph samples. The occlusive clamp at the knee (Figure 1) excluded lymph from the lower leg. Patent Blue Violet dye injected into the skin and muscle distal to the clamp (at rest or during exercise) was never seen in the lymph vessels central to the clamp. Also, dye injected in the skin above the clamp did not enter the deep lymph vessels. When dye was injected into the muscles above the clamp, the deep lymph vessels became colored within 5 minutes depending on the distance between injection site

and the isolated vessel. By such injections it was determined that the gracilis, semi-membranosus, semi-tendinosus, sartorius and vastus medialis muscles contributed to the lymph samples collected. The extent of the contribution of the other muscles and bone to the lymph was not determined. For the reasons stated above, we believe that predominantly muscle lymph was collected in these experiments.

Lymph Flow and Protein Concentration

Other studies have shown that lymph flow from the hindlimbs of anesthetized animals at rest is practically zero (8,97). Bach and Lewis (8), and Jacobsson and Kjellmer (97) used passive limb movements in order to collect control lymph samples in their preparations. In our studies, we increased the extracellular volume of the animal by I.V. infusions of saline (50 ml/kg). This maneuver caused only a small increase in lymph flow, and a gentle massaging action was used to "milk" lymph from the periphery towards the collection catheter. None of these procedures affected blood flow or the calculated vascular resistance in the hindlimb preparation.

A twofold increase in lymph flow during muscle contraction of the dog hindlimb (97), and rabbit hindlimb (8) has been reported. We found a threefold increase in lymph flow during hindlimb exercise. The difference here seems to be a result of the different frequencies of muscle contraction.

Contractions at 2/sec used by Jacobsson and Kjellmer (97) may not produce the same hemodynamic changes as contractions at 6/sec as used in our studies. Contractions at 20/sec used by Bach and Lewis (8) may actually retard lymph flow during the contraction period. Also, the difference in lymph flow rate could be the result of a different technique for passively increasing flow. The other workers passively flexed and extended the limb, while we applied a direct pressure with a massaging action by hand.

The protein concentration in lymph from muscle does not change during muscle contraction (8,83,97). This was confirmed by the present studies. Since the capillary filtration coefficient (CFC) increases two to threefold during exercise (115), since the net flux of fluid is from the vascular to extravascular space (13,98,115,128), and since capillary permeability is reported not to increase (6,115, 128), it might be expected that lymph protein concentration would decrease due to dilution with plasma water. However, as seen in Table 1, lymph protein concentration was the same before as during exercise. Protein transport was elevated. This could be explained if only a small portion of the capillary filtrate is drained off by the lymphatic system, and if the lymph vessels allow water but not protein to escape across their walls. Jacobsson and Kjellmer (97) showed that only 5-10% of the capillary filtrate is drained away from

active muscle by the lymphatics. However, the entire protein content of the capillary filtrate can be transported by the lymphatics. This suggests that protein becomes concentrated as the lymph moves along the lymph vessels, and that the bulk of the capillary filtrate is returned to the blood stream via transcapillary reabsorbtion (83,186).

An increased protein transport in the presence of an unchanged lymph protein concentration could also occur in another manner. It has been shown that the capillary filtration coefficient increases with exercise (115), while capillary permeability does not change (6,115,128). For this to obtain, capillary surface area must be increased (83). Thus many previously closed capillaries become open during exercise, produce lymph having the same protein concentration as before exercise, and give rise to the increased protein transport.

Osmolality, Potassium and Inorganic Phosphate Concentration in Lymph

As stated before, the main purpose of this group of experiments was to determine if potassium, osmolarity and inorganic phosphate concentration in lymph would increase during active hyperemia. Our findings indicate that these constituents of lymph may not be reflecting their interstitial fluid concentrations. This is readily understood in the case of potassium and osmolality for these constituents

appear to be released soon after the onset of stimulation and release wanes with continued muscle activity. In the present studies, the venous plasma potassium concentration and osmolality were higher at the 2nd minute after the start of exercise than at the 25th minute (Table 1). This means that the changes in potassium and osmolality occurred early in the course of the hyperemia, that the capillary membrane does not appear to retard the wash-out of these constituents from the interstitial space, and that the time period of the lymph collection (20-50 minutes) was too long to capture the changes. However, the lymph studies do support the fact that there is not a continual large release of potassium and other osmotic particles during continued activity. Potassium and osmolality are not only washed-out of the interstitial space early, but also their concentration may readily equilibrate across the early collecting ducts of the lymphatic system. This suggestion is supported by Jacobsson and Kjellmer (97) who noticed that when a lymphatic system is perfused with a solution containing increased amount of potassium, the perfusate collected after passage through the lymph vessels showed a decrease in potassium concentration. Furthermore, the lymph coming from contracting muscle may be diluted with lymph from non-contracting muscle, bone (152), or dead space lymph in the collecting system. In light of these observations, the small increase in potassium concentration found in the lymph at the end of the exercise

period may be a significant finding.

In respect to osmolality and potassium, these studies do not add new data on their possible involvement in the mediation of active hyperemia. However, these data do not detract from previous findings that potassium may be involved in the initiation of active hyperemia but not its maintenance (29,82,116,153,164), and that osmolality may contribute to the hyperemia for a short time immediately following its initiation (129,165).

On the other hand, our data do not support any important role for inorganic phosphate in active hyperemia of skeletal muscle. The venous plasma Pi did not change during nerve stimulation (Table 1). In 5 animals the venous plasma was sampled every 5 minutes for a 20-30 minutes exercise period, and no change in Pi appeared in any sample. There was only a small increase in lymph Pi (0.2 mM). If the interstitial concentration of Pi were greatly increased, and if there were a permeability barrier to prevent movement across the capillary membrane (92), much higher increases in lymph could be expected. Inorganic phosphate could possibly escape across the lymphatic wall, be sequestered in the interstitium, or undergo reuptake by the muscle cell. However, when Pi in the interstitium was artificially increased by IM injection, the lymph collected subsequently showed increased amount of Pi (Table 2). Since the venous

plasma taken at 25 minutes after the injection showed an increase in Pi (Table 2), it seems that Pi does cross the capillary membrane from the interstitium. Also, intra-arterial infusion of Pi raises lymph Pi (Table 3). A one-way diffusion barrier at the endothelial membrane seems to be unlikely. The time course of the concentration changes suggests that if the infusions were maintained for a longer period, the lymph Pi would be in equilibrium with the venous plasma Pi. This would mean bi-directional diffusion of Pi.

A second major objection to the hypothesis that Pi plays a major role in active hyperemia is the fact that it is very vaso-inactive. Final venous plasma concentrations of up to 12 times normal had no effect on vascular resistance (Tables 3, 4, and 5). The results were the same whether the hindlimb (muscle, bone and skin) or the isolated gracilis muscle was the test organ. This is in agreement with other studies (15,44,82,147) but in conflict with Hilton's findings (88). As pointed out before, Hilton's results showing that Pi was vasoactive, could be the simultaneous effects of increased hydrogen ions and osmolality. However, the hydrogen ion is not a good vasodilator (50,161, 164), and we failed to see any vascular effects when pH was lowered from 7.44 to 7.13 (Table 5).

Hilton's hypothesis that Pi is very importantly involved in the initiation and maintenance of active hyperemia,

was originally formulated because of his observation that in cats, a slow muscle exhibited little or no active hyperemia and produced no Pi, while a fast muscle exhibited active hyperemia and produced Pi (91). This association does not seem to hold in all cases, for the dog gracilis muscle (predominantly slow and intermediate fiber types) shows excellent active hyperemia but no increase in Pi (27,131). Furthermore, it is well-known to neurophysiologists that if the motor innervation of a slow muscle is switched with that of a fast muscle, the two muscles would exhibit each others characteristics when the re-innervation is completed.

In summary, the present studies indicate that the inorganic phosphate ion is not involved in active hyperemia in canine skeletal muscle. The small increase in Pi in lymph from exercising muscle could be due to leakage from red cells that escaped into the lymph. There was no increase in venous plasma Pi with exercise, and intra-arterial infusion of NaH_2PO_4 to raise lymph Pi concentration to 800% of normal had no effect on blood flow.

The Myogenic Response in the In Vitro Gracilis Muscle

Intramuscular Pressure

Although it is believed that intramuscular pressure increases during muscle contraction, there are few measurements to document these changes. Wells et al. (184) used a

22 or 26 gauge needle inserted into a muscle to record intramuscular pressure with a manometer. During maximal voluntary contraction in human leg muscles they measured pressures ranging from 8-90 mmHg. In one muscle (the soleus) pressures ranged from 25-90 mmHg depending on the site of placement of the needle. In the dog tibialis anterior muscle, maximal sciatic nerve stimulation (stimulus parameter not given) produded an 88 mmHg increase in intramuscular pressure. Mohrman and Sparks (139) used a small saline-filled balloon to measure intramuscular pressure during 1 sec of tetany in the dog gastrocnemius muscle. They found that intramuscular pressure so measured increased by 65-80 mmHg at a stimulation frequency of 16/sec. There was a great variability between dogs. For example, in one dog stimulation at 64/sec. produced an increase of 135 mmHg in intramuscular pressure, while in another, an increase of 400 mmHg was obtained with the same stimulation parameters.

Comparable results were obtained in our own experiments. Intramuscular pressure were measured with a Mikro-Tip pressure transducer implanted into the thickest portion of the muscle. With rhythmic muscle contractions (2/sec or 5/sec), intramuscular pressures ranging between 10 and 30 mmHg were recorded. Blood flow was usually increased 2-3 fold. However, there was no consistent relationship between the increase in blood flow and the magnitude of the increase in

intramuscular pressure. With 1 second periods of tetany at 16, 32, 64 pulses per second, intramuscular pressures of 75, 135, and 200 mmHg, respectively, were obtained in the same muscle. These values were very unpredictable from one preparation to the next, and on occasion contractions at 2/sec produced greater intramuscular pressures than contractions at 5/sec. With passive changes in muscle chamber pressure, the intramuscular pressure sensor recorded an equivalent change (Figure 13). This is in agreement with the studies of Brace and Guyton (30) who showed in an isolated dog hindlimb enclosed in a plethysmograph, that the full amount of a pressure change in the plethysmograph is transmitted to the total tissue pressure. Their designation "total tissue pressure" was measured by the balloon or needle method. It seems that the Mikro-Tip pressure transducer was a preferable method of measuring intramuscular pressure since gross bleeding and tissue damage would occur with the needle method, and a fluid filled balloon could cause a dampened response.

Vascular Transmural Pressure

Changes in vascular transmural pressure are proposed as the stimuli for the myogenic response (54). In the present studies, our main purpose was to see whether simulated pulsatile decreases in transmural pressure as may occur during rhythmic muscular contraction would elicit

myogenic relaxation of vascular smooth muscle. Vascular transmural pressure is not uniform throughout any vascular bed since intravascular pressure can range from systemic (100-150 mmHg) to venous (1-20 mmHg) depending on the organ and the surgical preparation. The vascular transmural pressure is the net distending pressure across the wall of the blood vessel (intravascular minus extravascular pressure). Since this is not directly measureable, a mean vascular transmural pressure is often used as an indication of vascular distension. This value is computed as one-half the sum of the inflow and outflow pressures. Obviously, the true vascular transmural pressure at any particular site would be higher or lower than the mean transmural pressure as one approaches the arterial inflow or venous outflow respectively. In an isolated muscle at rest extravascular pressure is assumed to be zero.

A mean transmural pressure of 70 mmHg is representative of the experiments in this study. The arterioles are the major resistance controls in a vascular bed and the distending pressure to which they are exposed may be equivalent to or greater than the mean transmural pressure. Thus when perfusion pressure was held constant electronically, or during natural flow perfusion, changes in muscle chamber pressure of 10-50 mmHg would change vascular transmural pressure at the level of the arteriole by a significant amount.

This may not be the case at constant flow perfusion since the intravascular pressure increased by the same amount when muscle chamber pressure was increased.

Effects of Passive Changes in Vascular Transmural Pressure

At constant pressure (Figures 14, 16, 18, 20, 22, and 24) and during natural flow perfusion (Figures 26 and 28), changes in muscle chamber pressure changed vascular transmural pressure by the same amount. The passive increases in intramuscular pressure (Figures 13 and 14) made to simulate changes that occurred during exercise did not affect vascular resistance significantly until the chamber pressure was 50 mmHg (Figure 15). If the mean vascular transmural pressure was 70 mmHg, a decrease in transmural pressure of 50 mmHg probably collapsed the vascular bed on the venous side up to the region of at least the terminal arterioles. This temporary obstruction of flow could account for the hyperemia seen after chamber pulsing was terminated. The studies of Staub et al. (174) indicate that this is a likely explanation. Using the dog calf muscles, they showed by angiography that even main arteries within the muscle can become occluded during muscular contraction.

In view of the fact that a decrease in vascular transmural pressure of approximately 35% (Figure 14, Panel C) did not significantly affect vascular resistance (Figure 15),

and that a decrease in vascular transmural pressure of approximately 70% (Figure 14, Panel D) caused a significant decrease in flow during the chamber pulsing (Figure 15), it seems reasonable to conclude that the change in vascular transmural pressure did not elicit a myogenic response. The hyperemia seen after termination of chamber pulsing (Figure 14, Panel D) was, most probably, a reactive hyperemia in response to the reduction in flow during pulsing. A comparison of Figure 8 where flow was purposely reduced slightly, and Figure 14, Panel D, would support this explanation. Thus it seems that although muscular contraction may indeed reduce vascular transmural pressure, the myogenic response does not contribute to active hyperemia. If there were a myogenic relaxation of vascular smooth muscle during muscular contraction, it should be evident before the series of contraction ceases. If there is a reduction of flow during muscle contraction, the effect may then be to contribute to the dilation of the early recovery period. This, in any event, would be a very minor factor in the over-all vascular response to muscle contraction since the maximum dilation is established early in the exercise period.

The experimental apparatus did not interfere with the ability of the muscle to respond to vasodilatory stimuli during chamber pulsing. Figures 26, 28, 30, and 32 illustrate that during muscle chamber pulsing, the muscle was capable

of responding to nerve stimulation. During simultaneous nerve stimulation and chamber pulsing, the hyperemia obtained was slightly less than with exercise alone (the difference was not statistically significant). In 2 muscles, papaverine (2 mg/min) was infused into the arterial inflow of the muscle to produce maximum vasodilation. Chamber pulsing alone decreased flow by the same percentage with as without papaverine. These observations lead to the conclusion that chamber pulsing caused only passive decreases in blood flow.

The hyperemia seen following a single pulse (50 mmHg) decrease in vascular transmural pressure (Figure 18, Panel C), is quite similar in time course and duration to that seen by Mohrman and Sparks (139) when they decreased vascular transmural pressure of an isolated muscle with a pressure cuff. In both the Mohrman and Sparks study and our present study, the hyperemia was preceded by a short decrease in arterial inflow. The question now is whether the flow decrease was sufficient and of long enough duration to cause vasodilator metabolites to be elaborated, or whether the effect was myogenically mediated. In our studies blood flow was decreased by about 15% for about 5 seconds. This could trigger metabolic changes which could cause the subsequent dilation. This situation would be equivalent to a reactive hyperemia following a 5 second period of partial arterial occlusion. The passive pressure change in the Mohrman and

Sparks study (139) was made over a period of 1 sec. A myogenic response is proposed to be responsible for the hyperemia following the pressure change, because it is thought that reduction of blood flow for 1 second or less would not cause elaboration of vasoactive metabolites, or a significant change in PO_2. However, the speed of metabolic reactions in vivo may be quite faster than the same reactions in vitro. It also seems reasonable to expect that if a single pulse (such as in Figure 18, Panel C) can elicit a dilation, a series of pulse (such as Figure 16, Panel D) should show some evidence of dilation during the pulsing, instead of only upon termination of pulsing. It seems then, that the hyperemia following passive decreases in vascular transmural pressure was a reactive hyperemia. In the case of the single pulse (Figure 18, Panel C), the reduction in flow was great but for a short duration. In the case of a series of pulses of the same magnitude (Figure 16, Panel D), the reduction in flow is not as great but the duration is longer. However, the myogenic response would function here to the extent that it is involved in reactive hyperemia (32, 106,144,179). Any contribution to exercise hyperemia on this basis could only be very minor, if present at all.

Smiesko (168) has described a myogenic response to transient changes in transmural pressure that is apparently different from the classical Bayliss phenomenon. He has

shown that there is a vasodilation in response to both a transient increase and a transient decrease in transmural pressure. Our data do not totally agree with Smiesko's findings. When we decreased vascular transmural pressure (Figure 18, Panel C) by 50 mmHg, a significant dilation followed within a few seconds. This agrees with Smiesko's results when he decreased perfusion pressure for 1 second, and can be a reactive hyperemia (in both cases) as discussed above. However, when we transiently raised vascular transmural pressure by creating a vacuum within the muscle chamber (Figure 20, Panel C), there was no vascular response. In a few cases there was a suggestion of a constriction but on the average there was no change. The vascular bed responded passively to this maneuver. When Smiesko increased vascular transmural pressure by a 1 second increase in perfusion pressure, he obtained a vasodilation of similar time course and magnitude as with the decrease in perfusion pressure. He also showed that the response appeared to be triggered by the ascending phase of the pressure change. It is interesting to note that the ascending phase of the pressure change when perfusion pressure was decreased would correspond to the release of a partial occlusion of the arterial inflow; the ascending limb of a pressure increase created by a sudden pressure pulse from an air-pressure chamber may traumatize blood cells, and release a bolus of vasodilator substances into the muscle inflow.

The effect of sustained decreases in vascular transmural pressure (Figures 22 and 23) seem to support our contention that the hyperemia seen after passive compression of the muscle is a reactive hyperemia. This situation does not seem likely during muscle contraction because flow increases almost immediately upon initiation of exercise.

The effect of a sustained increase in vascular transmural pressure (Figures 24 and 25) produced by creating a vacuum within the muscle chamber seemed to show evidence for a myogenic constriction of vascular smooth muscle during the period of the pressure change. This is not necessarily true because the same response could result from a washout of vasodilator metabolites that help to maintain the basal vascular tone. This is another instance when the postulated myogenic response cannot be separated from postulated metabolic control.

During constant flow perfusion (Figures 30 and 32), the vascular transmural pressure was not changed since the intravascular pressure increased by the same amount as the extravascular pressure. Thus this series of experiments did not contribute any information on the involvement of the myogenic response in active hyperemia.

VIII. SUMMARY AND CONCLUSIONS

1. Muscle contraction at rates representative of moderate exercise produced a threefold increase in blood and lymph flow, a significant increase in venous plasma osmolality and potassium levels, and no increase in venous plasma inorganic phosphate. Lymph from contracting muscle showed a small increase in potassium and inorganic phosphate which may be due to red cell contamination of the lymph. Lymph protein did not increase but protein transport was elevated.

These data do not add or detract from the hypotheses that increased interstitial potassium levels, and interstitial hyperosmolality play some role in active hyperemia. In the case of inorganic phosphate, the findings that venous plasma levels did not increase during muscle contraction, that there seem to be no capillary barrier to phosphate diffusion, and that exogenous phosphate was very vasoinactive, would speak against a role for inorganic phosphate in active hyperemia.

2. Muscle contraction at rates representative of moderate exercise caused an increase in intramuscular pressure of 10-30 mmHg. When perfusion pressure was held constant (as it would be in the intact animal), passive changes in

vascular transmural pressure of this magnitude do not cause any significant dilation. Higher pressure (pulsatile, non-pulsatile, transient or sustained) elicited a reactive hyperemia within a few seconds after the termination of the pressure change. These data do not give any support to the hypothesis that a myogenic relaxation of vascular smooth muscle contributes to active hyperemia.

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