ROLE OF METABOLICALLY LINKED CHEMICALS IN LOCAL REGULATION OF BLOOD FLOW: INTERACTION OF OXYGEN AND CARBON DIOXIDE IN SUSTAINED EXERCISE HYPEREMIA OF THE CANINE GRACILIS MUSCLE

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
DAVID FRANCIS STOWE
1974

This is to certify that the

thesis entitled

Role of Metabolically Linked Chemicals in Local Regulation of Blood Flow: Interaction of Oxygen and Carbon Dioxide in Sustained Exercise Hyperemia of the Canine Gracilis Muscle

presented by

David Francis Stowe

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology

Jerry B. Seal
Major professor

Date 7/26/74

O-7639

Ex
and an inc
ble for th
trations o
hydrogen a
venous eff
shows enha
other vasc
the concen
surroundin
the blood
metabolism
fairly con

Th
oxygen and
exercise h

ABSTRACT

ROLE OF METABOLICALLY LINKED CHEMICALS IN LOCAL REGULATION OF BLOOD FLOW: INTERACTION OF OXYGEN AND CARBON DIOXIDE IN SUSTAINED EXERCISE HYPEREMIA OF THE CANINE GRACILIS MUSCLE

By

David Francis Stowe

Exercise of skeletal muscle is associated with vascular dilation and an increase in muscle blood flow. The exact mechanism(s) responsible for the vasodilation is uncertain but it is known that the concentrations of certain metabolically linked chemicals (e.g., oxygen, hydrogen and potassium ions, and osmotically active particles) in the venous effluent from exercising muscle are altered and that this blood shows enhanced vasodilator properties when bioassayed in muscle, and other vascular beds. The metabolic theory proposes that changes in the concentration of one or more of these chemicals in the tissue fluid surrounding the resistance vessels result in active vasomotion so that the blood flow is adjusted to a level more appropriate to the rate of metabolism. In this way the ratio of blood flow to metabolism remains fairly constant and local tissue homeostasis is insured.

The purpose of this study was to evaluate the contributions of oxygen and hydrogen ions singly and in combination, to steady-state exercise hyperemia in canine gracilis muscle. Studies were carried

out on th
anesthet
what exte
ing the e
pH of the
muscle se
the venous
rection of
by alterin
placed in

In
tension an
while moni
mixture of
the arteria
monitored.
decreased p
during vari
role of sev
steady-state
and the cond
the blood d
stimulation

The
of venous bl

out on the collateral free, denervated muscle with the animal anesthetized. One series of experiments was designed to determine to what extent the enhanced vasodilator activity of the venous blood draining the exercising muscle is attenuated when the oxygen tension and/or pH of the blood are selectively corrected. The contralateral gracilis muscle served as the bioassay organ to test the vasodilator activity of the venous effluent before, and during exercise, without and with correction of the P_{O_2} and pH of the effluent. The latter was accomplished by altering the mixture of gas ventilating a gas exchange permeator placed in the venous effluent.

In another series, single and simultaneous reductions in oxygen tension and pH were produced in blood perfusing resting gracilis muscle while monitoring resistance. This was accomplished by altering the mixture of gas ventilating a lung (taken from another dog) placed in the arterial supply. Both arterial and venous effluent P_{O_2} and pH were monitored. In another series the degree of vasodilation produced by decreased P_{O_2} and pH in resting muscle was compared to that observed during various degrees of exercise. Finally, to assess the relative role of several metabolically linked factors in the maintenance of steady-state exercise hyperemia, the blood flow, gas tensions, pH, and the concentrations of potassium ions and water, were measured in the blood draining skeletal muscle during 60 minutes of sustained stimulation and during step increases in the level of stimulation.

The findings indicate that (a) the enhanced vasodilator activity of venous blood seen during steady state exercise can be completely

abolished
pre-exer
pH in the
the venou
produces
equal tha
concentra
sustained
the only m
cise is a
better wit
Th
activity o
decreases
oxygen and
taining ex
involved,
the blood
ured durin
that seen
minor role
hyperemia

abolished by simultaneously returning the oxygen tension and pH to pre-exercise levels, (b) simultaneous reduction of oxygen tension and pH in the blood perfusing resting muscle, so as to produce levels in the venous effluent similar to those observed during heavy exercise, produces marked vasodilation. The dilation, however, does not quite equal that seen during heavy exercise, (c) the increased potassium concentration and osmolality in the venous effluent disappear during sustained exercise but the changes in flow, pH and P_{O_2} do not, and (d) the only measured change in the venous effluent during mild steady exercise is a fall in P_{O_2}; during graded exercise, resistance correlates better with P_{O_2} than with pH, potassium, or osmolality.

These studies indicate that in the steady state the vasodilator activity of venous blood from exercising muscle is due to simultaneous decreases in oxygen tension and pH. Therefore, the concentrations of oxygen and hydrogen ions may be the most important determinants maintaining exercise hyperemia in skeletal muscle. Other factors may be involved, however, since induced reduction of oxygen tension and pH in the blood draining resting muscle to levels comparable to those measured during heavy exercise, failed to produce dilation comparable to that seen during heavy exercise. Finally, these studies suggest only minor roles for potassium and osmolality in steady-state active hyperemia of skeletal muscle.

ROLE OF METABOLICALLY LINKED CHEMICALS IN LOCAL REGULATION OF BLOOD FLOW: INTERACTION OF OXYGEN AND CARBON DIOXIDE IN SUSTAINED EXERICSE HYPEREMIA OF THE CANINE GRACILIS MUSCLE

By

David Francis Stowe

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1974

DEDICATION

To my friends who share my disparity
and to those who are not reproachful.

sincere a
his suppo
about the
Research A
course of
other mer
Burnell H
Bowdler.

S
his stimu

T
of Mrs. J
Chief Sur
Mrs. Marg

T
a special
administr

ACKNOWLEDGMENTS

The author wishes to take this opportunity to express his sincere appreciation to Professor Joe M. Dabney, Academic Advisor, for his support and encouragement and for the many stimulating discussions about the teaching of physiology, and to Professor Jerry B. Scott, Research Advisor, for his invaluable direction and advice during the course of these investigations. Appreciation is extended also to the other members of the writer's Guidance Committee, Drs. Francis J. Haddy, Burnell H. Selleck, Donald K. Anderson, Theodore M. Brody and Anthony J. Bowdler.

Special thanks are extended to my colleague, Dr. T. Lon Owen for his stimulating ideas and assistance during all phases of this study.

The author also acknowledges the skillful technical assistance of Mrs. Josephine Johnston, Laboratory Supervisor, Mr. Booker Swindall, Chief Surgical Technician, Mr. George Gamble, Surgical Technician and Mrs. Margo Smith, Laboratory Technician.

To Mrs. Amylou Davis, Graduate Affairs Secretary, is extended a special debt of gratitude for assistance in overcoming awesome administrative details.

TABLE OF CONTENTS

Figure

1. R
v
F

2. P
re
P
V
pa

3. Pr
co
ar
V_A
in

4. Pr
gr
A_G
lu

5. Re
il
ve
on
of

6. Av
va
an
6
th
re
il

7. Av
wi
ve
to

LIST OF FIGURES

Figure

8.

9.

10.

11.

12.

T

is largel

According

of local a

various va

and arteri

systems, t

continuall

vascular b

No

consequent

demonstrat

pressure

nerve trar

unequivoca

of blood f

into an al

Fi

believed t

INTRODUCTION

General Description of Vascular Control Systems

The distribution of blood flow in the peripheral circulation is largely regulated by alterations of vascular smooth muscle tone. According to current concepts, synergistic or antagonistic interactions of local and remote control systems regulate the blood flow to the various vascular beds by altering the radius of precapillary sphincters and arterioles. As a result of the balance of local and remote control systems, the vascular calibers in the different systemic circuits change continually, depending in part on the function of the particular vascular bed.

No one explanation for the phenomenon of vasomotion (and consequently the maintenance of local tissue homeostasis) has been demonstrated. The remote control mechanisms directed at controlling pressure are best defined. Several of their specific mediators, the nerve transmitters and the blood-born vasoactive hormones, have been unequivocally identified. The mechanisms responsible for local control of blood flow are, in general, more hypothetical and have been clumped into an all-inclusive multiple factor theory.

Figure 1 summarizes the remote and local mechanisms which are believed to control vascular smooth muscle tone. Vasoconstrictor fibers

Remote c

a. Vasocon
fib

b. Blood-b
excitator
inhibitor
influence

c. Vasodilat

Figure 1.

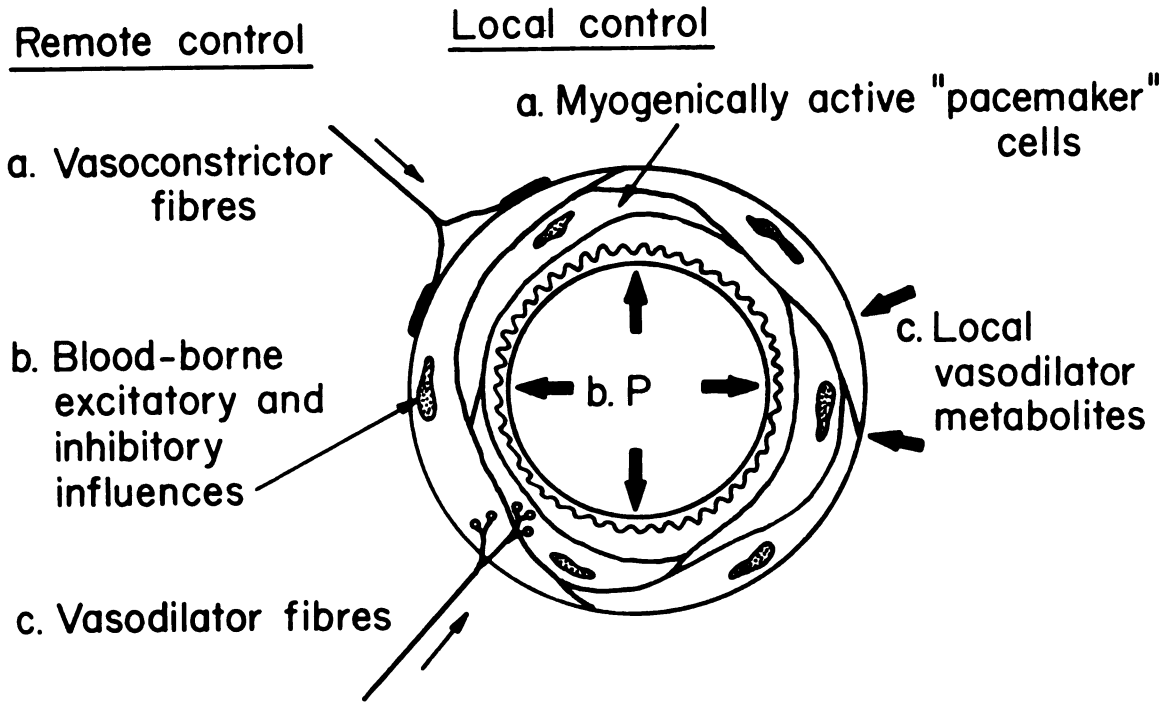


Figure 1. Remote and local mechanisms believed to control vascular smooth muscle tone. Modified from Folkow (46).

from t

catech

vascul

release

appears

other t

or at t

chemica

catechol

hormones

bradykin

demonstra

skeletal

flow thro

vascular

Temporary

associate

value upon

hyperemia.

varied ove

proportion

tively cons

from the adrenergic sympathetic nerve supply are well known to release catecholamines locally which act mainly on alpha receptors. In a few vascular beds, namely skeletal muscle, cholinergic sympathetic fibers release acetylcholine which causes vasodilatation. The latter response appears to be mediated through higher brain centers and may serve among other things to increase blood flow to skeletal muscle slightly before, or at the onset of, exercise. Blood-born vasoactive hormones and chemicals elaborated outside the vascular bed, such as the circulating catecholamines (norepinephrine and epinephrine), some polypeptide hormones (angiotensin and vasopressin), and the kinins (kallidin and bradykinin), are also well known to affect vascular smooth muscle tone.

Local Vascular Control Systems

Several forms of local regulation of blood flow have been demonstrated. Increased metabolic rate produced by activation of skeletal or cardiac muscle, for example, produces an increase in blood flow through the activated organ at a constant perfusion pressure; this vascular response is called active (exercise, functional) hyperemia. Temporary occlusion of arterial inflow to a vascular bed is usually associated with a transient increase in blood flow above the basal value upon release of the occlusion; this response is known as reactive hyperemia. When perfusion pressure to most systemic vascular beds is varied over the approximate range 70 to 200 mm Hg, there is a less than proportionate change in blood flow. This ability to maintain a relatively constant blood flow in face of a varying inflow perfusion pressure

is ca
react
regul
bolic
the T

these
mecha
activ
ciated
(73).
blood
contin
propag
transm
active
For ex
decrea
Simila
result
occlus
flow or
centrat
as subs
changes

is called autoregulation. These local responses, i.e., active and reactive hyperemia, and autoregulation, are manifestations of the local regulatory mechanisms to maintain a relatively constant ratio of metabolic rate to blood flow. Thus, like the remote controlling mechanisms, the local mechanisms act to maintain cardiovascular homeostasis.

Numerous theories or hypotheses have been advanced to explain these local regulatory responses; the exact nature of the mechanism or mechanisms responsible remain controversial. Vascular smooth muscle activity is thought to be controlled locally by chemical factors associated with tissue metabolism and myogenic reactions related to stretch (73). Blood viscosity and tissue pressure may also participate in local blood flow control (67). It is thought that the larger arterioles with continuous smooth muscle coats exhibit myogenic activity by cell to cell propagation of impulses originating in pacemaker cells. A change in transmural pressure across vascular smooth muscle cells may initiate active vasomotion as a sort of positive or negative stretch reflex. For example, exercise may increase tissue pressure which would cause a decrease in transmural pressure and subsequently produce vasodilation. Similarly, partial occlusion of the major artery to a vascular bed would result in a fall in transmural pressure in blood vessels distal to the occlusion and hence produce vasodilation. Finally, a change in blood flow or metabolism in a vascular bed is followed by changes in the concentrations of substances delivered to the tissue by the blood, as well as substances produced or released by the tissue. These chemical changes reportedly result in an adjustment of blood flow to a level

mo

tha

tic

pre

in

in

man

65)

fol

met

alt

mor

red

pre

(pa

cer

arte

infl

Presu

act b

smooth

flow re

tion (au

approxima

more appropriate to the rate of metabolism. It is now well established that muscular contraction reduces or abolishes sympathetic vasoconstriction in the vascular bed of skeletal muscle, i.e., local mechanisms predominate over remote mechanisms during exercise (24, 84, 142-144).

Although the myogenic response to stretch probably contributes in varying degrees to local blood flow regulation and tissue homeostasis in different vascular beds (44, 107), a very attractive explanation for many types of local control is based upon the metabolic hypothesis (63, 65). It is proposed that changes in metabolism or blood flow are followed by alterations in the concentration of oxygen and vasodilator metabolites in the tissue fluid surrounding the arterioles; these alterations result in active vasomotion that adjusts flow to a level more appropriate to the rate of metabolism. For example, during a reduction in flow to skeletal muscle, caused by a fall in perfusion pressure or occlusion of the arterial supply, substrate concentrations (particularly oxygen) are thought to fall and the concentrations of certain metabolites to rise in the tissue fluids surrounding the arterioles and precapillary sphincters. Following a reduction of the inflow pressure the flow increases to approximately the initial level. Presumably, the accumulated metabolites or lack of metabolic substrates act by inhibiting or attenuating the basal contractile state of vascular smooth muscle cells. Thus, the vascular radius increases and the blood flow returns to near the control level. In this form of local regulation (autoregulation) the ratio of blood flow to metabolism remains approximately constant and local tissue homeostasis is assured.

Likewise, a local increase in metabolism as a result of muscular contraction, for example, also causes vascular dilation with an increase in flow (active hyperemia). Again, changes in substrate and certain metabolite concentrations are believed to be importantly involved in this response.

The Metabolic Theory for Active Hyperemia in Skeletal Muscle

The metabolic hypothesis receives strong support from bioassay studies and chemical analysis of venous effluent. Bioassay studies show that the blood draining exercising skeletal muscle exhibits enhanced vasodilator activity. For example, if active hyperemia is induced in one muscle vascular bed by stimulating the muscle to contract, thereby changing its flow-to-metabolism ratio, similar directional changes in vascular resistance are promptly seen in a bioassay organ (65). This enhanced vasoactivity of the venous blood undoubtedly reflects to a large degree alterations in the vasoactivity of the tissue fluids in the donor organ, suggesting that the concentration of vasoactive agents in the tissue fluid changes sufficiently during local regulation to have an effect on blood vessels. Such bioassay studies clearly show that the concentration of a vasoactive substance(s) is altered in venous blood during exercise hyperemia of the regulatory (donor) muscle and that the substance(s) acts on the resistance vessels of the bioassay organ as well as on the regulatory muscle. However, it may be possible for a substance to escape this criteria if it acts on the vascular smooth

mus
di
re
the
st
ves
pa
do

alt
to
imp
may
as
sev
tra
res
of
blo
of p
tion
fluid
increa
exercis
during

muscle to effect vasodilation, but does not appear in the effluent in dilator concentrations due to rapid inactivation, reuptake or limited release. Also, it must be assumed, with some supporting evidence, that the effluent concentration of a substance closely reflects the interstitial fluid concentration in the vicinity of the affected resistance vessels. Although bioassay studies provide compelling evidence for participation of chemicals in local blood flow regulation, they alone do not firmly identify any specific mediator.

Chemical analysis of blood draining exercising muscle shows alterations in the concentration of certain vasoactive chemicals related to tissue metabolism. Many metabolically linked chemicals have been implicated in the hyperemia of exercise but the concentration of some may change coincidentally and not be vasoactive. In order to qualify as a mediator of active hyperemia any proposed chemical must satisfy several criteria: (1) The chemical must be found in altered concentrations in the tissue fluid or venous effluent during the vascular response. For example, it is well known that the oxygen tension and pH of blood draining exercising muscle drops, concomitant with increased blood flow (65). (2) The substance must be vasoactive within the range of plasma effluent or tissue concentrations found during local regulation when its concentration is artificially altered in the blood or fluid perfusing the resting organ (65). For example, the observed increase in hydrogen ion concentration of the venous effluent during exercise hyperemia, when mimicked in the venous effluent of the muscle during rest, must elicit a vasodilator response. (3) There must be a

reasonable temporal relationship between the concentration of the agent in the tissue fluid or venous blood and the change in resistance (65). For example, the concentration of the substance in the effluent must be rapidly altered at the onset of exercise, if it is involved in the initiation of exercise hyperemia, and/or must be continually altered while the exercise continues, if it is involved in the maintenance of exercise hyperemia. Similarly, if the hyperemia or vasodilation continues unabated, while the concentration of the substance is artificially altered in the blood perfusing the resting organ, then the substance may be involved in the maintenance of hyperemia.

To be an actual effector the substance must be shown to act directly on the vascular smooth muscle affecting resistance and not through release of intermediary substance(s) (1). It has been postulated, for example, that oxygen lack may have either a direct effect on vascular smooth muscle cells to attenuate oxidative phosphorylation and thus the contractile apparatus (31, 34), or that it stimulates the breakdown and release of various metabolites (16) such as adenosine or lactic and pyruvic acid which are dilators.

Several factors recently proposed in local regulation of blood flow which have not withstood the test of these criteria, at least in active hyperemia, are inorganic phosphate (6, 72) and prostaglandins (105). Several metabolically linked chemicals, however, remain as being more or less important factors in the regulation of blood flow during exercise. Currently, the most important of these appear to be oxygen, hydrogen ions, carbon dioxide, potassium ions, adenosine and its

nu
10
ce
in
of
Sir
cha
res
fac
the
eta
vas
som
exe
diff
work
(slo
the c
gastr
125).
restin
Red mus
energy f
in flow p

nucleotides, and osmolality (see reviews 1, 7, 17, 64, 65, 67, 68, 107, 108, 127, 151). Although all of these chemical factors satisfy certain of the outlined criteria, and thus may be directly or indirectly involved in exercise hyperemia, it seems highly unlikely that any one of these accounts completely for active hyperemia in skeletal muscle. Since the tissue and blood concentrations of several chemical factors change during the hyperemia, it has been postulated that the vascular responses result from the combined actions of two or more chemical factors (65). Some studies have shown that simultaneous changes in the concentration of vasoactive chemicals in the blood perfusing skeletal muscle interrelate in such a way as to reinforce their individual vascular effects (139-144). Also, recent studies have suggested that some factors may be important dilators during the initial phase of exercise, but not during later phases, and vice-versa (68, refs.).

The relative importance of the various dilator factors may differ depending on the type of skeletal muscle and the intensity of work performed. For example, comparative studies of blood flow in red (slow, tonic) soleus and white (fast, phasic) gastrocnemius muscles in the cat have shown that the soleus has a resting flow twice that of the gastrocnemius but exhibits a smaller exercise hyperemia (45, 75, 76, 125). During maximal dilation soleus flow increases about three times resting flow, whereas gastrocnemius flow increases about 10 to 15 fold. Red muscle contains more myoglobin and mitochondria and gets most of its energy from oxidative phosphorylation. Apparently, the smaller increase in flow provides an oxygen delivery and exchange in red muscle sufficient

for m
high
would
other
muscle
concen
higher

several
hyperem
evaluat
ions in
of the
the enha
contrala
natural
oxygen t
levels.
muscle) t
pH were m
evaluate
exercise
extent rec
resting gr
simultaneou

for maintaining a predominately aerobic metabolism even in situations of high work loads, whereas in white muscle, even low rates of contraction would lead to "oxygen debt" and a greater release of lactic acid and other metabolites. Perhaps the vascular bed of predominately white muscles dilates to a greater extent during exercise because the tissue concentration of oxygen is lower and the concentration of metabolites higher.

The purpose of this study was to elucidate further the role of several of the metabolically linked chemicals believed to mediate the hyperemia (or vasodilation) of exercise. Experiments were devised to evaluate the relative and combined contributions of oxygen and hydrogen ions in the metabolic control of blood flow during steady-state exercise of the canine gracilis muscle. Two series were designed to determine if the enhanced vasodilator activity of venous blood (by bioassay in the contralateral muscle) from exercising gracilis muscle (perfused at natural or constant flow) can be attenuated or abolished when the oxygen tension and/or pH of the blood are returned to the pre-exercise levels. In the second series (natural flow perfusion of the exercising muscle) the blood flow and the tensions of oxygen and carbon dioxide and pH were measured during a prolonged period of exercise (60 minutes) to evaluate the role of these factors in the maintenance of steady-state exercise hyperemia. A third series was designed to determine to what extent reduction of oxygen tension and/or pH of the blood perfusing resting gracilis muscle produce vascular dilation. In a fourth series simultaneous reductions in oxygen tension and pH were made in resting

gracilis muscle in order to mimic venous levels like those observed in blood from exercising muscle. The degree of vascular dilation produced in the resting muscle was compared to that produced in the exercising muscle at various levels of nerve stimulation. The potassium and osmolal concentrations were measured in gracilis muscle venous blood during these series to evaluate their roles in the genesis of the resistance changes observed.

SURVEY OF LITERATURE

Local Blood Flow Regulation: Early Studies and Theories

Gaskell (1877) made the first quantitative description of the flow changes that follow skeletal muscle contraction and suggested that the blood supply to active tissues could be regulated by chemical alterations affecting vascular tone (49). His further suggestion in 1880 that intermediary metabolites play a role in local regulation of blood flow probably originated from his observation that lactic acid painted on blood vessels produced dilation (50). Roy and Brown (1879) found evidence that the blood vessels carrying nutrients to the tissues contract or expand in accordance with the blood flow requirements of the tissues (130). They suggested that the quantity of oxygen or dilators determine the degree of vascular diameter. In 1901, Bayliss (9) first hinted at a role for pH in local regulation. He observed an increase in blood flow in the lower extremities of the frog when the perfusate was changed from a Ringer's solution with normal carbon dioxide to one saturated with carbon dioxide. But Bayliss' greatest contributions in the area of local regulation were his studies on reactive hyperemia, which formed the basis of the myogenic hypothesis (10, 11). He proposed that the vasodilation in the dog leg following relief of circulatory arrest produced by compression of the aorta for 8 seconds

w
s
t
a
a
t
p
a
t
a
t
l
t
h
a
m
t
v
m

h
t
f
w
o

was due to collapse of the arterial tree and lack of the stimulus of stretch acting upon the smooth muscle of the vessel wall. He did not think that deprivation of blood flow for 8 seconds could cause an appreciable accumulation of metabolites or lack of substrates. Lewis and Grant reported in 1925 that local vasodilation occurred in response to occlusion of vessels in the forearm and that this effect was independent of local nervous reflexes because the dilation was unaltered after nerve degeneration (96). They concluded that the vasodilation took place during the period of occlusion and that it was due to the action of metabolites, continuously formed and accumulating; the longer the occlusion, the greater the accumulation of metabolites, and the larger the vasodilation after release. Since the flush was bright red they thought that the dilation was not due to anoxia, but suggested histamine as a cause. Markwalder and Starling in 1913 (102), Dale (26), and Krogh (95) in 1929 also suggested that vasodilator metabolites accumulate in tissues and cause dilation. Krogh (95) found that decreasing the oxygen saturation and increasing carbon dioxide content caused vasodilation in rabbit ears, but failed to suggest that oxygen lack might have an effect on the circulation.

More reports on the possible roles for oxygen lack and increased hydrogen ion concentration in local regulation began to appear around the turn of the century. Verzar (1912) reported that decreasing the flow to resting cat skeletal muscle decreased oxygen uptake, although venous blood from that muscle still contained substantial amounts of oxygen (153). He suggested that oxygen consumption is limited by the

blood flow. Fleisch et al., in 1932, observed that local hypoxemia did not produce dilation in the cat hindlimb until the hypoxemia was severe but that a small increase in carbon dioxide concentration produced dilation (42). Therefore, they suggested that carbon dioxide, but not oxygen, was important in local regulation. In 1925, Hilton and Eichholtz (69) and in 1926, Gremels and Starling (58) using heart-lung preparations, found that perfusion of the coronaries with deoxygenated blood (about 40% saturation) produced a large increase in coronary blood flow. Flow also increased when lactic acid or carbonic acid was added to the blood perfusing the coronaries (58). Anrep and Saalfeld, in 1935, first used a bioassay technique to study active hyperemia (3). They perfused a dog gastrocnemius muscle with blood either from the arterial circulation or with its own venous blood collected during rest or rhythmic muscular contraction. Marked hyperemia accompanied the contraction. Neither venous blood re-perfused from the resting muscle nor blood collected during hyperemia and later re-perfused had vasodilator activity; but blood collected during vasodilation (restricted arterial inflow) and later re-perfused was a powerful vasodilator. They concluded that the vasodilator action must have been due to non-labile metabolites, but not oxygen since blood "reoxygenated" after collection during exercise at constant flow was still a good vasodilator. Kramer et al. (1939) noted that during steady-state exercise of the dog gastrocnemius muscle, the muscle blood flow was directly proportional to its rate of oxygen consumption (94). This was confirmed by Pappenheimer (1941) in the isolated perfused dog hindlimb (119).

Jalavisto et al., in 1948, measured the venous oxygen saturation and the blood flow to the gastrocnemius muscle of the dog during and following activity. They found that blood flow increased during the recovery period roughly in proportion to the oxygen deficit incurred during the period of muscular activity (78). This was similarly observed by Hilton (70).

In a typical experiment, Gollwitzer-Meier (1950) observed a 0.06 unit fall in pH of venous blood and a rise in flow to 370% of the control value with stimulation of the canine gastrocnemius for 3 minutes (54). Her study showed no correlation between venous blood pH during exercise and the magnitude of the hyperemia, but nevertheless suggested a possible minor role for hydrogen ions in active hyperemia. Kester et al. (1952) observed a 100% rise in blood flow when the pH of the dog hindlimb blood was locally reduced from 7.4 to 6.5 with buffered solutions (81). Deal and Green (1954) found that intra-arterial injections of solutions ranging in pH from 7.4 to 2.0 increased blood flow in the dog hindlimb (30). Diji (1959) observed dilation in hand vessels with high carbon dioxide application (32).

Studies implicating a role for potassium in active hyperemia began to appear in the thirties. Baetjer, in 1934, observed an increase in the concentration of potassium in the blood draining cat skeletal muscle during activation by motor nerve stimulation and during cross clamping of the aorta (4). Fenn (1937) reported a loss of potassium from cat, rat and frog skeletal muscle during exercise (41). In 1938, Katz and Linder found that increasing the potassium ion concentration

in blood perfusing the coronary circulation from 1.1 to 1.7 times the normal concentration produced a 52% increase in coronary blood flow, while increases from 1.5 to 2.5 times normal decreased coronary blood flow 63% (80). Dawes (1941) was the first to suggest a role of potassium in exercise hyperemia; he observed that small amounts of KCl (up to 10 mg in a 1% solution) injected locally increased canine or feline hindlimb flow (29).

With regard to osmolality, Barcroft and Kato, in 1915, suggested that the increase in metabolism during skeletal muscle exercise leads to an increased number of osmotically active particles in the tissue (8). Years later, Marshall and Shepard (1959) found that rapid local injections of 2 ml of 10-20% NaCl or 25-50% dextrose solutions into the femoral artery of the dog produced a prolonged two or three fold increase in flow through the hindlimb (103). Read et al., in 1960, observed that rapid intravenous injections of 1500 mOsm/kg solutions, produced peripheral vasodilation in dogs (124). Overbeck et al. demonstrated in 1961 that locally increasing forelimb osmolality by 100 mOsm/kg decreased forelimb vascular resistance to 71% of the control when flow was held constant (117). A direct physiological role for tissue osmolality in vascular control was first suggested by Mellander in 1967, when he presented evidence that venous osmolality was causally linked to the hyperemia of exercise (106).

More Recent Evidence for Participation of Specific Metabolically Linked Chemicals in Exercise Hyperemia

During the past 25 years a more systematic approach has been made to identify some of the metabolically linked vasoactive chemicals responsible for the genesis and maintenance of the hyperemia of exercise. From evidence based on the criteria outlined in the introduction for involvement of any particular factor, three general conclusions have gradually emerged over the past few years (65, 67, 68). First, no single metabolic factor appears to be able to explain exercise hyperemia; i.e., probably a combination of factors are involved. Second, the contribution of the various factors appears to change with the duration and intensity of exercise. Third, the relative contribution of the various factors may vary depending on the type of skeletal muscle (red, white or mixed) and the species.

The following two sections review evidence for the roles of oxygen, carbon dioxide, hydrogen ions and potassium ions and osmolality in exercise hyperemia. The first section relates to studies which demonstrate that vasodilation occurs when the concentrations of these vasoactive chemicals are altered in the arterial inflow of resting muscle vascular beds. The second section reviews evidence for participation of these factors in exercise hyperemia based on bioassay and chemical analysis of the tissue fluid or venous effluent during muscle contraction.

Vasoactivity of Metabolically Linked Chemicals Proposed as Mediators

Oxygen. Investigations by Fairchild et al. (40), Crawford et al. (25) and Ross et al. (128) added substantial support to the theory that moderate oxygen lack can cause vascular dilation. Ross et al. (128) showed that perfusing the resting dog hindlimb with blood in which the oxygen saturation was reduced stepwise to 0% caused up to a 3 to 4 fold increase in blood flow. The vasodilator effects of hypoxemia were verified in the dog forelimb by Daugherty et al. (28) and Scott et al. (134). It soon became evident that oxygen lack was importantly involved in, but could not wholly explain the vasodilation of exercise. Alterations of oxygen content over the range of normal arteriovenous differences had little effect on the dog forelimb and hindlimb vascular beds as reported by Molnar et al. (113). Stainsby and Otis (149) found that oxygen uptake by resting dog skeletal muscle was not altered by changes in blood flow except when the blood flow was reduced below a critical value. Daugherty et al. (28) observed that dog forelimb vascular resistance was unchanged when the oxygen tension in the perfusate was decreased from 120 to 30 mm Hg, but decreased 19% when the perfusate oxygen tension was reduced to 8 mm Hg.

More definitive studies on the relative role of oxygen lack in exercise hyperemia have appeared. Ross et al. (126) showed that the hyperemia accompanying contraction of the canine gastrocnemius was greater than that occurring at rest during perfusion with hypoxic blood, even though the effluent blood oxygen tensions were the same. They

found that during heavy exercise the increase in flow averaged 173% with a venous oxygen tension of 25 mm Hg, whereas venous blood perfused during rest resulted only in a 56% increase in blood flow with a venous oxygen tension of 23 mm Hg. Moreover, Scott et al. (136) demonstrated that constant flow perfusion of the dog hindlimb with hypoxic blood produced a moderate reduction in vascular resistance, but that the drop in resistance during exercise was larger even though the venous oxygen tension was not permitted to fall below the level immediately preceding exercise by the addition of oxygen via a donor lung interposed in the arterial supply. They found that the resting hindlimb showed a 17% drop in resistance when it was perfused with hypoxic blood so that the venous oxygen tension was 10 mm Hg. Increasing the oxygen tension of the perfusing blood during exercise (6 Hz) did not allow the venous oxygen tension to fall below 17 mm Hg; this procedure produced a 36% drop in resistance. In a series of papers, Skinner and his colleagues (142-144), reported that perfusion of the resting dog gracilis with hypoxic blood produced only a slight drop in vascular resistance, but perfusion with hypoxic, hyperkalemic, hyperosmotic blood increased both the rate and magnitude of the vasodilation. A consideration of these studies suggests, therefore, that oxygen contributes substantially in, but is not a complete explanation for exercise hyperemia.

The mechanism by which hypoxia alters vascular smooth muscle tone is not known. That oxygen acts causally through a direct effect on vascular smooth muscle to alter tone has been supported by many investigators (21, 31, 40, 69, 128, 141). The studies of Carrier et al.

(21) and Detar and Bohr (31) indicate good correlation between mechanical tension of the smooth muscle of isolated arterial segments and oxygen tension. Honig (75) has suggested that the oxygen sensitivity of vascular smooth muscle may be the result of an inhibition of smooth muscle myosin ATPase by inorganic phosphate and AMP. On the other hand, others have proposed that oxygen lack exerts an effect secondary to altered parenchymal tissue metabolism and release of vasodilator substances (14, 15, 34, 35). Berne (14, 15) observed breakdown products of adenine nucleotides in coronary sinus blood during cardiac hypoxia and proposed the hypothesis that oxygen lack leads to myocardial release of adenosine, a potent vasodilator in muscle. He suggested a similar hypothesis for skeletal muscle vascular beds. More recently, Berne et al. (16) found that the tissue concentrations of ADP, AMP, IMP, adenosine, inosine and hypoxanthine in rat skeletal muscle rose during exercise, especially when performed under ischemic conditions. Dobson et al. (33) examined the effluent draining dog hindlimb during severe, ischemic exercise and found adenosine but not adenine nucleotides.

Hydrogen ion. A causal relationship between pH and blood flow has been often demonstrated by locally altering the pH of blood perfusing resting muscle and noting the change in vasoactivity. Local increases in hydrogen ion concentration have been produced by either locally infusing acidic solutions or by increasing the carbon dioxide tension or content of the blood. Molnar et al. (114) found that intrabrachial infusion of various acids, which produced a fall in forelimb

venous pH on the average from 7.39 to 6.85, decreased small vessel resistance about 43%. Zsoter et al. (154) observed that a reduction of femoral venous blood pH from 7.4 to 7.2 by local infusion of acid solutions was associated with a 20% increase in canine hindlimb flow. Daugherty et al. (28) altered the pH of blood perfusing the dog forelimb by changing the ventilatory gas mixture of an extracorporeal lung placed in series with the forelimb. They observed that severe, local hypercapnic acidosis reduced pH from 7.58 to 7.19 and decreased forelimb resistance 24%. In a study by Kontos et al. (88), human forearm resistance decreased 43% on increasing venous carbon dioxide tension from 40 to 48 mm Hg by intrabrachial infusion of a saline solution with a carbon dioxide tension greater than 600 mm Hg. In another study by Kontos et al. (89), intra-arterial infusion of acid phosphate buffer solutions produced a fall in forearm venous blood pH from 7.34 to 7.24 and a rise in carbon dioxide tension from 42 to 52 mm Hg. This was accompanied by a 170% increase in forearm blood flow. Kontos (90) and Kontos et al. (91) more recently reported that hypercapnic acidosis produced a more pronounced vasodilation in human forearm than in the canine gastrocnemius muscle and that in man it was dependent on increased carbon dioxide tension, rather than decreased pH; this suggested the possibility that its action was brought about by decreased pH within vascular smooth muscle cells. Radawski et al. (123) compared skin and muscle vascular responses to various carbon dioxide tensions in the dog forelimb and reported that large reductions in arterial pH produce only a small drop in muscle vascular resistance and a moderate

drop in skin vascular resistance. Emerson et al. (39) recently demonstrated that large stepwise decreases in pH in blood perfusing dog gracilis muscle by intra-arterial infusion of isotonic lactic acid solutions produced only small stepwise decreases in muscle vascular resistance. The above studies indicate, therefore, that the hydrogen ion or carbon dioxide is locally vasoactive, high concentrations causing vasodilation, but that the induced change in pH must be quite large to produce substantial changes in resistance.

Potassium ion. Since Katz and Linder (80) and Dawes (29) early suggested that potassium is a vasodilator, many studies have shown subsequently that the resistance to flow decreases when potassium salts are infused into the arterial supply of the resting muscle, so that the venous plasma potassium ion concentration is elevated over the approximate range of 4 to 10 mEquiv/l (20, 23, 38, 48, 53, 60, 83, 85, 98, 117, 129, 132, 135, 139-144). Furthermore, a decrease in the local plasma potassium concentration below approximately 4 mEquiv/l (2, 23, 60, 62, 140) and an increase above approximately 10 mEquiv/l (29, 38, 48, 86) causes vascular constriction. Emanuel et al. (38) found that local infusion of 0.25 to 1.75 mEquiv K^+/min, as the chloride salt, produced dilation mainly in the small vessels of the dog forelimb. Overbeck et al. (117) observed a 22% fall in forelimb resistance during intra-arterial infusion of isotonic KCl into the dog forelimb; potassium ion concentration was estimated to have increased 1.4 mEquiv/l in the perfusing blood. Studies by Glover et al. (53) and Lowe and Thompson (98) showed that local intra-arterial infusion of KCl increased human

forearm blood flow. Kjellmer (83, 85) reported that local infusion of isotonic KCl, in amounts sufficient to raise feline calf muscle venous effluent potassium by 100%, produced a 40% fall in vascular resistance.

Using the isolated dog gracilis muscle preparation, Skinner and his group observed that moderate hyperkalemia (inflow potassium ion concentration of 6.5 mEquiv/l) produced vasodilation (77% of control), but to a lesser degree than that produced by muscular contraction (35% of control) (140, 141). However, for any degree of hypoxia, the degree and rate of vasodilation was greater or less depending on the concomitant potassium ion concentration and level of osmolality (143, 144). They found that blood, altered in a reservoir to make it oxygen deficient (41% saturated), hyperosmotic (352 mOsm/kg), and hyperkalemic (6.9 mEquiv/l), and perfused into a gracilis muscle, produced greater vasodilation (30% of control) than any one of the combination of any two of these. Brace et al. (20) reported that hyperkalemic (7.1 mEquiv/l) perfusion of the isolated dog forelimb initially decreased resistance, but that this response was followed by a gradual increase in resistance to a level above the control value within 5 minutes. Chen et al. (23) suggested that the vasodilation induced by potassium over the range 4 to 12 mEquiv/l results from stimulation of the electrogenic Na/K pump in the sarcoplasma of the smooth muscle cell since it is blocked by ouabain, a potent inhibitor of Na/K ATPase. Ouabain, however, had little effect on the magnitude of exercise dilation but did delay its onset and increase the time to

the steady-state response. These local infusion studies indicate that potassium is vasodilatory, but not to a great degree, and that the effect wanes with time.

Osmolality. Injection or infusion of hyperosmotic solutions into the vascular bed of resting muscle produces vasodilation (51, 55, 56, 59, 99-101, 102, 106, 109, 117, 118, 124, 136, 137, 142-144, 149, 150). Mellander et al. (106) have proposed that increased osmolality is a dominant factor in active hyperemia of skeletal muscle. They reported that local intra-arterial infusion of hypertonic glucose or xylose (about 330 to 340 mOsm/kg) into the feline calf muscles produces nearly the same degrees of venous osmolality and vasodilation as observed during heavy short-term (less than 5 minutes) exercise. Mellander, Lundvall and their co-workers have since expanded their studies to include man (99-101, 109). In the resting human forearm and leg, intra-arterial infusion of hyperosmotic solutions were found to increase venous osmolality by 15 to 30 mOsm/kg and blood flow 3 to 5 fold. Similar studies by Overbeck and Grega (118) showed that intra-brachial infusions of hypertonic dextrose or hypertonic NaCl increased cephalic venous plasma osmolality 10 to 23 mOsm/kg and reduced forearm vascular resistance 4 to 14%. They also found a positive linear correlation between the level of initial vascular resistance and magnitude of response to all hypertonic solutions. Scott et al. (136) and Scott and Radawski (137) showed that intra-arterial infusion of hyperosmotic solutions of NaCl and dextrose in the gracilis muscle (perfused at natural flow) produced about a 10% decrease in vascular resistance

corresponding to a 10 mOsm/kg increase in venous plasma osmolality; however, exercise dropped resistance 85% with an increase of venous osmolality of only 10 mOsm/kg. Gazitua et al. (51) found that a 40 to 50 mOsm/kg increase in venous plasma osmolality, produced by intra-arterial infusion of hyperosmotic solutions of dextrose, urea and NaCl into the dog forelimb, initially (at 2 minutes) decreased vascular resistance an average of 30%, but that continued infusion of any of these substances for 10 minutes resulted in a waning of the resistance toward control values and a complete return in the case of urea. Stainsby and Barclay (150) also reported that the initial rise in muscle and hindlimb flow during close arterial infusion of hyperosmotic urea waned toward the control level within 5 minutes. Skinner and Costin (142-144) observed that infusion of hyperosmotic glucose into the dog gracilis muscle increased venous plasma osmolality to 343 mOsm/kg and decreased resistance 25%. However, when the hyperosmolality was combined with oxygen deficiency (27% saturation) and hyperkalemia (7.6 mEquiv/l) resistance decreased 53%.

Recent Chemical and Biological Evidence Supporting a Role for Metabolically Linked Chemicals Proposed as Mediators

Additional evidence for the participation of various metabolically linked vasoactive chemicals in exercise hyperemia has been obtained from bioassay studies and studies showing increased levels of potassium and hydrogen ions, carbon dioxide and osmolality and decreased levels of oxygen in the tissues or venous effluent during exercise hyperemia.

Bioassay studies on the enhanced vasoactivity of venous blood draining exercising muscle provide compelling evidence that chemicals linked to metabolism play a role in exercise hyperemia. The early bioassay technique of Anrup and Saalfeld (3) was described on page 14. More recently, Ross et al. (126), using an improved reservoir-free system, found that perfusion of one resting canine gastrocnemius muscle (bioassay organ) with blood from the contralateral contracting muscle (donor or regulatory organ), produced a hyperemic response in the resting muscle. Similarly, Scott et al. (133) observed that perfusion of the dog forelimb with blood from the contracting hindlimb promptly lowered resistance in the forelimb. As shown by Scott et al. (138) and Radawski et al. (122), the same holds true when the donor organ is the gracilis muscle and the assay organ the hindlimb or contralateral gracilis. Thus, an immediate fall in resistance in the downstream bioassay organ indicates that the concentration of vasoactive chemicals from the upstream exercising muscle has changed sufficiently to affect the caliber of blood vessels in the bioassay organ. Bioassay studies alone, however, do not firmly establish any specific mediator of exercise hyperemia. Chemical analysis of the tissue fluid or venous effluent during exercise hyperemia is necessary to identify any specific mediator and permits a better evaluation of the relative role of these factors. Oxygen lack will be considered first.

Oxygen. During exercise, the oxygen saturation, content and tension of blood draining skeletal muscle decreases, and the blood flow or degree of vasodilation increases (5, 76, 78, 94, 112, 113, 115, 119,

126, 136, 141, 147, 148). Ross et al. (126) found that during stimulation of the sciatic nerve (1-4 Hz, 1 msec., 7-10 volts) the increase in blood flow averaged 173% with a venous oxygen tension of 25 mm Hg. Scott et al. (136) observed that faradic stimulation of the gracilis nerve (6 Hz, 0.6 msec., 6 volts) produced a 36% drop in resistance (constant flow preparation) when the venous oxygen tension was not permitted to fall below 17 mm Hg. Using a similar preparation, Skinner and Powell (141) found that at 5 minutes of stimulation (0.4 Hz), vascular resistance had dropped 60% while venous oxygen tension had dropped from 70 to 15 mm Hg.

Recent studies by Mohrman et al. (111) and Morhman and Sparks (112) indicate that the vasodilator response following a brief tetanus (32 Hz for one second) or sinusoidal exercise (0.5 to 1.0 Hz) of dog calf muscles parallels a fall in venous oxygen saturation, but that the response is probably too rapid during natural flow or high constant flow perfusion to be explained by changes in tissue oxygen content related to oxidative metabolism. Honig (76) reported that the time course of the hyperemia in dog gracilis muscle following contraction (2 Hz for 10 seconds) was poorly correlated with that of the oxygen tension in venous blood, since oxygen tension returned to control levels while post constriction hyperemia continued. Morganroth et al. (115) reported similar results for venous oxygen saturation following 20 and 60 minutes of exercise (4 Hz) of dog calf muscles. They observed that venous oxygen saturation and vascular conductance remained elevated during exercise, but that following exercise oxygen saturation returned to control with

a half-time five fold faster than vascular conductance. The two latter studies suggest that oxygen is not a mediator of prolonged vasodilation following exercise.

Hydrogen ion. Studies by Gollwitzer-Meier (54), Ross et al. (126), Kontos et al. (87), Kilburn (82), Scott et al. (137), Radawski et al. (122), Gebert and Friedman (52) and others (65) demonstrate that moderate to severe exercise causes an increase in blood flow and, in the steady-state, an increase in the hydrogen ion concentration of the venous blood. Ross et al. (126) showed that active hyperemia of the dog gastrocnemius muscle produced a rise in flow to 173% of the control level with only a 0.05 unit fall in pH and a 4 mm Hg rise in carbon dioxide tension in the effluent blood. Kontos et al. (87) found that exercise hyperemia of the human forearm could triple flow despite only a 0.03 unit fall in pH and a 5 mm Hg rise in carbon dioxide tension. Scott et al. (137) reported that skeletal muscle contraction of the dog hindlimb produced a rise in hindlimb flow to 270% of the control level with only a 0.07 unit fall in effluent blood pH. A comparison of these studies with those in which the hydrogen ion concentration was increased locally in resting muscle, shows that changes in venous blood pH correlate well with the direction but not the magnitude of the hyperemia.

The importance of hydrogen ions during the initiation and maintenance of exercise hyperemia has only recently been investigated. Gebert and Friedman (52) implanted hydrogen ion sensitive electrodes in the tissue of the rat gastrocnemius muscle. Contractions greater than one Hertz first transiently decreased hydrogen ion activity for

about one minute before increasing hydrogen ion activity. At lower contraction frequencies, hydrogen ion activity only decreased. Their findings, and those of Ross et al. (126) and Scott et al. (136), showing that the flow rises maximally within 30 seconds upon motor nerve stimulation, suggest that the hydrogen ion does not participate in the initiation of the dilation and that pre-existing hydrogen ions are first washed out before being added as exercise continues. Radawski et al. (122) suggested that the hydrogen ion may not be so important in the maintenance of prolonged exercise of the gracilis muscle, since the pH arteriovenous difference at the end of a 2 hour exercise (6 Hz, 1.6 msec., 3 volts) period was only 0.04 units. Morganroth et al. (115) reported that venous lactate concentration initially rose and then returned slowly to control values within a one hour exercise (4 Hz) period of dog calf muscles. Moreover, Tobin and Coleman (152) showed that marked hyperemia still occurs under conditions where effluent pH does not decrease (in part because lactic acid is not produced).

Potassium ion. The concentration of potassium in the blood draining skeletal muscle increases during exercise hyperemia (2, 82, 83, 85, 93, 110, 122, 135-137, 140, 141, 146). Kjellmer (83, 85) studied the efflux of potassium from cat limb skeletal muscle at various levels of somamotor nerve stimulation; increased contraction frequency was found to be correlated with increased venous plasma potassium ion concentration. He found that during maximum exercise dilation (constant flow) the venous plasma potassium ion concentration rose to a level twice that at rest and that infusion of potassium salt

solutions to resting muscle, at a level which produced venous potassium ion concentrations similar to that observed during graded exercise, produced vasodilation which was 25 to 65% of that seen during active hyperemia. Kilburn (82) showed that the exercising human forearm venous plasma contained 0.7 mEquiv/l more potassium than arterial blood. Skinner and Powell (140, 141) reported that active contraction of canine gracilis muscle produced increases in blood flow of 170% and 350% corresponding, respectively, with venous potassium ion concentrations of 0.8 and 0.95 mEquiv/l above the control level. Using a dog hindlimb preparation, Scott et al. (136) demonstrated a 270% increase in flow associated with a 0.8 mEquiv/l rise in potassium ion concentration in the venous effluent.

Sreter and Friedman (146) showed that the potassium content of rat gastrocnemius muscle decreased fast at first and then more slowly during a 60 minute exercise period. Scott et al. (136) showed that the source of increased potassium appearing in venous effluent during exercise was predominantly from skeletal muscle, because during perfusion of the canine gracilis with cell free solutions exercise caused venous potassium ion concentration to rise an average of 0.39 mEquiv/l above the control level. Regarding the temporal relationship between venous plasma potassium ion concentration changes during exercise, Scott et al. (136) and Scott and Radawski (137) showed that stimulation of the gracilis nerve (6 Hz, 0.06 msec., 6 volts) produced an 8 fold increase in gracilis muscle blood flow and a 1.2 mEq/l increase in potassium ion concentration within 60 and 10 seconds, respectively, of the onset of

exercise. With continued exercise (5 minutes) the arteriovenous difference for potassium waned toward the control level while the hyperemia continued unabated. Radawski et al. (122) observed that during a 2 hour period of gracilis muscle exercise (6 Hz, 1.6 msec., 6 volts, natural flow), venous potassium ion concentration waned toward the control level and was only 0.2 mEquiv/l higher at the end of the exercise period; vascular resistance fell 80% and remained at that level throughout exercise. Anderson et al. (2) demonstrated that reducing the potassium ion concentration (of the blood perfusing gracilis muscle with a hemodialyzer interposed in the arterial supply) from normal to hypokalemic during the dilation brought on by simulated exercise, did not change vascular resistance. Mohrman and Abbrecht (110) found that a one second tetanus produced dilation lasting 30 seconds and calculated that this could produce an increase in interstitial potassium within 10 seconds, which was sufficiently rapid to cause the accompanying vascular response.

A consideration of these studies on the role of potassium in exercise hyperemia suggests that potassium cannot contribute sufficiently to totally explain hyperemia since exercise is associated with up to a 10 fold increase in flow and only a small increase (about 1 mEquiv/l) in effluent plasma potassium ion concentration. Furthermore, these studies suggest that potassium may participate to a greater extent during the initiation than during the maintenance of exercise.

Osmolality. Mellander et al. (106) reported that active hyperemia (for 5 to 15 minutes) in the lower leg muscles of the cat results in a sustained 80% drop in vascular resistance associated with a 38 mOsm/kg rise in effluent plasma osmolality. Generally, resistance decreased and osmolality increased as a function of sciatic nerve stimulation. Lundvall (100) and Lundvall et al. (99) showed that exercise of the human forearm and leg produces an 85% decrease in resistance accompanied by a 17 mOsm/kg increase in venous plasma osmolality. They also showed a positive correlation between the degree of hyperosmolality and the magnitude of the dilation. In the studies of Mellander and Lundvall on cat and man the extent of vasodilation and venous plasma hyperosmolality induced by arterial infusion was only slightly lower than that observed in exercise.

Scott et al. (136) and Scott and Radawski (137) confirmed in the dog that exercise hyperemia of the gracilis muscle was associated with an increase in venous osmolality. However, they reported that at the end of one minute of exercise, an 8 fold increase in flow was associated with only a 9 mOsm/kg rise in osmolality. Furthermore, by the 5th minute of exercise, osmolality returned to pre-exercise levels, while flow remained elevated. Radawski et al. (122), using a similar preparation, found no arteriovenous difference in osmolality, but sustained hyperemia, at the end of a two hour exercise period. Similarly, Murray et al. (116) and Morganroth et al. (115) recently observed that exercise (4 Hz) of dog calf muscles (perfused at constant flow) for 60 minutes produced a sustained vasodilation, a 17 mOsm/kg increase in venous osmolality at

2 minutes, but no change by the 55th minute of exercise. Following exercise osmolality decreased below control levels while the flow was still elevated. Hilton et al. (74) noted that graded exercise of the cat gastrocnemius and soleus muscles produced changes in osmolality that did not correlate well with the degree of hyperemia.

Mellander et al. (106) suggested that the increase in tissue and venous osmolality during exercise is most likely due to parenchymal cell formation and release of osmotically active metabolites (such as hydrogen ions, phosphates, lactate and other Kreb's cycle intermediates) (121)). Hyperosmotic solutions appear to dilate primarily arteriolar and precapillary resistance vessels when applied topically to rat muscle, as shown by Gray (56). The studies of Johansson and Jonsson (82) suggest hyperosmolality may cause active vasodilation due to hyperpolarization of the vascular smooth muscle cell. Those of Gray (56) suggest passive vasodilation due to cellular dehydration, whereas those of Maxwell et al. (104) and Braasch (19) suggest decreased acto-myosin ATPase activity and decreased blood viscosity, respectively.

Although the vasodilation and increased venous osmolality which occurs subsequent to skeletal muscle exercise or local infusion of hyperosmotic substances clearly indicate that hyperosmolality is a factor in exercise hyperemia, many of the studies suggest that hyper-osmolality cannot completely explain the magnitude or time course of the vasodilation of exercise. As indicated above, Scott et al. (136) and Scott and Radawski (137) showed in the dog gracilis muscle that venous osmolality rises to a maximum in one minute whereas flow increases much

faster. The reports by Scott et al. (136), Scott and Radawski (137), Radawski et al. (122), Murray et al. (118), and Morganroth et al. (115) demonstrate that prolonged exercise of dog skeletal muscle is associated with a waning of the arteriovenous osmolality difference to control levels, while blood flow remains elevated. Scott et al. (136), Skinner and Costin (144) and Gazitua et al. (51) showed that exercise of canine skeletal muscle causes only a small increase in venous plasma osmolality (about 9 mOsm/kg) and a large increase in blood flow (up to 8 fold), whereas infusion of hyperosmolal solutions at rates which produce larger increases in venous osmolality (approximately 40 mOsm/kg), induces smaller increases in flow (2 fold). Furthermore, Gazitua et al. (51) and Stainsby and Barclay (150) found that the decrease in vascular resistance produced by close arterial infusion of some hyperosmotic solutions wanes toward the control values within several minutes.

STATEMENT OF PURPOSE

It is evident from this review that there is a paucity of information on the combined effects of chemical factors during exercise hyperemia. In particular, there appears to be no *in vivo* data on the possible interaction of oxygen lack and increased concentrations of hydrogen ions and/or carbon dioxide in the metabolic control of blood flow during exercise. In addition, the relative contribution of the various metabolites during exercise of different duration and intensity has only recently received attention. The studies described in this thesis were designed to evaluate the relative contribution of several of these metabolically linked factors (in particular oxygen and hydrogen ions or carbon dioxide), but also potassium ions and osmolality during moderate to heavy long-term exercise. Evidence for participation and interaction of these factors was obtained by bioassay of the vasodilator properties and chemical analysis of the blood draining exercising skeletal muscle perfused at natural and constant flow, and by chemical alteration of the blood perfusing resting skeletal muscle.

METHODS

All experiments were performed on mongrel dogs (15-20 Kg) of either sex, sedated with morphine sulfate (30 mg, subcutaneously), and anesthetized twenty minutes later by intravenous injection of a mixture of urethane (0.5 g/Kg) and alpha-chloralose (75 mg/Kg). The animals were artificially ventilated with a positive pressure respirator (Harvard Apparatus Co., Model 607, Dover, Mass.) at a volume and rate sufficient to maintain systemic arterial pH between 7.35 and 7.45. Following surgical procedures, heparin sodium (600 units/Kg) was administered intravenously to prevent blood coagulation. Supplemental doses of urethane-chloralose and heparin were given during the course of the experiment. Blood volume was maintained with dextran (6% w/v; average molecular weight, 75,000) in saline. All blood pressures mentioned were continuously monitored with pressure transducers (Statham Laboratories, low volume displacement model P23Gb, Hato Rey, Puerto Rico) and recorded on a direct writing oscillograph (Hewlett-Packard, model 7796A, Boston, Mass.).

Data were evaluated by the Student's t-test modified for paired replicates (145) except in one series of experiments where a 2-way analysis of variance was used (145). Initial non-experimental values served as statistical controls. In averaging data only the standard error of group means are displayed. The experiments were conducted in several series.

Bioassay Studies: Bilateral Gracilis

In each of 17 animals one gracilis muscle (regulatory gracilis, RG), was exposed and freed of connective tissue (Figure 2). All blood vessels communicating with this muscle except the major artery and vein were ligated. The gracilis nerve was severed and the distal stump carefully fixed under oil on platinum stimulating electrodes. Extracorporeal blood circuits were designed using polyethylene tubing, P.E. 190-220 (Intramedic, Becton, Dickinson and Co., Parsippany, N.J.), so that the RG artery was perfused at constant flow by diverting femoral artery blood through a pulsatile pump (Sigmamotor, Inc., model TM-5, Middleport, N.Y.). The RG vein was cannulated and the venous blood pumped at constant flow through a hollow fiber gas exchange permeator (type b/HFG-1, capillary tube surface area = 500 cm^2, Dow Chemical Co., Midland, Mich.) to the contralateral gracilis artery. The permeator rather than a lung was used in these experiments because bioassay (66, 133, 138) and chemical analysis (18, 22, 33, 47, 120) of venous blood suggest that one or more of the vasoactive adenyl compounds are released during exercise hyperemia. These compounds are rapidly degraded on passage through the lung, but presumably not on passage through the permeator. A bypass shunt permitted excess effluent blood to be diverted to the femoral vein. The contralateral muscle (assay gracilis, AG), was surgically isolated, including denervation, except for the major artery and vein. Isolation included occlusion of vessels at the sites of muscle origin and insertion.

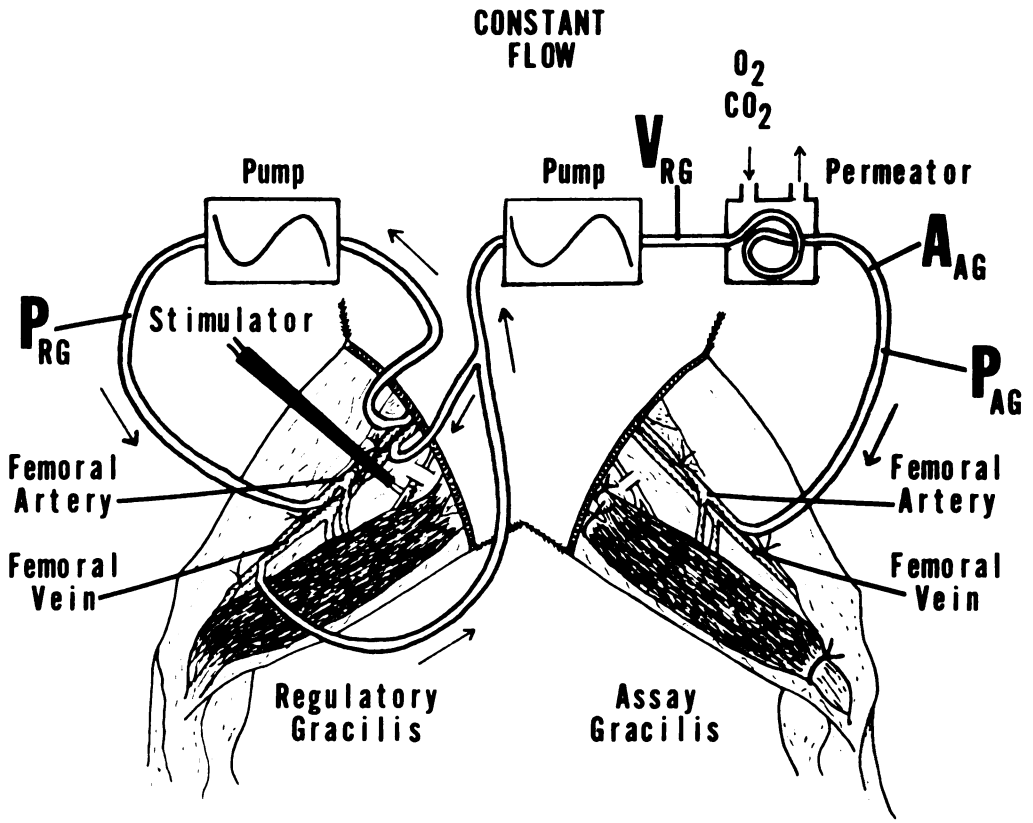


Figure 2. Preparation for constant flow perfusion of regulatory (RG) and assay (AG) gracilis muscles. P_{RG} and P_{AG} = perfusion pressures of RG and AG. V_{RG} and A_{AG} = RG effluent blood before and after passage through permeator.

Systemic blood pressure, regulatory gracilis vein pressure and regulatory and assay gracilis perfusion pressures (P_{RG} and P_{AG}) were monitored and recorded. The perfusion pressure of each muscle was adjusted initially to approximate systemic blood pressure by adjusting pump flows. RG flow was set at a rate equal to or slightly greater than that of the AG to assure that the regulatory muscle furnished the total blood supply to the assay muscle. The transit time for blood flowing between the two muscles varied between 1 and 3 minutes.

To produce muscle exercise the electrodes were connected to a square wave stimulator (Grass Instruments, model S-5, Quincy, Mass.) and the sectioned gracilis nerve was stimulated at a voltage of 6, duration of 1.6 milliseconds and a frequency of 1, 3 or 6 Hertz. Before, after and initially during exercise gas exchange across the permeator was prevented by occluding the inflow and outflow ports; thus, the resting AG was perfused with unaltered venous blood from the exercising RG. During exercise the gas permeator was ventilated randomly with selected oxygen, carbon dioxide, nitrogen mixtures at 37°C. Appropriate selection of the mixtures permitted selective corrections of gas tensions and pH of blood perfusing the AG to levels observed before exercise of the RG. In 12 animals, P_{O_2} and P_{CO_2} (pH) were corrected singly and simultaneously. In 5 additional animals only simultaneous corrections of P_{O_2} and P_{CO_2} (pH) were made.

Blood samples (1 to 3 ml) were drawn anaerobically upstream (V_{RG}) and downstream (A_{AG}) to the permeator during rest and during exercise with and without gas exchange for determination of P_{O_2}, P_{CO_2}

and pH, potassium ion concentration and osmolality. P_{CO_2} and pH determinations were made using potentiometric methods (Radiometer-Copenhagen, blood micro system, acid base analyzer, Copenhagen, Denmark). A calomel electrode served as the common reference electrode. P_{O_2} was determined by reduction of O_2 to H_2O_2 and measuring the resulting current flow. The P_{O_2} electrode was made of a platinum cathode and a silver-silver chloride anode placed in an electrolytic solution behind a polypropylene membrane. The P_{CO_2} and pH electrodes utilized a combination of a glass electrode and a silver-silver chloride electrode. The separate electrodes, encased in a common thermostatically controlled water filled jacket, allowed simultaneous measurement of these parameters at body temperature (38°C). Oxygen tension was also monitored continuously with a macroelectrode (Beckman Instruments, Inc., physiological gas analyzer, model 160, Fullerton, Cal.) interposed between the permeator and the assay muscle. Potassium ion concentration was determined by flame photometry (Beckman Instruments, Inc., model 105, Fullerton, Cal.) and plasma osmolality by the freezing point depression method (Advanced Instruments Osmometer, model 31L, Watertown, Mass.). RG vascular resistance (R_{RG}) was calculated by dividing the difference of P_{RG} and gracilis venous pressure by the flow and is expressed as mm Hg/ml/min/100 grams of wet tissue. AG resistance was calculated by dividing P_{AG} by flow, since AG venous pressure was not monitored.

In another series of animals (N = 12) the regulatory muscle artery was not pump perfused, but retained its natural connection with the circulation so that RG blood flow was allowed to vary (Figure 3).

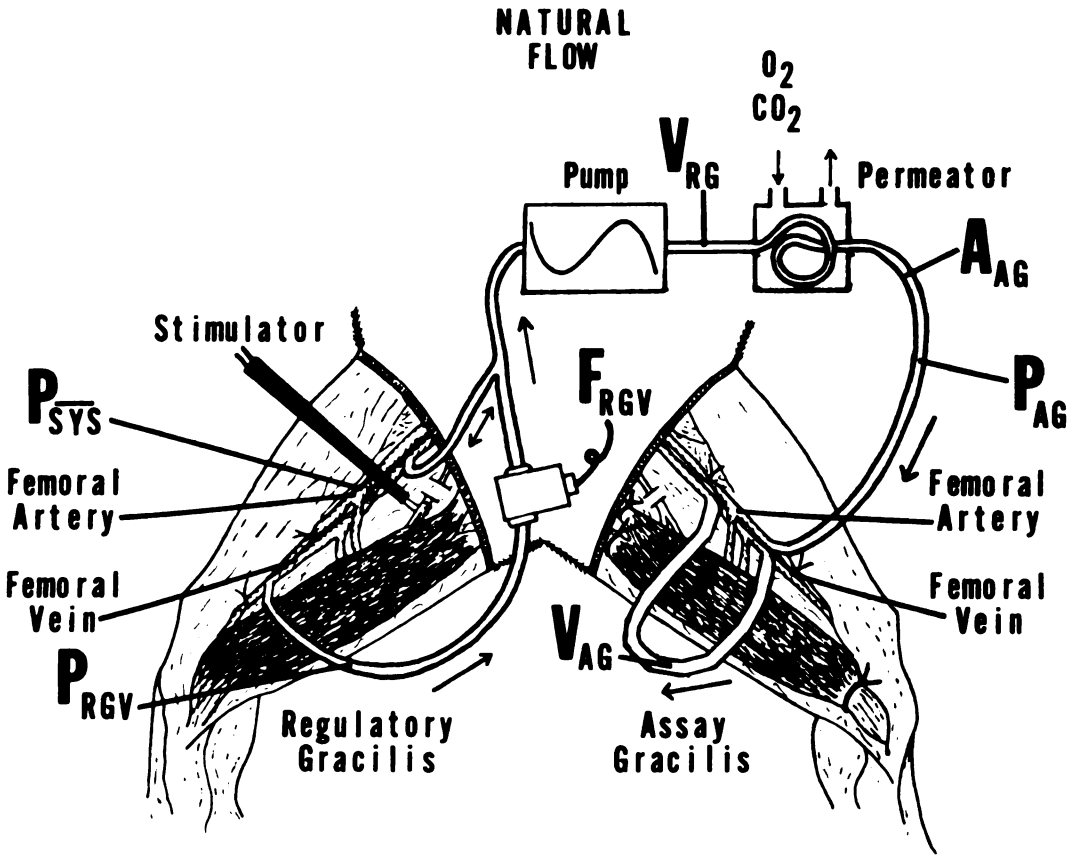


Figure 3. Preparation for natural flow perfusion of RG and constant flow perfusion of AG. P_{SYS} = systemic arterial blood pressure. F_{RGV} = RG vein flow. V_{AG} = AG effluent blood. P_{AG}, V_{RG} and A_{AG} as in Figure 2.

RG venous outflow was measured with an electromagnetic flowmeter (Biotronex Laboratory, model 610, BLC-2048-E04 probe, Silver Spring, Md.) and expressed in ml/min/100 grams. The AG was pump perfused at constant flow with RG venous blood after passing through the permeator. During rest, a small fraction of the blood supply to the AG was obtained by retrograde flow of systemic venous blood through a shunt connecting the femoral vein. The AG vein was cannulated in order to obtain venous samples (V_{AG}). To determine the relationship between chemical changes in venous blood and resistance to blood flow during prolonged exercise, the regulatory muscle was stimulated (6 Hz, 1.6 msec., 6 volts) for 60 minutes and its effluent blood was sampled at timed intervals (2, 5, 15, 30, 45, 60 minutes) for analysis as described above. The contralateral muscle (AG) in the same 12 animals, prepared as described above, was used again as a bioassay organ to test the vasoactivity of the RG venous effluent during exercise at natural flow and to determine to what extent correction of blood gases and pH influence its vasoactivity.

Exercise vs. Mimicked Exercise: Unilateral Gracilis

In this series the resistance response to reduced P_{O_2} and increased P_{CO_2} during rest was compared to that seen during exercise. In each of 17 animals a single gracilis muscle was surgically isolated except for the major artery and vein (Figure 4) as described above. The gracilis artery was perfused by diverting blood from a femoral artery through the pulmonary circulation of an isolated lung obtained from a 5-7 Kg dog. This procedure has been described in detail

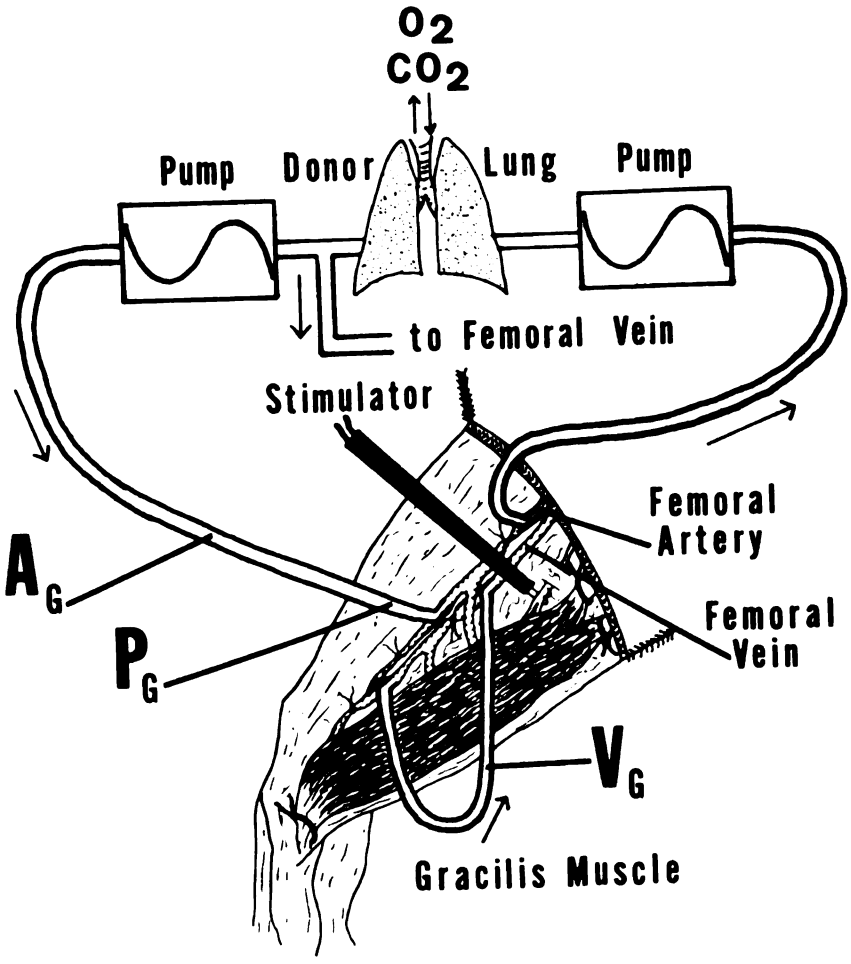


Figure 4. Preparation for constant flow perfusion of a single gracilis (G) muscle. P_G = G perfusion pressure. A_G = Blood perfusing G after passage through donor lung. V_G = G effluent blood.

previously (27, 134). In brief, femoral artery blood was pumped (Sigmamotor, model T-6SH) into the pulmonary artery of the donor lung. The pulmonary effluent flowed through a cannula tied in the partially preserved left atrium. Pulmonary artery and vein pressures were monitored in the isolated lung and maintained similar to the normal in vivo values by varying the rate at which femoral arterial blood was pumped into the lung. Excess pulmonary blood flow was diverted to a femoral vein. The lung was substituted for the permeator in this series because the permeator was not capable of producing the desired large reduction in blood P_{O_2} during a single pass. An extracorporeal circuit was also interposed in the gracilis venous outflow (V_G) to facilitate blood sampling.

In 8 animals the donor lung was ventilated (Harvard Apparatus Co., Dual Phase Control Respirator Pump, Millis, Ma.) in random order with mixtures of oxygen, carbon dioxide and nitrogen which produced gas changes in the blood perfusing the resting muscle such that the P_{O_2}, P_{CO_2} and pH of the venous blood resembled those in venous blood from heavily exercising muscle. Gracilis artery (A_G) and gracilis vein (V_G) blood was sampled before, during and after gas changes and analyzed as described above.

In 9 other experiments, the degree of vasodilation occurring during graded steady-state exercise was compared to that produced in the same resting muscle during perfusion with hypoxic-hypercapnic blood. To produce graded exercise the sectioned gracilis nerve was continuously stimulated at 6 volts, a duration of 1.6 milliseconds with step increases

in frequency every eight minutes as follows: 0.2, 0.4, 0.8, 1.6, 3.0, 6.0 Hz. During exercise, as during rest, the donor lung was ventilated with a control gas mixture (20% O_2, 5% CO_2, 75% N_2). Samples of gracilis venous blood (V_G) were taken just before each change in contraction frequency. With the muscle at rest, the ventilatory gas mixture was changed from the control mixture to 0% O_2, 20% CO_2, 80% N_2 in order to reduce arterial and thus, venous blood P_{O_2} and pH. This maneuver was conducted randomly either before or after graded exercise. The resistance and venous blood composition during mimicked exercise were compared to those during steady-state exercise.

RESULTS

Bioassay Studies: Bilateral Gracilis

Figure 5 presents representative data from one experiment. Exercise of the RG produced, within 2 minutes, a 52% decrease in RG perfusion pressure which was maintained throughout the 92 minute exercise period. The drop in RG perfusion pressure was associated with decreased P_{O_2} and pH and increased potassium ion concentration and osmolality in the RG venous effluent perfusing AG and a 45% decrease in AG perfusion pressure (panel 2). Since RG blood flow (14 ml/min) was greater than AG flow (12 ml/min), only RG effluent blood perfused AG. Exercise continuing, ventilation of the permeator with 100% O_2 returned P_{O_2} and pH of the blood perfusing AG and the AG perfusion pressure to approximately pre-exercise values (panel 3). At this time of exercise (46 minutes), RG venous plasma potassium ion concentration had stabilized slightly above control and osmolality had returned to the control level (permeator ventilation has no effect on these factors). Permeator ventilation was then terminated and RG venous blood P_{O_2} and pH and AG perfusion pressure again fell to approximately pre-ventilatory values (panel 4). Ventilation with 20% O_2 and 0% CO_2 increased pH slightly above control but did not affect P_{O_2}; concomitantly AG perfusion pressure rose slightly (panel 5). Ventilation with 95% O_2 and 5% CO_2 elevated P_{O_2} to the pre-exercise level and reduced pH to near the

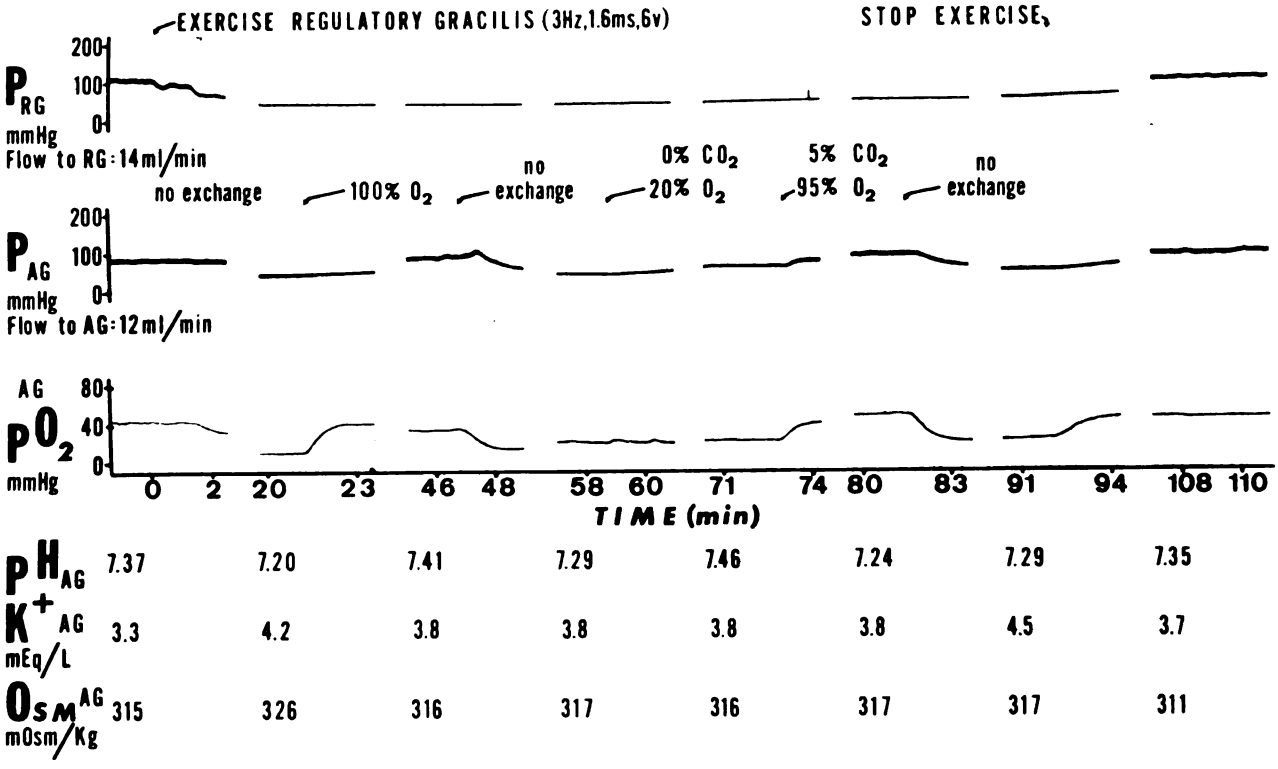
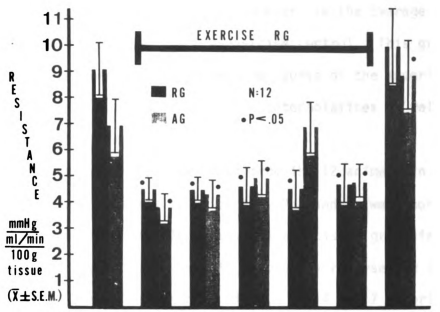


Figure 5. Representative tracing from the preparation illustrated in Figure 2 showing the effects of ventilating the permeator with various gas mixtures on P_{AG} and A_{AG} parameters during sustained exercise of RG. Arrows refer to time of gas exchange.

exercise level. In this animal AG perfusion pressure returned to the pre-exercise value (panel 6). Cessation of ventilation was again associated with reductions in P_{O_2} and pH of the blood perfusing AG and AG perfusion pressure returned to the initial exercise level (panel 7). All variables returned to or approached control values soon after exercise was terminated (panel 8).

Figure 6 presents average data from 12 such constant flow experiments. RG exercise produced a maintained 50% drop in RG resistance and a 45% drop in AG resistance associated with decreased P_{O_2} (49 to 19 mm Hg) and pH (7.37 to 7.20) and increased potassium ion concentration (3.6 to 4.5 mEq/l) and osmolality (306 to 316 mOsm/Kg) of RG venous blood perfusing AG. When the pH of the blood perfusing AG was returned to the pre-exercise level (7.39 vs. 7.37), while P_{O_2} remained at the exercise level (21 vs. 19 mm Hg), AG resistance increased significantly ($P < .025$) 13% above the exercise level. Correction of P_{O_2} (54 vs. 49 mm Hg), but not pH (7.23 vs. 7.37) increased AG resistance 29% ($P < .025$) above the exercise level. Simultaneous correction of both P_{O_2} and pH (44 vs. 7.37, respectively), however, completely abolished the drop in AG resistance (5th set of columns). At the time of simultaneous gas correction, RG venous blood potassium ion concentration and osmolality were also at pre-exercise levels (4.1 vs. 3.6 mEq/l and 306 vs. 306 mOsm/Kg, respectively). As will be shown below, this probably occurred spontaneously with time and independently of permeator ventilation. It is important to note, however, that AG resistance again dropped when permeator ventilation was terminated and this



pH	units	7.37 ±.014	7.20* ±.008	7.39 ±.016	7.23* ±.014	7.38 ±.016	7.29* ±.009	7.36 ±.010
N:12								
P_{O_2}	mmHg	49 ±3.2	19* ±2.1	21* ±1.9	54 ±5.2	44 ±3.5	21* ±1.9	40* ±1.6
A_{AG}								
K^+	mEq/L	3.6 ±0.13	4.5* ±0.06	4.2* ±0.10	4.2* ±0.10	4.1 ±0.11	4.1 ±0.13	3.8 ±0.14
N:8								
O_{SM}	mOsm/Kg	306 ±3.0	316* ±3.5	310* ±3.0	310* ±3.1	306 ±2.9	309* ±3.3	306 ±3.2

Figure 6. Average effects of ventilating the permeator with various gas mixtures (randomized) on AG resistance and A_{AG} parameters during sustained exercise (1 to 6 Hz, 1.6 msec., 6 volts) of RG. $\bar{X}$ ± S.E.M. represents the mean ± standard error of the group mean. P values relative to pre-exercise controls. Preparation illustrated in Figure 2.

was unassociated with large changes in potassium ion concentration and osmolality (6th set of columns). Following exercise the average AG resistance was 28% higher than the pre-exercise control. This gradual increase in vascular constriction during the course of the experiment may be due to sympatho-adrenal discharge of catecholamines or release of other pressor substances.

Figure 7 summarizes selected data from the 12 animals in Figure 6 plus 5 additional animals in which only P_{O_2} and pH were corrected simultaneously in the effluent blood from the exercising gracilis. Correction of both P_{O_2} and pH were found to completely reverse the fall in AG resistance associated with exercise of RG. In 14 of 17 experiments AG resistance completely returned to, or rose slightly higher than, pre-exercise values during simultaneous gas changes. RG venous potassium ion concentration was significantly elevated during the exercise period, but waned in the direction of the control level as exercise continued. The osmolality was significantly altered during the exercise periods before and after gas exchange, but not during correction of P_{O_2} and pH. Again the dilation in the assay muscle reappeared when the changes in P_{O_2} and pH were allowed to reoccur (4th set of columns).

Figure 8 shows data from a series of experiments (N = 12) in which the regulatory gracilis was naturally perfused. It is evident that steady-state exercise of the RG was quickly associated with a large initial (7 fold) increase in blood flow and reduction in resistance (90%) and that these changes waned only slightly during the 60 minute exercise period. Measured at 2 minutes of exercise, venous blood (V_{RG})

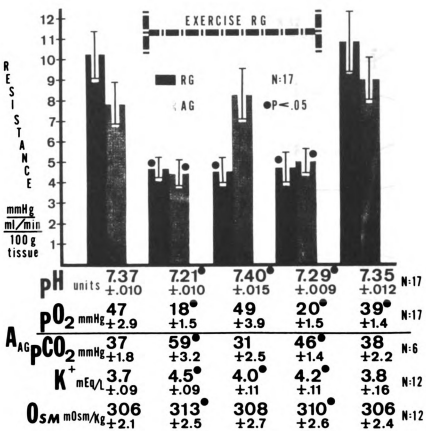


Figure 7. Average of the 12 experiments shown in Figure 6 together with 5 additional experiments in which the permeator was ventilated with only one gas mixture. P values relative to pre-exercise controls.

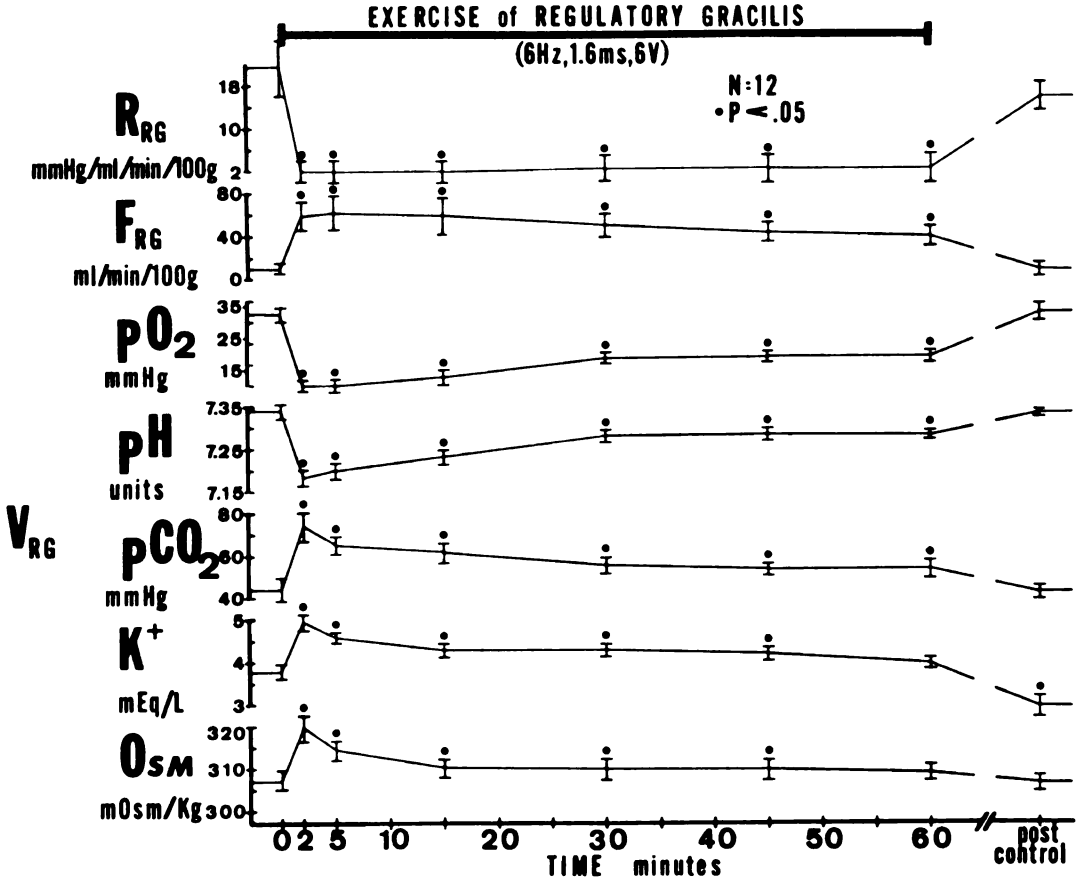


Figure 8. Average effects of exercise on RG resistance, flow and effluent blood parameters as a function of time. Preparation illustrated in Figure 3. P values relative to pre-exercise controls.

P_{O_2} and pH were markedly reduced and P_{CO_2}, osmolality and potassium ion concentration were elevated. The P_{O_2}, P_{CO_2} and pH changes tended to wane during exercise but did not attain pre-exercise levels after 60 minutes of exercise; P_{O_2} increased from 10 to 19 mm Hg, pH increased from 7.18 to 7.29 and P_{CO_2} decreased from 72 to 55 mm Hg. In contrast, the initial increase in potassium ion concentration (1.25 mEq/l) and osmolality (13 mOsm/Kg) tended to return to control more rapidly and neither were significantly different from pre-exercise levels after 60 minutes of exercise. At the end of the exercise period RG blood flow was still 5 times greater than before exercise and RG vascular resistance had increased only 4%. Following exercise, effluent plasma potassium ion concentration was 0.8 mEq/l lower than before exercise.

Figure 9 presents the findings on bioassay of the venous blood from the naturally perfused RG. The 12 animals used in this series were the same as those depicted in Figure 8. Since flow to the RG was allowed to increase it is unlikely that products of metabolism accumulated to the same extent in these studies as in the constant flow studies. As before, during those exercise periods when the permeator was not ventilated (columns 1, 2, 6 and 7), any changes in gas tensions and pH of the blood perfusing the AG were caused by the exercising upstream muscle (RG). In Figure 8 it was shown that all the blood parameters studied tended to wane to or toward control levels during the exercise period. This is reflected in columns 2 and 6 of Figure 9, which also shows that AG resistance rose 40% from the beginning to end of the exercise of RG. Column 6 of Figure 9 shows that A_{AG}--pH, P_{CO_2}

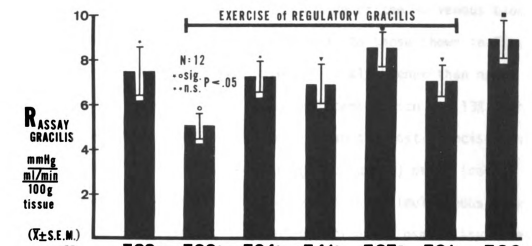
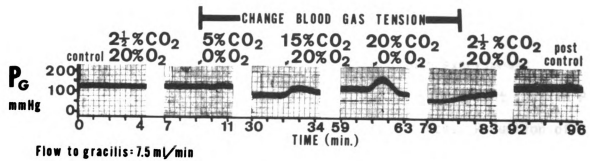


Figure 9. Average effects of ventilating the permeator with various gas mixtures (randomized) on AG resistance and A_{AG} parameters during exercise of RG (see Figure 8). Preparation illustrated in Figure 3. Statistical evaluation employed a 2-way analysis of variance with multiple comparison among means after the Students-Newman-Keuls test (145). Any row mean is significantly different from all other row means unless marked by a common symbol.

and osmolality returned to control levels during exercise of RG. The vascular response on changing both P_{O_2} and pH of the RG venous blood before it entered the AG artery were similar to those shown in Figure 6; induced changes of P_{O_2} and pH to levels slightly higher than non-exercising levels caused a rise in AG resistance which was 13% higher than the pre-exercise level and 5% lower than the post-exercise level (column 5). Single changes in either P_{O_2} (column 3) or pH (column 4) did not increase AG resistance to the extent as a simultaneous change in P_{O_2} and pH. The potassium ion concentration and osmolality were elevated during the pre-ventilatory exercise period (column 2). During the post-ventilatory exercise period (column 6) potassium ion concentration was slightly elevated and osmolality had returned to the non-exercise level.

Exercise vs. Mimicked Exercise: Unilateral Gracilis

Figure 10 shows data obtained from one representative experiment in which gas tensions and pH were altered sequentially and simultaneously in a single resting gracilis muscle. The arrows refer to the onset of the vascular response. The steady-state response to each change is shown on the left half of the subsequent panel. The tracings show that the perfusion pressure (P_G) dropped when either the inflow and thus, gracilis venous (V_G) blood P_{O_2} or pH was selectively reduced (left half of panels 3 and 4), but that the pressure drop was greater when pH and P_{O_2} were decreased simultaneously (panel 5). As has been shown



Flow to gracilis: 7.5 ml/min

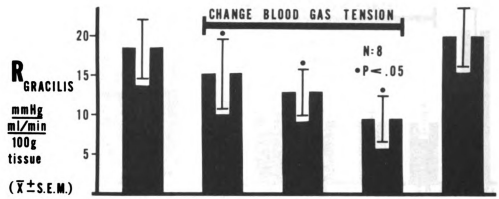
V_G	pO_2 mmHg	45	21	55	29	52
	pH units	7.43	7.38	7.17	7.06	7.38
	pCO_2 mmHg	20	28	59	88	28
	K^+ mEq/L	3.8	3.7	4.1	4.1	4.0
	O_{sm} mOsm/Kg H_2O	300	295	302	302	291

Figure 10. Representative data from the preparation illustrated in Figure 4 showing the effects of ventilating the donor lung with various gas mixtures on P_G and V_G parameters. Arrows refer to the onset of vascular response.

previously (42), the steady-state resistance response to carbon dioxide was preceded by a transient response of the opposite sign (panels 3 and 4). Induced changes in gas tensions of the blood perfusing the muscle did not appear to affect potassium ion concentration or osmolality in the effluent blood (V_G).

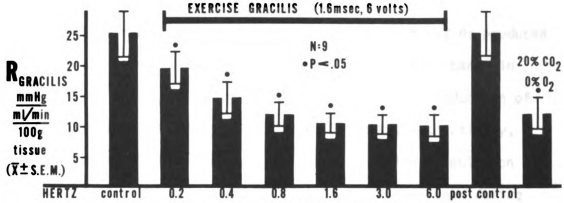
Figure 11 summarizes the data from 8 such experiments. Reduction of effluent blood pH (7.38 to 7.11) and a slight rise in P_{O_2} (52 to 69 mm Hg) produced an 18% fall in resistance. Reduction of P_{O_2} (52 to 21 mm Hg) but not pH (7.38 to 7.36) caused a 30% drop in resistance. When both pH (7.38 to 7.10) and P_{O_2} (52 to 23 mm Hg) were simultaneously reduced to levels observed during heavy steady-state exercise (see Figures 6, 7 and 12) vascular resistance dropped to 53% of the control level. The vasodilation induced by hypoxia alone was not significantly different than that produced by hypercapnia alone. However, hypercapnic-hypoxic blood perfusion produced a greater drop in resistance ($P < 0.05$) than perfusion with either hypercapnic or hypoxic blood. In 1 of 8 dogs, reduction of pH alone caused a small steady-state increase in resistance. Reduction of blood P_{O_2} alone produced vasodilation in all 8 animals. Effluent blood potassium ion concentration increased slightly from 3.3 to 3.8 mEq/l during the course of the experiments. Effluent blood osmolality increased slightly during hypercapnic-hypoxic perfusion.

Figure 12 shows the average effects of graded nerve stimulation on vascular resistance and on various vasoactive factors in the effluent blood (V_G). The last column shows the effects of simultaneous reduction



	pH units	7.38 .027	7.11• .022	7.36 .026	7.10• .019	7.34• .022
	pO_2 mmHg	52 3.7	69• 4.4	21• 1.4	23• 1.4	56 3.6
V_G	pCO_2 mmHg	27 2.2	71• 3.4	32 2.8	78• 6.3	33 2.6
	K^+ mEq/L	3.3 0.18	3.6• 0.18	3.4 0.12	3.7• 0.22	3.8• 0.18
	O_{SM} mOsm/Kg H_2O	302 1.5	304 2.7	304 2.1	308• 1.9	303 2.9

Figure 11. Average effects of ventilating the donor lung with various gas mixtures (randomized) on P_G and V_G parameters. Preparation shown in Figure 4. P values relative to pre-ventilatory change control.



V_G									
pH units	7.37 .017	7.35 .012	7.32* .013	7.27* .019	7.20* .015	7.16* .007	7.11* .015	7.31* .018	7.12* .018
pO_2 mmHg	51 3.3	41* 2.4	35* 2.4	30* 2.2	26* 1.8	23* 1.8	24* 2.0	49 3.0	23* 1.3
pCO_2 mmHg	36 4.2	39 4.3	42* 4.9	52* 4.9	66* 6.6	72* 5.5	83* 7.2	41 4.0	82* 5.9
K^+ mEq/L	3.5 0.14	3.6 0.13	3.8* 0.12	4.0* 0.12	4.5* 0.11	4.9* 0.11	5.1* 0.09	3.9* 0.11	3.6 0.17
O_{SM} mOsm/L	309 2.1	309 3.0	309 2.8	313* 2.4	317* 2.6	319* 2.4	319* 3.7	309 2.4	309 2.4

Figure 12. Average effects of step increases in contraction frequency and ventilation of the donor lung with hypoxic, hypercapnic gas on G resistance and V_G parameters. Preparation shown in Figure 4. P values relative to pre-exercise controls.

in pH and P_{O_2} during rest. Resistance and P_{O_2} decreased and osmolality increased as a function of stimulation frequency over the range 0.2 to 1.6 Hz and remained constant over the range 1.6 to 6.0 Hz; effluent blood pH decreased and P_{CO_2} and potassium ion concentration increased over the entire range of frequencies. Stimulation at 0.2 Hz produced a 24% decrease in resistance while the only significant change in effluent blood was a slight drop in P_{O_2}. During rest, reduction of the effluent blood pH and P_{O_2} to 7.12 and 23 mm Hg, respectively, reduced resistance only to the extent observed during stimulation at 0.8 Hz (P_{O_2}--30 mm Hg, pH--7.27) and not to the extent seen during stimulation at 6.0 Hz where the venous P_{O_2} (24 mm Hg) and pH (7.11) were like those produced by gas changes during rest. Osmolality and potassium ion concentration were unaffected during gas changes at rest but were elevated during stimulation above 0.4 Hz.

DISCUSSION

In brief, these studies show that the enhanced vasodilator activity of venous blood from the constant flow or naturally perfused canine gracilis muscle during steady-state exercise can be completely abolished by simultaneously returning the P_{O_2} and pH to pre-exercise levels. They also show that simultaneously induced changes in P_{O_2} and pH in the blood perfusing a resting gracilis muscle, to levels observed in venous blood from heavily exercising muscle, produce a fall in resistance which does not quite equal that seen during heavy exercise. Assuming that the enhanced vasodilator activity of the effluent blood during exercise is due to changes in the concentrations of metabolically linked vasoactive chemicals, these studies suggest that oxygen and hydrogen ions are important factors in the maintenance of active hyperemia of skeletal muscle.

It has been previously demonstrated by bioassay that the venous blood draining contracting skeletal muscle exhibits greater vasodilator activity than that draining resting skeletal muscle (3, 66, 122, 126, 133, 136, 138). Anrep and Saalfeld (3) found that venous blood collected from the dog gastrocnemius muscle during muscular activity and restricted arterial inflow was a powerful dilator when re-perfused later in the same muscle at rest. They concluded that the vasodilator action was due to a stable metabolite present in venous blood during exercise

but not during rest. Oxygenation of this blood did not abolish the vasodilator property, so they concluded further that oxygen lack was not a cause of the vasodilation. It may well be, however, that oxygen lack was a stimulus that caused vasodilation, particularly in blood collected from exercising muscle that was not re-oxygenated. Since they collected and only later re-perfused blood from exercising muscle, the vasodilatory property of the blood could have been due to potassium release from blood cells, independent of oxygenation. Using improved bioassay techniques investigators have more recently confirmed the marked vasodilator property of venous blood draining exercising muscle. The gastrocnemius (126), hindlimb (133) or gracilis (122) muscles of the dog have been used as the regulatory organ and the gastrocnemius (126), forelimb (133), hindlimb (138) or gracilis (122) muscles as the bioassay organ with similar results. Similarly, this study shows that exercise of the dog gracilis muscle produced a 50% fall in RG resistance when perfused at constant flow and a 90% fall in RG resistance when perfused at natural flow; this vasodilation in the regulatory gracilis was accompanied by a 45% and a 33% drop in resistance, respectively, in the assay gracilis perfused at constant flow. Clearly dilator factors are present in the effluent blood but the bioassay technique alone cannot identify the specific factor or factors eliciting this response.

As pointed out in several recent reviews (1, 7, 17, 64, 65, 67, 68, 107, 108, 127, 151), no single metabolic factor has been found which can entirely explain the hyperemia of exercise. The participation of oxygen in local regulation has been considered for many years and is considered here first.

Oxygen content, saturation and tension of blood draining contracting skeletal muscle has been shown to fall together with vascular resistance (5, 57, 76, 78, 94, 97, 111, 112, 115, 119, 126, 136, 141, 147) and oxygen lack has often been shown to produce vasodilation (28, 40, 42, 58, 61, 69, 95, 126, 134, 142-144). An evaluation of these studies, however, indicates that the magnitude of the dilation produced by oxygen lack is often considerably less than that produced by muscular exercise. In particular, the study by Ross et al. (126) showed that induced reduction of venous blood P_{O_2} (to 23 mm Hg) from resting muscle to levels obtained during exercise (25 mm Hg) produces only 31% of the flow increase observed with exercise. A related study by Scott et al. (136) demonstrated that perfusion of resting skeletal muscle with hypoxic blood produced a larger drop in venous oxygen tension (10 mm Hg) and a smaller drop in resistance (17%) than that produced by exercise (6 Hertz) when the venous oxygen tension was not allowed to fall to exercise levels by increasing the oxygen tension of the perfusing blood (oxygen tension: 17 mm Hg, resistance: 37% decrease). The present study supports the conclusion that oxygen is not wholly responsible for, but contributes significantly in, exercise hyperemia. It was observed that the enhanced vasodilatory activity of venous blood from exercising muscle is attenuated (29%) when the effluent blood P_{O_2} is singly corrected to pre-exercise levels (Figures 6 and 9) and that selective reduction of resting muscle effluent blood P_{O_2} to levels seen during heavy exercise produces a significant drop in resistance (30%) (Figure 11). In addition, effluent blood P_{O_2} remains

decreased during 60 minutes of exercise hyperemia (Figure 8) and is the only measured factor altered concomitant with exercise dilation at a low contraction frequency (Figure 12). On the other hand, correction of the P_{O_2} in the venous blood did not completely abolish its enhanced vasodilator activity (AG resistance increased 29% above the exercise level, see Figure 6), and reduction of P_{O_2} in resting muscle venous blood did not lower resistance to levels observed during heavy exercise (30 and 53% of pre-ventilatory or pre-exercise control, respectively. Compare Figures 11 and 12).

The participation of carbon dioxide or pH in local regulation has been also considered for many years. The pH of venous blood draining skeletal muscle drops during moderate to heavy exercise (52, 54, 66, 82, 87, 122, 126, 136, 137). A comparison of these studies with those in which the blood perfusing resting skeletal muscle was exposed locally to high CO_2 tensions or acids shows a good correlation between the hydrogen ion concentration and the direction but not the magnitude of the dilation. As the blood flow increases up to three times resting values during exercise, the pH decreases only .03 to .07 units (66, 122, 126, 136, 137). Induced changes in venous blood pH during rest must be very large to produce measurable changes in resistance (28, 30, 39, 54, 81, 88-91, 114, 123, 154). The present study concurs with these previous reports that pH or P_{CO_2} changes alone cannot account for most of the vascular response to exercise. We found that correction of the pH of the blood draining exercising muscle to pre-exercise levels only partially reduced (13% increase above the exercise level) the

vasodilation of the assay muscle (Figure 6). Reduction of the venous blood pH in resting muscle to the levels observed during heavy exercise produced a significant, but much smaller drop in resistance (18% of pre-ventilatory control) than during heavy exercise (53% of pre-exercise control, compare Figures 11 and 12). However, the pH, like P_{O_2}, remained depressed and the P_{CO_2} remained elevated during 60 minutes of steady-state exercise hyperemia (Figure 8).

The basis for the transient rise in vascular resistance during hypercapnic blood perfusion (Figure 10) is not known. Fleish et al. (42), who first reported it, suggested that carbon dioxide might have a double effect on vascular resistance; increased intravascular carbon dioxide concentrations might constrict arterial blood vessels, whereas increased tissue carbon dioxide concentrations might be vasodilatory. Hypercapnia might also produce a temporary increase in red blood cell size (chloride shift) and thus increase viscosity or increase the availability of molecular oxygen through the Bohr effect. Increased intravascular P_{CO_2} has been shown to increase perivascular P_{O_2} of the resistance vessels (37). Kontos et al. (92) have attributed the initial vasodilation of hypocapnic alkalosis to release of histamine but offered no explanation for the initial vasoconstriction of hypercapnic acidosis.

This study indicates that oxygen and hydrogen ions interact to play a major role in the maintenance of exercise hyperemia. Correction of both P_{O_2} and pH in the venous effluent of exercising muscle completely abolished its enhanced vasodilator activity (Figures 6, 7 and 9). Reduction of both P_{O_2} and pH in the blood perfusing resting muscle to

venous blood levels observed during steady-state exercise decreased resistance almost to the same degree as exercise itself which produced the same P_{O_2} and pH (Figure 12). Also, P_{O_2}, P_{CO_2} and pH levels were still significantly different from control levels during one hour of exercise hyperemia (Figure 8).

On the other hand, other studies suggest that oxygen and hydrogen ions may not participate in the initiation of exercise hyperemia. Hydrogen ion sensitive electrodes, implanted in rat gastrocnemius muscle, indicate that hydrogen ion activity transiently decreases before it increases with contractions in excess of one per second and only decreases with a contraction frequency of one per second (146). This suggests that the pre-existing hydrogen ions are first washed out before being added as exercise begins. Because flow rises very rapidly upon motor nerve stimulation (126, 136), the hydrogen ion may not participate in the genesis of exercise hyperemia (68). The time course of the change in oxygen saturation following a brief tetanus or "sinusoidal" exercise parallels the change in blood flow, but the vasodilator response appears to be too rapid during free flow and high constant flow perfusion to be explained by changes in tissue oxygen content (11, 112). In addition, the time course of the flow recovery following short term (10 seconds) (76) and long term (20-60 minutes) (115) exercise correlates poorly with the oxygen tension in the effluent blood.

A necessary assumption underlying any conclusions about this study is that the effluent blood P_{O_2} and P_{CO_2} or pH, whether altered by muscle exercise or by induced gas changes in the blood perfusing

resting muscle, closely reflect interstitial (perivascular) fluid gas tensions and pH. This assumption is supported by several studies which indicate that perivascular P_{O_2} is not much different from intravascular P_{O_2} at the level of the resistance vessels; i.e., oxygen diffuses not only from capillaries but also from pre-capillary vessels (sphincters and terminal arterioles), and that the diameter of these vessels is altered when perivascular P_{O_2} is changed (34-37, 77). It has also been demonstrated that increased intravascular P_{CO_2} produces significant dilation of large arterioles when P_{O_2} is held low and increases perivascular P_{O_2} of the resistance vessels, possibly due to the Bohr shift (37).

Potassium has been proposed to participate significantly in the genesis of exercise hyperemia because many studies have shown that exercise causes an initial loss of potassium from skeletal muscle (136-146) associated with increased venous potassium ion concentration (2, 4, 41, 82, 83, 85, 110, 122, 135, 137, 140, 141, 146) and because potassium itself is vasodilatory (23, 29, 38, 48, 53, 60, 80, 83, 85, 86, 98, 117, 135, 139-144). Several findings, however, indicate that potassium does not totally explain exercise hyperemia. Exercise can cause up to a 10 fold increase in flow and this is associated with only a small increase in effluent plasma potassium ion concentration (122, 135-137, 141). Although potassium is vasoactive when infused into resting muscle, the magnitude of the response is much less than that observed during exercise (83, 86, 136, 134-144). Moreover, the initial increase in the effluent potassium ion concentration wanes toward the control level

during prolonged exercise hyperemia (115, 122, 137). Tissue analysis shows that the total potassium content of skeletal muscle decreases rapidly in the first few minutes of exercise but then much more slowly during the next hour (146). Moreover, the initial dilation of skeletal muscle during hyperkalemic perfusion is replaced by a mild constriction within 5 minutes of continued perfusion (20). Finally, reduction of the inflow potassium ion concentration to a hypokalemic level during skeletal muscle exercise does not affect the magnitude of the exercise dilation (2).

This study confirms and strengthens previous reports suggesting that potassium cannot totally explain exercise hyperemia and that potassium plays only a relatively minor role during its maintenance. It was found that the enhanced vasoactivity of venous blood from exercising muscle can be abolished by simply correcting the P_{O_2} and pH (Figures 6, 7 and 9) and that induced large reductions of both P_{O_2} and pH in resting muscle can cause dilation nearly as great as heavy exercise itself (Figure 12). Also, during steady-state exercise hyperemia the potassium ion concentration, although initially elevated, rapidly and then slowly decreases to the pre-exercise level (Figure 8). However, as observed by Kjellmer (85) in the cat, increased contraction frequency was found to be correlated with increased venous plasma potassium concentration (Figure 12).

Osmolality has been proposed more recently as a mediator of active hyperemia (106) since an increase in osmolality occurs early during skeletal muscle exercise (76, 99-101, 106, 115, 116, 122, 136,

137) and perfusion of hyperosmotic blood to skeletal muscle decreases the resistance to flow (51, 55, 56, 59, 99-101, 103, 106, 109, 117, 118, 124, 136, 137, 142-144, 149, 150). However, like potassium, osmolality does not appear to participate importantly in steady-state exercise hyperemia. Infusion of hyperosmotic solutions into the arterial supply of resting skeletal muscle in the dog does not cause pronounced vasodilation, even when perfused in the highest concentration ranges seen in effluent blood during heavy exercise (51, 136, 137, 142-144, 149, 150). This is in contrast to that found in cat skeletal muscle (101, 106, 109) and man (99-101), perhaps reflecting differences in the type of muscle (mixture of red and white) and species. As with potassium, the venous effluent osmolal concentration progressively wanes during the course of exercise (115, 116, 122, 136, 137) and the vasodilation resulting from close arterial infusion of some hyperosmotic solutions wanes toward the control level within several minutes (51, 151).

The present study gives additional support to suggestions that osmolality is not greatly involved in the magnitude of active hyperemia, particularly during prolonged exercise. Again, the enhanced vasodilator activity of venous blood can be totally abolishing by correcting the P_{O_2} and pH without the necessity of correcting the osmolality (Figures 6, 7 and 9) and dilation of the resting muscle vascular bed to nearly the level seen during heavy exercise can be achieved by simultaneously reducing P_{O_2} and pH to levels seen during heavy exercise levels (Figure 12). Furthermore, osmolality, like potassium, rapidly and then more slowly decreases to the pre-exercise level while the hyperemia continues (Figure 8).

A number of recent observations show that increases in potassium ion concentration and osmolality occur rapidly at the onset of exercise and thus suggest that these factors are involved in the initiation of exercise dilatation. The potassium concentration in the venous plasma increases within the first 10 seconds of exercise (136). A one second tetanus (32 Hz) of dog calf muscles has been estimated to increase interstitial potassium levels within 10 seconds, which is sufficiently rapid to cause the accompanying vascular dilation lasting 30 seconds (110). In addition, Chen et al. (23) found that ouabain, which blocks the responsiveness of the vessels to potassium ions, has little effect on the magnitude of the exercise dilatation but delays the onset and increases the time to steady-state response. The increase in venous blood osmolality appears within one minute following the onset of exercise (136, 137). Although tissue fluid osmolality has not yet been determined during exercise hyperemia, it appears that muscle contraction is accompanied by vasodilation before the venous osmolality begins to rise. Once osmolality is elevated it may contribute with potassium in the genesis of exercise hyperemia.

In the past several years increased attention has been directed toward evaluating the interrelationships among the various chemical factors proposed to operate in active hyperemia. The studies by Skinner and his colleagues (139-144) suggest that exercise hyperemia may result may result from a combination of several factors which decrease vascular smooth muscle tone. They showed that perfusion of the resting canine gracilis muscle with hyperosmotic, hyperkalemic, hypoxic blood at levels

found in muscle venous blood during heavy short-term exercise increases both the rate of development and magnitude of the vasodilatation above that found when these factors are altered individually (142-144). In the present study experiments were designed to determine a possible interaction between oxygen and hydrogen ions or carbon dioxide in local control of blood flow during graded and heavy exercise of skeletal muscle. The data suggest that during long term exercise the concentrations of these substances are the most important determinants of blood flow regulation.

The mechanism of action of oxygen on peripheral resistance is not known. Oxygen has been proposed to regulate blood flow through a direct action on vascular smooth muscle cells in the arteriole wall and indirectly by the production and release of vasodilator metabolites from parenchymal cells (34). For example, Berne et al. (13-16) and Rubio et al. (131) have shown that oxygen lack leads to myocardial release of adenosine, which is a potent vasodilator. The vasodilator activity of adenyl compounds has been long known (12, 43).

Bioassay studies from this laboratory suggest the presence of AMP and/or adenosine in the blood draining skeletal muscle during ischemic exercise (66, 133, 138). On chemical analysis of this effluent blood, some investigators have shown increased levels of ATP (18, 22, 47, 120) and AMP (22). Dobson et al. (33) found higher levels of adenosine, but not its nucleotides. These findings seem to disagree with the present experiments showing that the enhanced vasodilator activity of venous blood from exercising muscle can be totally abolished by

correcting the P_{O_2} and pH. Two possibilities are offered which may explain the apparent absence of adenyl compounds in the blood perfusing the assay muscle: (a) These agents may be released only in severe ischemic exercise. Perhaps vasodilator concentrations of these agents were not produced under the conditions of exercise in this study. (b) The long circuit lag (1 to 3 minutes) for blood flowing between the two muscles may have allowed for a lessening of potency of these agents due to degradation in the blood. The possibility also remains that ischemia may cause release of these agents in vasodilator concentration in the bioassay as well as in the exercising muscle. Thus, correction of both P_{O_2} and pH could abolish the vasodilation in the bioassay muscle by preventing the local release of adenyl compounds in the bioassay muscle rather than by, or in addition to, the removal of the direct dilator effects of oxygen lack and high carbon dioxide concentration.

Factors other than oxygen and hydrogen ions are probably involved to a lesser extent in the maintenance of exercise hyperemia. Theoretically, the myogenic mechanism would be operative throughout the exercise period. Also, substances may act on vascular smooth muscle to contribute to vasodilation but not appear in the effluent in dilator concentrations due to rapid inactivation, reuptake or limited release. Finally, the roles of the potassium ion and osmolality in the maintenance of active hyperemia appear to be very minor.

With regard to the metabolic hypothesis the present study, as well as other recent studies, suggest that the vascular changes during

exercise hyperemia may be produced by several metabolites. The initial vasodilation with the onset of exercise may be due to combined increases in potassium ion concentration and osmolality. The maintenance of the dilation during steady-state exercise may result from increased hydrogen ion concentration and from decreased oxygen tension when oxygen consumption exceeds oxygen delivery.

SUMMARY AND CONCLUSIONS

Exercise of skeletal muscle is associated with a local increase in blood flow and changes in tissue metabolism and effluent blood chemistry. This hyperemia has been shown to override the vasoconstrictor effects of neural and humoral factors regulating vascular smooth muscle tone during rest. The purpose of this study was to elucidate further the relative contribution of oxygen and carbon dioxide (or hydrogen ions) in the metabolic control of blood flow during steady-state exercise of the denervated gracilis muscle in the anesthetized dog. In addition, the potassium and osmolal concentrations of effluent blood were measured to further evaluate their roles in the hyperemic response. Evidence for participation and interaction of these metabolically linked factors was obtained by bioassay and chemical analysis of the blood draining exercising muscle perfused at natural and constant flow, and by altering the gas tensions in the blood perfusing resting muscle by means of a gas exchange permeator or donor lung placed in the arterial supply.

These studies show: (a) The enhanced vasodilator activity of venous blood from the constant flow or naturally perfused muscle during steady-state exercise can be completely abolished by simultaneously returning the oxygen tension and pH to pre-exercise levels. Correction of potassium ion concentration and osmolality is not necessary. This

vasodilator activity is attenuated but not abolished when the effluent blood oxygen tension or pH is singly corrected to pre-exercise levels. (b) Simultaneously induced reductions in oxygen tension and pH in the blood perfusing resting muscle, to levels observed in venous blood from heavily exercising muscle, produce a vasodilatation which does not quite equal that seen during heavy exercise, but which is greater than that produced by reduction of oxygen tension or pH alone. (c) During exercise at constant stimulation frequency for 60 minutes, effluent blood oxygen tension and pH initially fall and then rise slowly but remain significantly lower than pre-exercise levels while the hyperemia remains unabated. On the other hand, potassium ion concentration and osmolality, although initially elevated, rapidly approach pre-exercise levels. (d) Mild exercise (0.3 Hz) at constant flow causes moderate vascular dilation (24% reduction in resistance) and is associated with only a slight drop in effluent blood oxygen tension. Resistance and oxygen tension continue to decrease as a function of contraction frequency during moderate exercise (0.2 to 1.6 Hz) but remain constant during heavy exercise (1.6 to 6.0 Hz). pH decreases and carbon dioxide tension and plasma potassium ion concentration increases as a function of frequency over the entire range. Osmolality is unchanged during mild exercise (0.2 to 0.8 Hz) but slightly elevated during moderate to heavy exercise (0.8 to 6 Hz).

These results suggest that the concentrations of oxygen and hydrogen ions (the classical metabolic factors) are the most important chemical determinants maintaining exercise hyperemia, and that potassium

ions and osmolality play only relatively minor roles in exercise hyperemia of long duration. It has been known for at least one hundred years that muscle exercise is associated with local hyperemia. Later researchers found this to be accompanied by changes in the concentration of metabolites in the tissue and venous effluent. The metabolic hypothesis was formulated and proposes that these changes result in an increase in blood flow so that the exercising muscle is better able to receive an adequate supply of nutrients and to eliminate products of metabolism. Today, we are still not clear on the mechanisms involved nor the relative contributions of these mechanisms. Knowledge of the basic mechanisms underlying exercise hyperemia not only contributes to a better understanding of the normal physiological state, but also establishes a foundation on which to treat rationally diseases of the cardiovascular system.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Altura, B.M. Chemical and humoral regulation of blood flow through the precapillary sphincter. Microvascular Res. 3:361-384, 1971.

2. Anderson, D.K., S.A. Roth, R.A. Brace, D. Radawski, F.J. Haddy and J.B. Scott. Effects of hypokalemia and hypomagnesemia produced by hemodialysis on skeletal muscle vascular resistance: Role of potassium in active hyperemia. Circ. Res. 31:165-173, 1972.

3. Anrep, G.V., and E. von Saalfeld. The blood flow through skeletal muscle in relation to its contraction. J. Physiol. (London) 85:375, 1935.

4. Baetjer, A.M. Diffusion of potassium from resting skeletal muscles following a reduction in the blood supply. Am. J. Physiol. 112:139, 1935.

5. Barcroft, H., B. Greenwood and R.F. Whelan. Blood flow and venous oxygen saturation during sustained contraction of the forearm muscles. J. Physiol. (London) 168:848, 1963.

6. Barcroft, H., T. Foley and R. McSweney. Experiments on liberation of phosphate from active human muscle, and in the action of phosphate on human blood vessels. J. Physiol. (London) 210:34P-35P, 1970.

7. Barcroft, H. An inquiry into the nature of the mediator of the vasodilation in skeletal muscle in exercise and during circulatory arrest. J. Physiol. (London) 222:99P-118P, 1972.

8. Barcroft, J., and T. Kato. Effects of functional activity in striated muscle and the submaxillary gland. Phil. Trans. Roy. Soc. Ser. B. Biol. Sci. (London) 207:149-182, 1915.

9. Bayliss, W.M. The action of carbon dioxide on blood vessels. J. Physiol. (London) 26:32P, 1901.

10. Bayliss, W.M. On the local reactions of the arterial wall to changes of internal pressure. J. Physiol. (London) 28: 220-231, 1902.

11. Bayliss, W.M. "Principles of General Physiology," 4th Ed., Longmans, Green & Co., London, (1924), pp. 703-704.

12. Bennett, D.W., and A.N. Drury. Further observations relating to the physiological activity of adenine compounds. J. Physiol. (London) 72:288, 1931.

13. Berne, R.M., J.R. Blackman and T.H. Gardner. Hypoxemia and coronary blood flow. J. Clin. Invest. 36:1101, 1957.

14. Berne, R.M. Cardiac nucleotides in hypoxia: Possible role in regulation of coronary blood flow. Am. J. Physiol. 204:317-322, 1963.

15. Berne, R.M. Metabolic regulation of blood flow. Circ. Res. 15 (Suppl. I): 261-268, 1964.

16. Berne, R.M., R. Rubio, J.G. Dobson, Jr., and R.R. Curnish. Adenosine and adenosine nucleotides as possible mediators of cardiac and skeletal muscle blood flow regulation. Circ. Res. 28 & 29 (Suppl. I):I-115-I-119, 1971.

17. Bohr, D.F. Vascular smooth muscle updated. Circ. Res. 32:665-672, 1973.

18. Boyd, I.A., and T. Forrester. The release of adenosine triphosphate from frog skeletal muscle in vitro. J. Physiol. (London) 199: 115-135.

19. Braasch, D. Red cell deformability and capillary blood flow. Physiol. Rev. 51:679-701, 1971.

20. Brace, R.A., J.B. Scott and D.K. Anderson. Time course and mechanisms of the vascular resistance response to hyperkalemia and hypokalemia. Physiologist 16:272, 1973.

21. Carrier, O., Jr., J.W. Walker and A.C. Guyton. Role of oxygen in autoregulation of blood flow in isolated vessels. Am. J. Physiol. 206:951-954, 1964.

22. Chen, W.T., B.H. Selleck and J.B. Scott. Evidence for participation of adenine nucleotides in exercise hyperemia. Fed. Proc. 31:379, 1972.

23. Chen, W.T., R.A. Brace, J.B. Scott, D.K. Anderson and F.J. Haddy. The mechanism of the vasodilator action of potassium. Proc. Soc. Exptl. Biol. Med. 140:820-824, 1972.

24. Costin, J.C., and N.S. Skinner, Jr. Competition between vasoconstrictor and vasodilator mechanisms in skeletal muscle. Am. J. Physiol. 220:462-466, 1971.

25. Crawford, D.G., H.M. Fairchild and A.C. Guyton. Oxygen lack as a possible cause of reactive hyperemia. Am. J. Physiol. 197:613, 1959.

26. Dale, H.H. Some chemical factors in the control of the circulation. Lancet 1:1179-1183, 1929.

27. Daugherty, R.M., Jr., J.B. Scott, J.M. Dabney, B.T. Swindall and F.J. Haddy. Direct effects of O_2, CO_2, nucleotides and ischemia on coronary resistance and ventricular force in the intact heart. Circulation 32:II-74, 1965.

28. Daugherty, R.M., Jr., J.B. Scott, J.M. Dabney and F.J. Haddy. Local effects of oxygen and carbon dioxide on limb, renal, and coronary vascular resistances. Am. J. Physiol. 213:1102-1110, 1967.

29. Dawes, G.S. Vasodilator action of potassium. J. Physiol. (London) 99:224, 1941.

30. Deal, C.P., and H.D. Green. Effect of pH on blood flow and peripheral resistance in muscular and cutaneous vascular beds in the hindlimb of the pentobarbitalized dog. Circ. Res. 2:148, 1954.

31. Detar, R., and D.F. Bohr. Oxygen and vascular smooth muscle contraction. Am. J. Physiol. 214:241-244, 1968.

32. Diji, A. Local vasodilator action of carbon dioxide on blood vessels of the hand. J. Appl. Physiol. 14:414, 1959.

33. Dobson, J.G., Jr., R. Rubio and R.M. Berne. Role of adenine nucleotides, adenosine and inorganic phosphate in the regulation of skeletal muscle blood flow. Circ. Res. 29:375-384, 1971.

34. Duling, B.R., and R.M. Berne. Possible mechanism for the participation of oxygen in local regulation of blood flow. Circ. Res. 27:669-678, 1970.

35. Duling, B.R., and R.M. Berne. Oxygen and the local regulation of blood flow: Possible significance of longitudinal gradients in arterial blood oxygen tension. Circ. Res. 28 & 29 (Suppl. I:I-65-I-69, 1971.

36. Duling, B.R. Microvascular responses to alterations in oxygen tension. Circ. Res. 31:481-489, 1972.

37. Duling, B.R. Changes in microvascular diameter and oxygen tension induced by carbon dioxide. Circ. Res. 32:370-376, 1973.

38. Emanuel, D., J.B. Scott and F.J. Haddy. Effect of potassium on small and large blood vessels of the dog forelimb. Am. J. Physiol. 197:637-642, 1959.

39. Emerson, T.M., Jr., J.L. Parker and G.W. Jelks. Effects of local acidosis on vascular resistance in dog skeletal muscle. Proc. Soc. Exptl. Biol. Med. 145:273-276, 1974.

40. Fairchild, H.M., J. Ross and A.C. Guyton. Failure of recovery from reactive hyperemia in the absence of oxygen. Am. J. Physiol. 210:490, 1966.

41. Fenn, W.O. Factors affecting the loss of potassium from muscles on stimulation. Am. J. Physiol. 119:307, 1937.

42. Fleisch, A., I. Sibul and V. Ponomarev. Kohlensaure und sauerstoffmangel als auslosende reize. Arch. Ges. Physiol. 230:814, 1932.

43. Folkow, B. The vasodilator action of adenosine triphosphate. Acta Physiol. Scand. 17:311, 1949.

44. Folkow, B. Description of the myogenic hypothesis. Circ. Res. 14 (Suppl. I):47, 1964.

45. Folkow, B., and H.D. Halicka. A comparison between "red" and "white" muscle with respect to blood supply, capillary surface area and oxygen uptake during rest and exercise. Microvascular Res. I-1014, 1968.

46. Folkow, B., and E. Neil. "Circulation." Oxford University Press, London (1971), p. 287.

47. Forrester, T., and A.R. Lind. Identification of adenosine triphosphate in human plasma and the concentration in the venous effluent before, during and after sustained contractions. J. Physiol. (London) 204:347-364, 1969.

48. Frohlich, E.D., J.B. Scott and F.J. Haddy. Effect of cations on resistance and responsiveness of renal and forelimb beds. Am. J. Physiol. 203:583-587, 1962.

49. Gaskell, T.W.H. On the changes of the blood stream through stimulation of their nerves. J. Anat. 11:360, 1877.

50. Gaskell, T.W.H. On the tonicity of the heart and blood vessels. J. Physiol. (London) 3:48, 1880.

51. Gazitua, S., J.B. Scott, B. Swindall and F.J. Haddy. Resistance responses to local changes in plasma osmolarity in three vascular beds. Am. J. Physiol. 220:384-391, 1971.

52. Gebert, G., and S.M. Friedman. An implantable glass electrode for pH measurement in working skeletal muscle. J. Appl. Physiol. 34:122-124, 1973.

53. Glover, W.E., I.C. Roddie and R.G. Shanks. Effect of intra-arterial potassium chloride infusions on vascular reactivity in the human forearm. J. Physiol. (London) 163:22P, 1963.

54. Gollwitzer-Meier, K. Blood pH and blood flow during muscular activity. Lancet 1:381-386, 1950.

55. Gray, S.D., J. Lundvall and S. Mellander. Regional hyperosmolarity in relation to exercise hyperemia. Acta Physiol. Scand. 73:11A-12A, 1968.

56. Gray, S.D. Effect of hypertonicity on vascular dimensions in skeletal muscle. Microvascular Res. 3:117-124, 1971.

57. Greenwood, B., M.F. Robards and A.E. Twite. Venous oxygen saturation during rhythmic contraction of the forearm muscles. J. Physiol. (London) 178:33, 1965.

58. Gremels, H., and E.H. Starling. On the influence of hydrogen ion concentration and of anoxemia upon the heart volume. J. Physiol. (London) 61:297-304, 1926.

59. Haddy, F.J. Local effects of sodium, calcium and magnesium upon large and small blood vessels of the dog foreleg. Circ. Res. 8:57, 1960.

60. Haddy, F.J., J.B. Scott, M.A. Florio, R.M. Daugherty, Jr., and J.N. Huizenga. Local vascular effects of hypokalemia, alkalosis, hypercalcemia, and hypomagnesemia. Am. J. Physiol. 204:202, 1963.

61. Haddy, F.J., and J.B. Scott. Effects of flow rate, venous pressure, metabolites, and oxygen upon resistance to blood flow through the dog forelimb. Circ. Res. 14 & 15 (Suppl. I):49, 1964.

62. Haddy, F.J., J.B. Scott and J.I. Molnar. Mechanism of volume replacement and vascular constriction following hemorrhage. Am. J. Physiol. 208:169, 1965.

63. Haddy, F.J. Role of chemicals in local regulation of vascular resistance. Circ. Res. 18 & 19 (Suppl. I):14, 1966.

64. Haddy, F.J., H.W. Overbeck and R.M. Daugherty, Jr. Peripheral vascular resistance. Ann. Rev. Med. 19:167-194, 1968.

65. Haddy, F.J., and J.B. Scott. Metabolically linked vasoactive chemicals in local regulation of blood flow. Physiol. Rev. 48:688-707, 1968.

66. Haddy, F.J., and J.B. Scott. Bioassay and other evidence for participation of chemical factors in local regulation of blood flow. Circ. Res. 28 & 29 (Suppl. I): I-86-I-92, 1971.

67. Haddy, F.J., and J.B. Scott. Active hyperemia, reactive hyperema and autoregulation of blood flow. In: Microcirculation, ed. by G. Kaley and B.M. Altura, University Park Press, Baltimore (1974).

68. Haddy, F.J., and J.B. Scott. Metabolic factors in peripheral circulatory regulation. Fed. Proc., (In Press), 1974.

69. Hilton, R., and F. Eichholtz. Influence of chemical factors on the coronary circulation. J. Physiol. (London) 59:413, 1925.

70. Hilton, S.M. Experiments on the post contraction hyperemia of skeletal muscle. J. Physiol. (London) 120:230-245, 1953.

71. Hilton, S.M. Local mechanisms regulating peripheral blood flow. Physiol. Rev. 42 (Suppl. 5):265, 1962.

72. Hilton, S.M., and G. Vrbova. Inorganic phosphate--a new candidate for mediator of functional vasodilitation in skeletal muscle. J. Physiol. (London) 206:29P, 1970.

73. Hilton, S.M., M. G. Jeffries and G. Vrbova. Functional specialization of the vascular bed of soleus. J. Physiol. (London) 206:543-563, 1970.

74. Hilton, S.M., O. Hudlicka and G. Vrbova. Further studies on the mediators of functional hyperemia in skeletal muscle. In: Vascular Smooth Muscle, ed. by E. Betz, Springer-Verlag, Berlin (1972), p. 75.

75. Honig, C.R. Control of smooth muscle actomyosin by phosphate and 5' AMP: Possible role in metabolic autoregulation. Microvascular Res. 1:133-146, 1968.

76. Honig, C.R., and J.L. Frierson. Mechanism of post-contraction hyperemia. Fed. Proc. 33:444, 1974.

77. Hutchins, P.M., R.F. Bond and H.D. Green. Participation of oxygen in the local control of skeletal muscle microvasculature. Circ. Res. 34:85-93, 1974.

78. Jalavisto, E., O. Mertens and W. Schoedel. Zur Steuerung der Muskeldurchblutung nach rhythmischer Tatigkeit. Arch. ges. Physiol. Pfluegers 249:167, 1948.

79. Johansson, B., and O. Johsson. Cell volume as a factor influencing electrical and mechanical activity of vascular smooth muscle. Acta Physiol. Scand. 72:456-468, 1968.

80. Katz, L.W., and E. Linder. Action of excess Na, Ca, and K on coronary vessels. Am. J. Physiol. 124:155, 1938.

81. Kester, N., A. Richardson and H. Green. Effect of controlled hydrogen ion concentration on peripheral vascular tone and blood flow in the innervated hindlimb of the dog. Am. J. Physiol. 169:678-687, 1952.

82. Kilburn, K.H. Muscular origin of elevated plasma potassium during exercise. J. Appl. Physiol. 21:675, 1966.

83. Kjellmer, I. Role of potassium ions in exercise hyperemia. Med. Exptl. (Basel) 5:56, 1961.

84. Kjellmer, I. On the competition between metabolic vasodilatation and neurogenic vasoconstriction in skeletal muscle. Acta Physiol. Scand. 63:450-460, 1965.

85. Kjellmer, I. The potassium ion as a vasodilator during muscular exercise. Acta Physiol. Scand. 63:460, 1965.

86. Kjellmer, I., and A. Odelram. The effect of some physiological vasodilators on the vascular bed of skeletal muscle. Acta Physiol. Scand. 63:94, 1965.

87. Kontos, H.A., D.W. Richardson and J.L. Patterson, Jr. Blood flow and metabolism of forearm muscle in man at rest and during sustained contraction. Am. J. Physiol. 211:869-876, 1966.

88. Kontos, H.A., D.W. Richardson and J.L. Patterson, Jr. Effects of hypercapnia on human forearm blood vessels. Am. J. Physiol. 212:1070, 1967.

89. Kontos, H.A., D.W. Richardson and J.L. Patterson, Jr. Vasodilator effect of hypercapnic acidosis on human forearm blood vessels. Am. J. Physiol. 215:1403-1405, 1968.

90. Kontos, H.A. Role of hypercapnic acidosis in the local regulation of blood flow in skeletal muscle. Circ. Res. 28 & 29 (Suppl. I:I-98-I-105, 1971.

91. Kontos, H.A., M.D. Thames, A. Lonnbana, C.O. Waltington and F. Jesse, Jr. Vasodilator effects of local hypercapnic acidosis in dog skeletal muscle. Am. J. Physiol. 220:1569-1572, 1971.

92. Kontos, H.A., D.W. Richardson, A.J. Raper, Z.V. Hassen and J.L. Patterson, Jr. Mechanisms of action of hypocapnic alkalosis on limb blood vessels in man. Am. J. Physiol. 223:1296-1307, 1972.

93. Knochel, J.P., and E.M. Schlein. Absence of active hyperemia in K-depleted skeletal muscle. Clin. Res. 19:87, 1971.

94. Kramer, K., F. Obal and W. Quensel. Untersuchungen Ueber den Muskelstoffwechsel des Warmeblueters. III. Mitteilung. Die Sauerstoffaufnahme des Muskels waehrend rhythmischer Tatigkeit. Arch. ges. Physiol. Pfluegers 241:717-729, 1939.

95. Krogh, A. "The anatomy and physiology of capillaries." Yale Univ. Press, New Haven, Conn. (1929).

96. Lewis, T., and R.T. Grant. Observations upon reactive hyperemia in man. Heart 12:73-120, 1925.

97. Love, A.H.G. The rate of blood flow and the oxygen saturation of the effluent blood following contraction of the muscles of the human forearm. Clin. Sci. 14:275, 1955.

98. Lowe, R.D., and J.W. Thompson. Effect of intra-arterial potassium chloride infusion upon forearm blood flow in man. J. Physiol. (London) 162:69P, 1962.

99. Lundvall, J., S. Mellander and T. White. Hyperosmolality and vasodilation in human skeletal muscle. Acta Physiol. Scand. 77:224-233, 1969.

100. Lundvall, J. Further studies on regional hyperosmolality in relation to exercise hyperemia. Acta Physiol. Scand. (Suppl.) 330:98, 1969.

101. Lundvall, J. Tissue hyperosmolality as a mediator of vasodilatation and transcapillary fluid flux in exercising skeletal muscle. Acta Physiol. Scand. (Suppl. 379):1-142, 1972.

102. Markwalder, J., and E.H. Starling. A note on some factors which determine the blood flow through the coronary circulation. J. Physiol. (London) 47:275-285, 1913.

103. Marshall, F.J., and J. Shepherd. Effect of injections of hypertonic solutions on blood flow through the femoral artery of the dog. Am. J. Physiol. 197:951-954, 1959.

104. Maxwell, L.C., D.F. Bohr and R.A. Murphy. Arterial actomyosin: effects of ionic strength on ATPase activity and solubility. Am. J. Physiol. 220:1871-1874, 1971.

105. McGiff, J.C., K. Crowshaw, N.A. Terragno and A.J. Lonigro. Release of prostaglandin-like substance into renal venous blood in response to angiotensin II. Circ. Res. 26 & 27 (Suppl. I):I-121-I-130, 1970.

106. Mellander, S., B. Johansson, S. Gray, O. Jonsson, J. Lundvall and B. Ljung. The effects of hyperosmolarity on intact and isolated vascular smooth muscle. Possible role in exercise hyperemia. Angiologica 4:310-322, 1967.

107. Mellander, S., and B. Johansson. Control of resistance, exchange, and capacitance functions in the peripheral circulation. Pharmacol. Rev. 20:117-196, 1968.

108. Mellander, S. Systemic circulation: Local control. Ann. Rev. Physiol. 32:313-344, 1970.

109. Mellander, S., and J. Lundvall. Role of tissue hyperosmolality in exercise hyperemia. Circ. Res. 28 & 29 (Suppl. I): I-39-I-45, 1971.

110. Mohrman, D.E., and P.H. Abbrecht. Time course of potassium release from skeletal muscle following brief tetanus. Fed. Proc. 32:374, 1973.

111. Mohrman, E.D., J.R. Cant and H.V. Sparks. Time course of vascular resistance and venous oxygen changes following brief tetanus of dog skeletal muscle. Circ. Res. 33:323-336, 1973.

112. Mohrman, E.D., and H.V. Sparks. Resistance and venous oxygen dynamics during sinusoidal exercise of dog skeletal muscle. Circ. Res. 33:337-345, 1973.

113. Molnar, J.I., H.W. Overbeck and F.J. Haddy. Local effects of oxygen and carbon dioxide upon dog forelimb vascular resistance. In: Proc. 22nd Intern. Congr. Physiol. Sci. Leyden, Vol. II, Excerpta Med. Found., Amsterdam (1962), p. 175.

114. Molnar, J.I., J.B. Scott, E.D. Frohlich and F.J. Haddy. Local effects of various anions and hydrogen ion on dog limb and coronary vascular resistances. Am. J. Physiol. 203:125, 1962.

115. Morganroth, M.L., D.E. Mohrman and H.V. Sparks. Prolonged vasodilation following fatiguing exercise. Fed. Proc. 33:357, 1974.

116. Murray, P.A., D.E. Mohrman, H.V. Sparks and A.J. Vander. Osmolality and the local control of skeletal muscular resistance. Fed. Proc. 33:377, 1974.

117. Overbeck, H.W., J.I. Molnar and F.J. Haddy. Resistance to blood flow through the vascular bed of the dog forelimb: Local effects of sodium, potassium, calcium, magnesium, acetate, hypertonicity and hypotonicity. Am. J. Cardiol. 8:533, 1961.

118. Overbeck, H.W., and G.J. Grega. Response of the limb vascular bed in man to intrabrachial arterial infusions of hypertonic dextrose or hypertonic sodium chloride solutions. Circ. Res. 26:717-731, 1970.

119. Pappenheimer, J.R. Blood flow, arterial oxygen saturation and oxygen consumption in the isolated perfused hindlimb of the dog. J. Physiol. (London) 99:283, 1941.

120. Parkinson, P.I. The effect of graded exercise on the concentration of adenine nucleotides in plasma. J. Physiol. (London) 234:72-74P, 1973.

121. Pernow, B., and J. Wahren. Lactate and pyruvate formation and oxygen consumption in the human forearm muscles during work of high intensity and varying duration. Acta Physiol. Scand. 56:267, 1962.

122. Radawski, D., W. Hoppe, R. Underwood and F.J. Haddy. Role of vasoactive substances in active hyperemia in skeletal muscle. Fed. Proc. 31:379, 1972.

123. Radawski, D., J.M. Dabney, R.M. Daugherty, Jr., F.J. Haddy and J.B. Scott. Local effects of CO_2 on vascular resistances and weight of the dog forelimb. Am. J. Physiol. 222:439-443, 1972.

124. Read, R.C., J.A. Johnson, J.A. Vick and M.W. Meyer. Vascular effects of hypertonic solutions. Circ. Res. 8:538-548, 1960.

125. Reis, D.J., D. Moorhead and G.F. Wooten. Differential regulation of blood flow to red and white muscle in sleep and defense behavior. Am. J. Physiol. 217:541-546.

126. Ross, J., Jr., G. Kaiser and F. Klocks. Observations on the role of diminished oxygen tension in functional hyperemia of skeletal muscle. Circ. Res. 15:473, 1964.

127. Ross, J., Jr. The regional circulation. Ann. Rev. Physiol. 33:445-473, 1971.

128. Ross, J.M., H.M. Fairchild, J. Weldy and A.C. Guyton. Autoregulation of blood flow by oxygen lack. Am. J. Physiol. 202:21-24, 1962.

129. Roth, S.A., D.K. Anderson, D.P. Radawski, J.B. Scott and F.J. Haddy. Effects of acute hypokalemia on resistance to blood flow through the gracilis muscle in the dog. Physiologist 12:343, 1969.

130. Roy, C.S., and J.G. Brown. The blood pressure and its variation in the arterioles, capillaries and smaller veins. J. Physiol. (London) 2:323, 1879.

131. Rubio, R., R.M. Berne and M. Katori. Release of adenosine in reactive hyperemia of the dog heart. Am. J. Physiol. 216:56-62, 1969.

132. Scott, J.B., D. Emanuel and F.J. Haddy. Effect of potassium on small and large blood vessels of the dog forelimb. Am. J. Physiol. 197:637, 1959.

133. Scott, J.B., R.M. Daugherty, Jr., J.M. Dabney and F.J. Haddy. Role of chemical factors in regulation of flow through kidney, hindlimb and heart. Am. J. Physiol. 208:813-824, 1965.

134. Scott, J.B., R.M. Daugherty, Jr., and F.J. Haddy. Effect of severe local hypoxemia on transcapillary water movement in dog forelimb. Am. J. Physiol. 212:847-851, 1967.

135. Scott, J.B., R.M. Daugherty, Jr., H.W. Overbeck and F.J. Haddy. Vascular effects of ions. Fed. Proc. 27:1403-1407, 1968.

136. Scott, J., M. Rudko, D. Radawski and F.J. Haddy. Role of osmolarity, K+, H+, Mg++, and O_2 in local blood flow regulation. Am. J. Physiol. 218:338-345, 1970.

137. Scott, J.B., and D. Radawski. Role of hyperosmolarity in the genesis of active and reactive hyperemia. Circ. Res. 28 & 29 (Suppl. I):I-26-I-32, 1971.

138. Scott, J., W. Chen, D. Radawski, D. Anderson and F.J. Haddy. Bioassay evidence for participation of adenosine and adenine nucleotides in local blood flow regulation. Proc. International Union Physiol. Sci. 9:504, 1971.

139. Skinner, N.S., Jr. Abolition of sympathetic vasoconstriction in dog skeletal muscle: role of K+ and reduced O_2 tension. Physiologist 10:307, 1967.

140. Skinner, N.S., Jr., and W.J. Powell, Jr. Action of oxygen and potassium on vascular resistance of dog skeletal muscle. Am. J. Physiol. 212:533-540, 1967.

141. Skinner, N.S., Jr., and W.J. Powell, Jr. Regulation of skeletal muscle blood flow during exercise. Circ. Res. 21:59-69, 1967.

142. Skinner, N.S., Jr., and J.C. Costin. Interrelationship between O_2, K+ and osmolality in regulation of skeletal muscle blood flow before and during sympathetic vasoconstriction. Fed. Proc. 29:266, 1970.

143. Skinner, N.S., Jr., and J.C. Costin. Interactions of vasoactive substances in exercise hyperemia: O_2, K+ and osmolality. Am. J. Physiol. 219:386-392, 1970.

144. Skinner, N.S., Jr., and J.C. Costin. Interaction between oxygen, potassium and osmolality in regulation of skeletal muscle blood flow. Circ. Res. 28 & 29 (Suppl. I):I-73-I-85, 1971.

145. Sokal, R.R., and F.J. Rohlf. "Biometry," W.H. Freeman and Company, San Francisco (1969).

146. Streter, F.A., and S.M. Friedman. The effects of adrenergic blockade on cationic exchanges in contracting muscle. J. Pharm. Exptl. Thera. 131:158-166, 1961.

147. Stainsby, W.N. Autoregulation of blood flow in skeletal muscle during increased metabolic activity. Am. J. Physiol. 202:273, 1962.

148. Stainsby, W.N., and A.B. Otis. Blood flow, blood oxygen tension, oxygen uptake, and oxygen transport in skeletal muscle. Am. J. Physiol. 206:858-866, 1964.

149. Stainsby, W.N., and M.J. Fregly. Effect of plasma osmolality on resistance to blood flow through skeletal muscle. Proc. Soc. Exptl. Biol. Med. 128:284-287, 1968.

150. Stainsby, W.N., and J.K. Barclay. Effect of infusions of osmotically active substances on muscle blood flow and systemic blood pressure. Circ. Res. 28 & 29 (Suppl. I): I-33-I-38, 1971.

151. Stainsby, W.N. Local control of regional blood flow. Ann. Rev. Physiol. 35:151-168, 1973.

152. Tobin, R.B., and W.H. Coleman. Family Study of phosphorylase deficiency in muscle. Ann. Intern. Med. 62:313, 1965.

153. Verzar, F. The influence of lack of oxygen on tissue respiration. J. Physiol. (London) 45:39-52, 1912.

154. Zsoter, T., L. Banderman and C.I. Chappel. The effect of "local" pH changes in blood flow in the dog. Am. Heart J. 61:777-782, 1961.