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THE ROLE OF CERTAIN CHEMICALS IN LOCAL REGULATION OF BLOOD FLOW

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CHAPTER I

INTRODUCTION

Remote as well as local mechanisms control the functions of the peripheral blood vessels. The local regulation of blood flow may be defined as the intrinsic capability of an organ to regulate its own blood supply according to its needs. The phenomenon of local regulation becomes apparent when arterial pressure, venous pressure or metabolic rate is altered in an organ, even if this organ is completely isolated (40, 71).

A change in the perfusion pressure in certain organs over the approximate range of 70 to 200 mm Hg produces a change in blood flow which is not in proportion to the change in pressure (1, 22). This intrinsic tendency of the organ to maintain a constant blood flow in the face of changing perfusion pressures is called autoregulation. When metabolic rate is increased, as during muscular exercise, the flow of blood increases even though the perfusion pressure remains constant. This response is called active hyperemia. Reactive hyperemia, on the other hand, is the term used to describe the increase in blood flow above basal levels that follows release of arterial occlusion. A mild elevation of venous pressure may produce a greater than proportionate fall in blood flow. This

reaction is known as veni-vasomotor reflex (76) or venous-arteriolar response (46).

It has been shown that the regulation of resistance to blood flow results from a change in the radius of the vessel rather than from a change in the viscosity of blood (48), as regulation also occurs during perfusion with noncellular fluids (71).

The exact mechanism that causes the change in vessel caliber resulting in autoregulation has not been elucidated. Several theories have been proposed to explain this phenomenon. The tissue pressure theory (40, 41, 66) suggests that the change of caliber of the vessel is due to a change in transmural pressure resulting from a rise or fall of tissue pressure which follows capillary filtration or absorption. Capillary filtration or absorption depends upon the rise or fall of perfusion pressure, respectively. It has been demonstrated, however, that autoregulation disappears after the vascular smooth muscle has been inactivated by means of cyanide or anoxic death (31, 52).

The weight of the evidence presented in the literature indicates that local regulation results from changes in the contractile state of the smooth muscle of the arterioles and possibly of the small arteries. The controversy in the field of local regulation revolves around the causative mechanism of the change in the radius of the vessels. In this broad area, an active vasomotion due to changes in transmural pressure, Bayliss effect, has been suggested (1, 22), which is known as the myogenic hypothesis (23). This hypothesis states that an increase in internal pressure in some way stimulates the vascular smooth muscle to contract, perhaps through a stretch reflex. A decrease in the transmural pressure lessens the stretch and the muscle relaxes.

Another group of hypotheses supporting the view of an active vasomotion can be designated collectively by the name of chemical theory. Under this general term would be included the metabolic and oxygen mechanisms. The latter hypothesis suggests that a decrease in oxygen tension in the tissues lessens the oxygen available to the vessel wall. This inadequate supply of oxygen weakens the arteriole and causes it to dilate. The metabolite theory proposes that the tissues produce vasodilating chemical materials whose concentration is directly related to the metabolic rate of the tissue and inversely related to the rate of blood flow through the tissue under study. The metabolic agents responsible for the vasodilation have not been positively identified, but the naturally occurring chemical substances that could locally alter the resistance to flow are numerous and range from simple ions to large and complex molecules. These substances include potassium, magnesium, hydrogen, CO_2, intermediates of the Kreb's cycle, and the adenyl compounds: AMP, ADP, ATP. All of these agents are potent vasodilators and are in some way involved in cell metabolism. The observation that venous blood from a regulating organ causes a response in a bioassay organ (67, 70) suggests that chemicals are involved in local regulation.

Local Effect of Oxygen

One of the earliest critical studies of the local effect of oxygen upon vascular beds appeared in 1932 when Fleisch *et al.* (21) perfused the innervated hindlimb and intestine of the cat with blood passed through an artificial lung ventilated with various gas mixtures. The authors found that only very severe hypoxemia, such as that obtained by ventilating the artificial lung with 100% N_2, produced significant vasodilation.

They thus concluded that oxygen is not important in local regulation of resistance.

Crawford et al. (10) perfused the hindlimb of the dog at constant pressure with arterial blood admixed with varying amounts of venous blood from a reservoir. As the concentration of venous admixture in the perfusate increased and the oxygen saturation lessened, they observed an increase in the femoral blood flow which reached a maximum of 250% when the oxygen saturation decreased from 91% to 32%. The investigators concluded that it was the lack of oxygen in the perfusing blood that caused the vasodilation. This study only shows, however, that venous blood is vasodilator with respect to arterial blood, and does not indicate why this is so.

Ross et al. (60) performed a similar study on the hindlimb of the dog varying the oxygen saturation of the perfusing blood from 100% to 0% while maintaining a constant P_{CO_2}. The perfusion was done at constant pressure with blood from a pulmonary vein of a lung which had been exposed to various gas mixtures. The authors observed a progressive increase in flow as the O_2 saturation of the blood gradually decreased from its peak value of 100% toward 0%. The maximal flow occurred in the intact animal when the oxygen saturation was 0% and occurred in the spinal-anesthetized animal when the oxygen saturation was 10%. The study concluded that oxygen is an important factor in the local regulation of resistance and that the vasodilator property of hypoxemia appeared to be independent of nervous control.

Fairchild et al. (20) recently reported their findings in an experiment in which they perfused the hindlimb of the dog with 100% O_2 saturated blood. After five minutes of femoral artery occlusion, the

hyperemic flow, which followed release of the occlusion, increased approximately four times the control value and then returned to its pre-occlusion value. This procedure was repeated, but the blood entering the limb during the reactive hyperemia phase contained no oxygen. The rate of flow following release of occlusion rose again approximately four-fold but failed to return to pre-occlusion levels for as long as the O_2 saturation in the infusate remained at 0%. The authors suggest that the vasodilation observed was probably the result of oxygen lack *per se*. For "if the legs had shown recovery from the reactive hyperemia after reperfusion with anoxic blood, this would have eliminated oxygen insufficiency *per se* as the cause of reactive hyperemia" (20).

Molnar *et al*. (53, 54), by contrast, observed little or no effect upon the resistance of the dog forelimb when the blood's oxygen content was lowered in volume from 14.6% to 10.5%. As they did observe vasodilation with venous blood relative to arterial blood, they concluded that the vasodilator property of venous blood is not dependent upon its oxygen content.

Daugherty (11) studied forelimb resistance in the dog after a drop in systemic P_{O_2} from 96 mm Hg to 30 mm Hg. The sympathico-adrenal effect was abolished by surgically ablating the adrenal glands, sectioning of both vagi and all forelimb nerves, and by bilateral carotid sinus nerve block. In such a preparation, he observed a 60 mm Hg drop in blood P_{O_2} during ventilation of the animal with a low oxygen mixture (8% O_2 in N_2). A small but significant drop in total limb resistance due to a decrease in small vessel resistance occurred during the period of hypoxemia. This author and his associates (11, 12, 14) also perfused the intact dog

forelimb and kidney with venous and arterial blood passed through an isolated lung from another dog ventilated with gas mixtures of low O_2 content and constant P_{CO_2}. A change in the perfusion pressure in this constant-flow system indicated a change in the organ's vascular resistance. These investigators observed that, upon reducing the blood's oxygen tension from a control value of 114 mm Hg to 2 mm Hg, the perfusion pressure in the forelimb was lowered from 100 mm Hg to 66 mm Hg while the pH remained constant. The same maneuver in the kidney did not significantly alter the perfusion pressure. They concluded, therefore, that severe local hypoxemia decreases limb resistance but has little effect upon renal resistance. Haddy et al. (33) demonstrated that passage of renal venous blood through the dog forelimb during partial renal artery occlusion causes an initial fall followed by a prolonged, sustained rise in forelimb resistance. Scott et al. (67) have further shown that injection of AMP or adenosine into the renal artery often produces a rise rather than a fall in renal vascular resistance. This group of investigators suggests that it is possible that the absence of a response to hypoxia by the kidney might be related to these two observations.

All investigators seem to agree that low oxygen content is probably the most potent stimulus in the production of a fall in the resistance of coronary vessels. The studies of Gremels and Starling (29), Hilton and Eichholtz (38), Berne et al. (4), and Guz et al. (30) agree that the local effect of hypoxia is dilation of the coronary vascular bed. However, the disagreement concerns the mechanism by which hypoxia elicits the vasodilation.

Starling (29) and his associates have suggested that non-volatile vasodilator metabolites are produced during hypoxia. Hilton and Eichholtz

(38) forwarded the idea that nonvolatile vasodilator materials are not concerned in the control of coronary blood flow, but that the hypoxic blood acts directly on the vessel walls. Berne et al. (4) found that if, during the period of hypoxia, the O_2 of coronary sinus blood did not fall in volume below 5.5% (regardless of the P_{O_2} level of the arterial blood), the coronary blood flow was unchanged. However, if the oxygen level of sinus blood fell below this level, the coronary bed dilated in proportion to the P_{O_2} decrease. Berne concluded that arterial blood P_{O_2} was not the critical factor in the vasodilation of hypoxemia, but that the myocardial tissue P_{O_2}, as reflected in the venous blood oxygen tension, was probably responsible by elaboration of vasodilator metabolites from the myocardium itself (55).

Haddy and associates (32, 53) have shown that a change in the oxygen content of the perfusing blood over the ranges of the usual arterio-venous differences, in the face of a constant pH, has little effect upon forelimb resistance, suggesting that the vasodilator property of venous blood cannot be explained totally by a lowered oxygen content. Hall and Sackner (37) report similar findings for the hindlimb.

Ross et al. (62) have demonstrated that perfusion of the gastrocnemius muscle with hypoxic blood produces an increase in flow that is not as great as that of exercise hyperemia even though the P_{O_2} of the venous blood is the same during both maneuvers. They suggest, therefore, that hypoxia per se is not a complete explanation for the dilation.

Local Effect of CO_2 and H^+

Although both CO_2 and H^+ are produced by tissues that are metabolically active and during periods of hypoxia, their vasodilator effects

fall far short of the magnitude seen during the physiological levels of exercise or during ischemia (12, 13, 38); as marked changes in resistance elicited by changes in metabolism or flow have been observed with minimal alteration in venous pH or CO_2 (63, 64, 67).

Fleisch et al. (21) perfused the hindlimb and intestinal vascular bed with blood exposed to 2% through 10% carbon dioxide and observed an increase in flow of 30% to 100% above the control. They, therefore, concluded that carbon dioxide, in contrast to oxygen, is an important factor in local regulation of blood flow. Hilton and Eichholtz (38) observed a similar rise in flow in the coronary bed when it was perfused with blood high in carbon dioxide tension, and also when lactic acid was added to the perfusate. Their conclusion, however, was that the vasodilator effect of carbon dioxide was secondary to the associated change in the pH of blood.

In an experiment on the isolated guinea pig heart perfused with a balanced ion solution, McElroy et al. (51) observed that a rise in the P_{CO_2} of the perfusate resulted in an increase in the coronary flow and a decrease in both heart rate and force of contraction. A lowered P_{CO_2} produced the opposite effect. Coronary flow remained unchanged, however, when the P_{CO_2} was raised, maintaining the pH constant. These authors also agreed that the hydrogen ion concentration rather than the carbon dioxide per se is responsible for the vasoactivity in their preparation.

Daugherty (14), in an intact dog heart preparation, perfused the coronary vascular bed with blood exposed to a gas mixture of 20% CO_2 - 20% O_2 in nitrogen. He observed that the perfusion pressure in this constant-flow preparation decreased from a control value of 112 mm Hg to

95 mm Hg. The force of myocardial contraction decreased from a control of 100 units to 70 units, as the pH decreased by 0.42 units.

Since hypercapnia affects the strength of myocardial contraction and, hence, the transmural pressure, it is difficult to assess the cause of the reduction in coronary resistance. Is such reduction secondary to the increase in transmural pressure or is it the result of the direct effect (either by CO_2 or H^+) upon the resistance vessels?

In other vascular beds, Daugherty et al. (12) observed that marked local hypercapnia resulted in a decreased forelimb and kidney resistance in the dog. Hypocapnia, on the other hand, had an opposite effect on these vascular beds, whereas the coronary bed seemed to be little affected by the decrease in P_{CO_2} (13). Molnar et al. (55) showed that intra-arterial infusion of sodium bicarbonate, at a rate that would not alter the pH, had little effect on forelimb vascular resistance but it did cause a slight resistance rise in the coronary vascular bed. Renal vascular resistance, however, is markedly elevated by intra-arterial infusion of sodium bicarbonate.

Role of K in Local Regulation

In 1934, Baetjer (2) observed an increase in the potassium ion concentration in the effluent blood from skeletal muscles activated by faradic stimulation of anterior roots. She also reported that venous blood obtained after cross-clamping the abdominal aorta of a rabbit for 5 to 23 minutes contained an elevated concentration of potassium ions (3). In 1938, Katz and Lindner (47) noted that low concentrations of potassium infused locally produced vasodilation in the coronary circulation, whereas high concentrations resulted in an elevation of the coronary resistance.

Three years later, Dawes (15) observed similar responses in skeletal muscle vasculature and, therefore, proposed that the potassium ion might be one of the factors responsible for the hyperemia of muscular exercise.

More recently, Kjellmer (49) also showed that the potassium ion concentration of venous blood rises during active hyperemia, and he suggested that potassium "locally released from activated skeletal muscles may be responsible for a considerable part, possibly the major part, of exercise hyperemia" (49).

Haddy and associates (35) have confirmed the vasodilator property of the potassium ion in limb and coronary beds and have additionally demonstrated the same effect in the intestine and kidney vascular beds (19, 35, 57).

Driscol and Berne (17) confirmed Katz and Lindner's (47) findings, but observed that an increased coronary blood flow induced by pressure work, asphyxia, dinitrophenol or epinephrine was not associated with a net increase of potassium ions from the myocardium.

Ponce-Zunido et al. (58), using an isolated cat heart preparation perfused with a balanced ion solution containing washed red blood cells, produced cardiac arrest by stopping perfusion. They noted that the perfusate returning from the heart upon reinstitution of perfusion contained a considerably higher concentration of potassium ions than the original perfusate.

Yonce (77) stated, however, that he failed to demonstrate any change in the K^+ concentration during reactive hyperemia of skeletal muscle. He presented no data to this effect.

Scott et al. (67) also reported that the renal vasodilation observed with partial artery occlusion was not associated with a rise in

the potassium ion in the renal venous blood. More recently, however, Daugherty et al. (personal communication) have at times observed a rise in the potassium ion concentration of the renal venous plasma above that of the arterial plasma when the kidney was perfused with blood very low in P_{O_2} (0-4 mm Hg).

Role of Magnesium in Local Regulation

Few studies on the local effect of magnesium upon vascular beds have been reported. Haddy (36) and his associates (24, 57, 68) demonstrated that the magnesium ion is an arteriolar dilator. They have shown that intra-arterial infusion of an isosmotic solution of $MgCl_2$ into the forelimb (24, 36), renal (24), and coronary vascular beds results in a decrease in resistance in all these beds. It has been further demonstrated that an intra-arterial infusion of $MgCl_2$ at concentrations with virtually no effect on limb resistance decreased measurably the pressor effect of intrabrachial injection of levarterenol (24, 36). The present author is not aware of any study reported in the literature concerning the role of the magnesium ion in active and reactive hyperemia.

Role of Adenosine Triphosphate in Local Regulation

It has long been known that adenine nucleotides are powerful vasodilators (18, 72, 73) and that they are intimately linked with energy metabolism. There also exists a close correlation between metabolic activity of skeletal muscle and its blood flow. It appears then quite logical to suppose that the adenine nucleotides might in some way be concerned with the local regulation of vascular resistance. As early as 1932, Rigler (59) attempted to show the relation of these compounds to local regulation of muscle blood flow. Although he suggested that

adenylic acid or derivatives of ATP are probably related to the hyperemia of muscular exercise, he did not present evidence to substantiate this hypothesis.

Wolfe and Berne (75) studied a large number of nucleotides and nucleotide derivatives and found that only the adenine nucleotides and adenosine were significant vasodilators. In the same study, they observed that ATP and adenosine diphosphate (ADP) have approximately the same vasodilator potency and that they are about four times more active than adenosine monophosphate (AMP). These authors ascribed the vasodilator property of uridine phosphate to a possible contamination of this compound with ATP.

Hilton (39), in a perfused muscle preparation, was unable to demonstrate ATP in the perfusate during muscle activation or at rest. In the coronary bed, Jacob and Berne (44, 45) and Berne (6) were unable to find nucleotides in the venous effluent during cardiac anoxia or in the normally oxygenated heart. Subsequently, when these investigators added C^{14} labeled adenosine to the perfusate used in an isolated cat heart preparation, they observed that this labeled compound was rapidly incorporated into myocardial ATP and ADP regardless of the state of oxygenation of the myocardium (44, 45).

In a more recent study, Berne (6) demonstrated inosine (deaminated adenosine) and hypoxanthine (another by-product of adenosine metabolism) in the venous outflow during myocardial hypoxia in the intact and isolated heart. Since much adenosine is potentially stored as muscle adenine nucleotides, Berne suggests that "these properties make adenosine a plausible candidate for a role in metabolic regulation of coronary blood flow" (5). He further proposes a scheme of action: "Reductions

in myocardial oxygen tension induced by reduction of CBF (coronary blood flow), by hypoxemia or by increased myocardial metabolic activity, lead to a breakdown of adenine nucleotides to adenosine, which diffuses out of the cells to produce dilation of the arterioles. This, in turn, increases CBF, which reaches a new steady state by virtue of an increase in myocardial P_{O_2} and reduction in nucleotide degradation, and by washout and enzymatic inactivation of adenosine by the increased CBF"(5).

No adenosine increase was found, however, by these investigators in ischemic skeletal muscle (7). ATP appears to be degraded to adenosine in the heart, but it follows a different pathway, bypassing adenosine via IMP, in the kidney and the skeletal muscle (7, 25, 26, 27, 43, 70).

A point often made against the theory that ATP might be an important factor in the local regulation of resistance is that ATP does not cross the cell membrane, at least not at a rate sufficient to produce vasodilation. Whittam (74) has shown that the human red blood cell is highly permeable to adenine and hypoxanthine and their ribosides, but is not permeable, apparently, to ATP. Conversely, Holton (42) reports that she could demonstrate ATP in the effluent perfusate (modified Ringer's solution) from a rabbit ear during and immediately after antidromic stimulation of the great auricular nerve or stimulation of the skin. Douglas et al. (16) noted the presence of AMP in venous effluent from perfused adrenal glands when these organs were stimulated with nicotine, acetylcholine, calcium and potassium. Gordon (28), furthermore, observed AMP in the renal venous blood after release of renal artery occlusion. Berne (5) stated that "if Conway and Cooke's (9) statement that skeletal muscle cells are permeable to AMP can be substantiated by experimental

proof, then it is conceivable that AMP may play a role in skeletal muscle similar to that hypothesized for adenosine in cardiac muscle."

In the present series of experiments, an attempt is made to elucidate the possible role of certain chemicals in the local regulation of vascular resistance. If the concentration of vasoactive metabolites is dependent upon their rate of production (metabolic rate) and the rate of their removal by the blood stream (flow rate), a given ratio of flow to metabolism (F/M), therefore, would be related to approximately the same degree of vasoactivity. This hypothesis was tested in the isolated hindlimb of the dog. The potassium and magnesium ions, as well as ATP and O_2, are studied in relation to their roles in vascular resistance changes under various conditions. Potassium, magnesium and ATP are determined in autoperfused and pump-perfused preparations during rest and during both active and reactive hyperemia. Potassium is also studied under conditions of severe hypoxemia. The local effect of O_2 upon vascular resistance is analyzed by perfusing an isolated limb with hypoxic blood and comparing the observed perfusion pressure to that seen when similar P_{O_2} levels are obtained with skeletal muscle activation. Furthermore, the ability of the hindlimb vascular bed to autoregulate in the virtual absence of O_2 in the perfusing blood is assessed.

CHAPTER II

MATERIALS AND METHODS

Flow to Metabolism Ratio

In this study, mongrel dogs of both sexes were used. They were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and ventilated with a mechanical respirator (Harvard Apparatus Co., model 607) via an endotracheal tube. The right hindlimb was surgically isolated at the hip joint leaving intact only the femoral artery. After heparinization (4 mg/kg), the femoral vein was cannulated and the effluent blood shunted to the contralateral femoral vein by means of a bypass circuit with a side arm through which flows could be measured directly with a graduated cylinder and a stop watch (Fig. 1). Femoral arterial and venous pressures were continuously recorded by a direct writing oscillograph utilizing 0-75 mm Hg resistance wire pressure transducer. Blood pH determinations were done with a Beckman, model 76 expanded scale, pH meter with a constant temperature block. Oxygen was measured by the Van Slyke manometric method. Skeletal muscles were activated by faradically stimulating the femoral and sciatic nerves at frequencies varying from 2 to 6 contractions per minute. The average stimulation voltage was 5.6v, and the duration was 0.5m sec. The perfusion rate was altered by partially occluding the femoral artery with a silk ligature to produce 2 or 3 levels of flow.

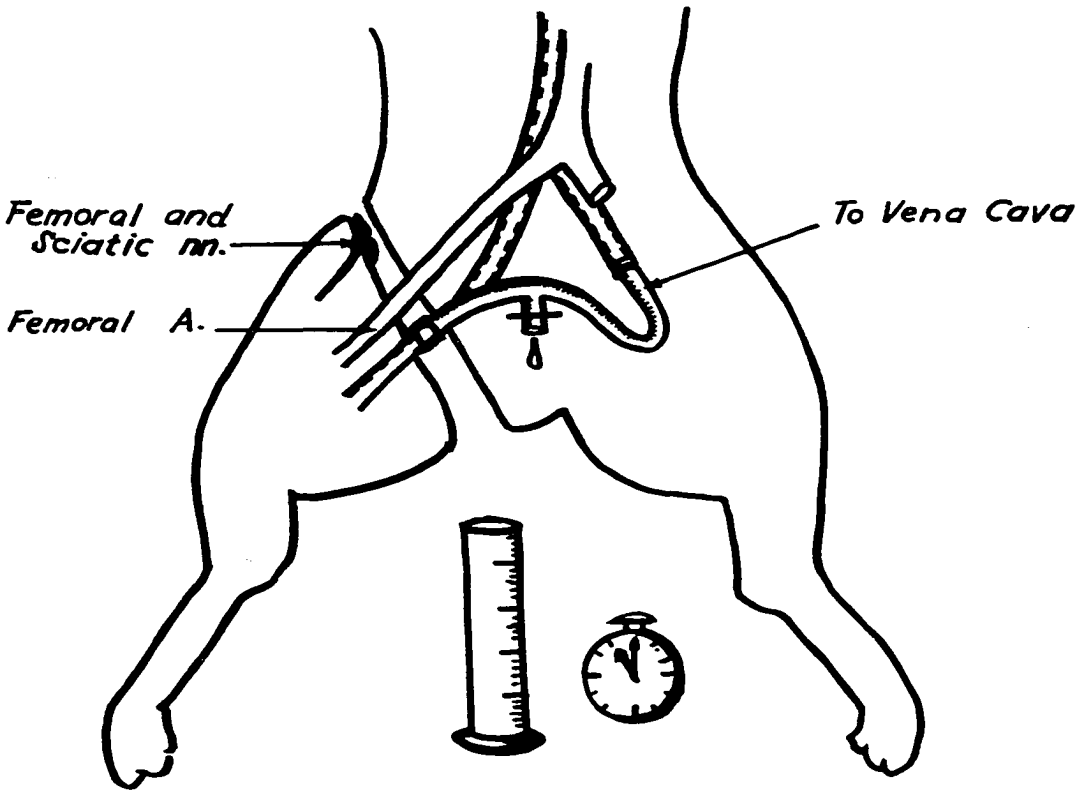

Figure 1. Diagramatic drawing of the isolated autoperfused hindlimb of the dog.

A standard sequence was followed in all experiments. Blood flow, pH, and arterial and venous oxygen content (vol.%) were obtained anaerobically during the control period, during skeletal muscle activation, during muscular activity with the femoral artery partially occluded, and during partial arterial occlusion with the limb at rest. At the completion of these maneuvers, a final control determination of these parameters was obtained. The limb was weighed at the end of the experiment. Resistance across the limb was calculated by dividing the arterio-venous pressure gradient by the measured flow. The resistance was expressed on a per kilogram basis by dividing the calculated resistance by the weight of the limb. The metabolic rate was calculated by obtaining the arterio-venous oxygen difference and multiplying it by the corresponding flow. This calculated oxygen consumption was also expressed on a per kilogram basis. No attempt was made to separate skin, bone and muscle weights. Flow to metabolic rate ratios were calculated. The closest pairs of F/M were selected and matched, and their respective resistances were compared both during and immediately after stopping muscle activity and releasing the arterial occlusion.

Potassium Study

In this series of nine experiments, the preparation was identical to the one described above. Venous blood was sampled for potassium ion determination by the flame photometric method using internal standard (Baird photometer, model KB4), and for pH measurements during the following sequential maneuver: 1) initial control period, 2) active hyperemia (simultaneous faradic stimulation of femoral and sciatic nerves at 3v, f: 4-5/sec., and 0.2 msec. duration, 3) skeletal muscle activation with

the femoral artery partially occluded, 4) active hyperemia after release of occlusion of the femoral artery, which was totally occluded for 3 to 5 minutes. Twenty consecutive samples were obtained during the interval between release of occlusion and 10-20 seconds later, 5) final control - approximately 10 to 15 minutes after the reactive hyperemia phase. In 3 experiments, femoral arterial blood was sampled simultaneously with venous blood for potassium determination during the above listed sequence.

The potassium ion was also studied using the isolated gastrocnemius muscle of the dog. The muscle was left in situ. The arterial and venous supply were isolated by ligating all branches of the popliteal artery and vein that did not directly supply this muscle. Perfusion of the muscle was done with homologous dog plasma in three experiments, and in two others, with a Dextran solution to which the chloride salts of calcium, magnesium and potassium were added in amounts sufficient to reach physiologic levels in the perfusate. Sodium bicarbonate was also added to shift the pH toward a normal blood value - 7.4. A gas mixture of 95% O_2 - 5% CO_2 was bubbled through the perfusates which were contained in a glass reservoir at constant temperature (37°C) by means of a water bath. The perfusate was then pumped (Sigmamotor pump, model T-6SH) at constant flow into the distal end of the divided popliteal artery. Thus a fall in the perfusion pressure would indicate a fall in resistance. The effluent solution was collected in a separate container (and not re-used) by means of a cannula introduced into the distal end of the divided popliteal vein. Samples were collected directly from this venous cannula. The muscle was activated by faradic stimulation of the

distal stump of the divided sciatic nerve with a current of 3v, a duration of 0.2 msec, and at a frequency of 4-6 contractions per second. Reactive dilation was studied by turning the perfusion pump off for periods of 2-4 minutes and observing the changes in perfusion pressure when the pump was turned on again.

The materials and methods for the study of potassium under anoxic conditions are described in the section on oxygen study, p. 20.

Adenosine Triphosphate Study

Two different preparations were used in this series of experiments. The first group of experiments was done on the isolated hindlimb of the dog such as described above, (Fig. 1), and the same experimental sequence was followed. A solution of ATP in saline of known concentration was infused with a constant infusion pump directly into the femoral artery by means of a polyethylene tubing introduced into the artery through a small side branch.

In the second group of experiments, the isolated gastrocnemius muscle of the dog was used. It was an identical preparation to the one described in the section on potassium study, p. 17. Homologous dog plasma and the modified Dextran solution were used as the perfusates. The experimental sequence in this second group of experiments was as follows: 1) initial control, 2) skeletal muscle activation by faradic stimulation of the distal end of the divided sciatic nerve, 3) control, 4) reactive dilation (re-institution of the perfusion after stopping perfusion for 2-4 minutes), and 5) final control. Perfusion pressure was constantly recorded with a direct writing oscillograph. Infusions of known concentrations of ATP in normal saline were accomplished by means

of a constant infusion pump (Harvard Apparatus Co., model 600-900) interposed between the Sigmamotor pump and the popliteal artery supplying the isolated muscle. Adenosine triphosphate was determined by the firefly method described by Beutler and Baluda (8), using the Turner fluorometer, model 110, and employing firefly tail extract from the Sigma Chemical Co. The following modifications by J. Doyle were introduced: a) The reconstituted firefly extract was diluted 1:2 and used in this dilution in all determinations. b) In the isolated pump-perfused muscle experiments in which the perfusate was the modified Dextran solution, 2 ml of the effluent was added to 2 ml of this diluted firefly extract. The perfusate was not boiled prior to adding to the firefly extract. c) When plasma was the perfusate, in the last 3 experiments presented in Table 6 (p. 42), the plasma was diluted 1:5 in Trisborate buffer and boiled for 5 minutes. Two ml of this solution was then added to 2 ml of the diluted firefly extract described in a). d) When whole blood was used, and in the first 5 experiments shown in Table 6, the method of Beutler and Baluda was used without modification.

Magnesium Study

The previously described isolated hindlimb and gastrocnemius muscle of the dog were used in this series of experiments. The experimental sequences in both methods were identical to those described for the potassium study. Magnesium was determined by the method described by Schachter (65) and by using the Turner fluorometer, model 110.

Oxygen Study

This study comprises a group of eight experiments in which the isolated hindlimb of the dog was used. Figure 2 illustrates the

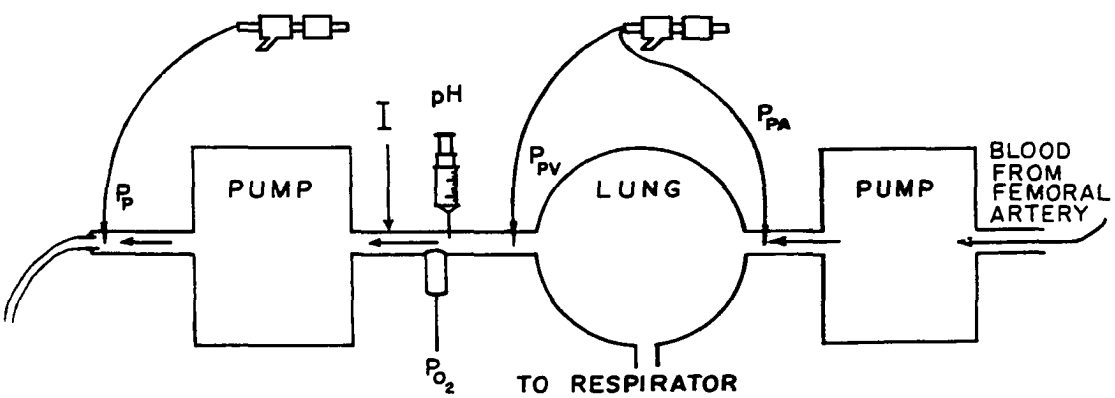

Figure 2. Schematic drawing of the extracorporeal circuit. P_{PA} = pulmonary artery pressure, P_{PV} = pulmonary vein pressure, P_{O_2} = oxygen tension probe, pH = site of sampling for arterial pH, I = injection of test compounds; P_p = perfusion pressure.

preparation diagramatically. The hindlimb of the dog was surgically separated from the animal. Blood from the femoral artery of the animal was pumped (Sigmamotor pump, model T-6SH) into the pulmonary artery of an isolated lung from another dog. Between the pulmonary vein of this isolated lung and the femoral artery of the isolated hindlimb, another Sigmamotor pump was interposed whose rate was synchronized with the first pump in order to maintain near normal pulmonary artery and vein pressures. The outflow from the femoral vein of the isolated limb was shunted to the contralateral femoral vein by means of a bypass rubber circuit into which an oxygen probe was placed. The P_{O_2} of the venous blood was continuously monitored by the oxygen electrode on a gas analyzer (Beckman, model 160, physiological gas analyzer) and was recorded by a direct writing oscillograph. The gas analyzer was calibrated at 37°C by bubbling two known oxygen-in-nitrogen gas mixtures through distilled water. An extracorporeal lung perfusion circuit was thus created, free of reservoirs, between the left femoral artery of the dog and its isolated hindlimb femoral artery.

Limb perfusion pressure approximated that of the systemic pressure. Flow, however, was constant throughout any given experiment except during the last experimental maneuver which tested the autoregulatory response in this vascular bed. Systemic pressure was measured through a catheter introduced in the carotid artery. Systemic, pulmonary artery and perfusion pressures were continuously recorded on a direct writing oscillograph by attaching their respective catheters to pressure transducers of 0-75 mm Hg resistance.

The isolated lung was ventilated with a mechanical respirator (Harvard Apparatus Co., model 607) through a tube introduced into the

main-stem bronchus. Blood was sampled from the extracorporeal system at points just beyond the isolated lung and at the exit from the femoral vein of the isolated limb for arterial and venous samples, respectively.

The experimental sequence used in this preparation was as follows: initially the isolated lung was ventilated with a gas mixture containing 20% oxygen, 5% carbon dioxide in N_2; after achieving the steady state, a control gas mixture of low oxygen content, 0-2% O_2 - 5% CO_2 in N_2, was used. The lung was then ventilated with a mixture containing 95% O_2 - 5% CO_2. After a high P_{O_2} was attained in the venous blood, the skeletal muscles were activated by faradically stimulating the distal ends of the divided femoral and sciatic nerves. The voltage varied from 2 to 5 volts, the duration was constant at 0.2 msec, and the frequency was adjusted to a rate sufficient to produce a fall in the P_{O_2} of the venous blood to a level as close as possible but not lower than that obtained during perfusion with blood with low oxygen content. After the steady state was achieved during the muscle activation maneuver, the nerve stimulation was discontinued and the isolated lung was once more ventilated with the same low-oxygen mixture used previously. Finally, the 20% O_2 - 5% CO_2 in N_2 gas mixture was used. After this portion of the experiment was completed, the autoregulatory capability of this vascular bed was tested in this manner: while the isolated lung was being ventilated with the 0% O_2 gas mixture, the flow through the limb was reduced by a fixed volume for any given experiment. Perfusion at this decreased rate continued for several minutes. The venous P_{O_2} was 0 mm Hg; the pump rate was then rapidly returned to the initial level. The constant recording of the perfusion pressure thus gave an indication of the ability of the vascular bed to autoregulate in the absence of O_2. This experiment was

repeated while perfusing the limb with gas mixtures containing 20% and 95% O_2, 5% CO_2 in N_2.

CHAPTER III

RESULTS

Flow to Metabolism Ratio

The relation of flow to metabolism ratio to vascular resistance in the hindlimb of the dog was studied in eight dogs. Figure 3 shows the average arterial pressure, flow and oxygen consumption for eight preparations. Essentially the same F/M ratio was achieved by both a reduction in flow by partial occlusion of the femoral artery, and by an increase in metabolism through simultaneous faradic stimulation of sciatic and femoral nerves. Thus, activation of the skeletal muscles increased the blood flow from a control value of 138 ml/min/kg to 188 ml/min/kg while the oxygen consumption rose from 2.8 ml/min/kg to 12.8 ml/min/kg, respectively, without altering perfusion pressure. The flow remained essentially unchanged immediately following cessation of stimulation. Femoral artery occlusion of sufficient degree to produce a reduction of perfusion pressure to 54 mm Hg resulted in a fall of flow to 54 ml/min/kg without changing the metabolic rate. Immediately after release of the partial arterial occlusion, the flow increased to 220 ml/min/kg. In addition, Figure 3 shows that during stimulation the metabolic rate increased disproportionately to flow; and, during partial arterial occlusion, the flow fell disproportionately to the metabolic rate.

Figure 4 presents the calculated values for F/M and their

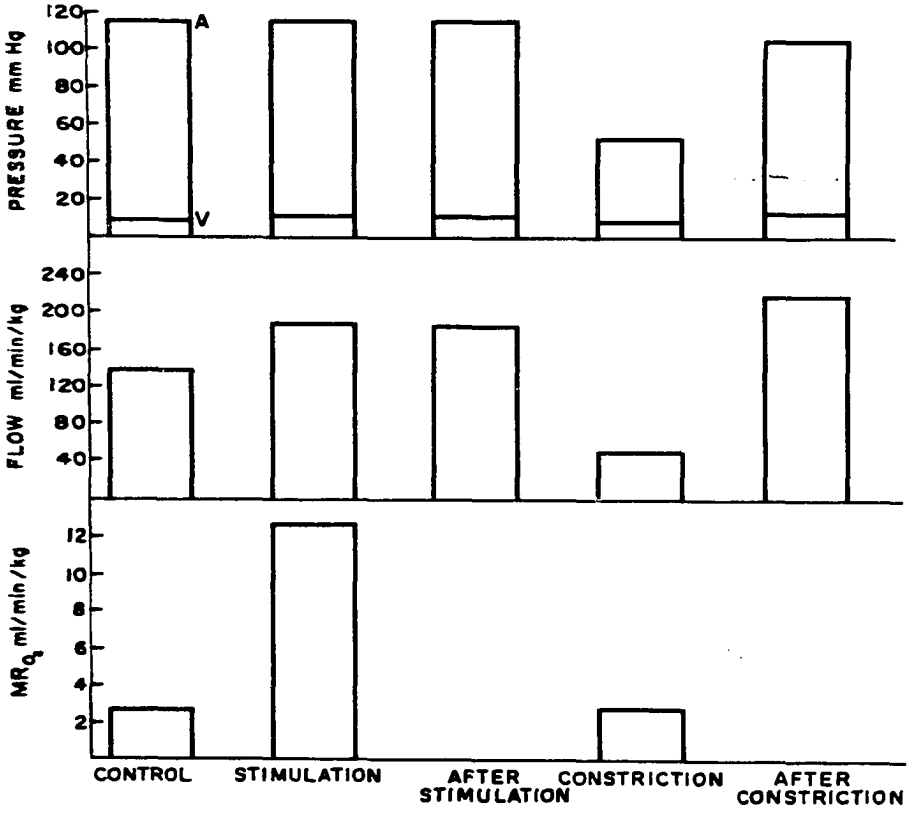

Figure 3. Average values of pressure, flow and metabolic rate (O_2 consumption) for 8 preparations during the experimental sequence.

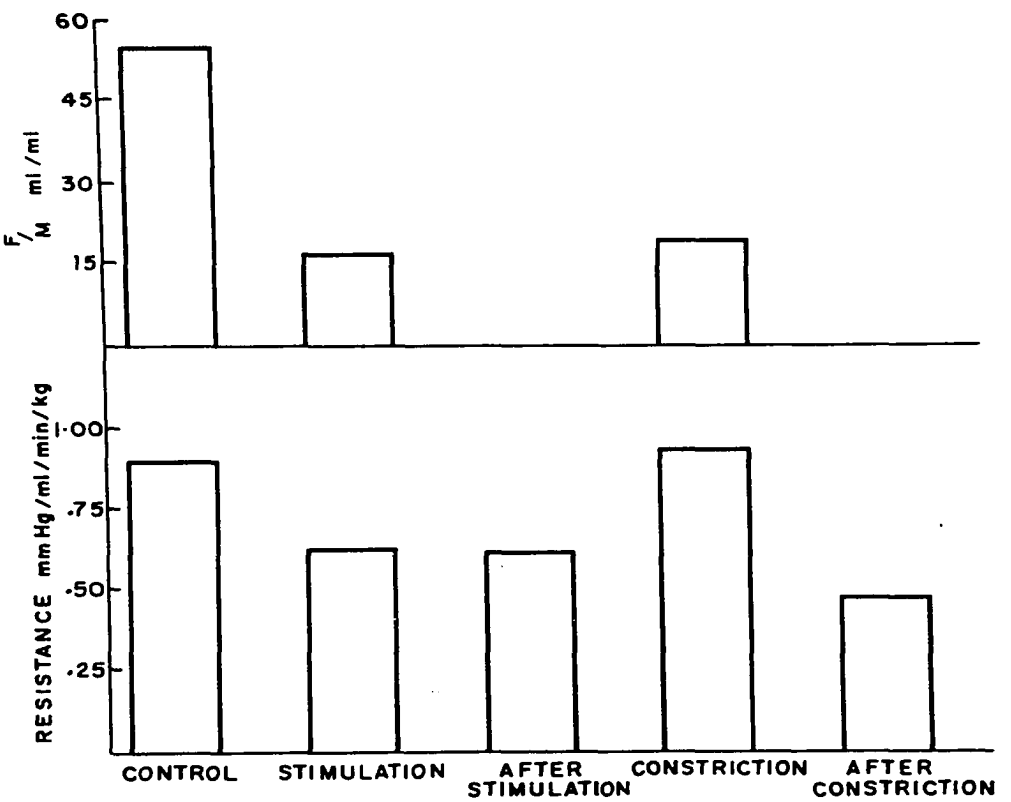

Figure 4. Average F/M ratios and their respective resistances for 8 preparations during the experimental sequence.

respective resistances. Resistance fell during muscular activation but rose during obstruction of inflow. Upon cessation of stimulation and release of the partially occluded artery, both resistances fell, however, the resistance was slightly less after releasing occlusion than after stopping stimulation. This was observed in seven of eight experiments.

Figure 5 depicts the resistances as a function of the F/M ratio. The two upper solid lines represent the values during occlusion and stimulation with both active and passive effects present; and the lower lines represent those values immediately following release of occlusion and cessation of stimulation--the active effect only. It is to be noted that, during muscular contraction, there is no passive effect on resistance; whereas, the passive effect during occlusion is obvious. It is also apparent that, for a given F/M, the resistance is lower immediately following release of occlusion than after cessation of stimulation. One of the mechanisms thought to be operational in the production of a lower resistance after release of the occluded artery was sympathetic vasoconstrictor fibers stimulation during faradic stimulation of the femoral and sciatic nerves. These experiments were repeated, therefore, in the Anectine paralyzed limb while stimulating the nerves with the same parameters used in the previous experiments. No vasoconstrictor effect could be thus demonstrated. Nevertheless, eight more experiments were carried out employing even lower voltage and duration; namely, 2.7 v and 0.2 msec. Three additional pairs of virtually identical F/M ratios were obtained. Figure 6 depicts the results of these three experiments. A slightly lower resistance than that observed after stopping stimulation once more followed release of the arterial occlusion. The passive effect of partial

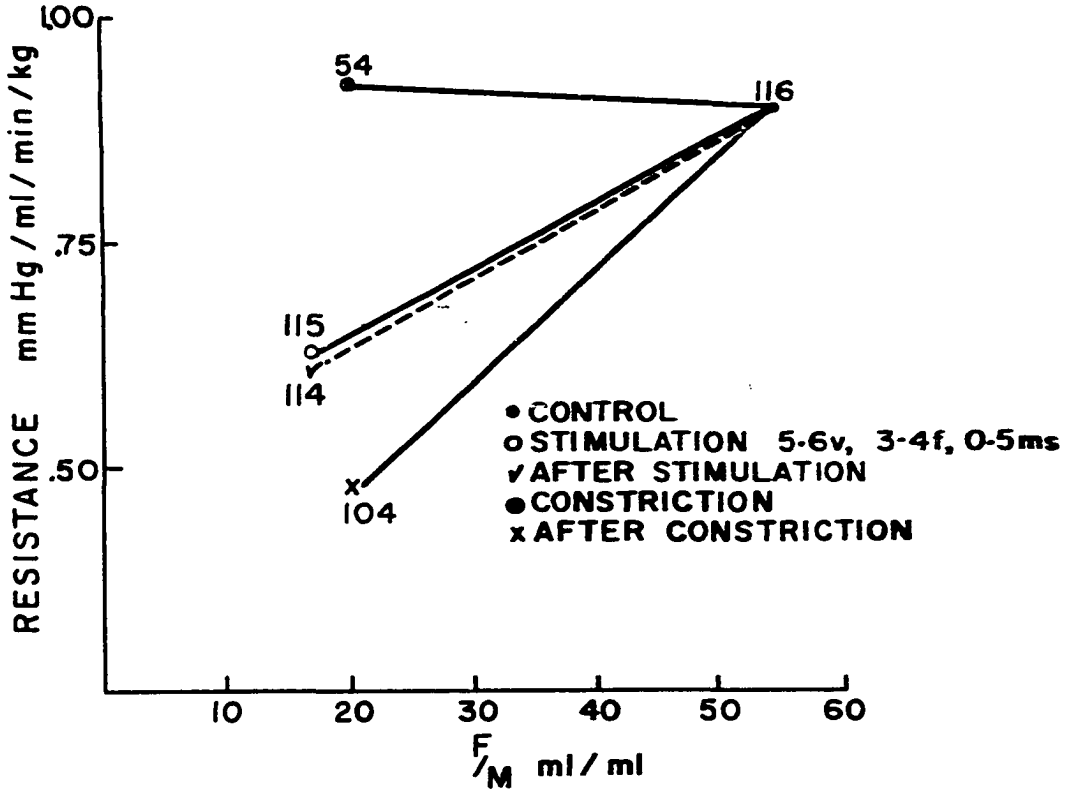

Figure 5. Average resistances for 8 experiments during the experimental sequence plotted as functions of F/M.

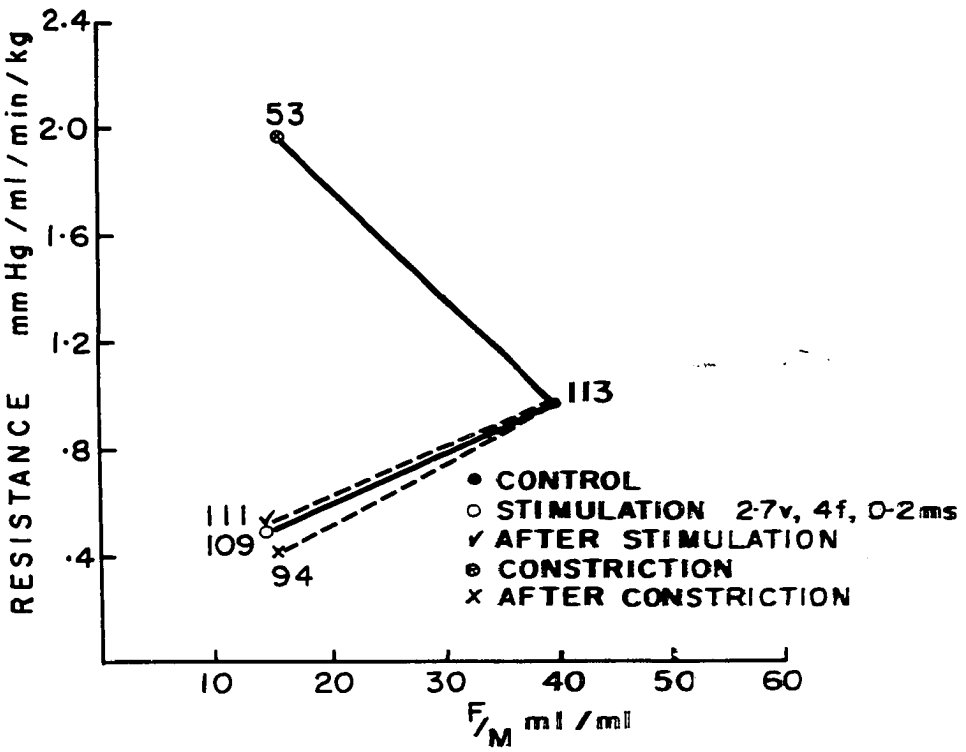

Figure 6. Average resistance for 3 experiments during experimental sequence plotted as functions of F/M.

occlusion is again obvious, but none is present during skeletal muscle activity.

Potassium Study

The findings in a representative experiment are demonstrated in Figure 7. The venous plasma $[K^+]$ in the initial and final control samples are essentially the same. During skeletal muscle activation, a good increase in flow was obtained. The pH decreased as expected and the $[K^+]$ rose from a control value of 3.00 mEq/L to 3.75 mEq/L.

In the next maneuver, muscular activity was maintained while the femoral artery was partially occluded with a silk ligature. There resulted a further drop in pH and a greater increase in $[K^+]$ 4.50 mEq/L.

During reactive hyperemia, the flow is comparable to that seen during exercise hyperemia. The pH, obtained approximately 5 seconds after release of the femoral artery occlusion, was lower than that obtained during active hyperemia. However, the $[K^+]$ was the same in all twenty samples collected during the reactive hyperemia phase, and this value was identical to that of the initial control.

The average values for nine experiments are shown in Figure 8. All parameters measured changed in the same direction as those shown in Figure 7. The pH changes are similar, again being slightly lower during reactive than during active hyperemia in seven of nine experiments. No change was observed in the $[K^+]$ of hyperemia blood in any of the twenty samples collected in each experiment. This value was identical to the initial control in all experiments. The final control level of the $[K^+]$ was slightly lower than the initial control due, probably, to dilution of the animal's blood with Dextran, as this solution was used to replace,

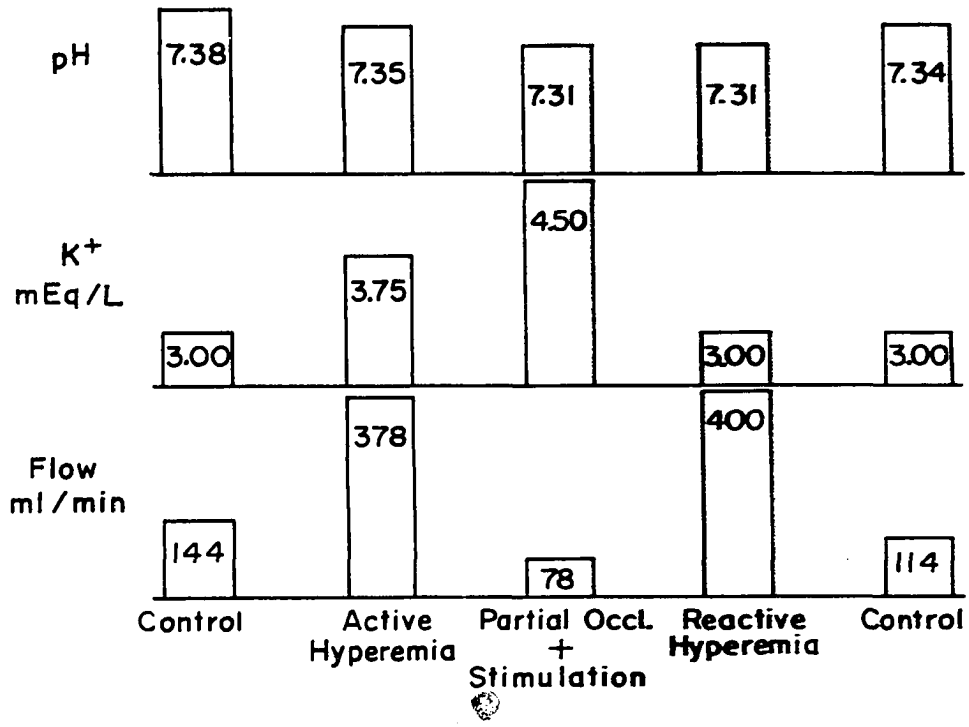

Figure 7. Values of pH, K^+ (mEq/L) and flow (ml/min) for one representative experiment during experimental sequence.

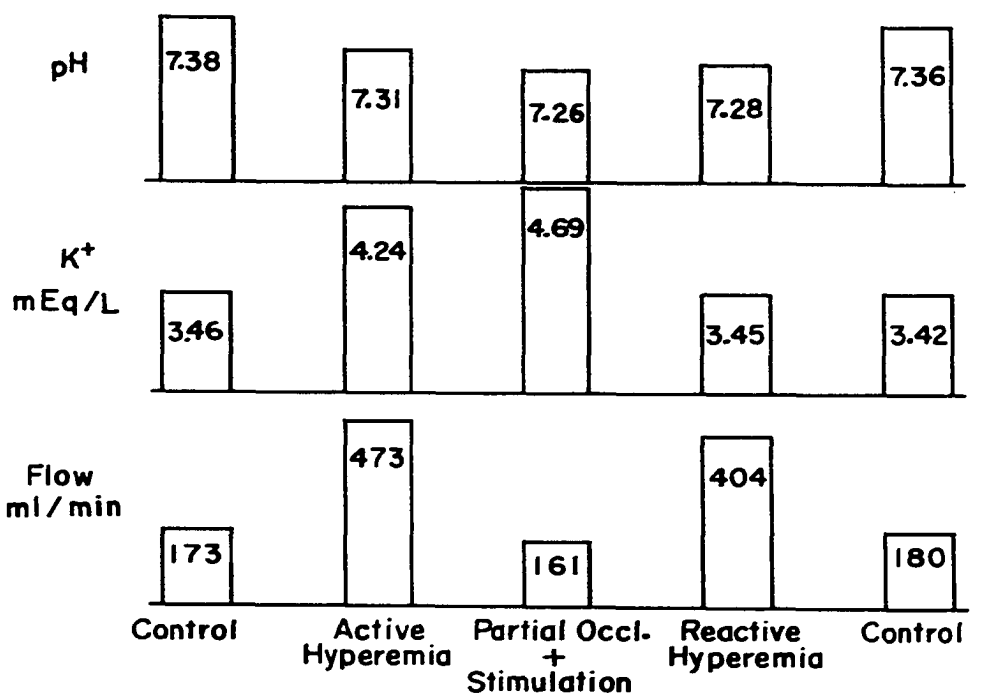

Figure 8. Average values of pH, K^+ and flow for 9 experiments during routine sequence.

ml for ml, the blood samples drawn for K^+ and pH determinations.

Furthermore, in three additional experiments, femoral arterial and venous K^+ were determined during each of the experimental maneuvers. Table 1 demonstrates these findings. The arterial K^+ concentration remained unchanged throughout the experiment, and its value was identical to the initial venous control and the reactive hyperemic blood.

The potassium ion concentration was also studied in the isolated, pump-perfused gastrocnemius muscle of the dog. The findings are similar to those observed in the isolated hindlimb preparation described above. The results are presented in Table 2. When either homologous plasma or modified Dextran solution was used as the perfusate, no change in K^+ concentration could be demonstrated in the effluent during the reactive dilation phase. Skeletal muscle activation, on the other hand, again produced a rise in the K^+ concentration of the cell-free perfusate. Perfusion pressure, as an indicator of the vascular resistance in this constant-flow preparation, fell from an average control value of 78 mm Hg to 55 mm Hg and 60 mm Hg during active and reactive dilation, respectively.

To further elucidate the question of a possible relation of hypoxemia to the release of K^+ from the tissues into the effluent blood, studies were carried out in the isolated, pump-perfused hindlimb of the dog. The perfusing blood was passed through an isolated lung from another dog and was exposed to a control gas mixture containing 20% O_2 - 5% CO_2 in N_2. The gas mixture was then changed to one containing no O_2: 95% N_2 - 5% CO_2. Arterial and venous blood from this system were sampled simultaneously for K^+ determination during perfusion with oxygenated and deoxygenated blood. Table 3 shows the results obtained.

TABLE 1

ARTERIOVENOUS PLASMA K^+ (mEq/L) DIFFERENCE DURING ACTIVE AND REACTIVE HYPEREMIA IN THE ISOLATED AUTOPERFUSED HINDLIMB OF THE DOG

	Control		Stimul.		St. + Con.		R.H.		Control	
Exp. No.	A	V	A	V	A	V	A	V	A	V
1	3.62	3.62	3.62	4.25	3.62	4.25	3.62	3.62	3.62	3.62
2	3.00	3.00	3.00	3.58	3.00	3.75	3.00	3.00	2.62	2.62
3	3.75	3.75	3.75	4.60	3.75	4.87	3.75	3.75	3.75	3.75
$\bar{X}$	3.46	3.46	3.46	4.14	3.46	4.29	3.46	3.46	3.33	3.33

A = arterial, V = Venous, R.H. = Reactive hyperemia

TABLE 2

PCTASSIUM ION CONCENTRATION (mEq/L) DURING ACTIVE AND REACTIVE DILATION IN THE ISOLATED PUMP-PERFUSED GASTROCNEMIUS MUSCLE OF THE DOG
PLASMA PERFUSATE

Exp. No.	Control	Stim.	Control	R.D.	Con.
1	5.37	5.42	5.37	5.37	5.37
2	4.37	4.60	4.37	4.37	4.37
3	4.87	5.60	4.87	4.87	4.87
$\bar{X}$	4.87	5.21	4.87	4.87	4.87
Modified Dextran perfusate					
1	5.00	5.50	5.00	5.00	5.00
2	5.00	5.37	5.00	5.00	5.00
$\bar{X}$	5.00	5.45	5.00	5.00	5.00

R.D. = reactive dilation

TABLE 3

PLASMA K^+ CONCENTRATION (mEq/L) IN THE ISOLATED PUMP-PERFUSED HINDLIMB OF THE DOG AT REST; WHOLE BLOOD PERFUSATE EXPOSED TO 20% O_2 AND 0% O_2

Exp. No.	20% O_2		0% O_2	
	A	V	A	V
1	2.75	2.75	2.75	2.75
2	2.50	2.50	2.50	2.50
3	3.25	3.25	3.25	3.25
4	3.00	3.00	3.00	3.00
$\bar{X}$	2.88	2.88	2.88	2.88

A = Arterial, V = Venous

It is evident that the K^+ concentration remained unchanged when the perfusing blood was deoxygenated although the perfusion pressure fell from a control value of 110 mm Hg to 69 mm Hg during anoxemia. The pH remained constant by the addition of 5% CO_2 to both gas mixtures.

Magnesium Study

The isolated hindlimb of the dog was used in ten experiments to determine changes in Mg^{++} concentration during active and reactive hyperemia. The experimental sequence was the same as that employed in the potassium study. The flow changes were also similar to those observed in K^+ experiments (Fig. 8, p. 33). Table 4 presents the Mg^{++} values for ten experiments. Although the difference in Mg^{++} concentration between control and active hyperemia plasmas is only 0.1 mEq/L, this difference is statistically significant. Conversely, the difference in Mg^{++} between reactive dilation and the controls is not significant.

The magnesium ion was also studied in the isolated, pump-perfused gastrocnemius muscle of the dog. In two such preparations, homologous plasma was the perfusate; and, in two others, a modified Dextran solution was used as the perfusing medium. Table 5 depicts the results. In contrast to the isolated limb in which the perfusate was whole blood, these experiments, utilizing cell-free perfusates, demonstrate no net change in the Mg^{++} concentration during any of the experimental maneuvers. The perfusion pressure, however, fell from an average control value of 80 mm Hg to 55 mm Hg during exercise, and to 59 mm Hg during the reactive dilation phase.

TABLE 4

PLASMA Mg^{++} (mEq/L) DURING ACTIVE AND REACTIVE HYPEREMIA IN THE ISOLATED, AUTOPERFUSED HINDLIMB OF THE DOG

Exp. No.	Control	Stimulation	Stimulation + Constriction	Control	R.H.	Control
1	1.3	1.5	1.6	1.3	1.6	1.3
2	1.3	1.5	1.5	1.3	1.4	1.2
3	1.7	1.8	1.8	1.7	1.8	1.8
4	1.4	1.5	1.8	1.3	1.6	1.3
5	1.7	1.8	1.8	1.7	1.7	1.7
6	1.5	1.5	1.6	1.5	1.4	1.5
7	0.9	1.0	1.1	1.0	1.1	1.0
8	1.8	1.9	2.0	1.8	1.8	1.8
9	1.6	1.6	1.7	1.6	1.6	1.6
10	1.6	2.0	1.6	1.5	1.6	1.6
$\bar{X}$	1.5	1.6	1.7	1.5	1.6	1.5

R. H. = reactive hyperemia

TABLE 5

MAGNESIUM ION CONCENTRATION (mEq/L) DURING ACTIVE AND REACTIVE DILATION IN THE ISOLATED, PUMP-PERFUSED GASTROCNEMIUS MUSCLE OF THE DOG. PLASMA PERFUSATE

Exp. No.	Control	Stimulation	Control	R.D.	Control
1	2.0	2.0	2.0	2.0	2.0
2	2.1	2.1	2.1	2.1	2.1
$\bar{X}$	2.1	2.1	2.1	2.1	2.1
Modified Dextran perfusate					
1	1.9	1.9		1.9	1.9
2	1.9	1.9		1.9	1.9
$\bar{X}$	1.9	1.9		1.9	1.9

R. D. = reactive dilation

Adenosine Triphosphate Study

ATP was studied in the isolated, autoperfused hindlimb of the dog. The results obtained are shown in Table 6. In the first five experiments, ATP was measured in both whole blood and plasma. In the last three experiments, a measurement in only plasma was obtained. Samples were obtained during the following experimental sequence: a) initial control, b) skeletal muscle activation, c) skeletal muscle activity with partial femoral artery occlusion, d) reactive hyperemia and e) final control period. The flow changes during each of these maneuvers closely parallel those observed in the K^+ experiments on the dog hindlimb (Fig. 8, p. 33).

In experiments 1 and 2, an ATP solution was infused into the femoral artery by means of a constant infusion pump. The infusion rate (0.39 ml/min, equivalent to 200 nM ATP/min.) was sufficient to increase flow by 65% and 125% in the respective experiments. No ATP was demonstrated in the venous plasma of the first experiment, and only less than 2% of the infused ATP was recovered in the venous effluent of the second experiment. An ATP solution was also infused in the isolated gastrocnemius muscle preparation. When the homologous plasma was the perfusate, no ATP was recovered in the effluent. In one study utilizing a modified Dextran solution as the perfusate, the recovery of ATP was again extremely low.

ATP was also studied in the isolated gastrocnemius muscle of the dog. In this constant-pump-perfused preparation, homologous plasma was used in the first six experiments and a modified Dextran solution in the last two. The findings are shown in that order in Table 7. It is evident from Table 7 that no ATP was demonstrated in the routine

TABLE 6

ADENOSINE TRIPHOSPHATE (nM/ml) DURING ACTIVE AND REACTIVE HYPEREMIA IN THE ISOLATED, AUTOPERFUSED HINDLIMB OF THE DOG

Exp. No.	Control W.B.	Control P	Stimulation W.B.	Stimulation P	Stimulation + Occlusion W.B.	Stimulation + Occlusion P	R,H. W.B.	R,H. P	Control W.B.	Control P
1	330	0.0	330	Tr.		11.4	340	22.8		
2	270	12.0	300	6.0	270	Tr.	270	6.0	270	Tr.
3	254	1.0	193	6.0	254	6.0	205	12.0	254	1.0
4	264	0.0	275	2.0	275	1.0	242	1.0	220	1.0
5	290	0.0	363	0.0	344	3.0	327	0.0	327	0.0
6		0.1		0.3		0.2		0.0		0.0
7		0.7		0.9		0.5		0.7		1.5
8		0.1		0.0		0.0		0.0		0.0

W.B. = whole blood, P. = plasma, R.H. = reactive hyperemia, Tr. = trace.

TABLE 7

ADENOSINE TRIPHOSPHATE (nM/ml) DURING ACTIVE AND REACTIVE DILATION IN THE ISOLATED PUMP-PERFUSED GASTROCNEMIUS MUSCLE OF THE DOG. PLASMA AND MODIFIED DEXTRAN PERFUSATES

Exp. No.	Control	Stimulation	Control	R.D.	Control
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	Tr.	0
4	0	0	0		
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0

R.D. = reactive dilation, Tr. = trace

experimental sequence. The vascular resistance during active and reactive dilation fell, however, as reflected in the fall in perfusion pressure. These values were approximately the same as those reported in the Mg^{++} study, p. 38.

Oxygen Study

The local effect of oxygen upon vascular resistance was studied in eight animals utilizing the isolated, pump-perfused hindlimb of the dog. The perfusing blood was exposed to various gas mixtures by means of an isolated lung from another animal.

Figure 9 depicts graphically the results of a representative experiment. The upper row of panels demonstrates the P_{O_2} of the venous blood during the experimental sequence. The middle row tracings demonstrate the respective perfusion pressures, and the lower row is a recording of the systemic pressure during this experimental sequence. It can be appreciated that although the P_{O_2} during skeletal muscle activity remained above the two controls, the perfusion pressure during this maneuver fell approximately 17 mm Hg below the control values.

The observed values for eight experiments are presented in Table 8. Under control, resting conditions, and perfusion of the limb with blood exposed to a low-oxygen gas mixture, the average venous P_{O_2} was 11 mm Hg, and the average perfusion pressure was 81 mm Hg. The limb was then perfused with blood exposed to 95% O_2 - 5% CO_2. The P_{O_2} rose to levels sufficiently high to permit skeletal muscle activation (faradic stimulation of femoral and sciatic nerves) at an average frequency of 1.3/sec without allowing the P_{O_2} to fall below the control levels of 11 mm Hg and 13 mm Hg, respectively. During muscular exercise,

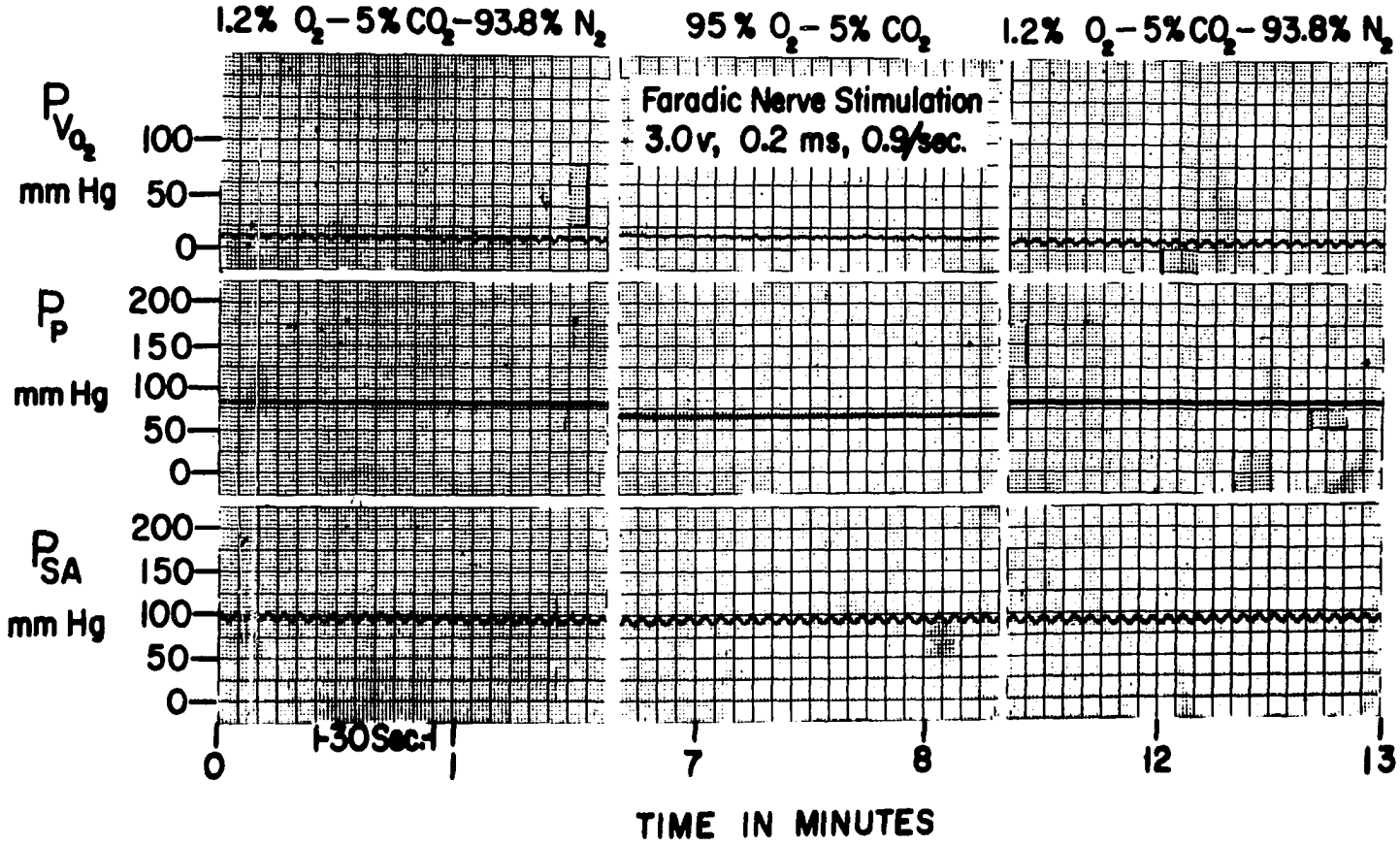

Figure 9. Local effect of low P_{O_2} (attained by perfusion with low P_{O_2} blood and by muscular activity) upon the vascular resistance of the isolated hindlimb of the dog. P_{VO_2} = venous P_{O_2}, P_P = perfusion pressure, P_{SA} = systemic pressure.

TABLE 8

EFFECT OF LOW P_{O_2} (ATTAINED BY ACTIVE EXERCISE AND BY PERFUSION WITH LOW P_{O_2} BLOOD) UPON THE VASCULAR RESISTANCE IN THE ISOLATED, PUMP-PERFUSED HINDLIMB OF THE DOG

Exp. No.	20% O2		Low % O_2		95% O_2		95% O_2 + Stimul.		Low % O_2		20% O_2	
	P_p	P_{O_2}	P_p	P_{O_2}	P_p	P_{O_2}	P_p	P_{O_2}	P_p	P_{O_2}	P_p	P_{O_2}
1	80	40	60	9	80	50	35	15	50	9	75	41
2	105	32	100	16	122	>160	115	20	112	16	--	--
3	90	56	80	9	95	>160	65	14	80	9	95	62
4	103	38	75	6	122	200	50	16	70	11	120	48
5	90	60	75	8	96	380	58	16	75	8	100	80
6	84	49	85	17	100	250	73	22	98	18	115	69
7	--	--	80	14	122	92	55	17	82	14	--	--
8	--	--	93	19	105	>160	73	24	95	20	--	--
$\bar{X}$			81	11			66	18	83	13		

P_p = perfusion pressure, P_{O_2} = partial O_2 tension

the average perfusion pressure was 66 mm Hg, and the P_{O_2} was 18 mm Hg.

Upon completion of skeletal muscle activity, the blood was again exposed to the low-oxygen gas mixture used in the initial control period. Both perfusion pressure and P_{O_2} returned to essentially the same values as those observed before skeletal muscle activation; namely, 83 mm Hg and 13 mm Hg, respectively.

Figure 10 demonstrates the capability of this vascular bed to autoregulate in the face of totally deoxygenated perfusing blood. Again, the upper row of panels shows the venous P_{O_2} when the perfusing blood was exposed to the various gas mixtures. The lowermost row is a recording of the systemic pressure during these periods. The middle row demonstrates autoregulation when the flow through the limb is raised from 42 ml/min to 160 ml/min during perfusion with blood exposed to the various gas mixtures.

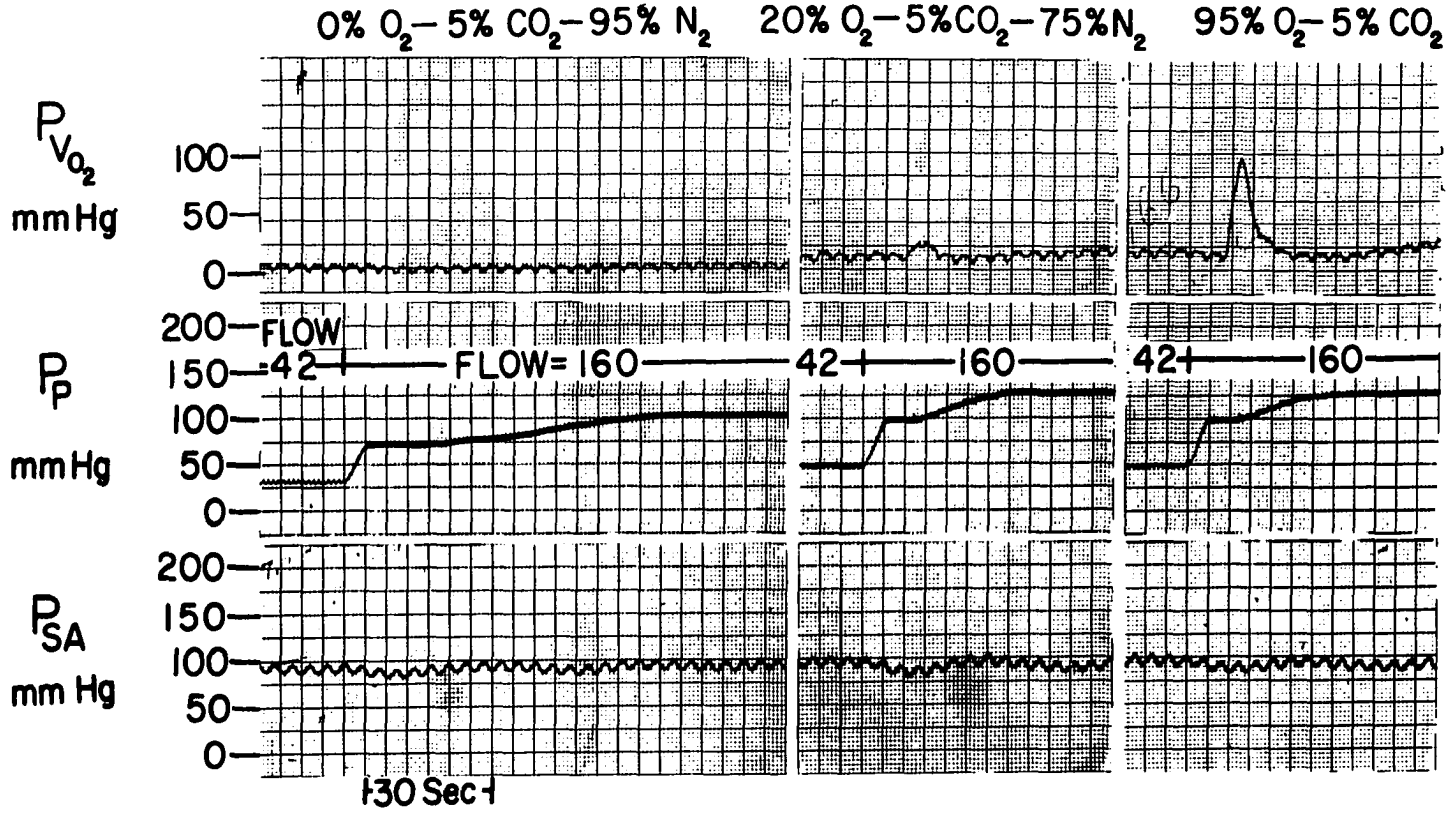

Figure 10. Autoregulatory response of the dog hindlimb vascular bed when perfused with blood exposed to 0%, 20%, 95% O_2. $P_{V_{O_2}}$ = venous P_{O2}, P_p = perfusion pressure, P_{SA} = systemic pressure; flow in ml/min.

CHAPTER IV

DISCUSSION

F/M vs Resistance

The chemical theory of local regulation of blood flow suggests that the contractility of vascular smooth muscle is determined by vasoactive chemicals whose concentrations depend upon the rate of flow through the vessels in question and upon the metabolic rate of the tissues supplied by these vessels. Therefore, the concentration of a given chemical will be determined, in the final analysis, by the ratio of the flow to the metabolic rate: F/M.

If the chemical theory of local regulation of resistance is correct, one would expect that a given F/M attained either by altering the flow or the metabolic rate would be related to the same resistance; that is, one would observe the same degree of active vascular dilatation or constriction. This idea was tested in the dog hindlimb by reducing flow through the isolated organ by partial femoral artery occlusion. The metabolic rate was altered by activating the skeletal muscles through faradic stimulation of femoral and sciatic nerves. In this manner, eight pairs of virtually identical F/M ratios were obtained and their respective resistances calculated. The resistance fell during skeletal muscle activation, but rose during partial artery occlusion. This rise in the latter was the result of passive vascular constriction secondary to a fall

in transmural pressure.

To examine the pure active changes, resistance was also determined immediately following release of the arterial occlusion and cessation of nerve stimulation. For any given pair of F/M, the respective resistances decreased to approximately the same level. However, resistance fell, after releasing the partial occlusion, to a level slightly below that observed immediately after faradic stimulation ceased. This slightly lower resistance level was observed in seven of eight experiments.

Why should partial occlusion produce a more active dilatation upon release of the obstruction than does skeletal muscle activity after stopping the faradic stimulation? The possibility of inadvertant vasoconstrictor fibers stimulation was investigated in the paralyzed limb preparation (56). Resistance did not rise under this condition when femoral and sciatic nerves were stimulated with the same parameters used in the non-paralyzed limb. The difference, therefore, between the active response following release of occlusion and cessation of stimulation does not seem to be related to autonomic nerve stimulation. There exists the possibility of a Bayliss response to explain at least partially this observation. The transmural pressure, being lower during the entire period of partial artery obstruction, would tend to relax the vascular bed. Upon release of the obstruction, this relaxation could result in a slightly lower resistance than that seen after exercise, inasmuch as transmural pressure decreases only intermittently during muscle contractions. A third mechanism could be a dilution of vasodilator substances in the extracellular fluid during muscular exercise as capillary pressure undoubtedly rose during stimulation and fell during

partial arterial occlusion. This study demonstrates that a given F/M ratio, obtained either by altering flow or metabolic rate, elicits approximately the same active changes in vessel caliber. The fact that the changes were not identical suggests that other factors may also be involved. The study also emphasizes the importance of passive changes in caliber in determining resistance.

Potassium

Many authors in the past have demonstrated the vasodilator properties of the potassium ion. Others showed an increase in the concentration of K^+ venous blood during muscular exercise (2, 49, 64). It has been, therefore, suggested that the K^+ may be, at least partially responsible for the hyperemia of muscular exercise (15, 49).

If the mechanism of vasodilation of active and reactive hyperemia is similar, one would also expect the potassium ion concentration of venous blood to rise during reactive hyperemia. In a paper published in 1935, Baetjer (3) reported that the reactive hyperemic blood obtained after cross-clamping the aorta of the cat for 5 to 23 minutes showed progressively increasing amounts of K^+. However, she does not describe her preparation and experiments in detail. Thus one cannot ascertain the level at which the aorta was cross-clamped nor estimate the effect upon K^+ release from visceral organs if flow to the adrenals, kidney and liver was impaired by occluding the artery above the celiac axis. Yonce (77) stated that no change in the potassium ion concentration results during reactive hyperemia but he offered no data.

The present study demonstrates that, during muscular exercise, the K^+ of the venous effluent rises as do the H^+ concentration and the

rate of flow. During reactive dilation, however, only the flow and the H^+ rise. These observations, therefore, suggest that K^+ probably does play a role in the vasodilation of exercise hyperemia but does not seem to be related to reactive vasodilation.

Since the pH of venous blood fell to the same degree during exercise and reactive dilation, and only during exercise did the K^+ increase (Fig. 7), it is suggested that the release of K^+ during active hyperemia is not related to the hydrogen ion concentration.

When the resting isolated hindlimb was perfused with extremely hypoxic blood, resistance fell but the potassium in the venous effluent did not increase. This experiment indicates that the decrease in resistance observed with severely hypoxemic blood is not mediated through K^+ release. It is possible that, in other vascular beds, lack of O_2 and K^+ release may be related. Haddy and associates (personal communication) have observed a rise in renal venous plasma K^+ above that in arterial plasma in approximately 50% of the cases in which the kidney was perfused with deoxygenated blood.

Magnesium

When the isolated, autoperfused hindlimb of the dog was used, the Mg^{++} concentration of the venous blood rose significantly during skeletal muscle exercise. However, when the isolated gastrocnemius muscle was perfused with cell-free fluids (plasma or modified Dextran), no change in Mg^{++} concentration could be demonstrated during any of the experimental maneuvers. There are two possible explanations for the observed findings. It is possible that Mg^{++} may be liberated from the dog red blood cell during muscular exercise, as the dog red cell contains

an estimated 3.7 mEq/L Mg^{++}. Next is the difference in the preparations themselves. The hindlimb is isolated by surgically severing large muscle groups and ligating bleeding points. In this process obviously many muscle cells are disrupted and injured. The dog skeletal muscle cell contains approximately 18 mEq/L Mg^{++}. The gastrocnemius muscle is left intact, in situ, relatively uninjured. It is therefore conceivable that during exercise of the injured muscle some Mg^{++} may find its way into the perfusing blood and thus elevate its concentration above that of the resting control. The fact that good active and reactive dilation were observed when perfusion was effected with cell-free perfusate indicates that the magnesium ion is not actually necessary for the vasodilation of exercise and reactive dilation. Conversely, the possibility that Mg^{++} may rise during exercise hyperemia suggests that this ion may play an active, although minor, role in the vasodilation of skeletal muscle activity.

Adenosine Triphosphate

The inability to demonstrate ATP at rest and during active and reactive dilation supports Hilton's (39) observations for the pump-perfused skeletal muscle and Berne and associates' (7) report on ischemic skeletal muscle. Jacob and Berne (44, 45) and Berne (6) were also unable to show the presence of ATP in the venous effluent of the coronary circulation during cardiac anoxia and in the normally oxygenated heart.

There are at least three possible explanations for the failure to find ATP in the present experiments. First, it is possible that there is no free ATP in plasma during any of the experimental maneuvers. Second, the analytical method used may not be sufficiently sensitive to detect the presence of ATP. Further, ATP may be rapidly degraded in the tissue cells,

and the by-products released, although retaining vasodilator properties, cannot be measured as ATP by the method employed.

Oxygen

This study indicates that local changes in oxygen tension alone cannot adequately explain the local regulation of blood flow in the voluntary muscle vascular bed.

A reduction in P_{O_2} to 18 mm Hg by active exercise of the hind-limb muscles resulted in a perfusion pressure of 66 mm Hg. Perfusion pressure fell, however, only to 81-83 mm Hg by deoxygenating the blood to levels of 11-13 mm Hg P_{O_2}. Assuming that the venous P_{O_2} is a fair measure of tissue P_{O_2}, this 16 mm Hg-pressure differential must be ascribed to some mechanism other than O_2 lack. The perfusing blood pH was kept constant by the addition of 5% CO_2 to all gas mixtures employed.

The obtained results confirm the observation reported by Ross et al. (61) that perfusion of the gastrocnemius muscle with hypoxic blood produces an increase in flow not as great as that of exercise hyperemia even though the P_{O_2} of the venous blood is the same during exercise and perfusion with hypoxic blood.

At least three factors might contribute to the greater fall in resistance observed during muscular activity. It has been shown in the preceding sections that the concentrations of K^+, Mg^{++} and H^+ increase during exercise, but K^+ and H^+ do not rise above control levels when perfusion is accomplished with deoxygenated blood.

The oxygen study also demonstrates that the vascular bed employed retains the ability to autoregulate in the virtual absence of oxygen from the perfusing blood. The response to a sudden increase in flow from 42

ml/min to 160 ml/min causes the vascular bed to gradually constrict and thus elevates resistance. During perfusion with the deoxygenated blood, this response, although definitely present, is sluggish and requires more time to reach a steady state level than it does when the blood is normally oxygenated. This observation does not tend to agree with that of Fairchild et al. (20) who reported that the vasodilated bed of the dog hindlimb failed to autoregulate during reactive hyperemia (blood flow increases of 400%) as long as the perfusing blood remained completely deoxygenated. It is possible that the failure of the hyperemic flow to return toward control levels resulted from a rate of flow which was insufficient to dilute or wash out the vasodilator substances being produced in the anoxic preparation. Anoxia per se may stimulate the production of vasodilator metabolites; and if the rate of production exceeds the rate of removal (rate of blood flow), the concentration of the metabolites may become sufficiently elevated to maintain maximal vasodilation for as long as the rate of flow remains low. In the present study, the rate of flow was apparently high enough to washout the vasodilator substances. Autoregulatory response, therefore, was observed.

Other vasoactive chemicals not investigated in this study, but which, nevertheless, are known to occur naturally in the living organism, may also actively participate in the local regulation of vascular resistance.

CHAPTER V

SUMMARY AND CONCLUSIONS

Several theories have been advanced to explain the mechanism of local regulation of blood flow. This study was carried out to test the chemical hypothesis and to elucidate the role that certain chemicals may play in the local regulation of vascular resistance.

Most of the experiments were performed on the surgically isolated, collateral-free, hindlimb of the dog. A few experiments were done on the isolated, pump-perfused gastrocnemius muscle of the dog utilizing homologous plasma or modified Dextran solution as the perfusing media. Reduction of the flow to metabolism ratio by either decreasing flow or increasing metabolism produced approximately the same changes in vascular resistance.

The K^+ concentration of venous effluent rose during exercise regardless of the type of perfusate used: blood, plasma or modified Dextran solution. But it did not change during the reactive dilation phase or during perfusion with totally deoxygenated blood. The Mg^{++} concentration rose during active dilation when the perfusate was whole blood, but remained unchanged throughout the experimental sequence during perfusion with cell-free perfusate.

ATP could not be demonstrated in venous effluent during any of the experimental maneuvers, regardless of the kind of perfusate used.

Under a specific experimental condition, skeletal muscle activity produced a greater fall in resistance than did perfusion with hypoxic blood, even though venous P_{O_2} was slightly higher during exercise.

Autoregulation in response to increased flow was observed during perfusion with totally deoxygenated blood.

These studies suggest that chemicals do play an active role in the local regulation of blood flow. During active hyperemia, O_2, H^+, K^+ and Mg^{++} seem to be involved; during reactive dilation, O_2 and H^+ but not K^+ or Mg^{++} appear to play an active role. These studies failed to provide evidence that ATP per se plays an active part in local regulation.

Other chemicals not investigated in this study may also be concerned with the local regulation of vascular resistance.

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