THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ACUTE LOCAL VASCULAR EFFECTS OF OXYGEN AND CARBON DIOXIDE

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

ROBERT M. DAUGHERTY, JR.

Oklahoma City, Oklahoma

1965

ACUTE LOCAL VASCULAR EFFECTS OF OXYGEN

AND CARBON DIOXIDE

APPROVED BY

DISSERTATION COMMITTEE

ACKNOWLEDGMENT

The author gratefully acknowledges the invaluable advice and encouragement of Dr. Francis J. Haddy in the accomplishment of this study. Any appreciable contribution that this study makes is due largely to his superb guidance.

The skillful assistance of Dr. J. B. Scott, Dr. J. M. Dabney, Mr. Sim Swindall, and Mr. Booker Swindall is also acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACUTE LOCAL VASCULAR EFFECTS OF OXYGEN AND CARBON DIOXIDE

CHAPTER I

INTRODUCTION

Resistance to blood flow is in part regulated locally by the tissues. This enables the tissue to vary its blood flow in proportion to its need for nutrients. The exact mechanism of local regulation of resistance has not been elucidated. The most plausible explanations which have been proposed are: 1) active vasomotion due to a change in transmural pressure (Bayliss response) (3) (22); 2) passive vasomotion due to a change in tissue pressure (33); 3) active vasomotion due to a change in concentration of vasodilating metabolites, including CO_2 and the adenyl compounds (5) (28) (29); and 4) active vasomotion due to a change in oxygen concentration (5) (10) (11) (28) (46) (47). There are a substantial number of studies concerning the possible role of the first two proposed mechanisms. The possible role of the last two mechanisms however, have not been adequately explored. For example, the local effects of hypoxia and hypercapnia on intact vascular beds have received very little attention. There do not appear to be any studies on the local effect of increasing the oxygen tension in an intact vascular bed.

Local Effect of Oxygen on Limb

The few studies available on the local effect of hypoxia in an intact limb vascular bed are not in complete agreement. Fleisch et al. (21) found that severe local hypoxia produced dilation in the perfused innervated hindlimb and intestine of the cat. The beds were perfused at constant pressure with venous blood which had been arterialized by pumping it through an artificial lung. Ventilation of the artificial lung with 100% nitrogen produced vasodilation. Fleisch et al. concluded that only severe local hypoxia would produce dilation and therefore it probably was not an important factor in local regulation.

Crawford et al. (11) noted a progressive increase in femoral artery blood flow at constant pressure while perfusing a dog's hindlimb with arterial blood containing increasing amounts of venous reservoir blood. As the venous blood concentration increased in the perfusate the oxygen saturation fell from 91% to 32% and associated with this was a 250% increase in femoral blood flow. Thus, they concluded that hypoxia is a powerful vasodilator.

In a similar study, Ross et al. (46) noted a progressive increase in hindlimb blood flow as the perfusing blood's oxygen saturation was decreased from 100% to 0%, while keeping P_{CO_2} constant. Intact and spinal anesthetized animals were studied. The hindlimb was perfused at a constant pressure with blood from the pulmonary vein of an isolated lung lobe which was ventilated with gases containing varying amounts of oxygen. The maximal increase in flow occurred during perfusion with blood having oxygen saturations of 0% and 10% in the intact and spinal anesthetized animals, respectively. In another study the same authors

suggested that the increase in flow following release of an arterial occlusion (reactive hyperemia) occurs because of hypoxia during the period of occlusion (47). When the hindlimb was perfused with 100% oxygenated blood, the flow upon release transiently increased to approximately four times the control level. However when the limb perfusate contained no oxygen, the flow increased upon release of the occlusion but did not return to the pre-occlusion level. In still another investigation, Carrier et al. (10) noted that the resistance to blood flow through isolated arterial segments decreased as a function of the oxygen tension of the perfusing blood over the range 100 to 30 mm Hg. Thus Crawford et al., Ross et al., and Carrier et al. not only concluded that O_2 lack is a powerful dilator but also that it is important in local regulation of blood flow.

On the other hand, Molnar et al. (40) could demonstrate little or no effect on dog forelimb resistance upon reducing the perfused blood's oxygen content from 14.6 vol. % to 10.5 vol. %. However, they did observe a vasodilating property of venous blood relative to arterial blood. They concluded that the vasodilatory activity of venous blood was independent of its oxygen content.

In an earlier study by the present author (12), it was demonstrated that a generalized fall in arterial P_{O_2} from 96 to 30 mm Hg produced a small fall in total forelimb resistance due to a lowering of small vessel resistance. The dog was ventilated with an 8% oxygen in nitrogen mixture while perfusing the forelimb at constant flow with arterial blood. The effect of the sympathico-adrenal system on the forelimb had been surgically removed.

Local Effect of Carbon Dioxide on Limb

There have been many studies on the effects of a generalized increase in CO_2 on the intact animal (8) (9) (13) (19) (35) (37) (38) (45) (51) (52). However, as with hypoxia, there are few studies on the clearly local effect of hypercapnia on a peripheral vascular bed. Fleisch et al. (21) noted a 30 to 100% increase in hindlimb and intestinal blood flow when ventilating the artificial lung with 2-10% carbon dioxide in air. Upon hypercapnic ventilation there was usually an initial transient constriction followed by dilation. However, they state that frequently only dilation occurred during the high CO_2 ventilation. The increase in flow was not related to the concentration of carbon dioxide in the mixture. The authors concluded that carbon dioxide was an important factor in local regulation of blood flow.

Molnar et al. (40) found that a local reduction in CO_2 content from 43.7 to 27.9 vol. % associated with a rise in pH from 7.21 to 7.50 produced only an irregular and insignificant rise in forelimb resistance. Switching from oxygenated venous blood to arterial blood, differing in pH by only 0.04 pH units, produced a significant rise in resistance. Thus they concluded that the vasodilator property of venous blood is not related to carbon dioxide. They also concluded that the local effect of carbon dioxide on the limb vascular bed is quite minimal.

In an attempt to examine the local effect of hypercapnia on forelimb vascular resistance, Fleishman et al. (20) administered CO_2 systemically after forelimb nerve block in conjunction with a local infusion of phentolamine. They found that ventilation with 20% CO_2 - 80% O_2 following hyperventilation returned forelimb resistance to its

control value. This finding was in a constricted limb subsequent to hyperventilation with an arterial pH of 7.64. The CO_2 lowered the pH to 7.07. They concluded that increasing the pH by decreasing CO_2 produced a significant effect. However, the effect of acidosis produced by increasing the CO_2 was minimal.

Crawford et al. (11) indicate that they used spinal anesthetized dogs to study the local effect of hypercapnia on the hindlimb. While no data was presented, they state that no significant or lasting change in femoral artery blood flow was produced upon ventilation with a 20% CO_2 mixture. Thus they concluded that CO_2 has no effect on the limb vasculature. However, the limb was not truly localized from the effects of systemically released catecholamines.

Litwin et al. (36) attempted to study the direct effect of CO_2 by administering a 10% CO_2 in air mixture to dogs following bilateral adrenalectomy and sympathetic blockade with bretylium bromide. They noted a 9% fall in hindlimb vascular resistance at constant flow upon hypercapnic ventilation. Vasodilation of the hindlimb also occurred upon CO_2 ventilation during an infusion of levarterenol subsequent to hexamethonium blockade. Only minimal dilation was noted in either preparation.

The present author in the previous study (12) also concluded that the local effect of CO_2 on the limb vascular bed is minimal. Ventilation of the animal with 20% CO_2 - 20% O_2 - 60% N_2 caused the blood pH to fall from 7.43 to 7.16 and produced only a 10% fall in forelimb resistance. However, the vasculature of the limb was partially dilated prior to hypercapnia due to local denervation and absence of the sympathico-adrenal system.

The local effect of CO_2 on the limb could be mediated through the concomitant change in hydrogen ion concentration. Deal and Green (15) noted a marked decrease in resistance in the bed supplied by the femoral and saphenous arteries upon locally lowering the blood pH with buffer solutions. However, the complicated buffer solutions and changes in tonicity make this study difficult to interpret. Molnar et al. (41) noted an appreciable dilation in the forelimb upon infusion of mineral acids. The drop in pH was not associated with changes in tonicity. The report of Deal and Green contains a review of other studies on the local effects of mineral acids.

Local Effect of Oxygen on Heart

In contrast to the lack of agreement concerning the effects of hypoxia on the limb, there is agreement with respect to the effect of hypoxia on the coronary vasculature. Hypoxia is considered to be a potent coronary dilator. In 1924, Hilton and Eichholtz (32) concluded that the reduced oxygen tension of hypoxia acted directly on the coronary vessel wall to produce relaxation. Blood in a Starling reservoir was replaced with blood deoxygenated by exposure to a vacuum. Perfusion with the deoxygenated blood, while ventilating with 100% N_2, reduced the oxygen saturation to near zero and produced a 5 fold increase in coronary blood flow. Ventilation with air increased the oxygen saturation to 99% and returned coronary flow to its control value. They concluded that coronary vascular dilation is inversely proportional to the oxygen saturation in the perfusing blood.

In a similar preparation Gremels and Starling (24) noted a progressive increase in coronary blood flow during a progressive

decrease in oxygen saturation from 93% to 8.5%. Utilizing the dog heart-lung preparation, they determined coronary flow by measuring coronary sinus outflow during ventilation with 5% O_2 in nitrogen and 100% N_2 mixtures. Associated with the fall in oxygen saturation was a small increase in the blood pH from 7.93 to 7.98. When the oxygen saturation fell below 40% the heart dilated. However, a large increase in coronary flow occurred prior to any change in heart volume. Dilation of the heart continued to increase as the oxygen saturation was decreased below 40%. Associated with the dilation was a decrease in cardiac output, an elevation of venous pressure, and a fall in arterial pressure. Thus myocardial strength began to decrease as the oxygen saturation was decreased below 40%. This was most marked at oxygen saturations below 10%.

Berne et al. (4) noted a large increase in coronary blood flow when coronary arterial oxygen content was reduced to at least half of its control value. Femoral arterial blood was deoxygenated by passing it through an isolated lung intermittently inflated with 5% CO_2 - 95% N_2. The deoxygenated blood was pumped into a reservoir and then into the left coronary artery at constant pressure. The animal was ventilated with ambient air throughout the experiment. Reducing the arterial oxygen content from 23.1 to 8.9 vol. % produced an increase in coronary flow and reduced the coronary sinus oxygen content from 12.5 to 2.2 vol. %. Coronary blood flow increased only when coronary sinus oxygen levels fell below 5.5 vol. %. The increase in flow was unrelated to the arterial oxygen content. Thus they concluded that the critical factor responsible for hypoxic coronary dilation is myocardial P_{O_2}, as reflected by the coronary sinus P_{O_2}.

Guz et al. (25) also suggested that the vasodilator response to hypoxia is secondary to a low tissue oxygen content. They decreased the oxygen capacity of hemoglobin perfusing an isolated rabbit heart by adding increasing amounts of Ringer-Locke solution. The coronary resistance fell despite a constant P_{O_2} at the arteriolar level as the hemoglobin concentration and therefore, oxygen content was reduced. They felt that the P_{O_2} of the perfusate did not fall until it had passed the site of principal coronary vascular resistance (arterioles) and reached the point where gas exchange occurs. Therefore, they concluded that the vasodilation is secondary to tissue hypoxia. They also measured the ventricular fiber tension generated per beat with a Walton strain gauge sutured to the wall of the left ventricle. The ventricular tension remained unchanged during hypoxia until the oxygen capacity of the coronary sinus blood fell below 2 vol. %. When the oxygen capacity was reduced below 2 vol. % the ventricular tension fell. The coronary blood flow was increased prior to the fall in force. Thus they concluded that the reduction in oxygen capacity had a direct effect on the coronary vasculature.

Bacaner et al. (2) monitored the left ventricular resting and contractile tension with a force gauge connected to the apex of an isolated heart. These investigators concluded that the contractile tension developed at any resting tension is proportional to the oxygen delivery to the myocardium over a wide range of variations in coronary blood flow and P_{O_2}.

Ebert et al. (16) used two separate extra-corporeal circuits to examine the direct effect of hypoxia on heart rate and myocardial

strength. While maintaining the systemic circulation under normal oxygenation, they noted a moderate fall in the strength of contraction after 14 minutes of perfusing the heart with blood that was 70% saturated with oxygen. The CO_2 of the perfusate was maintained constant. The oxygen content of the perfusate was controlled by gas mixtures entering an oxygenator through which blood from the aorta was routed into the left atrium.

Hackel and Clowes (26) attempted to study the local effect of hypoxia on coronary blood flow in the intact heart. They subjected dogs to 10% O_2 mixtures following bilateral adrenalectomy and thoracic sympathectomy while measuring coronary blood flow by the nitrous oxide desaturation technique. In the adrenalectomized sympathectomized as well as the intact control dogs, coronary blood flow increased during hypoxia. The increase in flow was not accompanied by tachycardia and a rise in aortic pressure in the former as it was in the intact dogs.

Since hypoxia consistently produces coronary vasodilation it might be expected that increasing the oxygen supply to the heart would produce constriction. However, the author is not aware of any studies on the vascular effects of locally increasing the oxygen tension in a bed. There have been many studies on the effects of a generalized increase as well as a decrease in oxygen concentration on coronary resistance and myocardial strength in the intact animal (5) (6) (13) (14) (18) (19) (31) (34) (35) (44). In addition, as will be pointed out in the discussion of the present report, a few of the studies described above failed to maintain a constant CO_2 concentration

while changing oxygen concentration. Also the effect of the sympathico-adrenal system on the heart was not completely removed in all studies.

Local Effect of Carbon Dioxide on Heart

The few studies on the local effect of carbon dioxide on coronary blood flow are not in complete agreement. Hilton and Eichholtz (32) and Gremels and Starling (24) ventilated the dog heart-lung preparation with increasing concentrations of CO_2 and noted an increase in coronary blood flow. Hilton and Eichholtz also lowered the pH of the perfusate with lactic acid and carbonic acid which produced a similar increase in flow. Thus they concluded that the effect of CO_2 on the coronary vasculature was via the associated change in hydrogen ion concentration.

In a truly local study, Nahas and Cavert (42) noted a fall in heart rate and cardiac output in a Starling heart-lung preparation upon switching from 5% CO_2 - 95% O_2 to a 10% CO_2 - 90% O_2 mixture. The inotropic and chronotropic activity progressively decreased as the per cent of CO_2 in the mixture was increased to 15, 20, and 30. Coronary sinus blood flow remained at or slightly above the control level until pH reached 7.25. When the pH fell below 7.25 a fall in perfusion pressure accompanied the fall in cardiac output and the coronary flow was reduced. In another *in vitro* study, McElroy *et al.* (39) produced an increase in coronary blood flow by increasing the P_{CO_2} of a balanced ion solution perfusing an isolated guinea pig heart. Associated with the increase in flow was a decrease in contraction amplitude and heart rate. A decrease in P_{CO_2} produced the reverse effects.

In an attempt to elucidate the direct effect of hypercapnia on myocardial contractile force and frequency, Greenfield and Ebert (23) have described a completely extrinsically cardiac denervated preparation. Ventilation with a 20% O_2 - 6% CO_2 in nitrogen mixture produced a fall in force and heart rate. However, it is doubtful if this represented a truly local effect because hypoxia produced an increase in force and rate, suggesting a sympathico-adrenal discharge.

The local effect of CO_2 on coronary resistance and myocardial strength could be mediated through the concomitant change in hydrogen ion concentration. When McElroy et al. (39) maintained the pH constant and changed only the P_{CO_2}, coronary flow was unaltered. Therefore, the authors concluded that the direct effects of CO_2 on coronary flow, heart rate and contraction amplitude are related to the pH change rather than the P_{CO_2} per se.

Local Effect of ATP and Adenosine on Heart

There have been several studies on the local effect of adenosine and ATP on the coronary vasculature (5). However, the author is not aware of any studies on the effect of adenosine or ATP on myocardial strength. Both chemicals are potent coronary dilators and have been proposed as candidates for a role in local regulation of blood flow.

Local Effect of Oxygen and Carbon Dioxide on Kidney

In contrast to the multiplicity of studies attempting to investigate the local effects of oxygen and carbon dioxide on the vasculature of the coronary and skeletal muscle, there is a paucity of studies on the renal vasculature. Emanuel et al. (17) investigated the effects of

acute variation of hydrogen ion concentration upon renal vascular resistance over both the acid and alkaline sides of pH 7.4. The kidney was isolated from the action of the sympathico-adrenal system by local denervation in conjunction with a local infusion of phentolamine. A generalized increase in hydrogen ion concentration by ventilation of the animal with 20% CO_2 - 80% O_2 had no effect on renal vascular resistance. Switching to air hyperventilation shifted the pH from 7.0 to 7.6 and produced a significant rise in resistance. There apparently have been no studies on the effect of a local lowering or elevation of O_2 to kidney.

In the present study an attempt was made to elucidate the effects of <u>local</u> changes in the concentrations of oxygen while holding CO_2 and pH constant and of CO_2 while holding O_2 constant on the intact coronary, renal, and forelimb vascular bed of the dog. The intact vascular bed was perfused at a constant rate with blood whose O_2 or CO_2 concentrations had been changed. This was accomplished by passing blood through an isolated lung which was ventilated with various CO_2-O_2-N_2 mixtures. The animal was maintained on ambient air ventilation. Thus the truly local individual effect of changes in O_2 or CO_2 concentrations could be studied in an intact bed without a sympathico-adrenal response.

CHAPTER II

METHODS

In the present study, the local effects of acute changes in the concentrations of oxygen and carbon dioxide on forelimb coronary and renal vascular resistance and left ventricular contractile force were studied in 50 anesthetized dogs. This was accomplished by passing the dog's femoral artery or vein blood through an isolated lung from another dog and perfusing it at a constant rate into the brachial, renal or left common coronary artery while measuring perfusion pressure. The oxygen or carbon dioxide tension of the perfusate was varied while maintaining one constant by ventilating the isolated lung with various mixtures of carbon dioxide, oxygen and nitrogen. All dogs were anesthetized intravenously with 30 mgm/Kgm of sodium pentobarbital and ventilated with a mechanical respirator (Harvard Apparatus Co., model 607) via an intratracheal tube.

Limb Perfused with Arterial Blood

In 11 animals, the effects of local changes in concentrations of O_2 and CO_2 on forelimb vascular resistance were studied. While lying on the right side, the skin of the dog's right forelimb was circumferentially sectioned about 3-5 cm above the elbow. At this level the right brachial artery, forelimb nerves, as well as the cephalic and brachial

vein, were dissected free. The remaining muscle and connective tissue at the elbow was encircled with three heavy cord ligatures, forcing all blood, with the exception of bone flow, to enter the limb through the brachial artery and to exit via the brachial or cephalic vein.

Following an intravenous injection of 4 mgm/Kg of heparin, an extracorporeal lung-perfusion circuit, free of reservoirs, was established between the right femoral artery and the right brachial artery (Figure 1). Femoral artery blood flowed into the pulmonary artery of an isolated right lung removed from another dog. An adjustable screw clamp was placed on the circuit between the femoral and pulmonary artery in order to maintain a near normal pulmonary artery pressure. The pulmonary venous effluent flowed through a cannula tied in the partially preserved left atrium past an oxygen tension probe (oxygen macroelectrode, Beckman Instruments, Inc., Spinco Division, Palo Alto, California) which was secured in the tubing. The flowing blood was then pumped (Sigmamotor pump, model T-6SH, Sigmamotor Inc., Middleport, N.Y.) at a constant rate into the brachial artery. Blood flow through the pump perfused limb was adjusted to a value which produced a perfusion pressure approximately equal to aortic pressure. However, flow was constant throughout any given experiment. A catheter was manipulated up the proximal segment of the brachial artery into the aorta for the measurement of aortic pressure. Aortic and brachial perfusion pressures as well as pulmonary artery and vein pressures were monitored continuously on a direct writing oscillograph by attachment of the catheters to 0-75 cm Hg resistance wire pressure transducers. The pulmonary artery and vein pressures were maintained similar to their

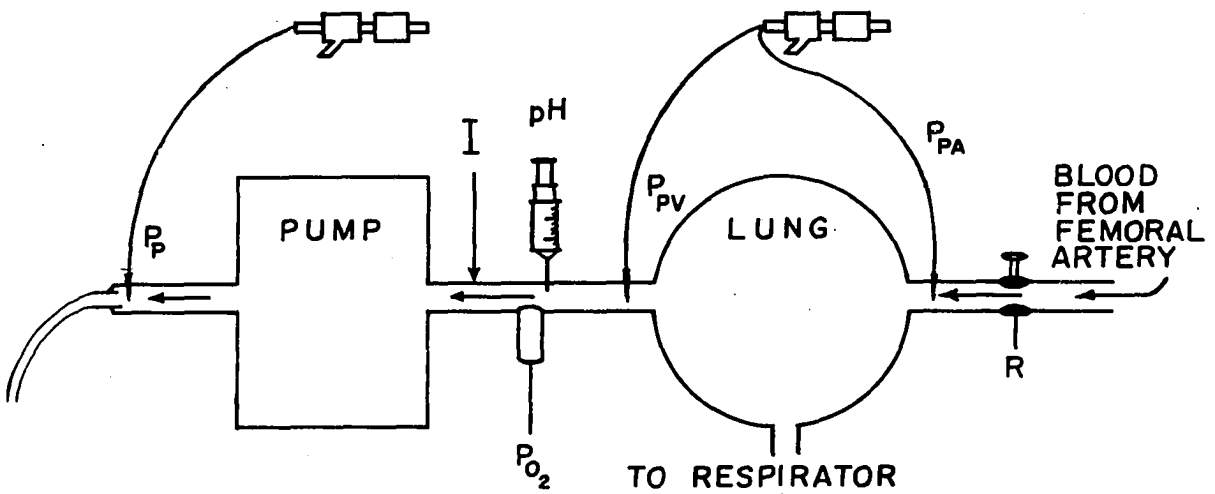

Figure 1. Schematic drawing of the extracorporeal circuit. R = adjustable resistance, P_{pA} = pulmonary artery pressure, P_{pV} = pulmonary vein pressure, P_{O_2} = oxygen tension (measured with a continuously recording probe), pH (site of sampling), I = injection site for adenyl compounds, P_p = perfusion pressure.

usual *in vivo* values by varying the adjustable screw clamp resistance. The isolated lung was ventilated with a second mechanical respirator via the main stem bronchus.

A standard experimental sequence was followed in each of 10 experiments. The isolated lung was ventilated with the mechanical respirator with air at a frequency and volume which produced a P_{O_2} and pH in the perfusing blood as near normal as possible. Without altering frequency or volume, in five dogs the lung was then ventilated sequentially with 100% N_2, air, 20% CO_2 - 20% O_2 - 60% N_2 and finally air again. In five separate animals, the ventilation sequence following the initial air control period was 100% N_2, 100% O_2, air, 20% CO_2 - 20% O_2 - 60% N_2, hyperventilation with air by increasing the frequency of the respirator and then finally ventilation with air at the original frequency and stroke. The isolated lung was ventilated with the gas mixtures by attaching a 100 liter Douglas bag filled with the appropriate mixture to the inlet of the respirator. Each ventilation period was terminated when the perfusion pressure, P_{O_2} and pH were stable.

The pH and P_{O_2} of the perfusate was measured throughout each experiment. The P_{O2} of the blood was continuously measured by the oxygen electrode on a gas analyzer (Beckman model 160 physiological gas analyzer) which was monitored on the direct writing oscillograph. Blood was sampled between the isolated lung and the pump at the end of each ventilation period for the measurement of pH (Beckman model 76 expanded scale pH meter with constant temperature block). The gas analyzer was calibrated at 37° C by bubbling two known oxygen in nitrogen mixtures through distilled water.

Heart Perfused with Arterial Blood

In 12 animals the effects of local changes in concentrations of O_2, CO_2, ATP, and adenosine and of ischemia on coronary vascular resistance and left ventricular contractile force were examined. The left common coronary artery was perfused with blood via the isolated lung. The isolated lung was prepared as described above. The heart was exposed through the third left intercostal space and a suture was passed around the left common coronary artery. Following heparinization the pulmonary venous effluent was pumped at a constant rate through a curved metal cannula inserted into the aorta via the left subclavian artery. Without stopping the pump, the cannula was manipulated down the ascending aorta into the mouth of the left common coronary artery and tied into position with the previously placed suture. Blood flow was adjusted to a value which gave a perfusion pressure similar to aortic pressure. Left ventricular contractile force was measured with a 120 ohm strain gauge arch (James L. Butterworth, P.O. Box 412, Charleston, S.C.) sutured to the surface of the left ventricle. Following an air control period, the lung was ventilated sequentially with 100% N_2, 100% O_2, air, and 20% CO_2 - 20% O_2 - 60% N_2, hyperventilation with air and finally with air at the original stroke and frequency. Coronary perfusion pressure, contractile force and P_{O2} of the perfusing blood were monitored continuously. Samples of the perfusate were taken at the end of each period for the measurement of pH. Upon completion of the above sequence, ATP 20 µg in 1 ml of saline and adenosine, 20 µg in 1 ml of saline were separately injected into the circuit just upstream to the perfusion pump. When the continuously monitored parameters were stable, myocardial

ischemia was produced by shutting off the blood pump for 15 seconds.

Kidney Perfused with Arterial Blood

In six additional dogs the effects of local changes in concentrations of O_2 and CO_2 on renal vascular resistance were examined. The isolated lung was prepared as previously described with the pulmonary venous effluent pumped into the left renal artery. The left kidney was exposed through a flank incision. After the administration of heparin the pump cannula was tied in the renal artery for perfusion at a constant rate. Flow was adjusted to produce a perfusion pressure similar to aortic pressure. Following an air control period the isolated lung was ventilated with 100% N_2, 100% O_2, air, 20% CO_2 - 20% O_2 - 60% N_2, air at an increased frequency (hyperventilation) and then air at the normal stroke and frequency. Perfusion pressure and P_{O_2} were continuously recorded and pH was measured at the end of each ventilation period.

In the 29 experiments described above, the perfusing blood was slightly alkalotic. Also it became evident that it was not possible to completely deoxygenate the blood because the ventilation system was not airtight. Therefore, a second group of experiments was performed using femoral venous blood as the perfusate. The pH was maintained at a more normal level during the control and hypoxic periods by adding 5% CO_2 to the gas mixtures. In addition, the Douglas bag method of ventilation was replaced by one which delivered gas to the ventilator at a slight positive pressure. The gas mixture was passed through a rubber tube directly from the compressed gas mixture tank to the respirator. A small rubber basketball innertube reservoir was

interposed in the tubing between the tank and the respirator in order to help maintain a constant gas flow. Femoral vein blood was pumped into the pulmonary artery of the isolated lung with a second pump. The remainder of the preparation was the same as described previously when femoral artery blood was used as the perfusate. Pulmonary arterial and venous pressures were maintained near normal by varying the speed at which the second pump pumped venous blood into the lung.

Limb Perfused with Venous Blood

In 7 animals the effects of hypoxia, hypocapnia and unaltered venous blood on forelimb vascular resistance were studied. Femoral vein blood was pumped through the lung which was ventilated sequentially with air, 5% CO_2 - 20% O_2 - 75% N_2, 5% CO_2 - 95% N_2, 5% CO_2 - 20% O_2 - 75% N_2, air, 5% CO_2 - 20% O_2 - 75% N_2, and finally with venous blood which was pumped through the circuit with the respirator off. Brachial perfusion pressure and P_{O_2} of the perfusing blood were continuously measured while pH was determined from a sample drawn from the perfusate at the end of each period. Simultaneous samples of blood were drawn downstream to the lung and from the brachial vein of the perfused limb at the end of the period of ventilation with 5% CO_2 - 20% O_2 - 75% N_2, 5% CO_2 - 95% N_2 and air for the measurement of oxygen content by the Van Slyke manometric method. The oxygen content of the venous blood during the period the respirator was shut off was also determined in 5 animals.

Heart Perfused with Venous Blood

In 6 animals the effects of local hypoxia, hypocapnia and

venous blood on coronary vascular resistance and ventricular contractile force were examined. The left common coronary artery was perfused at constant rate with oxygenated femoral venous blood as described above. The lung was sequentially ventilated with 5% CO_2 - 20% O_2 - 75% N_2, 5% CO_2- 95% N_2, 5% CO_2 - 20% O_2 - 75% N_2, air, and 5% CO_2 - 20% O_2 - 75% N_2. The respirator was then turned off and the coronary artery perfused with normal venous blood. Coronary perfusion pressure, left ventricular contractile force and P_{O_2} were continuously measured. Samples were drawn downstream to the lung at the end of each period for pH determinations. Samples of perfusate for measurement of oxygen content were obtained during ventilation with 5% CO_2 - 20% O_2 - 75% N_2 and 5% CO_2 - 95% N_2.

Kidney Perfused with Venous Blood

In 8 animals the effects of hypoxia, hypocapnia and venous blood on renal vascular resistance were studied. Femoral vein blood was pumped through the extracorporeal circuit into the renal artery at a constant rate as previously described. The isolated lung was sequentially ventilated with air, 5% CO_2 - 20% O_2 - 75% N_2, 5% CO_2 - 95% N_2, 5% CO_2 - 20% O_2 - 75% N_2 and air. This was followed by ventilation with 5% CO_2 - 20% O_2 - 75% N_2 and then the respirator was turned off. Renal artery perfusion pressure and P_{O_2} of the perfusing blood were continuously monitored while pH was measured from a sample of the perfusate obtained at the end of each period. The oxygen content of the perfusing blood was determined from a sample of the perfusate obtained at the end of the 5% CO_2 - 20% O_2 - 75% N_2, 5% CO_2 - 95% N_2 and respirator off periods.

When femoral vein blood was used as the perfusate in the latter 3 preparations the isolated lung was ventilated at a large stroke (500-600/min) and a fast frequency (20-25/min) during the entire experiment in order to achieve maximum changes in O_2 and CO_2 concentrations. Since blood flow to the vascular bed under study was constant in all cases, changes in perfusion pressure reflected a change in resistance.

The significance of the difference between values during control and test periods was evaluated by the Wilcoxon method of determining significance of difference between means of paired replicates.

CHAPTER III

RESULTS

Local Effect of O_2 and CO_2 on Limb

The effects of moderate local hypoxia and local hypercapnia on the forelimb during perfusion with alkalotic arterial blood are shown in Tables 1 and 2. Under these conditions, hypoxia and hypercapnia produced a slight fall in total forelimb resistance. Table 1 presents the average systemic and brachial artery perfusion pressure and the P_{O_2} and pH of the perfusing blood in 5 dogs during control, hypoxic, and hypercapnic ventilation of the isolated lung. By the sixth minute of ventilation with 100% N_2, the average P_{O_2} of the perfusing blood had fallen 74 mm Hg and this was associated with a reduction in brachial perfusion pressure below the control value in 4 of 5 preparations. The pH of the perfusate remained essentially unchanged. As would be expected with local changes, systemic arterial pressure was not affected. Replacement of the 100% N_2 with air caused the brachial perfusion pressure and P_{O_2} to return to their control values. Table 2 also shows that the effect of moderate local hypoxia on the forelimb vascular bed is dilation. Table 2 presents the average systemic and brachial artery perfusion pressure and the P_{O_2} and pH of the perfusing blood in 5 animals during sequential ventilation with

TABLE 1

EFFECTS OF LOCAL CHANGES IN ARTERIAL BLOOD CONCENTRATIONS OF O_2 AND CO_2 ON FORELIMB VASCULAR RESISTANCE

		t	P_S	P_p	P_{O_2}	pH
		min.	mm Hg	mm Hg	mm Hg	
Control		0	128	94	113	7.56
100% N_2		6	125	89	39	7.61
Control		12	122	98	105	7.58
20% CO_2	T	13	122	96	113	
20% CO_2	S	20	123	89	113	7.16
Control		28	126	114	109	7.52

t = elapsed time in minutes from first control, P_S = systemic arterial pressure, P_p = perfusion pressure in brachial artery at constant flow, P_{O_2} = oxygen tension of perfusing blood, pH = hydrogen ion concentration of perfusing blood, T = transient, S = steady state, N = 5, 3 of 5 were subjected to 7% O_2 in N_2 mixture between control and 100% N_2. Average blood flow = 128 ml/min.

TABLE 2

EFFECTS OF LOCAL CHANGES IN ARTERIAL BLOOD CONCENTRATIONS OF O_2 AND CO_2 ON FORELIMB VASCULAR RESISTANCE

		t	P_S	P_p	P_{O_2}	pH
		min.	mm Hg	mm Hg	mm Hg	
Control		0	132	118	102	7.48
100% N_2		3.2	134	112	29	7.58
100% O_2		6.6	134	136	≃650	7.56
Control		13.2	134	133	107	7.58
20% CO_2	T	14.2	135	124	117	
20% CO_2	S	17.2	130	101	123	7.19
Hyperventi-lation	T	18.4	131	112	103	
Hyperventi-lation	S	22.0	135	160	103	7.61
Control		26.2	133	156	105	7.53

t = elapsed time in minutes from first control, P_S = systemic arterial pressure, P_p = perfusion pressure in brachial artery at constant flow, P_{O_2} = oxygen tension of perfusing blood, pH = hydrogen ion concentration of perfusing blood, T = transient, S = steady state, N = 5, Average blood flow = 128 ml/min.

100% N_2, 100% O_2, air, 20% CO_2 - 20% O_2 - 60% N_2, hyperventilation with air and air with the respirator at control frequency and stroke. Three and two tenths minutes of ventilation with 100% N_2 produced a 73 mm Hg fall in the average P_{O_2} of the perfusing blood and this was associated with a 6 mm Hg fall in the average brachial perfusion pressure. The average pH rose 0.10 pH unit. These directional changes in perfusion pressure, P_{O_2} and pH occurred in all 5 animals. During ventilation of the isolated lung with 100% N_2, the average P_{O_2} of the perfusing blood was reduced 74 mm Hg in the 10 animals from Tables 1 and 2 while brachial perfusion pressure fell 6 mm Hg ($P = <0.01$). It can also be seen that ventilation with 100% O_2 produced a large rise in P_{O_2} while the average perfusion pressure rose 24 mm Hg. However, perfusion pressure remained elevated in the post-control period.

In addition, Table 1 shows that during ventilation with 20% CO_2- 20% O_2 - 60% N_2 the average pH of the perfusing blood fell 0.42 units associated with a 9 mm Hg fall in the average brachial perfusion pressure. Perfusion pressure fell in 4 and rose in one animal. There was a small rise in perfusate P_{O_2} during hypercapnia in all dogs. Previous studies (12) (7) suggest that the rise in oxygen tension during hypercapnia results from a shift of the oxyhemoglobin dissociation curve to the right, thus releasing more oxygen from hemoglobin. Table 2 shows that a similar fall in pH during hypercapnia produced a 32 mm Hg fall in perfusion pressure. Part of the difference in response to hypercapnia in the 2 groups might be explained by the higher limb resistance in Table 2. The average fall in pH of the perfusing blood during hypercapnia in the 10 animals from Tables 1 and 2 was 0.41 units ($P = <0.01$) associated

with an average fall in perfusion pressure of 20 mm Hg ($P = <0.01$). Table 2 also shows that during hyperventilation the pH rose to its pre-hypercapnia level and the average brachial perfusion pressure increased 48 mm Hg. This directional change in perfusion pressure occurred in 4 of the 5 preparations. However, the perfusion pressure did not return to the control level during the post-hyperventilation control period.

Table 3 shows the effect of severe local hypoxia and local hypocapnia on the forelimb during perfusion with venous blood at normal arterial pH in the airtight system. Under these conditions hypoxia produced severe dilation and hypocapnia produced constriction. The average systemic and brachial perfusion pressure and P_{O_2}, pH and oxygen content of the perfusing blood and oxygen content of the brachial vein blood during sequential ventilation of the isolated lung with the gas mixtures in 7 dogs is presented in Table 3. The isolated lung was ventilated at a stroke volume of 500-600 cc and a frequency of 20 strokes per minute. Upon switching from air to ventilation with the control mixture of 5% CO_2 - 20% O_2 - 75% N_2, a 0.53 unit fall ($P = <0.01$) in pH of the perfusing blood produced a 30 mm Hg fall in brachial perfusion pressure ($P = <0.01$). The P_{O_2} of the perfusate was unaltered. The simultaneous control oxygen content of the perfusing blood and brachial venous effluent was 16.0 and 14.7 vol. %, respectively. A fall in the P_{O_2} of the perfusing blood to 2 mm Hg and in oxygen content to 0.9 vol. % produced a 34 mm Hg fall in brachial perfusion pressure ($P = <0.01$). The pH of the perfusate remained essentially unchanged. Upon returning to the control 5% CO_2 - 20% O_2 - 75% N_2 mixture, the P_{O_2} as well as the perfusion pressure returned to the pre-hypoxic control levels. During

TABLE 3

EFFECTS OF LOCAL REDUCTION IN O_2 OR CO_2 CONCENTRATIONS OF ARTERIALIZED VENOUS BLOOD AND OF VENOUS BLOOD ON FORELIMB VASCULAR RESISTANCE

	t	P_S	P_p	P_{O_2}	pH	O_2 Content Lung	Limb
	min.	mm Hg	mm Hg	mm Hg		vol. %	
Air	0.0	103	130	117	7.88		
Control	4.7	105	100*	114	7.35*	16.0	14.7
Hypoxia	10.4	106	66*	2	7.37	0.9	0.8
Control	16.4	106	101*	112	7.35		
Air	23.6	99	176*	122	7.90	16.2	15.1
Control	27.3	97	99*	114	7.34		
Venous	33.3	95	85*	32	7.36	12.2	

t = elapsed time in minutes from first air ventilation, P_S = systemic arterial pressure, P_p = perfusion pressure in brachial artery at constant flow, P_{O_2} = oxygen tension of perfusing blood, pH = hydrogen ion concentration of perfusing blood, O_2 content, Lung = O_2 content of perfusing blood, O_2 content limb = O_2 content of brachial vein blood, Air = ventilation of isolated lung with ambient air, Control = ventilation of isolated lung with 5% CO_2 - 20% O_2 - 75% N_2, Hypoxia = ventilation of isolated lung with 5% CO_2 - 95% N_2, Venous = venous blood perfused into limb with respirator off, N = 7 for all parameters except P_{O_2}, N = 3, * P =<0.01 relative to preceding value, Average blood flow = 103 ml/min.

the succeeding 7.2 minutes of ambient air ventilation, the average pH of the perfusing blood rose 0.55 units ($P = <0.01$) and perfusion pressure rose 75 mm Hg ($P = <0.01$). There was a slight rise in perfusing blood P_{O_2} during the period of hypocapnia. The oxygen content of the perfusate and limb brachial vein was 16.15 vol. % and 15.05 vol. %, respectively. The pH of the perfusing blood and the perfusion pressure returned to their control values after 3.7 minutes of ventilation with the control (5% CO_2 - 20% O_2 - 75% N_2) mixture. When the respirator was turned off perfusion with the venous blood produced an 82 mm Hg fall in P_{O_2} of the perfusing blood ($P = <0.01$), associated with a 14 mm Hg fall in perfusion pressure ($P = <0.01$). The oxygen content of the venous blood was 12.19 vol. % while the pH did not change.

Local Effect of O_2 and CO_2 on Heart

Moderate hypoxia produced a small fall in coronary resistance but did not measurably affect ventricular contractile force. Hypercapnia in the steady state produced a reduction in resistance and force. The reverse changes were produced by lowering the CO_2. Table 4 shows the average systemic arterial pressure, left common coronary artery perfusion pressure, left ventricular contractile force, and P_{O_2} and pH of the perfusing blood in 11 dogs during sequential ventilation with the various gas mixtures. In addition, Table 4 shows the average systemic arterial pressure, coronary perfusion pressure and contractile force during intracoronary injections of ATP and adenosine and after local ischemia. Arterial blood was used as the perfusate. A 62 mm Hg fall in P_{O_2} ($P = <0.01$) during ventilation of 100% N_2 was associated with a 13 mm Hg fall ($P =< 0.01$) in coronary perfusion pressure. However, contractile force did not show a

TABLE 4

EFFECTS OF LOCAL CHANGES IN ARTERIAL BLOOD CONCENTRATIONS OF O_2, CO_2, ATP, AND ADENOSINE AND OF LOCAL ISCHEMIA ON LEFT CORONARY RESISTANCE AND CONTRACTILE FORCE

	t	P_S	P_p	F	P_{O_2}	pH
	min.	mm Hg	mm Hg	% of Control	mm Hg	
Control	0.0	101	107	100	92	7.64
100% N_2	2.0	101	94*	92	30*	7.67
100% O_2	6.0	101	105**	95	≈650**	7.65
Control	8.0	109	106	92	87	7.63
Control	0.0	106	112	100	89	7.65
20% CO_2 T	0.7	105	125*	90	91	
20% CO_2 S	3.0	101	95**	70*	107*	7.23*
Hyperventilation T	3.7	107	77**	88	93	
Hyperventilation S	7.0	102	101**	120*	76	7.80*
Control	10.0	97	116	93	76	7.66
Control	0.0	102	110	100		
ATP, 20 µg		102	77*	101		
Control	0.0	102	107	100		
Adenosine 20µg		102	81*	99		
Control	0.0	102	109	100		
Ischemia	0.3	102	72*	67		

t = elapsed time from control, P_S= systemic arterial pressure, P_p = perfusion pressure in left common coronary artery at constant flow, F = left ventricular contractile force, P_{O_2} = oxygen tension of perfusing blood, pH = hydrogen ion concentration of perfusing blood, n = 11 for N_2 - O_2 sequence, 9 for CO_2 - hyperventilation sequence, and 9 for ATP-adenosine-ischemia sequence, T = transient, S = steady state, Average blood flow = 123, 123, and 121 ml/min, respectively. *P = < 0.01 relative to the first control value and **P = < 0.01 relative to the preceding value.

regular change. Systemic pressure and the perfusing blood's pH were unaffected. Switching to 100% oxygen ventilation produced a large rise in perfusing blood P_{O_2} ($P = <0.01$) while coronary perfusion pressure rose 11 mm Hg to its control level. Ventricular contractile force and the pH of the perfusate were not changed by the high oxygen tension. Control air ventilation reduced the P_{O_2} to the control level while coronary perfusion pressure remained elevated.

Hypercapnia produced a 30% fall in contractile force ($P = <0.01$) but had a lesser effect on coronary perfusion pressure. The average pH of the perfusate fell 0.42 units ($P = <0.01$) upon ventilation with 20% CO_2 - 20% O_2 - 60% N_2. There was first a transient rise in perfusion pressure followed by a 17 mm Hg fall below the control value. Average perfusing blood P_{O_2} rose from 89 to 107 mm Hg ($P = <0.01$). Air hyperventilation raised the pH by 0.57 units ($P = <0.01$) and produced a further transient fall in perfusion pressure followed by an increase toward normal ($P = <0.01$ relative to hypercapnic value). However, in some experiments the perfusion pressure was still rising when the hyperventilation was terminated. Left ventricular contractile force rose to 20% above its initial control level ($P = <0.01$). The oxygen tension of the perfusing blood decreased 29 mm Hg from its hypercapnic level. Ventilation at a stroke and frequency similar to the control value lowered the perfusing blood's pH to 7.66 while brachial perfusion pressure continued to rise. The P_{O_2} was not changed. Ventricular contractile force fell 27% which was 93% of the control value.

Severe hypoxia produced a large fall in coronary resistance and ventricular contractile force. Table 5 presents the average systemic

TABLE 5

EFFECTS OF LOCAL REDUCTION IN O_2 OR CO_2 CONCENTRATIONS OF ARTERIALIZED VENOUS BLOOD AND OF VENOUS BLOOD ON LEFT CORONARY RESISTANCE AND CONTRACTILE FORCE

	t min	P_S mm Hg	P_P mm Hg	F % of control	P_{O_2} mm Hg	pH	O_2 content vol. %
Control	0.0	95	120	100	105	7.38	15.7
Hypoxia	2.0	62	63	70	8	7.42	0.4
Control	10.0	93	101	108	107	7.38	
Control	0.0	90	113	100	107	7.38	
Air	7.2	76	117	129	100	7.92	
Control	12.0	80	117		102		
Control		74	108	100	105		
Venous		71	76	74	23		

t = elapsed time in minutes from preceding control, P_S = systemic arterial pressure, P_P = perfusion pressure in left common coronary artery at constant flow, F = left ventricular contractile force, P_{O_2} = oxygen tension of perfusing blood, pH = hydrogen ion concentration of perfusing blood, O_2 content = O_2 content of perfusing blood, Control = ventilation of isolated lung with 5% CO_2 - 20% O_2 - 75% N_2, Hypoxia = ventilation of isolated lung with 5% CO_2 - 95% N_2, Air = ventilation of isolated lung with ambient air, Venous = venous blood perfused through lung with respirator off, N = 4, Average blood flow = 106 ml/min.

pressure, coronary artery perfusion pressure, left ventricular contractile force, and the P_{O_2}, pH and oxygen content of the perfusing blood during sequential ventilation with the gas mixtures and air in 4 animals. Venous blood was used as the perfusate in the airtight system. Two minutes after switching from the control 5% CO_2 - 20% O_2 - 75% N_2 mixture to 5% CO_2 - 95% N_2, the average P_{O_2} and oxygen content of the perfusing blood fell to 8 mm Hg and 0.4 vol. % respectively. This was associated with a 57 mm Hg fall in coronary perfusion pressure and a 30% fall in ventricular contractile force. Systemic arterial pressure fell 32 mm Hg, possibly because of the fall in ventricular force. The pH of the perfusate increased slightly in all experiments. During the eight minutes of post-hypoxic ventilation with the control mixture, all parameters returned to their control values. A 0.54 unit rise in the average pH of the perfusing blood upon switching from control to air ventilation was associated with a small rise in coronary perfusion pressure in 3 of the 4 preparations. Ventricular contractile force increased in all animals an average of 29%. Despite the increase in force, systemic pressure fell in all experiments an average of 14 mm Hg. A small decrease in oxygen tension occurred with the rise in pH. A return to the control mixture did not produce a regular change in systemic pressure, perfusion pressure or P_{O_2}. An 82 mm Hg fall in oxygen tension occurred when unaltered venous blood was perfused into the coronary artery. The fall in P_{O_2} was associated with a 32 mm Hg fall in perfusion pressures and a 26% fall in contractile force. Although coronary perfusion pressure was reduced in all 4 animals, the largest fall occurred in 3 in which the oxygen tension fell below 30 mm Hg.

Local Effect of ATP, Adenosine and Ischemia on Heart

Table 4 also shows that injections of ATP and adenosine into the coronary artery produced marked reductions in perfusion pressure but had no effect on contractile force. An injection of 20 μg of ATP or adenosine in 1cc of saline into the perfusion circuit upstream to the pump produced a rapid fall in perfusion pressure of 33 and 26 mm Hg, respectively ($P = <0.01$). Left ventricular contractile force was not altered by injection of the two adenyl compounds. However, a 15 second period of ischemia produced a 33% fall in force ($P = <0.01$). When flow was reinstituted by turning on the pump, perfusion pressure was 37 mm Hg below the control value ($P = <0.01$).

Local Effect of O_2 and CO_2 on Kidney

Moderate local hypoxia and local hypercapnia reduced renal vascular resistance. The reduction was very small in the case of hypoxia. Table 6 presents the average systemic arterial pressure, renal artery perfusion pressure and P_{O_2} and pH of the perfusing blood in 6 animals during ventilation with the various gas mixtures. The perfusate was arterial blood. A 70 mm Hg fall in the P_{O_2} of the perfusing blood during ventilation with 100% N_2 produced only a 9 mm Hg fall in average renal artery perfusion pressure. When the P_{O_2} of the perfusing blood was increased to approximately 650 mm Hg, the perfusion pressure rose well above the control value. However, when the P_{O_2} was returned to near control level by ventilation with air, the perfusion pressure continued to rise.

Reduction of pH from 7.57 to 7.16 by ventilation with 20% CO_2 -

TABLE 6

EFFECTS OF LOCAL CHANGES IN ARTERIAL BLOOD CONCENTRATIONS OF O_2 AND CO_2 ON RENAL VASCULAR RESISTANCE

		t	P_S	P_p	P_{O_2}	pH
		min	mm Hg	mm Hg	mm Hg	
Control		0.0	110	94	103	7.57
100% N_2		4.0	111	85*	33*	7.60
100% O_2		7.5	109	100**	≈650**	7.57
Control		12.6	108	108	93	7.57
20% CO_2	T	13.5	108	112	107	
20% CO_2	S	17.5	107	84**	122*	7.16*
Hyper-ventilation	T	18.3	107	90	104	
Hyper-ventilation	S	22.8	104	109	88**	7.72*
Control		26.3	106	135**	90	7.53

t = elapsed time in minutes from first control, P_S = systemic arterial pressure, P_p = perfusion pressure in renal artery at constant flow, P_{O_2} = oxygen tension of perfusing blood, pH = hydrogen ion concentration of perfusing blood, T = transient, S = steady state, N = 6, Average blood flow = 104 ml/min, *P = <0.01 relative to preceding control, **P = <0.01 relative to preceding value.

20% O_2 - 60% N_2 produced a small transient rise in perfusion pressure followed in the steady state by a 24 mm Hg decrease ($P = <0.01$). The P_{O_2} of the perfusate increased 15 mm Hg ($P = <0.01$). Elevation of the pH to 7.72 upon switching to air hyperventilation caused renal artery perfusion pressure to climb 27 mm Hg above the hypercapnic level. A 34 mm Hg fall in P_{O_2} ($P = <0.01$ relative to hypercapnic value) accompanied the elevation of pH. However, when pH was returned to the control value by ventilation at the control rate and stroke, perfusion pressure continued to rise.

A large fall in oxygen tension produced irregular changes in renal vascular resistance. Unaltered venous blood also did not produce a regular change in resistance. Table 7 presents the average systemic arterial pressure, renal artery perfusion pressure and P_{O_2}, pH and O_2 content of the perfusing blood in 8 animals during sequential ventilation with the gas mixtures utilizing the airtight system with venous blood as the perfusate. In addition, the effects of perfusing unaltered venous blood is presented. A reduction in average pH of the perfusing blood from 7.94 to 7.28 ($P = <0.01$) upon switching from air ventilation to the 5% CO_2 - 20% O_2 - 75% N_2 mixture caused a 41 mm Hg drop in average renal artery perfusion pressure ($P = <0.01$). P_{O_2} of the perfusate increased 14 mm Hg ($P = <0.01$) above the control value. Reduction of the P_{O_2} and O_2 content of the perfusate to 6 mm Hg and 0.7 vol. % respectively, ($P = <0.01$) by ventilation with 5% CO_2 - 95% N_2 did not produce a regular change in perfusion pressure. The perfusion pressure increased in 5 and decreased in 3 animals. Elevation of the P_{O_2} to near pre-hypoxic level by ventilation with the 5% CO_2 - 20% O_2 - 75% N_2 control

TABLE 7

EFFECTS OF LOCAL REDUCTION IN O_2 OR CO_2 CONCENTRATION OF ARTERIALIZED VENOUS BLOOD AND OF VENOUS BLOOD ON RENAL VASCULAR RESISTANCE

	P_S mm Hg	P_p mm Hg	P_{O_2} mm Hg	pH	O_2 Content vol. %
Air	90	151	116	7.94	
Control	91	110*	130*	7.28	13.5
Hypoxia	88	115	6*	7.31	0.7*
Control	87	124	123*	7.26	
Air	80	208*	113*	7.96*	11.0
Control	84	106	123		
Venous	82	100	26*		

P_S = systemic arterial pressure, P_p = perfusion pressure in renal artery at constant flow, P_{O_2} = oxygen tension of perfusing blood, pH = hydrogen ion concentration of perfusing blood, O_2 content of perfusing blood, Air = ventilation with ambient air, Control = ventilation with 20% O_2 - 5% CO_2 - 75% N_2, Hypoxia = ventilation with 5% CO_2 - 95% N_2, Venous = respirator off, *P = <0.01 relative to preceding value, N = 8 for air-control-hypoxia sequence, N = 6 for control-venous blood sequence.

mixture again produced no regular change in perfusion pressure. An elevation of pH from 7.26 to 7.96 ($P = <0.01$) produced an 84 mm Hg ($P = <0.01$) rise in renal artery perfusion pressure. A 10 mm Hg ($P = <0.01$) fall in perfusate P_{O_2} occurred during the period of hypocapnia. A 97 mm Hg ($P = <0.01$) fall in P_{O_2} of the perfusing blood produced by perfusion with the respirator off was associated with a varied response in perfusion pressure. There was no correlation between direction of response of perfusion pressure and the P_{O_2} of the perfusing blood.

CHAPTER IV

DISCUSSION

The following discussion will be divided into sections concerning the effect of altering either the oxygen or carbon dioxide concentration in the intact limb, heart and kidney. In addition a section covering the effects of the adenyl compounds and ischemia on the heart will be included. Finally, the possible role of O_2 and CO_2 in the local regulation of blood flow through the limb, coronary, and renal vasculature will be briefly explored.

Local Effect of O_2 on Limb

This study shows that local hypoxia produces forelimb vasodilation. Further, the magnitude of dilation is greatest when the oxygen content is almost zero. A large local rise in oxygen tension above the normal value produces an equivocal rise in forelimb vascular resistance. The finding of dilation of the forelimb vascular bed during hypoxia in the present study appears to differ from the studies of some in terms of magnitude of change. Ross *et al.*(46) noted a 175% increase in hindlimb blood flow from control during a reduction in oxygen saturation from 100% to 55%. In the present study a fall in oxygen tension from 117 mm Hg (16.0 vol.%) to 2 mm Hg (0.9 vol. %) produced only a 34% fall in resistance. However, the hindlimb resistance in the study of Ross *et al.* prior to hypoxia was quite high whereas it was relatively

normal in the present study. One would expect a greater dilation in a bed with a high control resistance than in a bed with normal resistance. Also it is possible that blood allowed to stand in a reservoir, as in the study of Ross et al., will acquire vasodilating properties. However, it is difficult to compare the study of Ross et al. with the present investigation since the limb in the former was perfused at constant pressure while in the latter it was at constant flow. Crawford et al. (11) also reported a large reduction in resistance to blood flow during local hypoxia in the hindlimb achieved by dilution of arterial blood with venous blood. This study was not specific for oxygen because venous blood differs from arterial blood in other respects. Haddy et al. (29) and Hall and Suckner (30) suggest that venous blood has vasodilating properties which are independent of oxygen. Therefore the large reduction in resistance noted by Crawford et al. might have been due to factors other than changes in oxygen concentration. Molnar et al. (40) found that lowering the oxygen content from 14.6 to 10.5 vol. % had no effect on forelimb resistance. The lack of an effect was most likely due to the small change in oxygen content. In an earlier study. (12), the present author noted only an 8% drop in the forelimb vascular resistance during moderate hypoxia. However, the forelimb vascular bed was already dilated prior to hypoxia due to local denervation and absence of the sympathico-adrenal system. A bed with low resistance will dilate less than a bed with normal resistance. Fleisch et al.(21) reported findings similar to those in the present study. They noted only moderate dilation in the innervated cat hindlimb during severe hypoxia. The dilation noted by these investigators might have been greater if

the pH of the infusing blood had been controlled during the hypoxia.

Since hypoxia produced dilation of the forelimb vasculature one might expect an increase in oxygen to cause constriction. When the oxygen tension was elevated to high levels (≈650 mm Hg), there occurred only an equivocal rise in forelimb vascular resistance. It should be noted however, that even a 5 fold increase in O_2 tension cannot produce an appreciable increase in the oxygen content. The present author is not aware of any other reports on the local effect of increasing the oxygen tension on a peripheral vascular bed.

Local Effect of CO_2 on Limb

This study shows that local hypercapnia produces vasodilation in the dog forelimb while hypocapnia produces vasoconstriction. The response during hypocapnia was greater than that during hypercapnia. The findings of the present study are not in complete agreement with those of Crawford et al. (11), Fleishman et al.(20), Litwin et al. (36) and of the present author in a previous study (12). Crawford et al. and Fleishman et al. noted no significant change in hindlimb or forelimb resistance during hypercapnia. Litwin et al. and the present author noted only a small fall in limb resistance during ventilation with high CO_2. In these 4 investigations the vascular bed of the limb was probably dilated, as indicated by a low resistance, prior to hypercapnia. In addition, oxygen concentration was not controlled by Crawford et al. or Fleishman et al. Further, the phentolamine in the study of Fleishman et al. and the bretylium in the work of Litwin et al. might have effects on a vascular bed independent of adrenergic and ganglionic

blockade, respectively. As a result, the local vascular bed's response to CO_2 would be altered. Also it is possible that systemically administered CO_2 will stimulate the release of non-catecholamine vasoactive substances which are not blocked by phentolamine or bretylium. Finally, the vascular bed of the limb, in the report of Crawford et al., was not truly localized from the effect of a sympathico-adrenal response. Fleisch et al. (20) produced local hypercapnia in an innervated limb by ventilating an artificial lung with 2-10% carbon dioxide in air mixtures. In some experiments 0.5-1% carbon dioxide in air mixtures produced up to a 30% increase in flow while in other dogs changes in CO_2 over physiologic levels caused the flow to increase up to 100%. In the present study, a 24% fall in forelimb vascular resistance occurred during the local administration of 20% CO_2 - 20% O_2 - 60% N_2. Thus the findings of the present study agree more closely with those of Fleisch et al. than with any other report. Fleisch et al. however, did not measure the oxygen concentration of the perfusing blood.

The large increase in forelimb resistance produced by hypocapnia in the present study is not in agreement with the study of Molnar et al. (40). They failed to observe a consistent rise in resistance while a 58% rise occurred in the present study over a comparable pH range. Part of the difference in response might be accounted for by the fact that Molnar et al. used reservoir blood and that the control limb resistances were elevated above those in the present study. On the other hand, the vasoconstriction produced by the local hypocapnia in the present investigation is in agreement with the study of Fleischman et al. (20) in the nerve blocked-phentolaminized limb. However,

Fleischman et al. did not control or measure O_2 concentration.

Local Effect of O_2 on Heart

This study shows that the local effect of hypoxia on the intact heart is reduction in coronary resistance and myocardial strength. The reduction in resistance as well as in strength is greatest at the lowest oxygen tension. Increasing the oxygen tension had no effect on coronary resistance or myocardial strength. In terms of direction, these findings are in agreement with the findings of others. Hilton and Eichholtz (32) reported that in the heart-lung preparation coronary flow increases as a function of decreasing oxygen saturation. This was confirmed by Gremels and Starling (24) who also reported that myocardial strength begins to decrease at an arterial oxygen saturation of 40% and falls progressively as the saturation is reduced below this value. However, in the latter two studies the CO_2 concentration was not controlled as indicated by the fact that the perfusing blood used by Gremels and Starling was severely alkalotic. Working with the intact heart, Berne et al. (4) also noted an increase in coronary blood flow during local hypoxia. However, they concluded that the coronary dilation is related to myocardial P_{O_2} rather than to the arterial P_{O_2}. Guz et al.(25) in an in vitro heart also concluded that the critical factor producing coronary dilation is the myocardial hypoxia. The latter investigators did not observe an effect on myocardial strength until the oxygen capacity of coronary sinus blood reached 2 vol. %. In the latter study the pH of the perfusing solution was maintained constant. In another paper in which pH was also maintained constant, Ebert et al.(16) re-

reported a moderate fall in myocardial strength at a local oxygen saturation of 70%. Hackel and Clowes (26) observed a large increase in coronary blood flow upon the administration of 10% O_2 - 90% N_2 to adrenalectomized and thoracic sympathectomized dogs. Part of the large increase in flow noted by Hackel and Clowes might have been related to changes in CO_2 or to some systemic effect. As mentioned previously, there apparently are no studies on the effects of a local increase in oxygen tension.

Local Effect of CO_2 on Heart

The present study shows that, in the steady state, local hypercapnia causes coronary dilation and reduction in myocardial strength. However, reduction in CO_2 produces little effect on resistance while increasing strength. In terms of direction of change the findings during hypercapnia are in agreement with those of other investigators. Gremels and Starling (24) noted a 60% increase in coronary blood flow during hypercapnia in the heart-lung preparation. Hilton and Eichholtz (32) also noted an increase in coronary blood flow during hypercapnia. pH changes were similar to those of the present study. The oxygen saturation remained constant in these two heart-lung studies. Nahas and Cavert (42) did not control the oxygen at a normal level during hypercapnia in the Starling heart-lung preparation but they nevertheless found a small fall in coronary resistance. However, they did not observe a fall in cardiac output, with venous and arterial pressures held constant, until the pH had reached 7.25. In the present investigation, strength was reduced by 30% at a pH of 7.23. It is difficult

to compare the report of McElroy et al. (39) with the present study since in the former, blood was not used as the perfusate. However, they did note a rise in flow and a fall in strength on elevation of CO_2 and the reverse changes during a reduction of P_{CO_2}. Apparently, the only data available on the effect of a local reduction of CO_2 on the heart are those of the present study and those from the study of McElroy et al.

Local Effect of Adenyl Compounds and Ischemia on Heart

The present study shows that intra-coronary injections of ATP and adenosine produce coronary vasodilation but have no measurable effect on myocardial strength. A 15 second period of local ischemia produces a reduction in strength and, upon restoring flow, a reduction in coronary resistance. The coronary vasodilator effect of ATP and adenosine have been reviewed by Berne (5). However, no previous reports are available concerning the effect of adenyl compounds on myocardial strength. The reduction in resistance during a reduction in flow is in agreement with the findings of Scott et al. (50) and others. Further the finding that a reduction in coronary flow reduces myocardial strength has previously been reported by Bacaner et al. (2).

Local Effect of O_2 on Kidney

In the present study a moderate fall in local P_{O_2} produced a small but significant reduction in renal vascular resistance. However, severe local hypoxia failed to produce a measurable effect on resistance. A large increase in O_2 tension produced equivocal results. The author is not aware of any truly local studies on the effect of changing oxygen concentration on the renal vasculature.

Local Effect of CO_2 on Kidney

This study shows that a local increase in CO_2 produces moderate renal vascular dilation. A local reduction in CO_2 produces renal vascular constriction. The findings of Emanuel et al. (17) on the denervated phentolaminized kidney during systemic hypercapnia are not in agreement with those of the present study. However, their failure to find renal vascular dilation might have been due to one or more factors: 1) oxygen saturation was not controlled, 2) phentolamine might have affected the action of CO_2 on the vasculature through effects independent of adrenergic blockade, 3) systemic administration of CO_2 might have released non-catecholamine vasoactive substances and 4) the kidney was apparently dilated prior to hypercapnia. On the other hand, Emanuel et al. (17) reported that a local reduction of the CO_2 produced renal artery constriction. Similar findings were observed in the present study as the pH increased from 7.26 to 7.96.

O_2 in Local Regulation of Blood Flow in Limb

Despite the significant fall in resistance produced during hypoxia in the present study, it is unlikely that local changes in oxygen concentration alone represents the major mechanism for local regulation of blood flow in the limb. Significant dilation did not occur until the P_{O_2} of the perfusing blood was lowered to 29 mm Hg. Thus as pointed out in 1932 by Fleisch et al. (21), the degree of hypoxia must be severe to produce dilation. Similarly, a large rise in oxygen tension produced only an equivocal change in limb resistance. Rudko et al. (48) (49) observed that femoral venous blood

oxygen content fell to 8.8 vol. % during autoregulation in the isolated dog hindlimb produced by reducing arterial perfusion pressure to 54 mm Hg. In addition, Rudko et al. (48) (49) noted a 36% increase in femoral venous blood flow during an increase in hindlimb metabolic rate (active hyperemia) while the femoral venous blood oxygen content fell from 12.2 to 7.6 vol. %. Thus it would appear that the magnitude of change in local oxygen concentration required to produce a significant change in limb vascular resistance is much greater than the oxygen change observed during autoregulation or active hyperemia in the limb. However, it is possible that the phenomenon of reactive hyperemia of complete occlusion might be explained by hypoxia because the P_{O_2} could fall to these low levels during the period of occlusion (47).

CO_2 in Local Regulation of Blood Flow in Limb

The present study also supports the concept that a change in CO_2 is not the major mechanism for local regulation of blood flow in the limb. Scott et al. (50) observed no change in femoral venous blood pH upon sudden elevation of femoral artery blood flow from a low to a high value which was followed by a rise in hindlimb vascular resistance (autoregulation). Further, during active hyperemia which produced a 174% increase in femoral blood flow, Rudko et al. (48) (49) has observed only an average 0.07 unit fall in femoral venous blood pH. In addition, Rudko et al. noted a similar small fall in venous pH following release of complete occlusion of the femoral artery (reactive hyperemia). In the present investigation, in one set of experiments local administration of 20% CO_2 produced a pH fall from 7.58 to 7.16 but did not produce

a significant fall in limb resistance (Table 1). While in a separate group of 5 dogs, a pH fall from 7.58 to 7.19 produced a 32% fall in limb resistance (Table 2). Therefore, it is doubtful that changes in CO_2 concentration per se can explain the mechanism for local regulation of blood flow in the limb.

O_2 in Local Regulation of Coronary Resistance and Myocardial Strength of the Heart

Coronary resistance and ventricular contractile force were reduced during severe local hypoxia in the present study. However, it is unlikely that changes in oxygen per se represent the only mechanism for local regulation in the heart. A fall in P_{O_2} to 30 mm Hg produced only a minimal reduction in coronary resistance and myocardial strength. However, a large fall in oxygen tension and content from 105 mm Hg (15.74 vol. %) to 8.5 mm Hg (0.36 vol. %) produced a large reduction in both resistance and strength. These findings are similar to those of others. A large rise in P_{O_2} had no measurable effect on coronary resistance or myocardial strength. Scott et al. (50) observed an increase in coronary resistance and myocardial strength upon sudden elevation of left common coronary artery blood flow by an amount which produced only a small increase in coronary sinus blood P_{O_2}. A reduction in flow produced only a small fall in coronary sinus P_{O_2} with large reductions in resistance and strength (autoregulation). Thus it is unlikely that the local regulation of coronary resistance and myocardial strength can be explained entirely by changes in oxygen concentration. However, as suggested above for the limb, the reactive hypere-

mia and reduction of strength following complete arterial occlusion in the heart might be explained by a large fall in oxygen during the period of occlusion.

CO_2 in Local Regulation of Coronary Resistance and Myocardial Strength of the Heart

This study also suggests that local changes in carbon dioxide concentration alone cannot offer a complete explanation for local regulation of coronary blood flow and myocardial strength. A short period of ischemia produced a 34% fall in both coronary resistance and force whereas elevation of CO_2 caused force to fall 30% but resistance decreased only 15%. Thus, although accumulation of CO_2 will produce the same reduction in force as seen during ischemia, it fails to produce the same changes in resistance. Furthermore, a local reduction of CO_2, while producing an increase in force, failed to raise coronary resistance. Scott et al. (50) did not observe any change in coronary sinus blood pH during autoregulation of coronary resistance and myocardial strength produced by elevation or reduction of coronary flow. Therefore, it is doubtful that changes in CO_2 concentration would be the major factor in local regulation of coronary resistance and myocardial strength.

ATP and Adenosine in Local Regulation of Coronary Resistance and Myocardial Strength of the Heart

The present study does not indicate that ATP or adenosine alone would explain the effects of changes in coronary flow on coronary resistance and myocardial strength. The adenyl com-

pounds produced a significant fall in coronary resistance but failed to mimic the fall in force seen during ischemia.

However, it is possible that the response to changes in coronary flow might result from a combination of changes in the concentrations of the adenyl compounds and CO_2. ATP and adenosine had a large effect on coronary resistance while changes in CO_2 had a large effect on myocardial strength. Thus, the combined effect of changes in the concentrations of the adenyl compounds and CO_2 would mimic the response observed with changes in coronary flow. Indeed, the effects of hypoxia might result indirectly from such a combination of changes in adenyl compounds and CO_2. Berne et al. (4) (5) has suggested that the coronary dilation of severe hypoxia results from an increase in the concentration of adenosine.

O_2 in Local Regulation of Blood Flow in Kidney

The findings in this study do not support the concept that local changes in the concentration of oxygen is an important factor in the local control of renal blood flow. Scott et al. (50) noted only a 4 mm Hg fall in renal venous blood P_{O_2} during autoregulation of the kidney in response to renal artery constriction. Also autoregulation still occurred when the drop in P_{O_2} was prevented by ventilation with a 100% oxygen mixture. In the present investigation, a 70 mm Hg fall in oxygen tension produced only a 9% reduction in renal vascular resistance while a fall from 130 mm Hg (13.5 vol. %) to 6 mm Hg (0.7 vol. %) had no regular effect. Also venous blood with a P_{O_2} of 26 mm Hg had no effect on resistance. Further, a large rise in P_{O_2} produced equivocal

changes in renal vascular resistance. Therefore, it is difficult to assign a change in oxygen concentration as being very important in local regulation of renal blood flow.

CO_2 in Local Regulation of Blood Flow in Kidney

A change in carbon dioxide tension does not seem to completely explain local regulation of renal blood flow. While a local change in CO_2 concentration produced a significant change in renal vascular resistance the pH of the renal venous blood remains constant during renal autoregulation (50). Further an increase in renal vascular resistance in response to an increase in renal artery blood flow (autoregulation of resistance) occurs despite ventilation of the animal with 20% CO_2 - 80% O_2 (27). Thus, it appears that changes in CO_2 concentration per se cannot explain the mechanism for local regulation of blood flow in the kidney.

CHAPTER V

SUMMARY AND CONCLUSIONS

In 50 anesthetized dogs the acute direct effect of changes in oxygen or carbon dioxide concentration on the intact forelimb, coronary and renal vascular bed as well as on left ventricular contractile force were studied. The concentration of oxygen or carbon dioxide in femoral arterial or venous blood was altered by passing the blood through an isolated lung which was ventilated with various mixtures of carbon dioxide, oxygen and nitrogen. The blood was then pumped at a constant rate into either the brachial, left common coronary, or renal artery. Since the changes in O_2 and CO_2 were purely local, the preparation obviated interference from indirect actions on the vascular bed via the sympathico-adrenal system. It also permitted a change in oxygen concentration while holding carbon dioxide reasonably constant and a change in CO_2 in the absence of a large change in O_2 concentration. Thus the direct effect of a change in one could be separated from that of the other. The observation of the direct effect of high oxygen tension on a vascular bed has not previously been reported nor have the effects of truly local changes in O_2 or CO_2 on the renal vascular bed been reported.

Ventilation of the isolated lung with 5% CO_2 - 95% N_2 reduced the perfusing blood P_{O_2} to 2 and 8 mm Hg causing a significant reduction

in forelimb and coronary artery resistance, respectively. A similar degree of local hypoxia had no regular effect on renal vascular resistance. Left ventricular contractile force fell during the severe hypoxia. Ventilation of the isolated lung with 100% O_2 did not produce a measurable effect on vascular resistance of the forelimb, heart or kidney nor did hyperoxia affect contractile force. Ventilation of the isolated lung with 20% CO_2 - 20% O_2 - 60% N_2 produced an average 0.42 unit fall in the perfusing blood pH and caused a significant fall in limb, coronary and renal vascular resistance. Contractile force fell greatly during hypercapnia. A large fall in CO_2 concentration produced an increase in limb and renal vascular resistance. The hypocapnia produced equivocal changes in coronary resistance but force increased significantly. Intracoronary injections of ATP and adenosine reduced coronary resistance without affecting force. Myocardial ischemia of 15 seconds, achieved by shutting off the pump, produced a reduction in force and upon re-establishment of flow a reduction in coronary resistance. These findings have been discussed in relation to the mechanism of local regulation of blood flow and myocardial strength.

REFERENCES

1. Aviado, D. M., Jr., A. Cerletti, J. Alonis, P. H. Bulle, and C. F. Schmidt. Effects of anoxia on pressure, resistance, and blood volume of pulmonary vessels. Am. J. Physiol. 169:460, 1952.

2. Bacaner, M., M. Visscher, F. Lioy, R. Stish, and H. Ballin. Oxygen delivery and myocardial performance. The Physiologist. 6 (3):133, 1963.

3. Bayliss, W. M. On the local reactions of the arterial wall to changes in internal pressure. J. Physiol. 28:220, 1902.

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

5. Berne, R. M. Regulation of coronary blood flow. Physiol. Rev. 44:1, 1964.

6. Bernthal, T. Chemo-reflex control of vascular reactions through the carotid body. Am. J. Physiol. 121:1, 1938.

7. Bock, A. V., H. Field Jr., and G. S. Adair. The oxygen and carbon dicxide dissociation curves of blood. J. Biol. Chem. 59:353, 1924.

8. Boniface, K. J. and J. M. Brown. Effect of carbon dioxide excess on contractile force of the heart, in situ. Am. J. Physiol. 172:752 1953.

9. Brickner, E. W., E. G. Dowds, B. Willitts, and E. E. Selkurt. Mesenteric blood flow as influenced by progressive hypercapnia. Am. J. Physiol. 184:275, 1956.

10. Carrier, O., Jr., J. R. Walker, and A. C. Guyton. Oxygen: Its role in local autoregulation. The Physiologist. 6 (3):155, 1963.

11. 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.

12. Daugherty, R. M., Jr. The acute local effect of hypoxia and hypercapnia on the vascular bed of the dog forelimb. Thesis in partial fulfillment of the requirement for the degree of Master of Science, 1964.

13. Downing, S. E., J. H. Mitchell, and A. G. Wallace. Cardiovascular response to ischemia, hypoxia, and hypercapnia of the CNS. Am. J. Physiol. 204:881, 1963.

14. Downing, S. E., J. P. Remensnyder and J. H. Mitchell. Cardiovascular response to hypoxic stimulation of carotid bodies. Circ. Res. 10:676, 1962.

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

16. Ebert, P. A., W. G. Austen, L. J. Greenfield, H. W. Bender, and A. G. Morrow. Direct and reflex effects of hypoxia, hypotension and hypothermia on the heart. Am. J. of Med. Electronics. 3:162, 1964.

17. Emanuel, D. A., M. Fleishman, and F. J. Haddy. Effect of pH change upon renal vascular resistance and urine flow. Circ. Res. 5:607, 1957.

18. Feinberg, H., A. Gerola, L. N. Katz, and E. Boyd. Effect of hypoxia on cardiac oxygen consumption and coronary flow. Am. J. Physiol. 195:593, 1958.

19. Feinberg, H. A. Gerola, and L. N. Katz. Effect of changes in blood CO_2 level on coronary flow and myocardial O_2 consumption. Am. J. Physiol. 199:349, 1960.

20. Fleishman, M., J. Scott and F. J. Haddy. Effect of pH change upon systemic large and small vessel resistance. Circ. Res. 5:602, 1957.

21. Fleisch, A., I. Sibul and V. Ponomarev. Kohlensaure und Sauerstoff mangel als auslosende Reize. Pfluger's Archiv fur Physiologie. 230: 814, 1932.

22. Folkow, B. Intravascular pressure as a factor regulating the tone of small vessels. Acta Physiol. Scand. 17:289, 1949.

23. Greenfield, L. J. and P. A. Ebert. Cardiac denervation effect in hypoxia and hypercapnia. Arch. of Surg. 87:717, 1963.

24. Gremels, H. and E. H. Starling. On the influence of hydrogen ion concentration and of anoxemia upon the heart volume. J. Physiol. 61:297, 1926.

25. Guz, A., G. S. Kurland and A. S. Freedberg. Relation of coronary flow to oxygen supply. Am. J. Physiol. 199:179, 1960.

26. Hackel, D. B. and G. A. H. Clowes Jr. Coronary blood flow and myocardial metabolism during hypoxia in adrenalectomized-sympathectomized dogs. Am. J. Physiol. 186:111, 1956.

27. Haddy, F. J., J. Scott, M. Fleishman and D. Emanuel. Effect of change in flow rate upon renal vascular resistance. Am. J. Physiol. 195:111, 1958.

28. Haddy, F., J. Scott, J. Dabney, R. Daugherty, G. Rahe, T. Russell and G. Earl. Local regulation of blood flow. Clin. Res. 12:69, 1964.

29. 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. Cir. Res. Supplement on Autoregulation of Blood Flow 8, 1964.

30. Hall, G. H. and M. A. Suckner. Pulmonary inactivation of endogenous vasodilator material. The Physiologist 6 (3):195, 1963.

31. Harrison, T. R. and A. Blalock. The effects of severe anoxemia of short duration on the cardiac output of morphinized dogs and trained unnarcotized dogs. Am. J. Physiol. 80:169, 1927.

32. Hilton, S. M. And F. Eichholtz. The influence of chemical factors on the coronary circulation. J. Physiol. 59:413, 1925.

33. Hinshaw, L. B., S. B. Day, and C. H. Carlson. Tissue pressure as a causal factor in the autoregulation of blood flow in the isolated perfused kidney. Am. J. Physiol. 197:309, 1959.

34. Kahler, R. L., A. Goldblatt, and E. Braunwald. The effects of acute hypoxia on the systemic venous and arterial systems and on myocardial contractile force. J. Clin. Invest. 41:1553, 1962.

35. Katz, A. M., L. N. Katz and F. L. Williams. Regulation of coronary flow. Am. J. Physiol. 180:392, 1955.

36. Litwin, J., A. H. Dil, and D. M. Aviado. Effects of hypercapnia on the vascular resistance of the dog's hindlimb. Pfluger's Archiv. Fur Die Gesamte Physiologie. 277:387, 1963.

37. Manley, E. S., Jr., C. B. Nash and R. A. Woodbury. Cardiovascular responses to severe hypercapnia of short duration. Am. J. Physiol. 207:634, 1964.

38. McArdle, L., I. C. Roddie, J. I. Shepherd, and R. F. Whelan. Effect of inhalation of 30% CO_2 on peripheral circulation of a human. Brit. J. Pharm. 12:293, 1957.

39. McElroy, W. T., A. J. Gerdes and E. B. Brown, Jr. Effects of CO_2, bicarbonate, and pH on the performance of isolated perfused guinea pig hearts. Am. J. Physiol. 195:412, 1958.

40. Molnar, J., H. Overbeck and F. Haddy. Local effects of oxygen and carbon dioxide upon dog forelimb vascular resistance. Proc. Int'l. Union of Physiol. Sci. 2:175, 1962.

41. Molnar, J. I., J. B. Scott, E. D. Frohlich, and F. J. Haddy. Local effects of various anions and H^+ on dog limb and coronary resistance. Am. J. Physiol. 203:125, 1962.

42. Nahas, G. G. and H. M. Cavert. Cardiac depressant effect of CO_2 and its reversal. Am. J. Physiol. 190:483, 1957.

43. Nahas, G. G., M. B. Visscher, G. W. Mathes, F. J. Haddy and H. R. Warner. Influences of hypoxia on the pulmonary circulation of non-narcotized dogs. J. of Appl. Physiol. 6:467, 1954.

44. Penna, M., L. Soma and D. M. Aviado. Role of carotid and aortic bodies in mediating the increase in cardiac output during anoxemia. Am. J. Physiol. 203:133, 1962.

45. Richards, J. B. and S. N. Stein. Effects of CO_2 exposure and respiratory acidosis on adrenal 17-hydroxycorticosteroid secretion in anesthetized dogs. Am. J. Physiol. 188:1, 1957.

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

47. Ross, J. M., H. M. Fairchild and A. C. Guyton, Oxygen lack as a cause of post-occlusion hyperemia. The Physiologist 5 (3): 206, 1962.

48. Rudko, M. J. Scott and F. Haddy. Flow to metabolism ratio (F/M) and vascular resistance (R). Fed. Proc. 24:458, 1965.

49. Rudko, M. Personal Communication.

50. 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, 1965.

51. Wiggers, C. J. A study of the direct effect of hypercapnia on the contraction of the mammalian ventricle. Am. J. Physiol. 90:230, 1929.

52. Wiggers, C. J. Cardiac adaptations in acute progressive anoxia. Ann. Int. Med. 14:1237, 1941.