Unclassified

CIRCULATORY EFFECTS OF ACUTE AND CHRONIC HYPOXIA

Final Progress Report

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May 3, 1974

Supported by
U.S. Army Medical Research and Development Command
Washington, D.C. 20314

Contract No. DADA-17-67-C-7090

Medical College of Virginia
Health Sciences Division
Virginia Commonwealth University
Richmond, Virginia 23298

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Virginia Commonwealth University Medical College of Virginia 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Health Sciences Division Richmond, Virginia 23298	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DADA17-67-C-7090
8c. ADDRESS (City, State, and ZIP Code) Washington, DC 20314	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. -- / PROJECT NO. -- / TASK NO. -- / WORK UNIT ACCESSION NO --

11. TITLE (Include Security Classification)

CIRCULATORY EFFECTS OF ACUTE AND CHRONIC HYPOXIA

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13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final Progress Report	FROM 2/1/67 TO 12/31/72	1974 May 3	23

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
--	--		
--	--		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

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20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller	22b TELEPHONE (Include Area Code) (301) 663-7325 / 22c. OFFICE SYMBOL SGPD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

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I. Circulatory effects of hypoxia in animals.

1. This work was carried out in anesthetized and unanesthetized dogs and in anesthetized cats. The aim of the work was to identify the mechanism of important circulatory effects of acute and chronic hypoxia. The results of specific investigations are summarized below:

The possibility that mechanisms secondary to the increased ventilation may contribute significantly to the circulatory responses to systemic hypoxia was explored in anesthetized dogs. In 14 spontaneously breathing dogs systemic hypoxia induced by breathing 7.5% oxygen in nitrogen increased cardiac output, heart rate, mean arterial blood pressure, and femoral arterial flow, and decreased systemic and hindlimb vascular resistances. In 14 dogs whose ventilation was kept constant by means of a respirator pump and intravenous decamethonium, systemic hypoxia did not change cardiac output, femoral arterial flow, or limb vascular resistance; it significantly decreased heart rate and significantly increased systemic vascular resistance. In seven spontaneously breathing dogs arterial blood P_{CO_2} was maintained at the resting level during systemic hypoxia. The increase in heart rate was significantly less pronounced but the other circulatory findings were not different from those found during hypocapnic hypoxia. Thus, mechanisms secondary to increased ventilation contribute significantly to the circulatory responses to systemic hypoxia. Hypocapnia accounts partly for the increased heart rate, but not for the other circulatory responses.

2. The importance of reflexes from the lungs in the circulatory response to hypoxia was examined in anesthetized dogs. The right lung was denervated by surgical means and

infiltration of its hilum with procaine and the left cervical vagus was cut. It was shown that this procedure produced afferent pulmonary vagal denervation, whereas afferent and efferent vagal innervation of the heart were preserved. Before the left vagus was cut, e.g., when the vagal afferents from the left lung were intact. hypoxia produced tachycardia, increase in blood flow through the ascending aorta (electromagnetic flowmeter), and decrease in systemic vascular resistance. After the left vagus was cut, e.g., when pulmonary afferent vagal denervation was complete, these responses to hypoxia were abolished. The results support the view that reflexes from the lungs having their afferent limb in the vagus and elicited by the hyperventilation associated with hypoxia contribute to the tachycardia and increase in cardiac output seen during hypoxia in the anesthetized dogs.

3. The role of beta-adrenergic receptor stimulation in circulatory response to hypoxia was studied in dogs. Breathing 7% oxygen in unanesthetized dogs produced increases in heart rate, cardiac output, and mean arterial blood pressure. The tachycardia and elevated cardiac output were abolished by propranolol. The circulatory response to hypoxia could not be reproduced by intravenous infusions of isoproterenol, epinephrine, or a mixture of isoproterenol and norepinephrine. The effect of administration of a small dose (0.075 mg/kg) of propranolol on the circulatory response to hypoxia was distinctly different from that on the response to catecholamine infusion. Bilateral adrenalectomy did not modify the response to hypoxia in anesthetized dogs. In dogs with cardiac denervation following cardiac autotransplantation, hypoxia produced increases in heart rate and cardiac output which were markedly reduced by propranolol, but not modified by bilateral adrenalectomy. Autotransplanted dogs following cardiac reinnervation responded to hypoxia before and after propranolol like the unanesthetized dogs. The results suggest that stimulation of cardiac beta-adrenergic receptors is a major factor in the production of tachycardia and increased cardiac output

during hypoxia. In a normal dog this is the result of increased activity of cardiac sympathetic nerves, rather than circulating catecholamines. In the denervated dogs circulating catecholamines are responsible for cardiac beta-adrenergic receptor stimulation. This difference between normal and denervated dogs is probably due to the absence of reflex control of the heart and to the hypersensitivity of the denervated heart to catecholamines.

4. Circulatory response to systemic hypoxia, induced by breathing 7.5% oxygen, was determined in dogs with open chest whose ventilation was maintained constant, before and after inactivation of the carotid chemoreceptors by the injection of acetic acid into the caroti arteries. Before chemoreceptor inactivation, hypoxia produced bradycardia, increase in mean arterial blood pressure, and no significant change in aortic blood flow. Following carotid chemoreceptor inactivation, the response to hypoxia was modified so that arterial blood pressure did not change significantly and heart rate and aortic blood flow increased significantly. In another series of dogs with open chest and constant ventilation, the respons to hypoxia was studied before and after intravenous administration of atropine. Before the administration of atropine hypoxia produced bradycardia, increase in mean arterial blood pressure, and no change in aortic blood flow, while after atropine hypoxia produced significant increases in heart rate, aortic blood flow, and mean arterial blood pressure. These results show that the carotid chemoreceptors are active during systemic hypoxia and that they exert vasoconstrictor and cardiodepressant effects.

5. Arterioles approximately 40 microns in diameter on the surface of the cat's brain are observed through a cranial window sealed into the skull. A microscope equipped with a beam-splitting device facilitates continual, accurate recording of vascular diameter. Alterations in inspired oxygen concentration (F_{IO_2}) had the effects on cerebral arteriolar diameter shown in the table below, which shows average results in eight cats, anesthetized with pentobarbital.

INTRAVASCULAR HYPOXIA

F_{IO_2}	21%	40%	12%	7%
Equivalent Altitude, Ft.	0	---	15,000	25,000
P_{IO_2} mm Hg	150	286	85	50
PaO_2 mm Hg	95	200	55	27
Arterial Diameter % of Control	100	97	108	131

These results show minimal changes in cerebral arteriolar diameter with 12% oxygen in inspired air, equivalent to 15,000 feet above sea level. Additional experiments w varying tensions of oxygen in the cerebrospinal fluid surrounding the blood vessels were per formed by equilibrating the artificial spinal fluid which is continuously perfused through the space under the glass window with 6% oxygen (which provides normal cerebrospinal fluid P_O and subsequently in each cat with 35% oxygen and with pure nitrogen. The results of such experiments in nine cats are shown in the table below.

EXTRAVASCULAR HYPEROXIA AND HYPOXIA

$\%O_2$ Equilibrated with CSF	6	35	0
P_{O_2} of CSF mm Hg	52	230	4
Arterial Diameter % of Control	100	92	113

Marked extravascular hypoxia, presumably affecting the smooth muscle of the arterioles whose diameters were observed, had modest but significant dilator effect on arteriolar diameter. Thus both intravascular as well as extravascular hypoxia involve cerebral arterioles. The response to external hypoxic shows that O_2 may be involved in the lower regulation of brain vessel caliber.

Further experiments determining the effects of exercise, of systemic hypoxia induced by breathing 7-9% oxygen, and of ischemia induced by reducing arterial inflow to the muscle are underway.

6. We determined the interaction between hypoxia and hypercapnia in cats breathing room air, 10 per cent carbon dioxide in air; 10 per cent carbon dioxide - 6% oxygen; and 10 per cent carbon dioxide in air. Each of the four gas mixtures were breathed for ten minutes. The diameter of arterioles on the surface of the brain was measured photographically six to eight times during the last five minutes of exposure to each gas mixture. The results are shown in the table below, in which each entry represents the mean ± standard error of diameter of 10 arterioles in seven cats. The mean diameters in each experimental condition are significantly ($p<0.025$) different from each other.

	Control	10% CO_2-Air	10% CO_2-6% O_2	10% CO_2-Air
Diameter, microns	25.9 ± 2.8	43.3 ± 7.3	56.5 ± 7.2	50.0 ± 7.5
PaO_2, mm Hg	106 ± 5.6	109 ± 7.0	29 ± 2.2	115 ± 6.2
$PaCO_2$, mm Hg	41 ± 2.3	86 ± 5.1	90 ± 5.6	100 ± 3.9

Hypercapnia dilated the arterioles. There was further significant increase in arteriolar diameter when hypoxia was added to hypercapnia, indicating that hypoxia has a dilator effect over and above the effects of severe hypocapnia.

7. Five healthy mongrel dogs chronically instrumented with an electromagnetic flowmeter probe around the ascending aorta and cannulas at several intravascular sites were exposed to a five-day period of breathing 10% oxygen in a chamber. Each animal was allowed one month in the chamber breathing air to adjust to the experimental surroundings. Two fairly distinct phases of the response to hypoxia could be distinguished. During the early phase which lasted 24 hours, hypoxia produced increases in heart rate, cardiac output, pulmonary and systemic arterial blood pressures and in pulmonary vascular resistance. Systemic vascular resistance was unchanged while left atrial pressure decreased. During this phase the animals became hypocapnic and arterial blood pH rose significantly. During the rest of the hypoxic period, cardiac output, heart rate and arterial blood pH returned to the control values while pulmonary arterial and systemic arterial pressures and pulmonary vascular resistance remained elevated. Systemic vascular resistance rose significantly above its control value and left atrial pressure remained below control. The experiment was repeated during administration of the antihistamine promethazine in a dose which blocked the vascular responses to exogenous histamine both during room air breathing and during hypoxia. The responses to hypoxia were substantially unchanged except that systemic vascular resistance did not rise and heart rate remained elevated throughout the hypoxic period. We conclude that histamine is likely not a mediator of the vascular responses to hypoxia and particularly of the pulmonary vasoconstrictor response to hypoxia.

II. Circulatory effects of hypoxia in man.

These investigations were carried out in normal human volunteers. Their aim was to uncover the mechanisms of important circulatory effects of acute hypoxia and to determine the possible role of hypoxia in the physiological regulation of blood flow. The specific

investigations carried out are summarized below:

1. The role of hypocapnia in the circulatory response to acute hypoxia was investigated in 18 healthy men. Cardiac output increased by 76%, heart rate increased by 25%, and arterial pressure did not change significantly in 9 subjects who breathed 8% oxygen in nitrogen for 7-8 min. Addition to this inspired gas mixture of sufficient carbon dioxide to raise arterial pCO_2 to its control value reduced the circulatory changes, but raised arterial oxygen tension from an average of 37 to 52 mm Hg as a result of increased ventilation. Abolition of hypocapnia without change in arterial oxygen tension, by reducing oxygen concentration from 9 to 7% when CO_2 was added to inspired gas, produced no change in the circulatory responses to hypoxia in 12 subjects. Thus, hypocapnia does not appear to be responsible for the increase in cardiac output, heart rate, and forearm blood flow which accompany acute arterial hypoxia.

2. The circulatory response to breathing low-oxygen-containing gas mixtures for 7 min was examined in 33 experiments on 7 unanesthetized, trained dogs previously fitted with a flowmeter probe on the ascending aorta, and in 35 experiments on 26 young, normal human volunteers. In the dog, hypoxia caused tachycardia, increase in aortic blood flow, hypertension, and hypocapnia. In man, hypoxia was associated with tachycardia, increases in stroke volume and cardiac output, decreases in systemic vascular resistance and arterial blood P_{CO_2}, but with no change in mean arterial blood pressure.

3. In 17 healthy men, beta-adrenergic blockade reduced significantly the tachycardia and the elevation of cardiac output associated with inhalation of 7.5% oxygen for 7 to 10 minutes.

Hypoxia did not increase plasma concentrations of epinephrine or norepinephrine in six subjects. Furthermore, blockade of alpha and beta receptors in the forearm did not modify the vasodilation in the forearm induced by hypoxia, providing pharmacologic

evidence that hypoxia of the degree and duration used was not associated with an increase in the concentrations of circulating catecholamines in man.

Part of the increase in cardiac output and heart rate during acute hypoxia in man is produced by stimulation of beta-adrenergic receptors, probably by cardiac sympathetic nerves. The mechanism of the vasodilation in the forearm during hypoxia remains uncertain.

4. Hypoxia, induced by 7-12% oxygen breathing, produced vasodilatation in the intact or in the phenoxybenzamine and propranolol treated forearm of human volunteers when arterial blood P_{O_2} decreased below 45 mm Hg, or when deep forearm venous blood P_{O_2} decreased below 35-40 mm Hg.

Circulatory arrest of the forearm following alpha and beta adrenergic receptor blocka was followed by greater increases in blood flow and greater decreases in forearm vascular resistance during CO_2 breathing than during room air breathing. The increased flow following ischaemia was maintained at a high level until CO_2 administration was stopped.

The vasodilator response following ischaemia of the human forearm, produced by digital occlusion of the brachial artery, was compared to that produced by hypercapnia or hypoxia or a combination of the two, produced by breathing the appropriate gas mixtures. The forearm was pre-treated with phenoxybenzamine and propranolol to produce alpha and beta adrenergic receptor blockade. For equal increases in deep forearm venous blood P_{CO_2} the vasodilator response to hypercapnia averaged 60% of that following ischaemia. For equal decreases in deep forearm venous blood P_{O_2} the vasodilator response to hypoxia averaged 26% of that produced by ischaemia. The vasodilator response to ischaemia was not modified by breathing 100% oxygen to maintain the deep forearm venous blood P_{O_2} at a level above that seen with the circulation free during room air breathing. Combined hypoxia and hypercapnia of equal severity as those produced by ischaemia resulted in a vasodilator response which averaged 64% of that produced by ischaemia.

III. Development of a method for the determination of intracellular oxygen tension in muscle.

1. The aim of this research was to develop and validate a method for determining intracellular oxygen tension in muscle, based on the determination of oxygen saturation of myoglobin. This method will be used to explore the mechanisms of regulation of intracellular P_{O_2} in this tissue and the changes it undergoes in response to commonly occurring physiological interventions.

2. Earlier studies on this project were carried out with a modified Beckman Model B spectrophotometer. This instrument was modified so that the existing optical path was replaced with a bifurcated bundle of fiberoptics light guide through which the light from the light source of the instrument could be taken to the muscle and the reflected light from the muscle taken back to the phototube of the instrument. Because this instrument is a single beam spectrophotometer, it was necessary with changes in wavelength to change the slit width. The instrument was only marginally meeting our requirements and it was subsequently replaced with another modified spectrophotometer. The instrument we are now using is a Perkin-Elmer Model 124 doublebeam grating spectrophotometer which was modified for these experiments. The range of wavelengths of this instrument is from 190-700 mμ and it measures up to 2.0 absorbance units. The instrument can be set to have bandwidths of 0.5-2 mμ. We are usually using a 1.0 mμ bandwidth. The modification of this instrument was similar to the one described above for the Beckman spectrophotometer. The usual optical path of the instrument was replaced with a bifurcated fiberoptics light guide, with the fibers randomly distributed in it. Through an appropriate arrangement of mirrors and lenses the light from the monochromator is focused on one limb of the fiberoptics catheter and thereby taken to the muscle or specimen, while the reflected light from the muscle is returned through the other limb of the fiberoptics catheter and focused on the

sensing element of the photoelectric cell.

An early problem with this instrument was the low intensity of the reflected light. Most of our data were taken with a 48 watt sealed beam automatic head lamp as an external source of light. At this power the light intensity was marginal and we were forced to design modifications which resulted in considerable improvement. These modifications had to do with the arrangement of the light path in the instrument; they resulted in considerably improved efficiency and eliminated the noise problems which were associated with the marginal light intensity. The noise level was further alleviated by averaging the signal from the output terminals of the spectrophotometer through feeding it into a filter connected to an analogue computer. The probing end of the fiberoptics light guide has one of two configurations. In one configuration the measurements are taken on the surface of the muscle. The placement of the catheter is facilitated by the construction of a plastic circular platform with a flat base. The center of this platform is perforated and leads to a vertical tube through which the fiberoptics catheter is inserted. The platform is sutured into the muscle at its edges and helps retain the fiberoptics probe on the surface of the muscle without exerting undue pressure on the tissue. In the second configuration the end of the fiberoptics catheter is inserted into a 16-gauge needle which can then be introduced into the substance of the muscle.

The derivation of the basic equations used in the calculations of the intracellular P_{O_2} in muscle are as follows: We assume that light reflected from the muscle obeys Beer's law. For the validation of this assumption see below. This being the case for each absorbing substance the following relationship obtains:

$$R_{ij} = e_{ij} c_i \ell$$

where:

$R_{ij} = \log (I_o/I)_{ij}$

I_o = intensity of incident light

I = intensity of reflected light

e_{ij} = molar extinction coefficient of substance i at wavelength j

ℓ = effective path length

We recognize that the processes which the light undergoes in an experiment of this sort do not consist of simple reflectance but probably represent a more complicated process involving not only reflectance but also absorbance, scattering and difraction. One cannot therefore accept a priori that Beer's law would be obeyed. We sought to validate that this was indeed the case in in vitro experiments. An early difficulty was that clear solutions do not have reflectance. This problem was solved by placing the solutions in a specially constructed cell whose wall facing the probe was represented by a mirror. Another way, equally effective, was to prepare the solutions in a suspension of calcium carbonate. Ideally, we wished to use myoglobin for this validation. However, this was impractical, mainly because myoglobin in solution undergoes a slow denaturation. Because of the critical importance of demonstrating that truly Beer's law was obeyed, we chose to use a more stable system which, in other respects, was entirely comparable to myoglobin. Such a system makes use of pH indicators. We used for this purpose bromthymol blue and phenol red. We prepared solutions of these indicators in gelatin to simulate the consistency of muscle and adjusted the pH to cover a wide range of values with phosphate buffer. We compared the spectrophotometrically determined concentration with the concentration of

the indicator based on the known pH of the solution. There was linearity between the reflectance determined spectrophotometrically and the concentration of either indicator confirming that Beer's law is obeyed.

From the reversibie combination of myoglobin (Mb) with oxygen to form oxymyoglobin (MbO_2):

$$MbO_2 \rightleftharpoons Mb + O_2$$

It follows that:

$$k = \frac{(Mb)\ (O_2)}{(MbO_2)}$$

Since from Henry's law:

$$P_{O_2} = k_H\ (O_2)$$

and

$$P_{O_2} = k_H\ (O_2) = \frac{k_H k\ (MbO_2)}{(Mb)} = K\ \frac{(MbO_2)}{(Mb)}$$

Therefore, the P_{O_2} at the site where myoglobin resides, e.g. in the cytoplasm of the muscle cells, can be determined from the ratio of oxymyoglobin to reduced myoglobin.

A point of major concern was the possibility that the presence of hemoglobin might seriously interfere with the spectrophotometric determination of the oxygen saturation of myoglobin. Assume that the measurements are taken in a medium which contains both hemoglobin and myoglobin. For convenience the following subscripts are assigned for identification as shown in Table 1:

TABLE 1

substance	subscript
Mb	1
MbO_2	2
Hb	3
HbO_2	4

Then the reflectance at wavelength j will be as follows:

$$R_j = \left[e_{1j} (Mb) + e_{2j} (MbO_2) + e_{3j} (Hb) + e_{4j} (HbO_2) \right] \ell$$

We make the implicit, but reasonable assumption, that the effective pathlength is th same for each absorbing substance. If one takes into account that the total amount of hemoglobin (Hb_0) is:

$$(Hb_0) = (Hb) + (HbO_2)$$

and the total amount of myoglobin (Mb_0) is:

$$(Mb_0) = (Mb) + (MbO_2)$$

The reflectance may be written as follows:

$$R_j = \left[e_{1j} (Mb_0) + (e_{2j} - e_{1j}) (MbO_2) + e_{3j}(Hb_0) + (e_{4j} - e_{3j}) (HbO_2) \right] \ell$$

If wavelength j is chosen so that $e_{4j} - e_{3j} = 0$, i.e. this wavelength is an isosbestic point for hemoglobin, then it is evident that the reflectance will depend only on the total amounts of hemoglobin and myoglobin and on the oxygen saturation of myoglobin. A suitable choice of wavelength where this occurs is 565 mμ which is an isosbestic point for hemoglobin and at the same time gives near maximum differences between the absorptio of myoglobin and oxymyoglobin. The superiority of the modern spectrophotometers with

their narrow bandwidths over the older instrument used by Millikan can be appreciated. The narrow bandwidth permits exact choice of wavelengths so that a difference of even 10 mμ between the isosbestic points of hemoglobin and myoglobin can be resolved.

Assume that the measurements are taken under three different conditions: 1) When the intracellular P_{O_2} is zero and hence the concentration of oxymyoglobin is zero; 2) when the intracellular P_{O_2} is so high that the myoglobin is completely saturated: and 3) at some intermediate point between the two at which the determination of intracellular P_{O_2} is desired. For convenience, the table below lists the three conditions and the corresponding reflectances:

TABLE 2

condition	reflectance
$(MbO_2) = 0$	R^o
$(MbO_2) = (Mb_0)$	R^x
$(Mb_0)>(MbO_2)>0$	R

Then the following relationships are obtained:

$$R_j^o - R_j = -\ell\,(e_{2j} - e_{1j})\,(MbO_2)$$

$$R_j^o - R_j^x = -\ell\,(e_{2j} - e_{1j})\,(Mb_0)$$

By dividing these two equations and taking into account the relationship between P_{O_2} and oxygen saturation of myoglobin it follows that:

$$\frac{(Mb_0)}{(MbO_2)} = \left[\frac{R_j^o - R_j^x}{R_j^o - R_j}\right]$$

and

$$\frac{1}{P_{O_2}} = \frac{1}{K}\left[\frac{R_j - R_j^x}{R_j^o - R_j}\right]$$

By rearranging it follows that:

$$P_{O_2} = K\left[\frac{R_j^o - R_j}{R_j - R_j^x}\right]$$

We examined the validity of this equation in the calculation of P_{O_2} in an in vitro system. We placed a 2% solution of myoglobin in phosphate buffer in the cell whose wall was made up by a mirror to provide reflectance. The fiberoptics probe was placed in the cell and at first the myoglobin was reduced by the addition of sodium dithionite. Subsequently, low concentrations of oxygen were bubbled through the myoglobin solution to raise the oxygen tension. Measurements of oxygen tension were taken with a polarographic electrode and simultaneous estimations of P_{O_2} were made from the oxygen saturation of myoglobin. This was a difficult experiment, because the slow denaturation of myoglobin does not permit proceeding progressively to higher levels of oxygen tension. Instead, one has to proceed from full desaturation to any given level of saturation and take the measurements as quickly as possible. The addition of albumin and lowering the temperature to retard denaturation do not completely solve this problem. We have recently devised a new

method for the preparation of dog myoglobin which results in considerable curtailment of the rate at which this denaturation occurs. We have made some preliminary determinations using dog myoglobin prepared in this fashion at 37°C, but we are not yet certain about the reliability of the measurements or about their reproducibility. The results demonstrated satisfactory agreement between measured and calculated P_{O_2}.

Our early experience with actual determinations of the oxygen saturation of myoglobin in muscle showed that under certain circumstances significant alterations in background reflectance occur. It was necessary therefore, to correct for these changes. The correction we employed makes use of the changes in reflectance at known isosbestic points for myoglobin-oxymyoglobin. Such isosbestic points occur at wavelengths of 588 and 548 mμ. Instead of using the observed reflectances at 565 for the calculation of P_{O_2} we used a corrected reflectance which was the sum of the observed reflectance at 565 plus the difference between the reflectance at 548 in the control period and the reflectance at 548 during the condition where a change in background was expected to occur. As a check of the correctness of this method, the reflection corrections determined at 548 were applied in the same manner to reflectances measured at another isosbestic point, namely 588 mμ. If the correction is appropriate then this should result in correction of all reflectances observed at 588 to a constant corrected value. This indeed proved to be the case.

Our measurements have been taken in the gracilis muscle of the anesthetized dog. The animal is anesthetized with intravenous pentobarbital and either allowed to breathe spontaneously or ventilated with a positive pressure respirator. Heparin is used as anticoagulant. The arterial supply and the venous outflow of the gracilis muscle are identified and dissected free. The arterial supply is cannulated with an arrangement

which permits either perfusion of the muscle naturally through its own arterial supply with the interposition of a short piece of plastic tubing or perfusion from a reservoir at constant pressure. The venous outflow is cannulated and the blood flow measured with a cannulating type of electromagnetic flowmeter probe. The nerve of the muscle is dissected free and an electrode placed on it for subsequent stimulation. The fiberoptics probe is placed either on the surface of the muscle, or the needle type probe inserted within its substance. All collaterals from the muscle are isolated tied and divided. This preparation permits the determination of blood flow to the muscle and the determination of oxygen consumption based on the Fick principle, from the blood flow and the arteriovenous oxygen difference. Determinations of the spectrum of the muscle are made at rest with free blood supply, during prolonged tetanic contraction (50 cycles/sec) with the blood flow occluded to give zero oxygen saturation of myoglobin, and when 100% saturation of myoglobin was obtained during perfusion of the muscle from a reservoir containing blood with 2mg% of sodium cyanide. This is used to inhibit the metabolism of the muscle and allow the intracellular oxygen tension to rise to levels which will fully saturate myoglobin. During the infusion of cyanide the outflow from the gracilis was diverted to a beaker, so that the cyanide did not enter the general circulation. That inhibition of muscle metabolism is complete as shown by the fact that there is no demonstrable A-V difference for oxygen. Higher concentrations of cyanide were used in our earlier experiments in an effort to ascertain that full inhibition would occur. This was later found to be unnecessary and in addition it was disadvantageous because it produced changes in background from an alteration in the muscle, the nature of which we were not able to determine.

Despite the choice of wavelengths to eliminate the possible effects of changes in the saturation of hemoglobin we were concerned that, under certain conditions, alterations in the concentration of total hemoglobin would occur that might disturb the determination of the oxygen saturation of myoglobin. Fortunately, we found out that the probe apparently does not "see" hemoglobin. Muscle perfused with blood or with oxygenated plasma showed no appreciable change in its spectrum. Furthermore, the intraarterial administration of indocyanine green produces no change in reflectance at a wavelength set to give maximum reflectance for this substance.

3. We have so far carried out determinations of the resting oxygen tension on the surface of the gracilis muscle of the anesthetized dog. Also, determinations were carried out within the muscle. We found no difference between surface values and values within the muscle. The average resting P_{O_2} in the muscles examined was 5.4 mm Hg (range 0.7-16.5 mm Hg). This value corresponds closely to the one found by Whalen and that found by Coburn and Mayers. The calculation of these values, however, makes use of equilibrium constants for myoglobin published in the literature, which were determined at room temperature. These constants were then adjusted, on the basis of thermodynamic considerations, to a temperature of 37°C. We are presently engaged in determining the equilibrium constant for dog myoglobin at 37°C and in the future calculations will make use of values so obtained in our laboratory. One of our major interests was the possibility of providing evidence for or against the hypothesis that intracellular oxygen tension in the muscle is the determining factor for the regulation of blood flow in response to alterations in metabolic rate. It is assumed according to this hypothesis that the intracellular oxygen tension in muscle determin the rate of production of vasoactive metabolites. These metabolites diffuse outside the cell and affect the tone of the vascular smooth muscle, thus adjusting blood flow to the prevailing

metabolic rate. We found that during tetanic contraction of the muscle produced by stimulation at rates of 50 cycles/sec intracellular P_{O_2} rapidly declined and approached zero. Our most exciting finding was the observations that for each muscle we could find a rate of contraction, usually between 0.5 and 3 cycles/sec, at which intracellular P_{O_2} actually increased. This observation makes untenable the hypothesis that regulation of blood flow during mild muscular contraction occurs primarily as a result of decreases in intracellular oxygen tension with resulting increase in the production of vasoactive metabolites. However, it does not exclude a variation of this hypothesis which would assume that the production rate of vasodilator metabolites is determined by the metabolic rate of muscle irrespective of any associated changes in oxygen tension.

Significance of This Research as Related to the Mission of U.S.A. Research and Development Command

We are studying alterations in the circulation induced by hypoxia similar to that encountered at high terrestrial altitudes such as the passes between India and China. Understanding of the mechanisms responsible for altered circulatory function appears to be a valuable basis for improved management, both preventive and therapeutic, of the illnesses of high altitude, including blurred consciousness and acute pulmonary edema.

PUBLICATIONS

I. Complete papers published

1. Kontos, H.A., Mauck, H.P., Jr., Richardson, D.W., and Patterson, J.L., Jr.: Mechanism of circulatory responses to systemic hypoxia in the anesthetized dog. American Journal Physiology. 209:397, 1965

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5. Richardson, D.W., Kontos, H.A., Raper, A.J., and Patterson, J.L., Jr.: Modification by beta-adrenergic blockade of the circulatory responses to acute hypoxia in man. Journal of Clinical Investigation. 46:77, 1967.

6. Kontos, H.A., Levasseur, J.E., Richardson, D.W., Mauck, H.P., Jr., and Patterson, J.L., Jr.: Comparative circulatory responses to systemic hypoxia in man and in unanesthetized dog. Journal of Applied Physiology. 23:381, 1967.

7. Kontos, H.A., and Lower, R.R.: Role of beta-adrenergic receptors in the circulatory response to hypoxia. American Journal of Physiology. 217:756, 1969.

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9. Kontos, H.A., Richardson, D.W., Raper, A.J., and Patterson, J.L., Jr.: Contribution of hypercapnia and hypoxia to the vascular response to ischaemia. Clinical Science. 39:203, 1970.

II. Complete papers in press

1. Levasseur, J.E., Kontos, H.A., Richardson, D.W., and Patterson, J.L., Jr.: Circulatory effects of prolonged hypoxia before and during antihistamine administration. Journal of Applied Physiology (in press).

III. Abstracts published

1. Richardson, D.W., Kontos, H.A., Shapiro, W., and Patterson, J.L., Jr.: Role of hypocapnia in circulatory responses to acute anoxia. Clinical Research. 12:192, 1964.

2. Richardson, D.W., Kontos, H.A., and Patterson, J.L., Jr.: Modification of circulatory responses to hypoxia by beta-adrenergic blockade. Clinical Research. 13:76, 1965.

3. Kontos, H.A., Levasseur, J.E., Richardson, D.W., Mauck, H.P., Jr., and Patterson, J.L., Jr.: Comparative circulatory responses to systemic hypoxia in man and unanesthetized dog. Clinical Research 15:27, 1967.

4. Kontos, H.A., and Lower, R.R.: Role of beta-adrenergic receptors in the circulatory response to hypoxia. Circulation 26 (Supple. II):164, 1967.

5. Raper, A.J., Kontos, H.A., and Patterson, J.L., Jr.: Effect of hypoxia on pial precapillary vessels. Fed. Proc. 31:401, 1972.

6. Levasseur, J.E., Kontos, H.A., Richardson, D.W., and Patterson, J.L., Jr.: Circulatory effects of prolonged hypoxia before and after antihistamine. Physiologist 15:196, 1972.