Journal: **Respiratory Physiology**

Date: **Nov 1987**
Vol: **70 (2)** Pgs: **143-58**

Article
Title: Skeletal muscle oxygen availability during respiratory acid-base disturbances in cats.

Author: Hampson NB

Imprint:

Neil Hampson (neil.hampson@vmmc.org)
42306 N Caledonia Way
Anthem, AZ

Phone: **206-817-9945**

UHMS

Respiration Physiology (1987) **70**, 143–158
Elsevier

RSP 01342

Skeletal muscle oxygen availability during respiratory acid–base disturbances in cats*

Neil B. Hampson, Frans F. Jöbsis-VanderVliet and Claude A. Piantadosi

Departments of Medicine and Physiology, Duke University Medical Center, Durham, NC 27710, U.S.A.

(Accepted for publication 12 June 1987)

Abstract. Respiratory acid–base disorders elicit physiological responses that alter O_2 delivery to various tissues. We have used a near infrared (NIR) optical technique to monitor cytochrome a,a_3 oxidation state, tissue O_2 store (relative hemoglobin plus myoglobin oxygenation), and regional blood volume in intact resting skeletal muscle during respiratory acid–base disturbances in anesthetized cats. Hypercapnic acidosis and hypocapnic alkalosis were produced in separate groups of animals by ventilation with increasing concentrations of CO_2 (n = 13) or hyperventilation (n = 8). Respiratory acidosis decreased oxygen availability to hindlimb muscle while respiratory alkalosis did not change tissue oxygenation. Inspired CO_2 progressively decreased muscle blood volume, cytochrome a,a_3 oxidation level, and muscle oxygen store. These optical responses were greatly attenuated both by pre-treatment with bretylium and by hemorrhagic hypotension, suggesting mediation through sympathetic vasoconstriction. Metabolic acidosis, produced by intravenous HCl infusion (n = 8), did not reproduce the hindlimb optical responses mediated by CO_2. These experiments demonstrate that hypercapnic acidosis significantly decreases oxygen supply to resting skeletal muscle in the anesthetized cat, probably via neuroregulatory responses to CO_2 which do not depend on changes in arterial $[H^+]$ in the tested pH range.

Cytochrome oxidase; Muscle; Near infrared spectrophotometry; Oxygen consumption

Organ blood flow and thereby, tissue oxygen availability, is redistributed in many pathophysiological states (Bond, 1982). Most experimental techniques used for measuring tissue oxygen delivery are invasive, slow, or insensitive to changes in intracellular oxidative metabolism (Heistad and Abboud, 1974). The importance of monitoring oxygen utilization at the tissue level during these pathophysiological conditions has been long recognized, but satisfactory methods have not been widely available.

Recently, near infrared (NIR) multiple wavelength spectrophotometry has been found to be a sensitive, noninvasive technique for *in vivo* assessment of tissue oxygen sufficiency during hypoxia (Jöbsis-VanderVliet, 1985). The approach, first described by Jöbsis (1977), allows continuous monitoring of regional blood volume changes,

---

* Preliminary results reported in: *Adv. Exp. Med. Biol.* 200: 523–530 (1986).

Correspondence address: Neil B. Hampson, M.D., Box 31122, Duke University Medical Center, Durham, NC 27710, U.S.A.

hemoglobin saturation, and changes in the reduction-oxidation (redox) state of the NIR copper band of cytochrome a,a_3 in intact tissue (Jöbsis-VanderVliet, 1985; Piantadosi and Jöbsis-VanderVliet, 1985; Piantadosi *et al.*, 1986). Cytochrome a,a_3 (cytochrome *c* oxidase), the terminal member of the mitochondrial respiratory chain, catalyzes the final step of electron transport to oxygen and reduces about 90% of the oxygen consumed by tissues. The optical density of cytochrome a,a_3 characteristically varies as a function of its redox level, which is itself determined by changes in the availability of oxygen, substrate, and ADP. The redox state of brain cytochrome a,a_3 monitored *in vivo* correlates well with local tissue oxygen tension (Kreisman *et al.*, 1981), while the oxidation level of the NIR band of cytochrome a,a_3 in intact skeletal muscle decreases linearly with hindlimb oxygen consumption as muscle oxygen delivery declines (Hampson *et al.*, 1986).

Regulation of blood flow and oxygen delivery to skeletal muscle has been extensively investigated because mammalian skeletal muscle comprises as much as 40% of body mass, has a blood supply that can increase 10–15 fold with exercise, and has a total oxygen consumption that can increase as much as 60 fold (Shepherd, 1983; Hudlicka, 1985). It is a primary target for vasomotor reflexes concerned with maintenance of general cardiovascular homeostasis, receiving 15–80% of the cardiac output under various conditions (Mellander and Johansson, 1968). Furthermore, physiological adjustments in oxygen transport may be considerably modified by respiratory acid–base status. Previous studies of the effects of changes in arterial P_{CO_2} on O_2 transport to resting muscle have given conflicting results (Fleishman *et al.*, 1957; Richardson *et al.*, 1961; Whalen and Nair, 1970; Goldstick, 1973; Brice and Welch, 1985). Since the invasive nature of these studies may have altered the dynamics of skeletal muscle blood flow and oxygen utilization, we monitored tissue oxygen delivery and mitochondrial oxygen availability in intact resting skeletal muscle during controlled changes in respiratory acid–base status using the noninvasive NIR optical method. Our results indicate, in agreement with Whalen and Nair (1970), that primary respiratory acidosis in the anesthetized cat decreases hindlimb muscle oxygen availability. The muscle responses to CO_2 appear to be mediated primarily via sympathetic vasoconstriction and the new NIR optical technique is a sensitive way to follow such vasomotor adjustments.

Methods

Animals. Adult cats of both sexes weighing 2–4 kg were anesthetized with intraperitoneal injections of sodium pentobarbital (38 mg/kg). A tracheal tube was inserted and polyethylene cannulae were placed in left brachial and right femoral arteries and veins. Femoral artery blood pressure was continuously monitored using a Statham P23Dc pressure transducer (Statham Instruments, Hato Rey, PR). Animals were paralyzed with gallamine triethiodide (5 mg/kg i.v.) and mechanically ventilated using a positive pressure respirator. Tidal volume was adjusted during initial ventilation with room air to maintain Pa_{CO_2} near 30 Torr, the normal Pa_{CO_2} of the awake cat (Sørensen,

1967). Anesthesia and muscular paralysis were maintained by constant infusion of sodium pentobarbital ($5\ mg \cdot kg^{-1} \cdot h^{-1}$) and gallamine triethiodide ($3.75\ mg \cdot kg^{-1} \cdot h^{-1}$) in dextrose solution (total infused volume 1 ml/h) via the brachial vein. Blood samples from the brachial arterial catheter were used to measure blood gases and pH (Model 513 pH/blood gas analyzer; Instrumentation Laboratory, Lexington, MA). The femoral venous catheter was used as necessary to infuse other agents. Rectal temperature was maintained near 37 °C with external heating. The hindlimb was immobilized and the hair removed to facilitate optical monitoring of the intact skeletal muscles.

Near infrared optical monitoring. NIR light (700–1000 nm) is transmitted with relative ease through biologic tissues, being absorbed primarily by the oxidized copper atoms of cytochrome a,a_3 and the heme moieties of oxyhemoglobin and deoxyhemoglobin (Jöbsis, 1977; Jöbsis-VanderVliet *et al.*, 1987). Differences in these three absorption spectra in the NIR range make it possible to illuminate intact tissue with selected wavelengths of NIR light and separately monitor changes in cytochrome a,a_3 redox level, tissue blood (hemoglobin) volume (tBV), and relative hemoglobin saturation (tHb_{O_2} and tHb) within the small blood vessels and capillaries (Jöbsis-VanderVliet, 1985). Preliminary experiments to monitor the copper band of cytochrome a,a_3 utilized only two wavelengths selected on the basis of the *in vitro* absorption spectrum of the purified enzyme. Subsequent tissue measurements of the three absorption spectra have led to the development of an improved multiwavelength NIR instrument capable of noninvasive, continuous trend monitoring of oxygen availability at the tissue level (Jöbsis-VanderVliet, 1985; Jöbsis-VanderVliet *et al.*, 1987). The technique has been used successfully in brain cortex (Jöbsis-VanderVliet, 1985), skeletal muscle (Piantadosi and Jöbsis-VanderVliet, 1985; Hampson *et al.*, 1986) and both tissues simultaneously (Piantadosi *et al.*, 1986).

In this study, NIR light of three wavelengths (775, 815, and 914 nm) produced by a single incandescent source and three grating monochromators (H10 V-IR, Instruments S.A., Metuchen, NJ) was pulsed with a slotted chopping wheel and conducted in sequence to the tissue by means of a fiberoptic bundle. The hindlimb muscles (primarily biceps plus gracilis) were transilluminated by applying the fiberoptic bundle gently to the skin and transmitting light through the muscles to a photomultiplier tube (Hamamatsu R936, Hamamatsu City, Japan). The photocurrents generated were sequentially integrated for each wavelength and stored for signal processing. Independent hemoglobin and cytochrome a,a_3 copper signals were derived using algorithms generated during development of the method (Jöbsis-VanderVliet *et al.*, 1987). The output signals were filtered, amplified, and displayed on a multichannel recorder.

The NIR optical signals contain only a minor contribution from skin relative to hindlimb muscle (Piantadosi *et al.*, 1986). Skin contains relatively little cytochrome a,a_3 and, therefore, would not be expected to affect monitoring of cytochrome a,a_3 oxidation level in an underlying muscle. The skin also contributes little to the NIR hemoglobin signals because the double thickness of skin between the optrodes comprises less than

10% of the interoptrode distance and its intravascular volume is small compared to that of an equal thickness of muscle. Because the NIR absorption spectra of hemoglobin and myoglobin are so similar (Churg and Makinen, 1978), the oxygenated and deoxygenated fractions of each cannot be separated *in vivo*. Optical contributions from changes in the two pigments are therefore treated as a sum when monitoring skeletal muscle tissue (tHb + Mb and tHb_{O_2} + Mb_{O_2}) (Piantadosi *et al.*, 1986). A shift in oxygen saturation by either of the two pigments cannot be analyzed in terms of its optical contribution relative to the other. For convenience, we refer to changes in the tHb_{O_2} + Mb_{O_2} signal as changes in the O_2 store. If the concentration of myoglobin in the optical field is constant over the course of the experiment, adding the two signals (Hb + Mb) + (Hb_{O_2} + Mb_{O_2}) cancels changes in myoglobin oxygenation, allowing changes in relative tissue blood (hemoglobin) volume (tBV) to be measured. This linearity with respect to changes in concentration is a result of expressing the data in log form.

The three algorithms used are of the general form:

$$\Delta[m] = a\ (\Delta OD_{775\ nm}) + b\ (\Delta OD_{815\ nm}) + c\ (\Delta OD_{914\ nm}),$$

where [m] is the concentration of the molecular species measured; a, b and c are weighting coefficients; and ΔOD is the total change in optical density at that wavelength. The term variation in density (v/d) has been substituted for optical density in the solution for each of the three algorithms because different weighting coefficients are used at each of the three measuring wavelengths. Therefore, a change in the sum of the three weighted factors in each algorithm is not a true optical density change. However, variations in density are expressed in log form and, therefore, the units are proportional to the concentrations of the molecular species being monitored. Although absolute concentration cannot be measured because of unquantified variables such as optical path length, changes in relative concentration caused by experimental manipulations can be compared between animals in terms of v/d units with the understanding that these biophysical variables are affected by changes in light scattering arising from minor differences in the geometric arrangement of the optical fibers between experiments. One v/d unit has been defined as a 10-fold change in a signal computed through its algorithm (Jöbsis-VanderVliet, 1985).

Changes in the NIR signals also may be expressed by dividing the signal change produced with a specific metabolic transition by the total signal for that experiment. This approach normalizes all data to fractions or percentages of a total labile signal (TLS) and is useful to compare the cytochrome redox responses from different experiments (Piantadosi *et al.*, 1986). In the present experiments, TLS has been defined as the signal difference between the baseline cytochrome a,a_3 oxidation level at normoxic control (100% oxidation) and complete cytochrome a,a_3 reduction after death (0% oxidation). When an exact TLS cannot be determined, as often happens for the hemoglobin signals, optical data are reported only in relative optical units (v/d).

Experimental protocols. Baseline optical and physiological parameters were measured while the animals were ventilated with room air. After establishing stable optical baselines, separate groups of animals were exposed to hypercapnic acidosis (n = 13) and hypocapnic alkalosis (n = 8). Hypercapnia was produced using progressively increasing concentrations of inspired carbon dioxide (3%, 5%, 10%, and 15%) at constant oxygen concentration (20% O_2, balance N_2). Each CO_2 exposure was 15 min long, a time period sufficient for stabilization of the optical signals at new steady states. Because of the results of the CO_2 exposures, metabolic acidosis was induced in 8 separate cats by intravenous infusion of 0.25 N HCl at a rate of approximately 3.5 meq $\cdot$ kg^{-1} $\cdot$ h^{-1}. This infusion lowered arterial pH to 7.00–7.07 over 60 to 90 min. Animals were ventilated with room air throughout the acid infusion. After achieving the desired degree of acidosis, arterial pH was normalized with intravenous sodium bicarbonate (1 meq/ml). These animals were then ventilated with 15% CO_2 (20% O_2, balance N_2) prior to terminating the experiments.

Six additional animals were exposed to hypercapnic acidosis before and after the administration of the sympatholytic drug, bretylium tosylate (Bretylol; Arnar-Stone Laboratories, McGaw Park, IL). Animals were ventilated with 15% CO_2 (20% O_2, balance N_2) for 15 min and then returned to room air. After recovery, intravenous bretylium (10 mg/kg in 5 ml normal saline) was infused over 20 min. The animals were allowed to stabilize for 60 min and then exposed to a second hypercapnic stress. This bretylium protocol is sufficient to produce complete sympathetic blockade in the cat (Boura and Green, 1959).

Separate heparinized animals were exposed to CO_2 before and after controlled arterial hemorrhage to demonstrate that baroreceptor responses would override the CO_2 response. These cats were ventilated with 15% CO_2 (20% O_2, balance N_2) for 10 min, returned to room air ventilation, bled 1.0 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} to a total of 20 ml/kg, and again ventilated with the same hypercapnic gas mixture for 10 min.

Hypocapnia was produced separately by increasing ventilatory frequency to a rate sufficient to lower arterial P_{CO_2} to less than half of control value. Hypocapnia was maintained for 30 min, and then the arterial P_{CO_2} was returned to normal by decreasing the respiratory frequency. As a control experiment, NIR monitoring was performed in 4 other animals while arterial pH was increased by infusing sodium bicarbonate (1 meq/ml) intravenously at a rate chosen to raise pHa over 60 to 100 min to 7.60 or above.

Statistical analysis. Summarized data for groups of animals in specific protocols are presented as mean $\pm$ standard error of the mean. When appropriate, statistical significance of change from baseline control value was tested using Student's *t*-test. One-way ANOVA was utilized to determine the significance of successive changes caused by graded hypercapnic acidosis. A P value of < 0.05 was accepted as significant.

Results

Physiological parameters measured during baseline periods and after each acid–base perturbation are shown in table 1. Stepwise increases in inspired CO_2 in hypercapnia experiments decreased pHa by an average of 0.42 units. This pH effect was closely reproduced in control acid infusion experiments. Hyperventilation raised pHa by an average of 0.21 units. This effect was also achieved by sodium bicarbonate infusions in four control alkalosis experiments. Arterial P_{CO_2} remained stable during both metabolic acidosis and alkalosis protocols. Hypocapnic alkalosis was the only perturbation that produced a significant change in Pa_{O_2} from baseline. Mean arterial blood pressure (MABP) progressively rose during hypercapnia, fell with hyperventilation, and did not change during metabolic acidosis or alkalosis. Rectal temperature remained near 37 °C throughout all experiments.

Hypercapnic acidosis significantly decreased the oxidation level of muscle cytochrome a,a_3. In fact, increases in arterial carbon dioxide tension resulted in rapid progressive changes in all 3 NIR signals recorded from the intact skeletal muscles.

TABLE 1

Arterial blood gas tensions, pH, and mean arterial blood pressure in experimental acid–base disturbances in cats.

Experimental group	pHa	Pa_{CO_2} (mm Hg)	Pa_{O_2} (mm Hg)	MABP (mm Hg)
Hypercapnic acidosis (n = 13)				
Baseline	7.38 ± 0.01	27.7 ± 1.1	102.2 ± 3.4	111 ± 11
3% CO_2 15 min	7.27 ± 0.01*	35.9 ± 1.1*	103.5 ± 3.3	120 ± 11*
5% CO_2 15 min	7.20 ± 0.01*	45.6 ± 1.3*	103.3 ± 2.5	127 ± 11*
10% CO_2 15 min	7.07 ± 0.01*	65.6 ± 2.1*	104.3 ± 2.2	137 ± 11*
15% CO_2 15 min	6.96 ± 0.01*	93.6 ± 2.2*	102.7 ± 2.3	158 ± 12*
Metabolic acidosis (n = 8)				
Baseline	7.33 ± 0.01	29.1 ± 0.9	89.5 ± 3.3	112 ± 7
After HCl infusion	7.03 ± 0.01*	31.4 ± 1.1	91.1 ± 5.1	114 ± 9
After pH normalization	7.33 ± 0.01	30.7 ± 0.6	89.2 ± 3.7	110 ± 9
15% CO_2 15 min	6.96 ± 0.01*	88.8 ± 3.5*	92.6 ± 3.6	154 ± 5*
Hypocapnic alkalosis (n = 8)				
Baseline	7.39 ± 0.01	28.5 ± 0.4	102.3 ± 1.8	115 ± 11
Hyperventilation	7.60 ± 0.02*	13.5 ± 0.5*	124.2 ± 2.7*	91 ± 10*
Metabolic alkalosis (n = 4)				
Baseline	7.39 ± 0.01	26.8 ± 0.4	109.6 ± 5.6	135 ± 4
After $NaHCO_3$ infusion	7.60 ± 0.01	29.7 ± 0.7	102.4 ± 8.1	134 ± 8

Values are mean ± SEM for each group of animals. Values noted by * are significantly different ($P < 0.05$) from preceding baseline value.

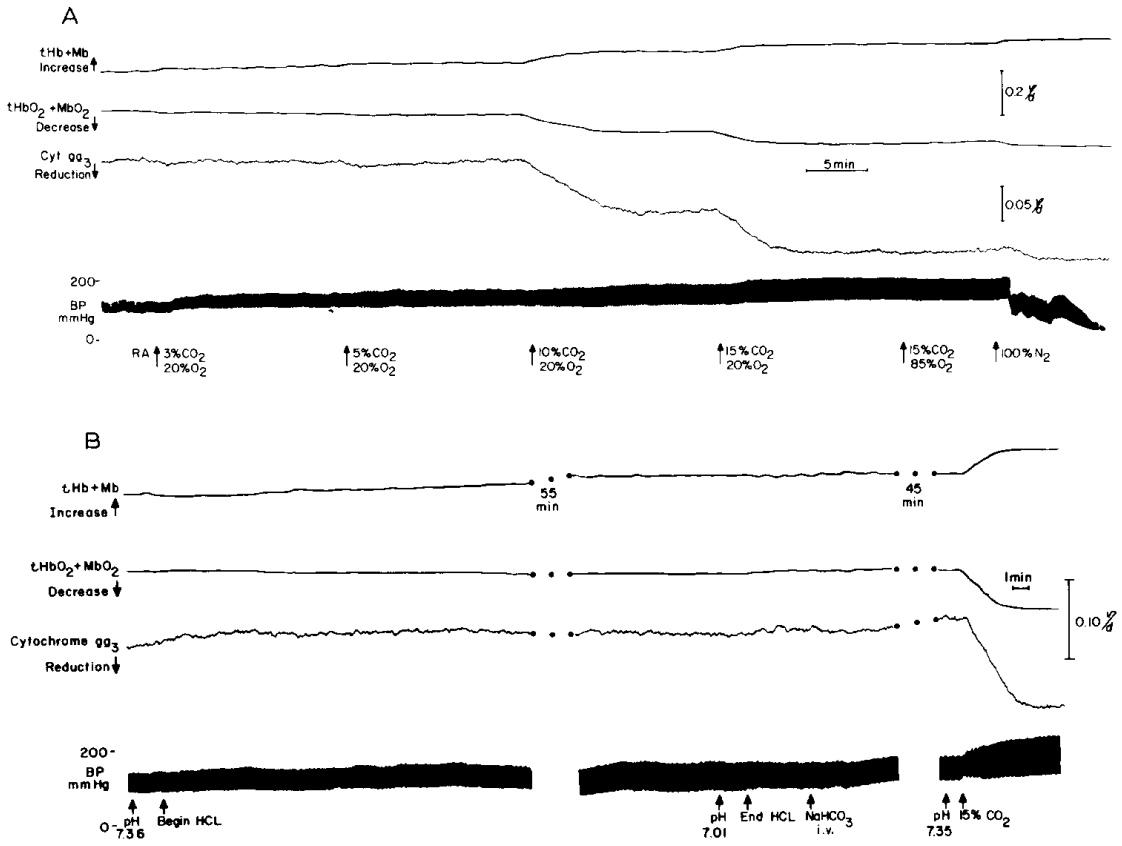

Fig. 1. (A) Near infrared optical and blood pressure responses to progressive hypercapnia in a typical animal. Definition of abbreviations: tHb + Mb = tissue deoxyhemoglobin plus deoxymyoglobin, tHb_{O_2} + Mb_{O_2} = tissue oxyhemoglobin plus oxymyoglobin, BP = arterial blood pressure, v/d = variation in density. (B) Near infrared optical and blood pressure responses to metabolic acidosis followed by hypercapnic acidosis in a typical animal. For definition of abbreviations, see panel A.

Figure 1A shows optical responses to graded hypercapnia in the hindlimb muscles of a typical animal. Hypercapnia increased the relative amount of tHb + Mb and simultaneously decreased the amounts of tHb_{O_2} + Mb_{O_2} and oxidized cytochrome a,a_3 in intact muscle tissue. Optical responses from a typical animal during hydrochloric acid infusion are shown in fig. 1B for comparison. In contrast to the changes seen with hypercapnic acidosis, tHb + Mb, tHb_{O_2} + Mb_{O_2}, and cytochrome a,a_3 signals all remained stable during acid infusion. After pH normalization, the NIR optical parameters responded normally to 15% CO_2 ventilation, demonstrating preservation of the muscle response to hypercapnia.

Small changes in skeletal muscle cytochrome a,a_3 redox level in the direction of enzyme oxidation were seen during the hypocapnia studies, but were not statistically significant. Hypocapnic alkalosis did not produce changes in the muscle hemoglobin or myoglobin signals. Neither control group (*i.e.* metabolic acidosis and alkalosis) showed significant changes in the optical parameters, although progressive metabolic alkalosis did cause a small increase in tissue blood volume. Mean changes in cytochrome a,a_3 oxidation level in all four types of protocols are summarized in fig. 2.

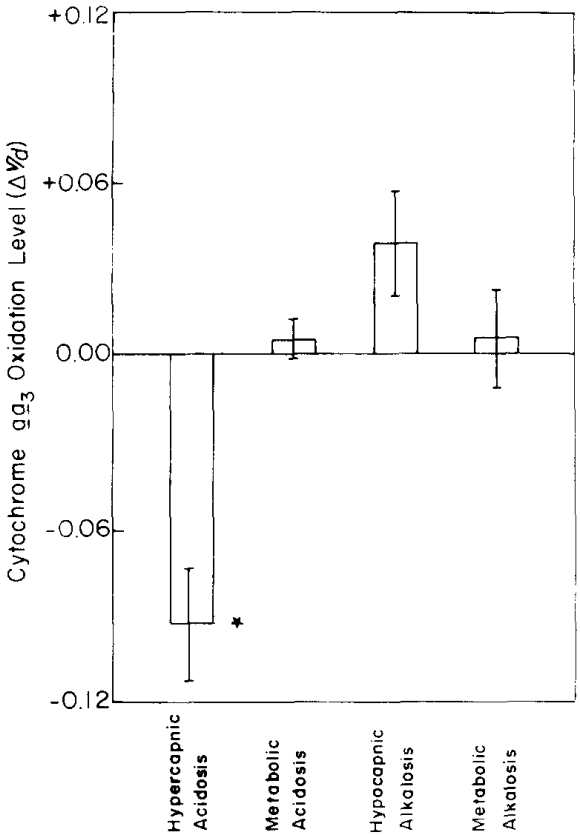

Fig. 2. Change in skeletal muscle cytochrome a,a_3 oxidation level during respiratory acid–base disturbances. Control data for metabolic pH changes are also provided for comparison. The data are means ± SEM (* = $P < 0.01$). Definition of abbreviation: v/d = variation in density (explained in Methods).

Figure 3A summarizes hemoglobin, myoglobin, and blood volume optical data from 13 hypercapnia experiments. Statistically significant increases in tHb + Mb and decreases in tHb_{O_2} + Mb_{O_2} occurred at each level of hypercapnia. Tissue blood volume (tBV) in the muscle also fell progressively with increasing hypercapnia. Decreases in cytochrome a,a_3 oxidation level caused by hypercapnia are displayed in fig. 3B as a percentage of the total labile signal between normoxic, normocapnic baseline and death. Increasing hypercapnic acidosis produced a progressive fall in skeletal muscle cytochrome a,a_3 oxidation level, reaching a minimum value of 57.5 ± 5.7% below control during ventilation with 15% CO_2 ($P < 0.001$) (fig. 3B).

The effects of hypercapnia on skeletal muscle after systemic administration of bretylium were studied in separate animals. Physiological data from these six experiments are summarized in table 2. Arterial pH and P_{CO_2} responses to ventilation with 15% CO_2 in room air were similar before and after bretylium administration. As in previous hypercapnia experiments, ventilation with CO_2 increased MABP above normocapnic values in intact animals (122 ± 7 to 153 ± 7 mm Hg; $P < 0.01$). After bretylium, however, hypercapnia caused MABP to fall from 114 ± 4 to 102 ± 8 mm Hg in the same animals ($P < 0.05$). Figure 4 shows optical responses from a representative

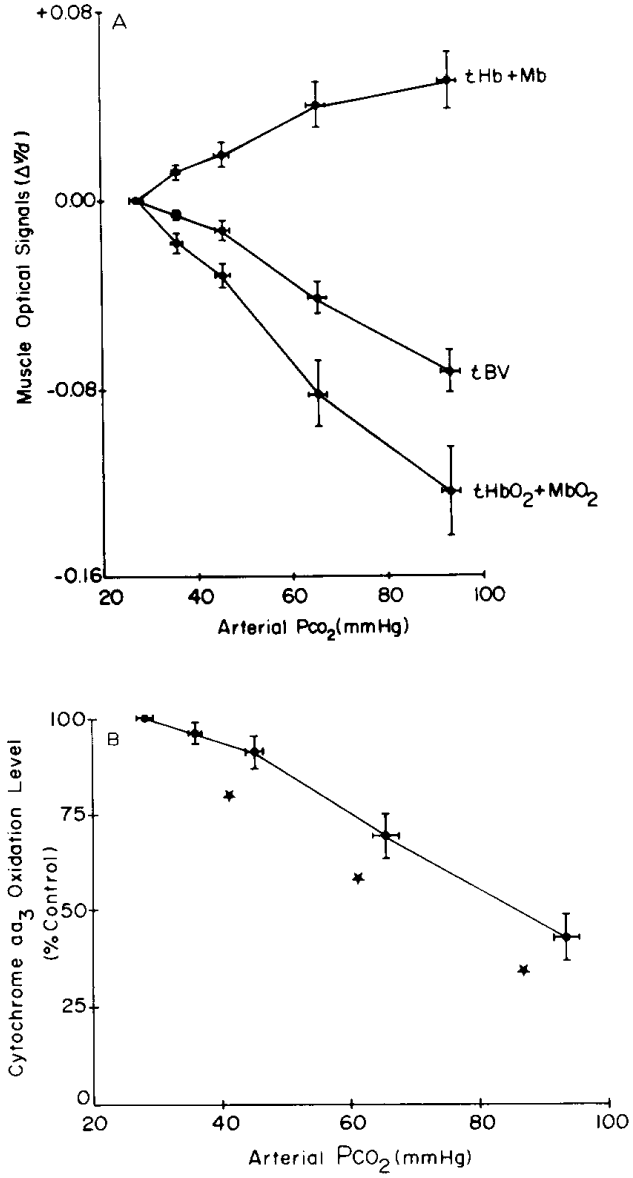

Fig. 3. (A) Change in skeletal muscle deoxymyoglobin plus deoxyhemoglobin (tHb + Mb), oxyhemoglobin plus oxymyoglobin (tHb_{O_2} + Mb_{O_2}), and tissue blood volume (tBV) as a function of hypercapnia. The data are the means ± SEM of results from 13 animals. All points represent significant changes ($P < 0.025$) from preceding values. (B) Change in skeletal muscle cytochrome a,a_3 oxidation level as a function of hypercapnia. The data are the means ± SEM of results from 13 animals. Values denoted by * represent significant changes ($P < 0.05$) from preceding value.

experiment and fig. 5 displays summarized NIR optical data from the 6 animals. Ventilation with CO_2 prior to bretylium increased resting muscle tHb + Mb, decreased tHb_{O_2} + Mb_{O_2}, decreased tBV, and decreased cytochrome a,a_3 oxidation level. These responses were attenuated after treatment with intravenous bretylium.

The muscle responses to CO_2 were also attenuated when the animal's blood volume was decreased by hemorrhage before CO_2 exposure. A representative experiment is shown in fig. 6 and demonstrates typical muscle optical responses to hypercapnia before

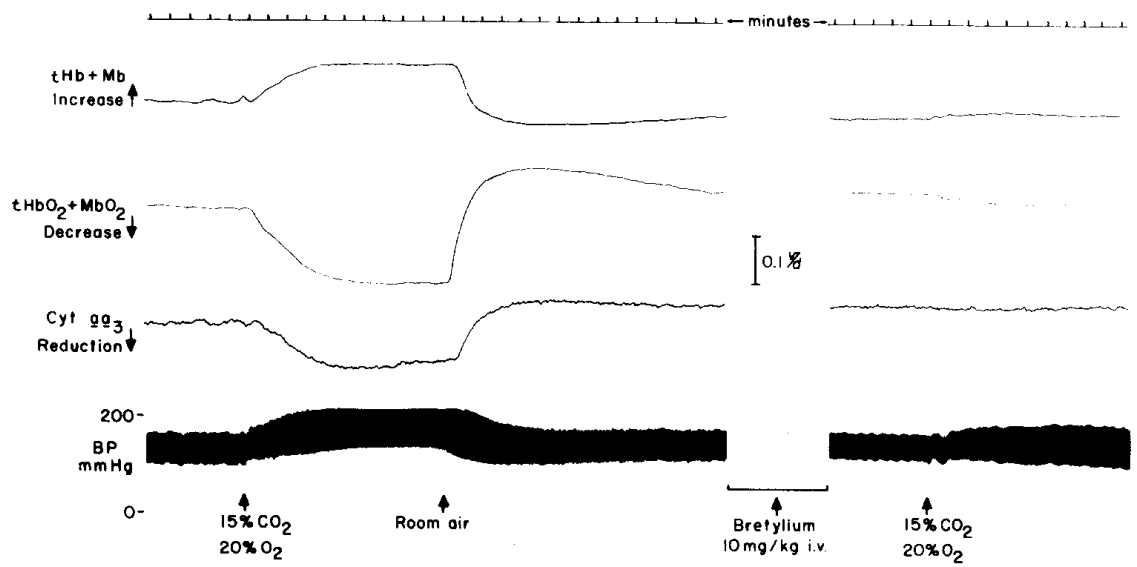

Fig. 4. Near infrared optical and blood pressure responses to hypercapnia before and after intravenous administration of bretylium tosylate in a typical animal. For definition of abbreviations, see fig. 1A.

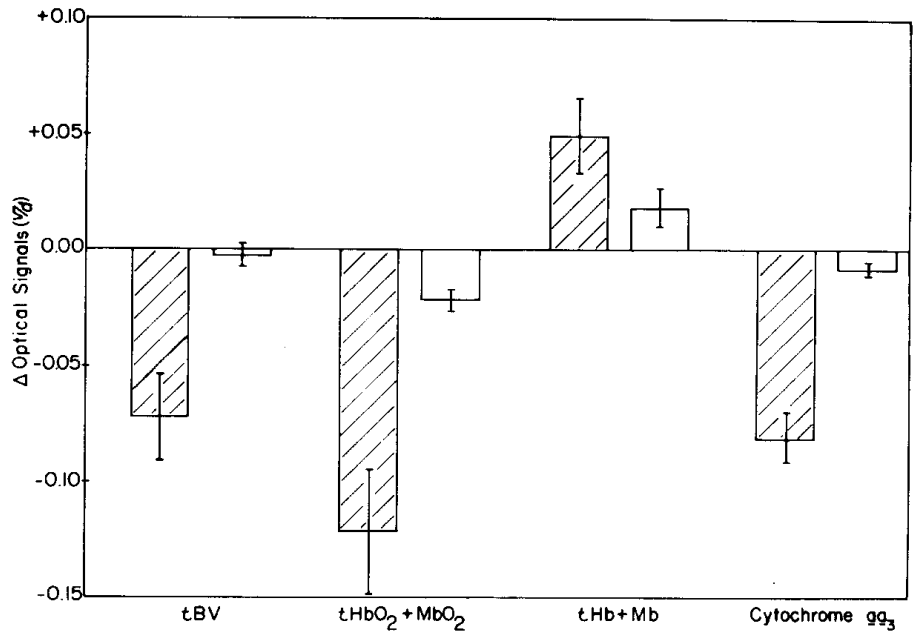

Fig. 5. Summarized optical responses to hypercapnia from hindlimb skeletal muscle of 6 animals before (hatched bars) and after (open bars) administration of bretylium tosylate. The data are means ± SEM of changes from baseline due to hypercapnia. For cytochrome a,a_3, a positive change represents an increase in oxidation level and a negative change a decrease. Attenuation of responses following bretylium is statistically significant ($P < 0.05$) for all optical parameters except tHb + Mb. For definition of abbreviations, see fig. 3A.

TABLE 2

Arterial blood gas tensions, pH, and mean arterial blood pressure in cats exposed to hypercapnia before and after administration of bretylium.

Ventilation	pHa	Pa_{CO_2} (mm Hg)	Pa_{O_2} (mm Hg)	MABP (mm Hg)
Baseline room air	7.36 ± 0.02	29.2 ± 1.4	96.2 ± 5.3	122 ± 7
15% CO_2 15 min	7.01 ± 0.01*	78.1 ± 1.3*	90.2 ± 4.2	153 ± 7*
RA after bretylium	7.35 ± 0.03	28.6 ± 1.2	88.0 ± 5.9	114 ± 4
15% CO_2 15 min	7.01 ± 0.01*	77.0 ± 1.1*	84.2 ± 4.8	102 ± 8*

Values are mean ± SEM for 6 animals. Values noted by * are significantly different ($P < 0.05$) than immediately preceding baseline value.

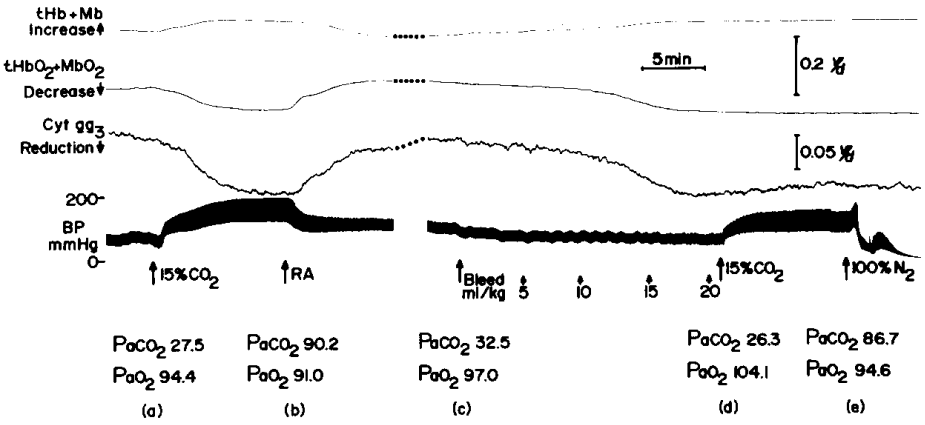

Fig. 6. Near infrared optical and blood pressure responses to hypercapnia before and after arterial hemorrhage demonstrating ablation of the muscle response to CO_2 by hypovolemia. Values for Pa_{CO_2} and Pa_{O_2} are reported during initial ventilation with room air (RA) (a), after 10 min of ventilation with 15% CO_2 in RA (b), during subsequent RA ventilation (c), following 20 ml/kg hemorrhage (d), and after a subsequent 10 min period of 15% CO_2 ventilation (e). For definition of other abbreviations, see fig. 1A.

hemorrhage (Piantadosi *et al.*, 1986). After 20 ml/kg of hemorrhage, MABP had fallen to 48 mm Hg, accompanied by an increase in tHb + Mb, a decrease in tHb_{O_2} + Mb_{O_2}, and a decrease in cytochrome a,a_3 oxidation level. Neither ventilation with 15% CO_2 in air nor ventilation with N_2 subsequently decreased muscle cytochrome a,a_3 oxidation level or further changed the hemoglobin + myoglobin signals.

Discussion

NIR optical monitoring is a relatively new way to noninvasively assess oxygen sufficiency in intact tissues. The technique is a qualitative trend monitor based upon the characteristic of cytochrome a,a_3 oxidation level to vary as a function of tissue

oxygenation *in vivo* (Jöbsis, 1974; Kreisman *et al.*, 1981). The NIR band of the copper atom, which is redox-linked to cytochrome *a*, also undergoes continuous, O_2-dependent redox adjustments. Although there is no available standard against which to compare these *in situ* redox events, the technique applies algorithms that accurately describe the separate absorbance spectra of Hb, Hb_{O_2} and cytochrome a,a_3. These spectra, readily obtained *in vitro*, have been identified *in vivo* by independently varying the concentrations of each of the three molecular species. Such *in vivo* spectra vary slightly from the *in vitro* because of minor light scattering by tissue in the NIR region. Using the *in vivo* spectra to derive the algorithms minimizes these light scattering effects. Each algorithm can then be tested by varying the concentration of one or both of the other two species by physiological manipulations which do not affect the third. The ability of the algorithms to generate specific biological signals is often confirmed from independent responses within particular experiments. Examples of this can be seen in figs. 1B and 4 where the hemoglobin + myoglobin signals move independently from the cytochrome signal and vice versa.

In the present experiments, hypercapnic acidosis was the only acid–base disturbance that produced significant changes in cytochrome a,a_3 redox state in intact resting skeletal muscle. Carbon dioxide progressively decreased muscle cytochrome a,a_3 oxidation level, a change that could be explained by several mechanisms. Hypercapnia could have decreased available mitochondrial oxygen relative to electron flow through the cytochrome chain, increased electron flow relative to available oxygen, or decreased in the affinity of cytochrome oxidase for O_2. A CO_2-related change in the O_2 affinity of the enzyme is unlikely in part because it is without precedence with respect to CO_2. The current optical evidence also indicates that CO_2-mediated muscle cytochrome a,a_3 redox responses may be explained largely by decreased oxygen delivery to the tissue. During hypercapnia, both $tHb_{O_2} + Mb_{O_2}$ and tBV decreased, suggesting that less intracellular availability of oxygen caused the decrease in cytochrome a,a_3 redox level.

Previous investigations of the influence of acid–base disturbances on skeletal muscle blood flow and oxygen delivery frequently evaluated only local effects of pH and CO_2 in efforts to describe the mechanisms of functional hyperemia. This work has been summarized recently in excellent reviews (Shepherd, 1983; Hudlicka, 1985). It is well known, however, that blood flow to innervated skeletal muscle is under both local and central control with net regulation resulting from the interplay between these often opposing mechanisms (Heistad and Abboud, 1974; Shepherd, 1983). Previous studies investigating blood flow to innervated muscle or limb have reported that hypercapnic acidosis causes total flow to decrease (Goldstick, 1973) or remain unchanged (Fleishman *et al.*, 1957; Richardson *et al.*, 1961). Systemic hypocapnic alkalosis has been variously reported to increase (Richardson *et al.*, 1961; Goldstick, 1973), decrease (Brice and Welch, 1985), or not affect intact muscle or limb blood flow (Fleishman *et al.*, 1957). Systemic metabolic acid–base disturbances have equally unclear effects, with fewer reports in the literature. Earlier studies used diverse and sometimes traumatic techniques which could be expected to contribute to disparate conclusions regarding the effect of pH and CO_2. Techniques such as plethysmography are noninvasive but can

be non-specific as well, combining, for example, changes in skin and muscle blood flow when monitoring a limb (Heistad and Abboud, 1974).

Even sensitive and specific measurements of single organ or tissue blood flow may not adequately assess 'nutritional' blood flow (Heistad and Abboud, 1974). Total organ blood flow will not be a true indicator of nutritional perfusion if both nutritive and non-nutritive circulations exist within a tissue. Effective non-nutritional blood flow in skeletal muscle happens under a variety of physiological circumstances (Hyman *et al.*, 1959; Rosell and Uvnäs, 1962), although there is little evidence for true anatomic arteriovenous shunts in skeletal muscle. These observations lead to concepts of 'physiological' or 'functional' shunting which can occur through vessels of any size having reduced exchange capacity (Renkin and Rosell, 1962; Mellander and Johansson, 1968). Thus, stimuli that increase total blood flow to a tissue may have very different effects on nutritional blood flow (Rosell and Uvnäs, 1962; Heistad and Abboud, 1974), making it necessary to assess nutritive flow if the impact of changes in oxygen delivery are to be known accurately. This problem may be alleviated in part by the ability of NIR multiwavelength spectrophotometry to monitor changes in both the amount and oxygenation of hemoglobin within a tissue. Our NIR data indicating that systemic hypercapnic acidosis reduces muscle tissue oxygen availability are consistent with the results of Goldstick (1973) and with microelectrode observations that tissue P_{O_2} falls in cat skeletal muscle with hypercapnia (Whalen and Nair, 1970). In the latter study, hypercapnia caused muscle venous P_{O_2} to rise as muscle P_{O_2} fell, further supporting the concept of functional shunting in the tissue. The relation between decreasing blood volume and falling cytochrome a,a_3 oxidation level with hypercapnia in the present study also supports the concept that the NIR signal correlates well with muscle nutrient vessel flow (Piantadosi *et al.*, 1986).

Ventilation with CO_2 in these animals caused both hypercarbia and systemic acidosis; therefore, the reduction responses in muscle cytochrome a,a_3 could be attributed either to increases in CO_2 or increases in $[H^+]$ ion. Simple metabolic acidosis, however, did not change the NIR optical signals significantly, indicating that carbon dioxide itself modified the muscle oxidative metabolic parameters. Effects of hypercapnic acidosis in other tissues have similarly been ascribed to CO_2 (Lockett, 1967; McGinn *et al.*, 1967). The reduction in muscle nutrient vessel perfusion was not attributable to changes in systemic pressure because arterial blood pressure progressively rose with increasing hypercapnia, as expected (Goldstick, 1973). This increase in pressure occurs when CO_2 stimulates carotid, aortic, and central chemoreceptors, leading to constriction of sympathetic resistance vasculature (Downing *et al.*, 1963; Parker *et al.*, 1975). The importance of adrenergic stimulation to the muscle responses to hypercapnia was clearly shown by the great attenuation of the CO_2 response after administration of the peripheral sympatholytic agent, bretylium tosylate. Sympathetic vasoconstrictor nerve stimulation also has been shown to decrease outward capillary filtration rate and reduce resting oxygen consumption in muscle (Renkin and Rosell, 1962). This sympathetic effect probably decreases the number of open nutrient capillaries and is consistent with the effect of hypercapnia demonstrated in our studies. The skeletal muscle effects of

hypercapnic acidosis were also attenuated in these experiments by hemorrhagic hypotension. Hemorrhage is a potent stimulus for sympathetic vasoconstriction in skeletal muscle (Bond, 1982), and thus our results provide further evidence that the response of muscle to hypercapnia is mediated through a sympathetic mechanism. It should also be noted that the sympathetic responses to CO_2 may have been exaggerated in our animals for two reasons. First, controlled ventilation during hypercapnia may have prevented vasodilator reflexes normally activated during spontaneous lung inflation. Second, it is remotely possible that barbiturate-anesthesia suppressed putative vasodilator responses more than vasoconstrictor responses to CO_2.

It is noteworthy that metabolic alkalosis caused a small increase in muscle blood volume without changing cytochrome a,a_3 oxidation level during a small number of control studies. The mechanism for this increase in tissue blood volume is not clear but could relate to the depressant effect of metabolic alkalosis on carotid body chemoreceptor activity (Pokorski and Lahiri, 1983). Sympathetic fibers innervating vessels to muscle show tonic activity at rest (Mellander and Johansson, 1968) and a decrease in their discharge rate may increase the number of open arterioles and/or capillaries (Chen *et al.*, 1981). Possible reasons for the oxidation level of cytochrome a,a_3 not to increase with blood volume include (1) skeletal muscle cytochrome a,a_3 is near-totally oxidized at rest, (2) the increase in blood volume seen was non-nutrient, (3) an intracellular effect of metabolic alkalosis counterbalanced an increase in oxygen delivery to prevent further oxidation, or (4) net oxygen availability did not actually increase because of an increase in the oxygen affinity of hemoglobin due to alkalosis. Further studies will be necessary to discern among these possibilities. Interestingly, this effect was not seen during hyperventilation, although the Pa_{O_2} actually rose and MABP declined in those experiments. This divergence in cytochrome a,a_3 and tissue blood volume changes not only raises several interesting questions about tissue oxygen utilization, but once again confirms the independent behavior of the NIR cytochrome signal.

In summary, noninvasive NIR multiple wavelength spectrophotometry was found to be sensitive to changes in muscle tissue oxygenation during respiratory acidosis produced by high levels of inspired CO_2. We have demonstrated in intact resting cat skeletal muscle that (1) hypercapnic acidosis decreases muscle blood volume, O_2 store and cytochrome a,a_3 oxidation level in anesthetized cats, (2) the decrease in muscle cytochrome a,a_3 oxidation level suggests a decrease in intracellular oxygen supply, and (3) this response is mediated via sympathetic activation by CO_2.

Acknowledgements. The writers thank Craig D. Marshall, Tom M. Hemstreet, and Todd D. Slayton for their excellent technical assistance in this project. We also thank Ms. Margaret Grafton for preparing the illustrations for this manuscript. This paper was supported in part by NHLBI Grant N01-HR-34002 and by a grant from R.J. Reynolds, Co.

References

Bond, R. F. (1982). A review of skin and muscle hemodynamics during hemorrhagic hypotension and shock. *Adv. Shock Res.* 8: 53–70.

Boura, A. L. A. and A. F. Green (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Br. J. Pharmacol.* 14: 536–548.

Brice, A. G. and H. G. Welch (1985). Effect of respiratory alkalosis on skeletal muscle metabolism in the dog. *J. Appl. Physiol.* 58: 658–664.

Chen, I. I. H., R. L. Prewitt and R. F. Dowell (1981). Microvascular rarefaction in spontaneously hypertensive rat cremaster muscle. *Am. J. Physiol.* 241: H306–H310.

Churg, A. K. and M. W. Makinen (1978). The electronic structure and coordination geometry of the oxyheme complex in myoglobin. *J. Chem. Phys.* 68: 1913–1925.

Downing, S. E., J. H. Mitchell and A. G. Wallace (1963). Cardiovascular responses to ischemia, hypoxia, and hypercapnia of the central nervous system. *Am. J. Physiol.* 204: 881–887.

Fleishman, M., J. Scott and F. J. Haddy (1957). Effect of pH change upon systemic large and small vessel resistance. *Circ. Res.* 5: 602–606.

Goldstick, T. K. (1973). Oxygen autoregulation in skeletal muscle. *Adv. Exp. Med. Biol.* 37A: 441–449.

Hampson, N. B., P. A. Lee, F. F. Jöbsis-VanderVliet and C. A. Piantadosi (1986). Skeletal muscle cytochrome a,a_3 oxidation level correlates with hindlimb oxygen consumption during controlled hemorrhage in cats. *Clin. Res.* 34: 412A.

Heistad, D. D. and F. M. Abboud (1974). Factors that influence blood flow in skeletal muscle and skin. *Anesthesiology* 41: 139–156.

Hudlicka, O. (1985). Regulation of muscle blood flow. *Clin. Physiol.* 5: 201–229.

Hyman, C., S. Rosell, A. Rosen, R. R. Sonnenschein and B. Uvnäs (1959). Effects of alterations of total muscular blood flow on local tissue clearance of radio-iodide in the cat. *Acta Physiol. Scand.* 46: 358–374.

Jöbsis, F. F. (1974). Intracellular metabolism of oxygen. *Am. Rev. Respir. Dis.* 110 (Suppl.): 58–63.

Jöbsis, F. F. (1977). Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science* 198: 1264–1267.

Jöbsis-VanderVliet, F. F. (1985). Noninvasive, near infrared monitoring of cellular oxygen sufficiency *in vivo*. *Adv. Exp. Med. Biol.* 191: 833–842.

Jöbsis-VanderVliet, F. F., C. A. Piantadosi, A. L. Sylvia, S. K. Lucas and J. H. Keizer (1987). Near infra red monitoring of cerebral oxygen sufficiency: I. Spectra of cytochrome *c* oxidase. *Neurol. Res.* (in press).

Kreisman, N. R., T. J. Sick, J. C. LaManna and M. Rosenthal (1981). Local tissue oxygen tension – cytochrome a,a_3 redox relationships in rat cerebral cortex *in vivo*. *Brain Res.* 218: 161–174.

Lockett, M. F. (1967). Effects of changes of P_{O_2}, P_{CO_2}, and pH on the total vascular resistance of perfused cat kidneys. *J. Physiol. (London)* 193: 671–678.

McGinn, F. P., D. Mendel and P. M. Perry (1967). The effects of alteration of CO_2 and pH on intestinal blood flow in the cat. *J. Physiol. (London)* 192: 669–680.

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

Parker, P. E., J. M. Dabney, J. B. Scott and F. J. Haddy (1975). Reflex vascular responses in kidney, ileum, and forelimb to carotid body stimulation. *Am. J. Physiol.* 228: 46–51.

Piantadosi, C. A. and F. F. Jöbsis-VanderVliet (1985). Near infrared optical monitoring of intact skeletal muscle during hypoxia and hemorrhagic hypotension in cats. *Adv. Exp. Med. Biol.* 191: 855–862.

Piantadosi, C. A., T. M. Hemstreet and F. F. Jöbsis-VanderVliet (1986). Near-infrared spectrophotometric monitoring of oxygen distribution to intact brain and skeletal muscle tissues. *Crit. Care Med.* 14: 698–706.

Pokorski, M. and S. Lahiri (1983). Relative peripheral and central chemosensory responses to metabolic alkalosis. *Am. J. Physiol.* 245: R873–R880.

Renkin, E. M. and S. Rosell (1962). The influence of sympathetic adrenergic vasoconstrictor nerves on transport of diffusible solutes from blood to tissues in skeletal muscle. *Acta Physiol. Scand.* 54: 223–240.

Richardson, D.W., A.J. Wasserman and J.L. Patterson (1961). General and regional circulatory responses to change in blood pH and carbon dioxide tension. *J. Clin. Invest.* 40: 31–43.

Rosell, S. and B. Uvnäs (1962). Vasomotor nerve activity and oxygen uptake in skeletal muscle of the anesthetized cat. *Acta Physiol. Scand.* 54: 209–222.

Shepherd, J.T. (1983). Circulation to skeletal muscle. In: Handbook of Physiology. Section 2. Vol. III, edited by J.T. Shepherd and F.M. Abboud. Baltimore, MD, Williams and Wilkins, pp. 319–370.

Sørensen, S.C. (1967). Arterial P_{CO_2} in awake cats calculated from gas tensions in subcutaneous pockets. *Respir. Physiol.* 3: 261–265.

Whalen, W.J. and P. Nair (1970). Skeletal muscle P_{O_2}: effect of inhaled and topically applied O_2 and CO_2. *Am. J. Physiol.* 218: 973–980.