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NL-339 (3/77)

A PERFUSED RAT HINDQUARTER SYSTEM FOR EXAMINING THE RELATIONSHIP BETWEEN MUSCLE TEMPERATURE AND OXYGEN UPTAKE

by

Harold William Burton

A Thesis
submitted to the Faculty of Graduate Studies
through the Faculty of
Human Kinetics in Partial Fulfillment
of the requirements for the Degree
of Master of Human Kinetics at
The University of Windsor

Windsor, Ontario, Canada

1978

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DEDICATION

To Mom

ACKNOWLEDGEMENTS

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ABSTRACT

A perfused rat hindquarter system was developed to examine the specific interrelationship between muscle temperature and oxygen uptake. Twenty-five male, Wistar rats were divided into five equal groups. Each group was examined for oxygen uptake under one of the following muscle temperature conditions: 36, 38, 40, 42 or 44°C. The data at 42 and 44°C were not acceptable because of marked tissue edema which led to increased hemicorpus weight and frequently low PaO_2 values which resulted in a disruption in the normal resting state of the muscle. The results from the remaining three groups (36, 38 and 40°C) indicated a significant increase ($P < .01$) in muscle oxygen uptake in response to elevations in temperature. In addition the curve that represented this increased oxygen consumption was quadratic. This information suggests that at a critical temperature level some mechanism within the muscle cell triggers an increased rate of oxygen consumption. These data have implications toward future calculations of post-exercise oxygen consumption. Since muscle temperature is elevated during recovery from exercise the relative contribution of the resulting increased levels of mitochondrial respiration to the total recovery oxygen uptake must be considered.

CHAPTER I

INTRODUCTION

At the onset of exercise the working muscle quickly exhausts its available oxygen, ATP and CP stores and must rely primarily on the glycolytic production of ATP for muscle contraction. Before a steady level of oxygen uptake is achieved the muscle accumulates an energy deficit that must be repaid after the exercise is terminated (5, 19, 20, 29, 34, 35, 52). Post exercise oxygen consumption does not return immediately to resting levels but remains elevated for a period of time, depending on the intensity and duration of the exercise (20, 29, 34, 35, 52). The rate of recovery oxygen uptake declines rapidly at first and then more slowly, finally approaching resting levels.

This phenomenon was designated as oxygen debt when first identified (20) and was separated by early investigators into two distinct components. The initial rapid decline in the rate of oxygen consumption was identified as the alactic portion and was attributed to the replenishment of immediately available energy sources such as ATP, CP, oxygen stores and dissolved oxygen in the blood plasma (19, 34). The slow component of oxygen debt was identified as the lactic portion of the total debt and was associated with the conversion of blood and tissue lactate to glucose and glycogen in the liver or terminal oxidation of lactic acid by the

liver, kidney, or heart (19, 34).

This simplistic view of oxygen debt has recently been challenged (8, 29, 35, 52). Several investigators (8, 29, 35, 45) have indicated the failure of this simple definition to account for such factors as: 1) oxygen cost of elevated ventilation and myocardial contraction after exercise; 2) oxygen required to supply energy for tissue repair, and to correct imbalances caused by metabolite turnover and electrolyte shifts; and 3) elevated oxygen consumption caused by increased core and muscle temperatures. In view of these added dimensions the term "oxygen debt" is no longer sufficient to define the period of increased O_2 consumption after exercise. Hereafter post-exercise O_2 consumption will be referred to as recovery oxygen uptake.

Studies with man (3, 11, 48, 49) and animals (9) have demonstrated that muscle temperature increases rapidly during exercise and remains elevated in recovery. In addition, experiments with isolated rat skeletal muscle mitochondria have clearly shown that temperatures above 40°C resulted in increased rates of oxygen consumption. Collectively, these studies strongly suggest that elevated internal muscle temperature may play a significant role in recovery oxygen uptake and should be considered as a contributing factor in future studies of this phenomenon. However, the specific interrelationship between temperature and oxygen consumption in mammalian skeletal muscle during and after exercise remains

to be evaluated in intact muscle tissue. Until recently it was not possible to investigate the systematic effect of temperature on muscle respiration. During exercise performance several intervening factors give rise to alterations in whole body oxygen consumption and ATP production. These factors, as previously mentioned, include: 1) tissue damage and repair; 2) elevated myocardial contraction; 3) increased ventilation; 4) metabolite and electrolyte imbalances; 5) lactic acid production; and 6) replenishment of ATP, CP and oxygen stores. These "whole body" effects on post exercise oxygen consumption must be eliminated. To establish a direct relationship between internal muscle temperature and respiration a system in which temperature and the rate of oxygen supply can easily be controlled and measured, must be used.

Several investigators have used a perfused rat hindquarter system to study metabolic performance in skeletal muscle tissue employing whole blood (32, 47) or washed red blood cells (24) as oxygen transporters. In this study the procedure and apparatus were simplified by eliminating the red blood cells and substituting a standard, oxygenated Krebs-Henseleit bicarbonate buffer. This perfusion medium has been successfully used in isolated perfused rat heart studies in this laboratory and several others (39, 41), but the reliability of the cell-free Krebs-Henseleit buffer as a perfusate in rat hindquarters has not been tested.

The intent of this study was to: 1) establish a

perfused skeletal muscle preparation in which arterial and venous PO_2, as well as muscle temperature could simultaneously be measured; and 2) systematically investigate the effect of internal muscle temperature on tissue oxygen consumption.

Hypotheses

1) H:o The system for perfusing rat hindquarters will not serve as a viable instrument for detecting variations in oxygen consumption in rat skeletal muscle in response to changes in internal muscle temperature.

H:a The perfusion system will serve as a viable instrument.

2) H:o There will be no increase in cell respiration in rat skeletal muscle in response to increasing muscle temperatures.

$H{:}a_1$ There will be a positive linear relationship between oxygen uptake and muscle temperature.

$H{:}a_2$ Initially cellular respiration will increase in a linear fashion in response to elevations in muscle temperature, but at some critical temperature the slope of the VO_2/temperature curve will change.

Definition of Terms

Energy Deficit: The oxygen, ATP and CP stores used for energy production after contractile activity in the muscle begins and before a steady level of oxygen uptake is reached.

Recovery Oxygen Uptake: 1) The total amount of oxygen used to replete oxygen and energy stores and to reconvert or

oxidize lactates produced during an exercise bout. 2) The excess oxygen used as a result of exercise causing a general disturbance of the resting condition of the body. This includes oxygen used as a result of elevated levels of ventilation and myocardial contraction, electrolyte shifts, tissue damage, metabolite turnover and elevated core and muscle temperature.

State 3 Respiration: The rate of mitochondrial oxygen consumption proceeds at the maximum rate in the presence of phosphate, ADP (phosphate acceptor) and substrate. This is referred to as "active respiration" (30).

State 4 Respiration: ADP is lacking in the system (no phosphate acceptor) and the rate of mitochondrial oxygen consumption is at its lowest rate. This is referred to as the "resting state of respiration" (30).

CHAPTER II

METHODS

Care and Preparation of Animals

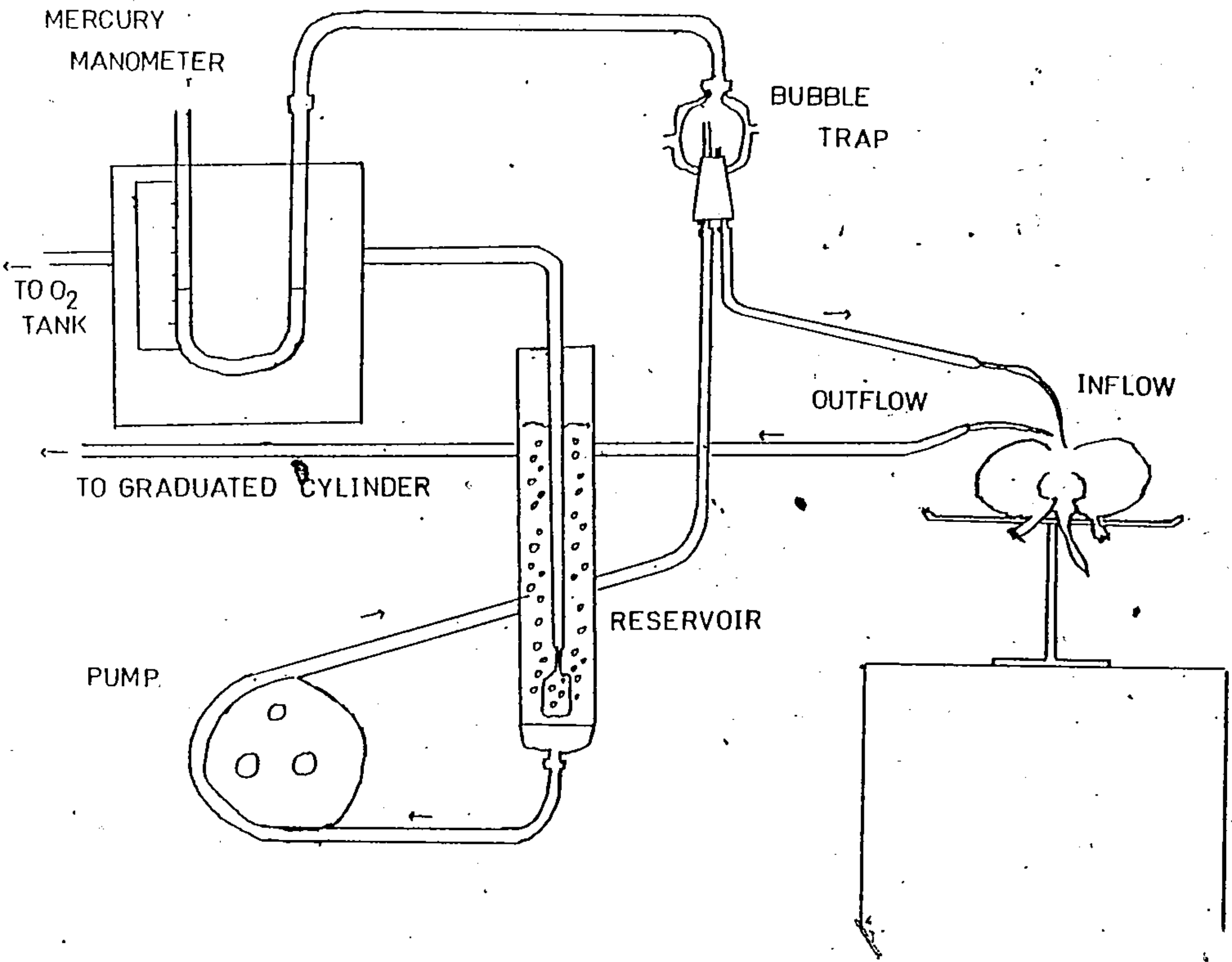
Twenty-five male Wistar rats obtained from Woodlyn Farms in Guelph, Ontario and weighing approximately 220 gm were used for the study. The animals were housed in a temperature controlled room (25°C) and were provided with water and Purina Rat Chow, ad libitum.

The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (25mg/100g) 30 minutes after being heparinized (1 unit/g) with sodium heparin to prevent blood clotting in the muscle tissue during the surgical procedures.

The Perfusion Apparatus

The perfusion apparatus was a modification of that described by Jefferson (24)(Fig. 1). The system was housed in a 36" x 30" x 24" wooden chamber with a sliding plexi-glass door on one side. A variable speed peristaltic pump, protruding from one end of the chamber, drew the perfusate from a 250 ml glass reservoir through a coarse glass filter and delivered it to a glass bubble trap located 60 cm above the pump. The bubble trap was joined to a mercury manometer for easy monitoring of perfusion pressure and to an aortic cannula (21 gauge hypodermic needle) located below the trap and attached

FIGURE 1. A SCHEMATIC REPRESENTATION OF THE PERFUSION APPARATUS



to the animal tray. The sharp bevelled tip of the hypodermic needle was blunted to prevent puncturing of the artery. All connective tubing in the perfusion system was 3/8" o.d., 1/4" i.d., Tygon.

A 10 ml graduated cylinder which received venous outflow from the hindquarter, was secured to a retort stand outside the perfusion chamber. During each experiment the venous cannula was connected to a rubber tube which delivered venous outflow through an opening in the chamber wall into the reservoir. Clamping the tube and timing the drip rate with a stop watch provided an easy and accurate method of calculating the rate of flow of the perfusate through the hindquarter.

Since the buffer was not recirculated an extra supply in a 2000 ml pyrex container was kept within the chamber to assure uniform buffer temperature. When required, perfusate from the 2000 ml reservoir was drawn from outside the chamber, through a length of Tygon tubing into a 50 cc plastic syringe. This amount of buffer was then transferred through a similar section of Tygon into the 250 ml container. This procedure limited the number of times the chamber had to be opened and prevented heat loss. The perfusate in both the 2000 ml and 250 ml containers was gassed with a water-saturated mixture of 95% O_2, 5% CO_2, using fine sandstone filters.

To heat the chamber uniformly, four 150 watt light bulbs were attached to the back and one end of the box with

a small fan circulating the heated air. These bulbs were connected to a micro-switch and wafer-thermostat which turned the lights on and off as they controlled temperature. A mercury thermometer suspended directly over the preparation provided convenient observation of the temperature within the chamber.

Limitations of Perfusion Chamber

During the experiments problems in temperature control caused by the design of the perfusion chamber became evident. When the sliding plexi-glass door was opened to allow for extraction of arterial and venous samples, heat escaped from inside the chamber and a slight reduction (0.5°C) in tissue temperature was noticed. When the door was closed again the muscle temperature soon rose to the desired level and was stable by the next sampling time. A smaller door that gives access to a sliding animal tray and allows for easier sampling is necessary for convenience and to help maintain a stable internal temperature. In addition to this, better insulation of the chamber walls is required since it was necessary to keep the environment within the box slightly higher (2-3°C) than the muscle temperature to maintain the desired level.

Perfusion Medium

A standard Krebs-Henseleit bicarbonate buffer, freshly prepared each day, was the basic perfusion medium. It contained the following salts in millimolar concentrations: NaCl, 118; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; Mg $SO_4 \cdot 7H_2O$, 1.2:

KH_2PO_4,1.2; Na_2EDTA,0.5 . This mixture was gassed for 15 minutes then $NaHCO_3$ (to a final concentration of 25mM) was added. The NaCl, with 15mM glucose added, and $NaHCO_3$ were prepared daily while the other salts were obtained from stock solutions.

Surgical Technique

The abdomen was opened by making a horizontal midline incision then two vertical incisions along either side of the abdominal cavity. The following vessels were ligated with silk thread: coeliac artery; renal, suprarenal, spermatic and iliolumbar arteries and veins; and rectum. The visceral organs were removed and a loose ligature placed around the inferior vena cava just above the right iliolumbar vein. After clamping the vena cava above, then below the ligature, a small cut was made with scissors between the clamps and a 12 gauge cannula was inserted and tied in place.

In the pre-heated perfusion chamber the chest cavity was opened by making a vertical incision along the left side of the anterior thorax. After placing a loose ligature around the descending aorta, just above the diaphragm, the aorta was clamped with a small hemostat one inch proximal to the diaphragm. A small incision was then made between the clamp and the ligature, the aortic cannula was inserted and tied securely.

After each perfusion was completed the animal was transected just above the position of the aortic cannula and the remaining viscera and all fat deposits were extracted.

The tail and feet were removed, then the animal was skinned and weighed.

Experimental Procedure

The 25 animals were divided into five equal groups. Each group was tested at one of the following muscle temperatures: 36, 38, 40, 42, or 44°C.

After each animal was anesthetized, the procedures for evisceration and placement of the venous cannula were completed outside the perfusion chamber. The corpus was then transferred to the animal tray inside the pre-heated chamber for placement of the aortic cannula. Since the animal was exposed to anoxic conditions after the chest was opened and before perfusion was started with the oxygenated buffer, it was important that these final surgical procedures be done quickly. As soon as the aortic cannula was secured, perfusion at a rate of 17-21 ml/min was started and continued for at least thirty minutes before samples were extracted for analysis.

Muscle temperature was monitored constantly by a YSI500, 18 gauge thermistor probe inserted to a depth of 1 cm in the vastus medialis muscle and connected to a YSI Model 36TU Tele-Thermometer. Since the temperature of the interior dropped considerably each time the sliding plexiglass door was opened, it was necessary to keep the inside temperature 2-3°C higher than the pre-selected muscle and buffer temperatures to maintain the required temperature levels. If the desired muscle temperature was attained

thirty minutes after the beginning of perfusion, one arterial and one venous sample were removed with 2cc glass syringes, within 10 seconds of each other. Other identical samples were drawn 10 minutes later. If the necessary muscle temperature was not reached after 30 minutes an additional perfusion period of not more than 15 minutes was allowed before the first sample was taken. No samples were taken after 60 minutes of perfusion because of possible deterioration of the preparation. The arterial samples (2 ml) were drawn through gum rubber tubing positioned in the arterial line of the system, just before entry into the animal and venous samples (2 ml) were taken through a hypodermic needle inserted 3 cm into the outflow tube. All samples were immediately capped and later analyzed for PO_2, PCO_2, and pH at the Grace Hospital Chemistry laboratory using a Copenhagen model A-1 Acid-Base Analyzer.

Oxygen consumption was expressed in micromoles of O_2/min/100 gm of hindquarter, wet weight, and was calculated according to the following equation:

$$\text{umoles } O_2 \text{ consumed/min/100g hemicorpus} =$$

$$\frac{(\text{ul}O_2/\text{ml.arterial} - \text{ul/ml venous})}{22.4(\text{ul}O_2/\text{umole})} \times \frac{\text{flow(ml/min)x100}}{\text{hemicorpus weight}}$$

$$\text{ul}O_2/\text{ml} = \frac{24.P}{760}$$

Where: 24 = solubility of O_2 in buffer (ul/ml medium)

760 = mmHg

P = O_2 tension in medium (mmHg)

Test for Stability of Krebs-Henseleit Buffer

Since there is no evidence documenting the stability of the PO_2 in a Krebs-Henseleit buffer when exposed to high temperatures, some preliminary experiments were undertaken to investigate changes in the PO_2 of this buffer when heated between 36 and 44°C. In three experiments, deviations in PO_2 were measured as the buffer circulated through the perfusion system. Two ml samples were extracted by syringe when a stable buffer temperature was reached and were analyzed for PO_2 with an IL Model 113 pH/blood gas analyzer.

Tests for Stability of Hindquarter Preparation

Some additional preliminary investigations were conducted to examine lactic acid production and stability of O_2 consumption at 36°C over a 75 minute perfusion period. Since either washed red blood cells (32, 47) or whole blood (24) were used as oxygen carriers in all the literature examined, it was necessary to carry out these experiments to investigate the stability of the hindquarter preparation using the Krebs-Henseleit buffer as a perfusion medium. Lactic acid production was measured in six animals weighing between 550-650 gm. Two ml samples were extracted from the venous outflow at four 15 minute intervals after a 15 minute pre-wash. The samples were stored frozen and later analyzed colorimetrically for lactic acid concentration according to the method of Pryce (44). Oxygen consumption was examined in these same animals at three 30 minute intervals. In all cases separate two ml arterial and venous samples were

analyzed for PO_2 with the IL pH/blood gas analyzer.

In addition, another parameter was investigated to secure further evidence supporting the viability of this technique of rat hindquarter perfusion. Samples of tissue were quickly excised from the vastus medialis and the belly of the gastrocnemius muscles in three separate groups of animals. The third group consisted of animals used in experiments at 36°C (1), 38°C (3), and 40°C (4). The muscle samples were frozen immediately in liquid nitrogen and later analyzed for glycogen content according to the method of Lo, Russel and Taylor (31). The three groups were tested according to the following protocol:

GROUP	CONDITION	N
1	Normal Resting	5
2	0 Min. Perfusion (after anoxia)	5
3	40 Min. Perfusion	8

CHAPTER III

RESULTS

Stability of Krebs-Henseleit Buffer

Since an adequate oxygen delivery system is critical to maintain metabolic homeostasis in the muscle tissue it was necessary to conduct a preliminary investigation that would demonstrate the Krebs-Henseleit buffer PO_2 responses to temperature. These results are given in Figure 2. PO_2 values in the buffer decreased when temperature was elevated but still remained high (greater than 600 mmHg) even at $44^{o}C$. On the basis of these results it was decided that the oxygenated Krebs-Henseleit buffer was a sufficient perfusion medium to examine oxygen uptake responses to elevations in muscle temperature.

Oxygen Consumption Lactate Production and Glycogen Depletion

The results of experiments indicating lactic acid production and tissue oxygen uptake in the larger animals are given in Figure 3 (Raw Data available in Appendix A) and Table 1 respectively. Lactic acid production decreased after the initial anoxic period and remained at low levels throughout the total perfusion period. Oxygen uptake did not vary significantly during a sixty minute period after 15 minutes of pre-wash. These data indicate the metabolic stability of this hindquarter preparation at a normal resting muscle temperature of $36^{o}C$.

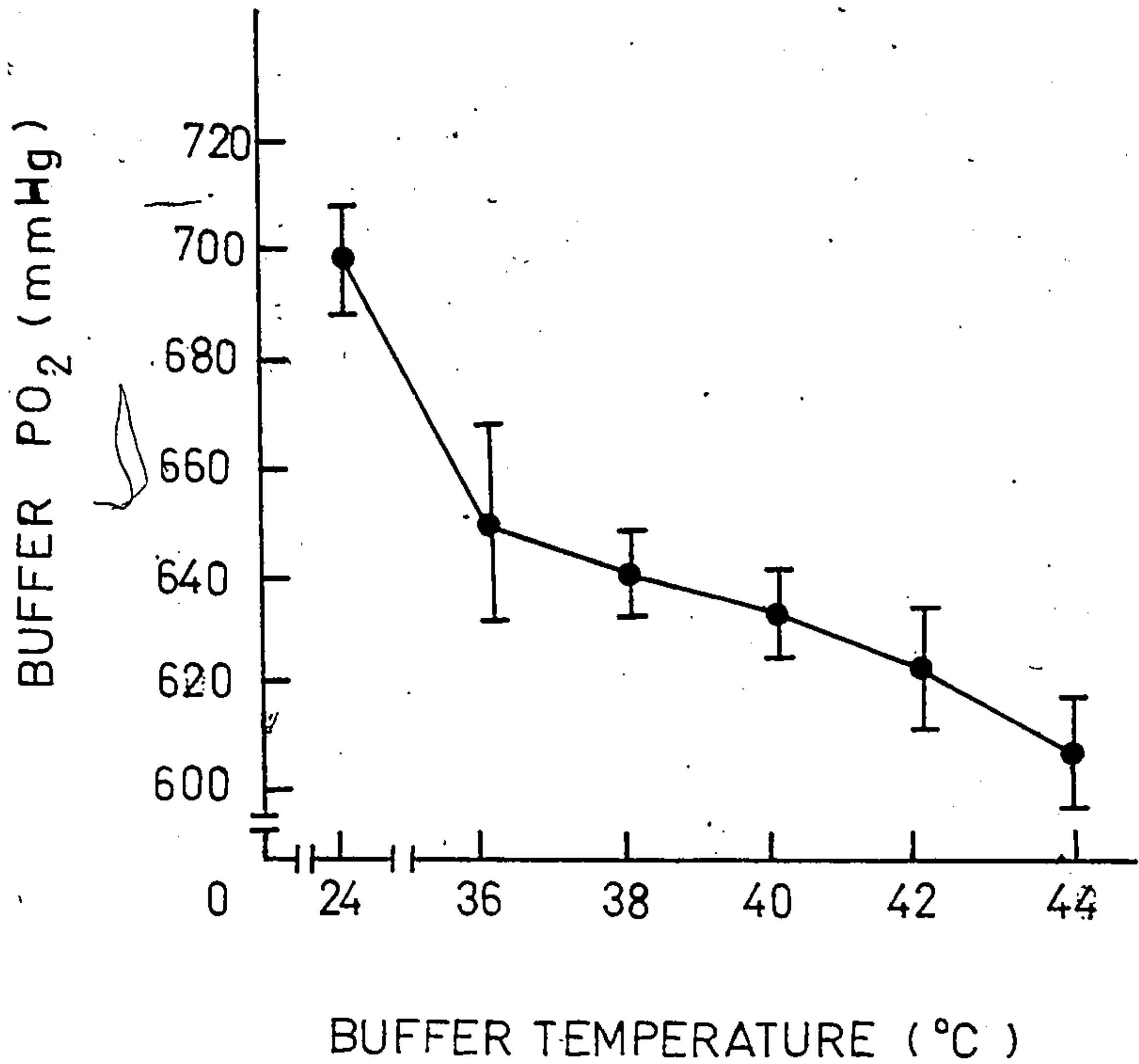


FIGURE 2. CHANGES IN KREBS-HENSELEIT BUFFER PO_2 (± SEM) IN RESPONSE TO ELEVATIONS IN TEMPERATURE.

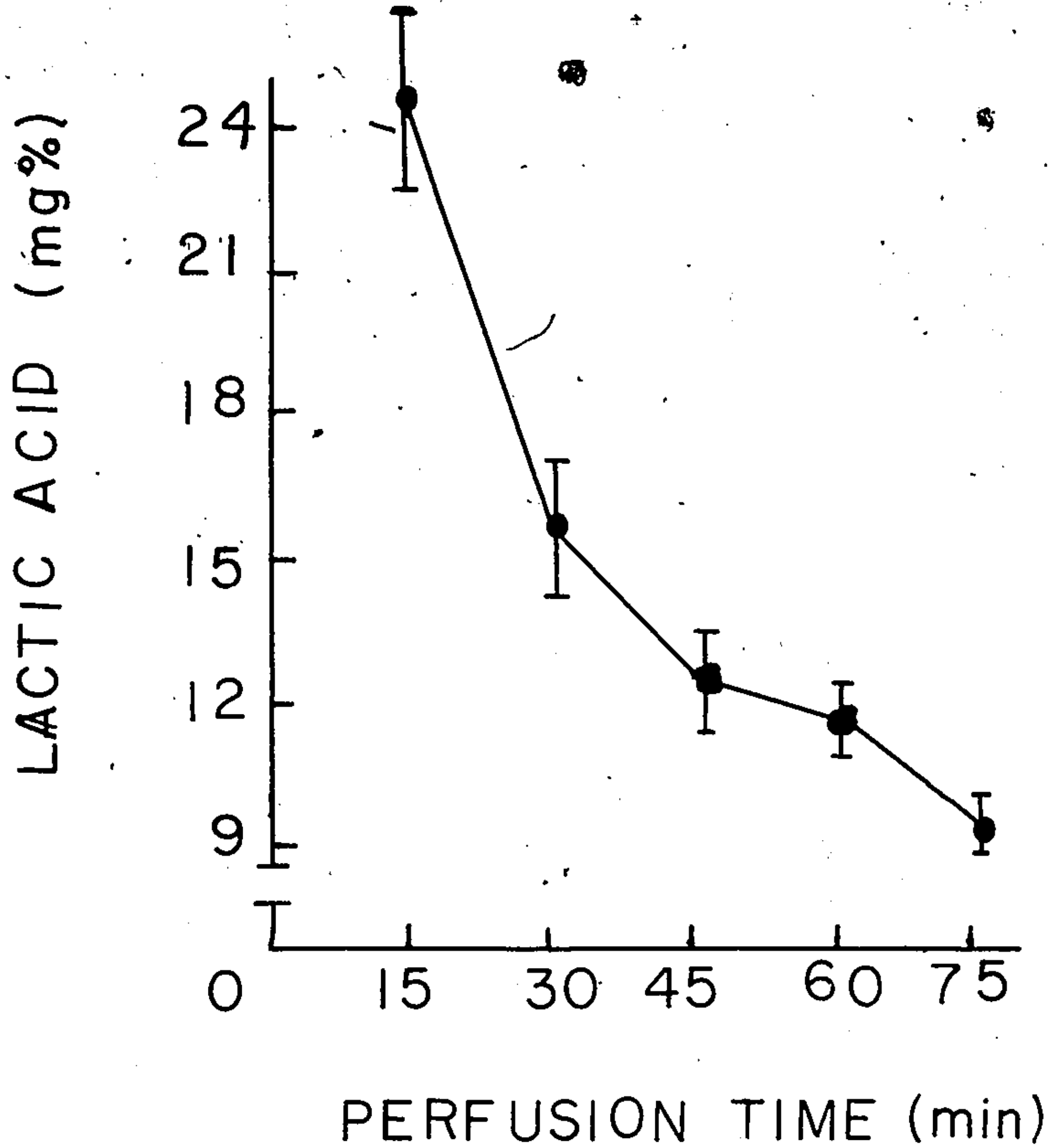


FIGURE 3. LACTIC ACID PRODUCTION (± SEM)
IN LARGE ANIMALS (n=6)

TABLE 1

CHANGES IN $\dot{V}O_2$ DURING PERFUSION IN LARGE ANIMALS (umoles/min/100g)

ANIMAL	15 Min.	45 Min.	75 Min.
1	4.78	5.10	4.16
2	4.49	4.83	4.83
3	4.29	4.33	4.56
4	6.43	5.90	5.16
5	4.25	4.09	3.73
$\bar{x}$	4.85	4.85	4.49
s	0.74	0.67	0.50
SEM	0.18	0.17	0.13

Figure 4 represents the total glycogen depletion in the vastus medialis and gastrocnemius muscles of three separate groups of animals (Raw Data in Appendix B). There was a 3.5% reduction in glycogen content from normal resting values to the start of perfusion (0 minutes) in the vastus medialis. This depletion represents the amount of glycogen used for energy production as a result of tissue exposure to anoxic conditions, lasting 1-1.5 minutes, during the final stages of the surgical procedure. After 40 minutes of hindquarter perfusion a further 21% depletion of glycogen stores was evident in this muscle. There was quite a different pattern of glycogen reduction in the gastrocnemius with a 25.5% depletion from rest to 0 minute and a subsequent 14% drop from 0-40 minute of perfusion.

Oxygen Uptake in Response to Temperature Increases

The oxygen uptake values from the two groups tested at 42 and 44°C are not included in the results because of a disruption of the normal state of the muscle tissue (sample available in Appendix C). Marked tissue edema at these temperatures resulted in an increased hemicorpus weight, markedly lowering the oxygen uptake values. Extremely low PaO_2 in the perfusion medium led to disruptions in metabolic performance of the tissue. These problems were characterized in most cases by one or more of the following conditions: low pH in venous outflow, low PvO_2 (less than 30 mmHg); high PvCO2 .

Changes in oxygen consumption in response to

increases in muscle temperature in the remaining three groups (36, 38, 40°C) are presented in Figure 5 (Raw Data in Appendix C). A one-way ANOVA procedure indicated a significant difference ($P < .01$) and a subsequent trend analysis demonstrated that the $\dot{V}O_2$/temperature curve is quadratic in nature ($P < .01$).

ANOVA Table for Oxygen Uptake

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCE
Between	25.39	2	12.70
Within	15.08	12	1.26
Total	40.47	14	F=10.08

TREND ANALYSIS

Coefficients for Sums of Squares

Linear: c_1	-1	0	1
Quadratic: c_2	1	-2	1

	Σc^2	C	$D = n\Sigma c^2$	C^2/D
Linear	2	15.76	30	8.28
Quadratic	6	4.04	90	.18

$F_{lin} = 6.57$ ($P < .05$)

$F_{non\ lin} = 13.55$ ($P < .01$)

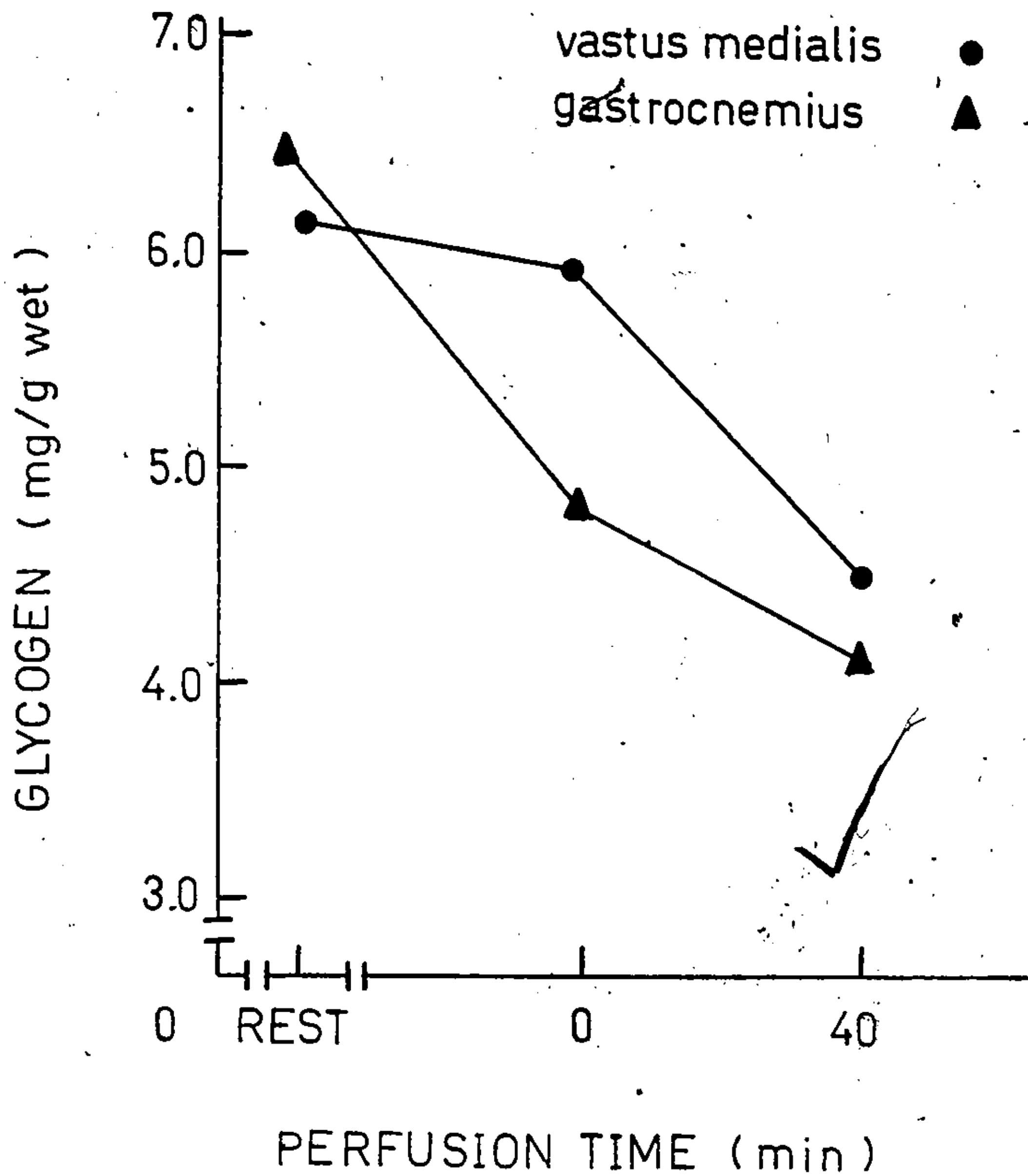


FIGURE 4. GLYCOGEN CONTENT IN SMALL ANIMALS AT REST (n=4), 0 MIN. OF PERFUSION (n=4) AND AFTER 40 MIN. OF PERFUSION (n=8)

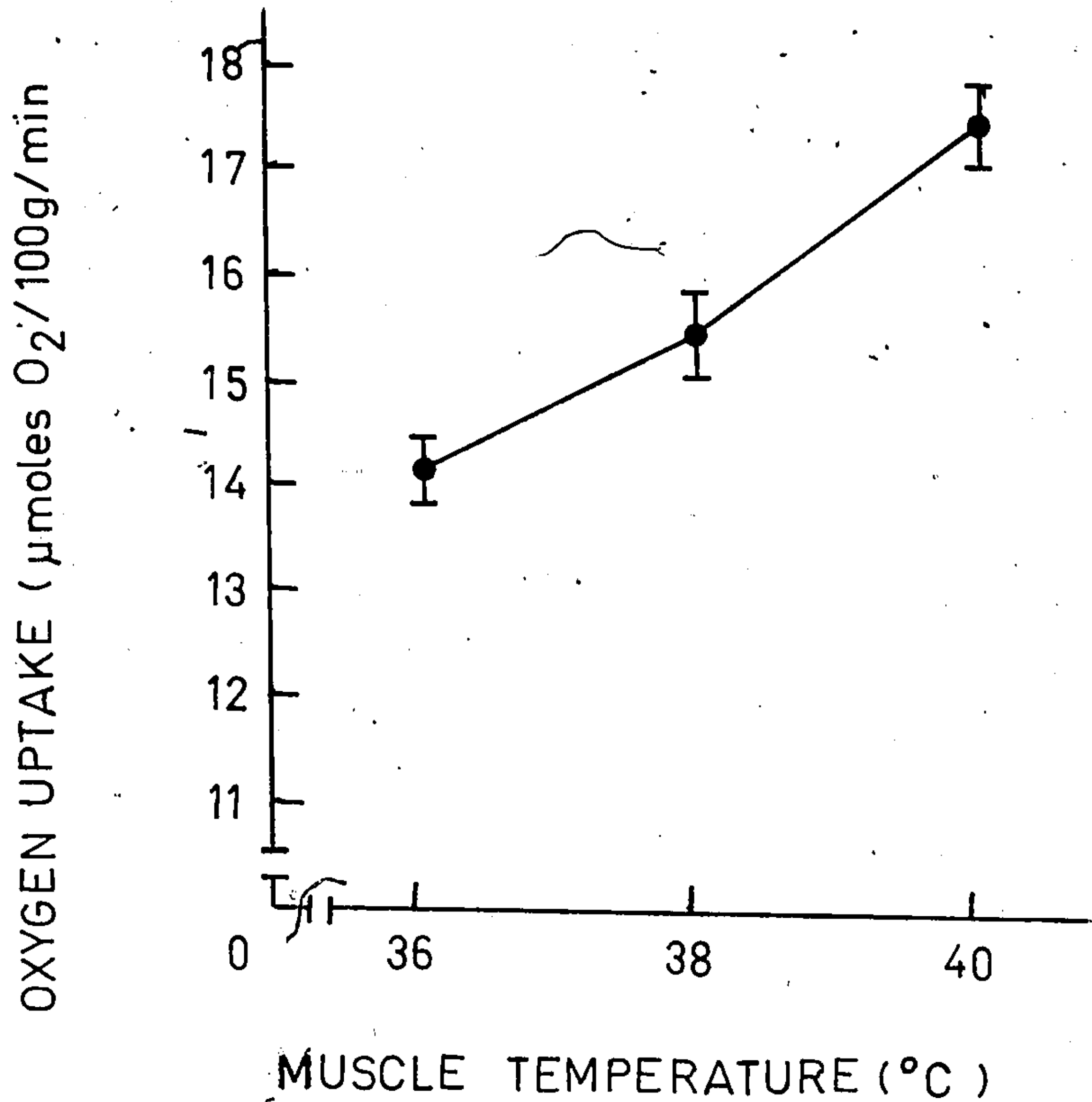


FIGURE 5. CHANGES IN OXYGEN UPTAKE (± SEM) IN RESPONSE TO ELEVATIONS IN TEMPERATURE (n=5)

CHAPTER IV

DISCUSSION

Several investigators have used a variety of isolated perfused muscle systems to examine the metabolic performance of muscle tissue (23, 32, 47). To answer the question proposed in this study it was necessary to develop a model in which muscle temperature and oxygen delivery to the muscle could easily be monitored and controlled.

The perfused rat hindquarter system described within this text was adapted and designed specifically to provide a simple and reliable technique for examining the relationship between internal muscle temperature and oxygen consumption. To demonstrate the ability of this technique to maintain metabolic stability in the preparation, the Krebs-Henseleit buffer was tested for fluctuations in PO_2 when exposed to high temperatures and the hindquarter was examined for lactic acid production and changes in cell respiration during perfusion. In addition to this, glycogen depletion in two representative muscle groups was tested after exposure to anoxia and after 40 minutes of perfusion.

The results of the preliminary tests on buffer PO_2 deviations indicated initially that the Krebs-Henseleit buffer was an adequate perfusion medium in terms of oxygen carrying capacity within a temperature range of 36-44°C. The information gained on experiments on $\dot{V}O_2$ fluctuations during

75 minutes of perfusion (large animals) gave further evidence that the Krebs-Henseleit buffer was able to support a viable preparation. A steady level of oxygen uptake was evident over this perfusion period and in addition the PvO_2 values in all five animals tested were greater than 100 mmHg after 30 minutes of perfusion suggesting that the muscle tissue was in a state of normoxia. The a-v difference in PO_2 did not vary appreciably in samples taken at different times from any single animal and it was demonstrated here that a sufficiently high PaO_2 (greater than 450 mmHg) provided adequate oxygen delivery to the muscle tissue.

Lactic acid production and oxygen consumption were examined in preliminary experiments using animals considerably larger (550-650 g) than those used in the final experiments (approximately 220 g). These larger animals were selected for easy location, separation, ligation and cannulation of blood vessels but smaller animals were used when the $\dot{V}O_2$ of the larger animals repeatedly fell within a range much lower than reported in similar preparations in the literature (15, 22, 42, 47, 51). The information obtained from these preliminary experiments on larger animals was still considered valuable as tests for viability since the data on lactic acid production and oxygen consumption demonstrated a very stable preparation after 30 total minutes of perfusion.

Past investigators who used isolated perfused muscle systems to examine the metabolic performance of skeletal muscle tissue used either whole blood from the animal itself

or washed red blood cells obtained from another mammalian source (24, 32, 47) to ensure sufficient oxygen carrying capacity. The reliability of these two techniques is well documented (24, 47). In the present study the cell-free, standard Krebs-Henseleit bicarbonate buffer was used based on these preliminary experiments demonstrating fluctuations in circulating buffer PO_2 in response to changes in temperature between 36 and 44°C and lactic acid production and $\dot{V}O_2$ measurements during perfusion. Furthermore, a phosphate buffer such as the Krebs-Henseleit, was more convenient since it was easy to prepare and eliminated the complex apparti needed for whole blood oxygenation.

It became evident, however, as the experiments proceeded that the Krebs-Henseleit buffer was an inadequate perfusion medium for examining $\dot{V}O_2$ responses to high temperatures. At 42 and 44°C the PaO_2 was extremely low in some cases and was reflected by one or more of the following conditions: 1) severely depressed PvO_2 or pH values; and 2) high $PvCO_2$. In addition to this, the pH in the effluent frequently fell below 7.0 and in one case as low as 6.7 in experiments performed at 36, 38 and 40°C. These findings illustrate the ineffectiveness of the buffering capacity of the perfusate.

The initial resting levels of glycogen found in the belly of the gastrocnemius muscle corresponded to values found in the literature for male Wistar rats weighing between 200-300gm (2,12). Although no reports of glycogen levels in

the vastus medialis could be located it was assumed that the initial resting values found in this study represented a normal population since the S.E.M. was very small (.17).

Depletion of glycogen stores in the vastus medialis during 40 minutes of perfusion, and to a lesser extent in the gastrocnemius may have indicated inadequate perfusion and subsequent lack of oxygen in some tissue areas. Since lactic acid production and $\dot{V}O_2$ were found to be stable, however, this small reduction in glycogen probably did not contribute to any severe instability in the hindquarter. These divergent glycogen reduction patterns in the two muscles in response to anoxia cannot be explained at this time.

Harken (1976) has demonstrated the relationship between $\dot{V}O_2$ and pH in an isolated canine hindlimb. He found a positive linear relationship between $\dot{V}O_2$ and pH at pH values from 6.9 to 7.6. His data are supported by several other investigators who also observed a cellular depression of $\dot{V}O_2$ as H^+ ion concentration increased. If this is the case in the present experiments all the $\dot{V}O_2$ values should be slightly higher under normal pH conditions. There should be little total effect on the slope of the $\dot{V}O_2$/temperature curve, however, since there was no definite trend in pH fluctuations under the three temperature conditions.

The depressed pH values found in these experiments are much lower than can be expected in a physiological system (21, 33, 46) but several investigators (10, 18, 26, 36), all demonstrated viable muscle preparations between 6.9 and 7.6

pH units. In the present study, all pH values except 1 were within this range so it can be assumed that the only effect of these low values would be a depression of $\dot{V}O_2$ at each temperature. Under normal pH conditions an elevated $\dot{V}O_2$ would be expected, but the response of $\dot{V}O_2$ to temperature elevations would probably not change. In the type of perfusion system used in this study the buffering capacity of blood would be an advantage in controlling pH.

Several investigators support the contention that increased core and muscle temperatures resulting from moderate or severe exercise account for a significant but unknown fraction of post-exercise oxygen consumption (8, 29, 35, 52). It can be assumed that the well known Q_{10} effect contributes somewhat to the elevated levels of oxygen uptake. However, Brooks et al (8) raised a question of respiratory inefficiency in the muscle tissue based on their studies with isolated muscle mitochondria. Their findings demonstrated a linear response of states 3 and 4 respiration to elevated temperature between 25 and 37°C but implied greater respiration rate at temperatures above 37°C. They offered a theory that explained this inefficient use of oxygen and attributed it mainly to nonconservative state 4 respiration which increased by 200% between 37° and 45°C whereas state 3 respiration increased by only 60%. According to this hypothesis, the mitochondrial ATPase activity is stimulated as muscle temperature increases. The greatest increase in state 4 respiration was noticed between 40-45°C which coincided with a linear decrease in ADP:O ratio.

An increase of 22.4% in total cellular respiration between 36° and 40°C was noticed in the present study. Barclay et al (3) also demonstrated an increase in muscle oxygen uptake as a direct result of internally produced temperature increases. They proposed that muscle temperature could be used to estimate oxygen uptake of a given muscle group. Their findings demonstrated that muscle temperature increases, which were directly related to stimulation rate, could account for a 23-40% increase in oxygen uptake in twitch contractions and 51-63% increase in tetanic contractions. This evidence, coupled with the findings of Brooks et al support the contention that muscle temperature is a significant contributing factor to elevated levels of oxygen consumption during and after an exercise bout. Any future calculation of recovery oxygen uptake must include an estimation of the contributing effect of muscle temperature.

The results from the present study support the findings of Brooks et al (8) and Barclay et al (3), since there was a significant increase in oxygen uptake in relation to elevations in muscle temperature. The slope of the $\dot{V}O_2$/temperature curve increased and this suggests that some mechanism, possibly that proposed by Brooks et al, triggers an increased rate of cellular respiration in response to an elevation in tissue temperature past some critical level. In these experiments this critical level is between 38° and 40°C.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The perfused rat hindquarter system described here was designed specifically to examine the relationship between $\dot{V}O_2$ and muscle temperature. It was hypothesized that: 1) the perfusion technique would be reliable and the standard Krebs-Henseleit bicarbonate buffer would be an adequate perfusion medium for testing $\dot{V}O_2$ responses to temperature elevations; and 2) the response of cellular respiration between 36° and 44°C would be linear up to some temperature level, then increase at a greater rate.

To test these hypotheses, oxygen consumption by the isolated hindquarter was measured after 30 and 40 minutes of perfusion in five groups of rats; each group being studied under one of the following temperature conditions: 36, 38, 40, 42 or 44°C. The results from experiments at 42° and 44°C were not acceptable because of a disruption in the normal metabolic state of the muscle and a marked tissue edema which caused an increase in the final hemicorpus weight. However, the $\dot{V}O_2$ data from the three remaining temperatures (36, 38, 40°C) indicated that cellular respiration increased significantly between 36° and 40°C and this increase was quadratic in nature. These results suggest that some mechanism, possibly an increase in mitochondrial ATPase activity, as proposed by

Brooks et al (8), triggered an increased response of oxygen utilization in the cell, at some critical temperature level.

Conclusions

As hypothesized, there was a significant non-linear response of $\dot{V}O_2$ to increases in muscle temperature. The perfusion technique and the Krebs-Henseleit buffer were limiting in these experiments, however, since the metabolic performance of the muscle tissue was disrupted at 42° and $44^{o}C$.

Recommendations for Further Study

Because of problems in the buffering capacity and oxygen solubility of the Krebs-Henseleit buffer it is suggested that washed red blood cells, from another mammalian source, be used in combination with the Krebs-Henseleit buffer. In this case, to conserve the perfusate and to oxygenate the erythrocytes, a recirculating buffer system and a rotating flask for buffer oxygenation need to be incorporated into the perfusion apparatus.

To avoid fluctuations in environmental temperature within the perfusion chamber, a small sliding door that provides easy access to the preparation and better insulation along the inside of the chamber are necessary.

Once these modifications are complete an examination of $\dot{V}O_2$ under a wider range of temperature conditions (34°-$44^{o}C$), than was possible here, would provide a clearer description of the response of cellular respiration to changes in muscle temperature.

To examine the question of the relative contribution of muscle temperature to recovery oxygen uptake in future studies, it will be necessary to test the muscle performance under conditions more closely approaching a real physiological situation. Measuring the $\dot{V}O_2$ response of a skeletal muscle during and after several different rates of contraction would further delineate the relationship between recovery oxygen uptake and muscle temperature.

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APPENDIX A

LACTIC ACID PRODUCTION IN LARGE ANIMALS

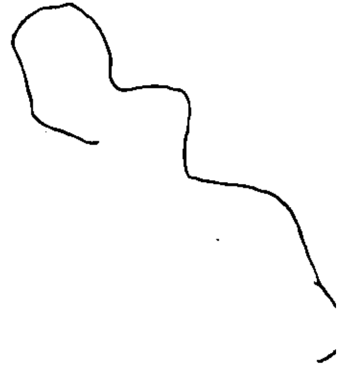

TABLE 2

LACTIC ACID PRODUCTION IN LARGE ANIMALS
(mg%)

ANIMAL	15 Min.	30 Min.	45 Min.	60 Min.	75 Min.
1	17.1	18.8	17.9	18.8	11.9
2	47.6	18.5	11.5	10.9	6.5
3	16.8	8.4	7.0	7.6	9.9
4	23.0	12.5	12.5	9.6	10.5
5	16.1	19.0	15.9	13.0	10.1
$\bar{x}$	24.12	15.44	12.96	11.98	9.78
s	12.0	4.05	3.77	3.84	1.78
SEM	3.0	1.01	.94	.96	.45

APPENDIX B

CHANGES IN GLYCOGEN CONCENTRATION IN SMALL ANIMALS

TABLE 3

CHANGES IN GLYCOGEN CONCENTRATION
IN GASTROCNEMIUS MUSCLE (Mg/gm wet)

ANIMAL	NORMAL REST	ANOXIC	40 MIN. PERFUSION		
			40°C	38°C	36°C
1	6.21	3.71	3.57	5.74	5.33
2	6.60	SAMPLE RUINED	3.70	4.57	
3	6.25	3.78	2.34	4.14	
4	SAMPLE RUINED	6.17	4.00		
5	7.07	5.77			
$\bar{x}$	6.53	4.86	3.40	4.81	
s	.35	1.12	.63	.68	
SEM	.12	.37	.21	.34	

TABLE 4

CHANGES IN GLYCOGEN CONCENTRATION
IN VASTUS MEDIALIS MUSCLE (Mg/gm wet)

ANIMAL	NORMAL REST	ANOXIC	40 MIN. PERFUSION		
			40°C	38°C	36°C
1	5.71	4.14	4.99	5.24	4.15
2	5.71	6.00	5.42	4.33	
3	5.91	6.33	5.15	4.50	
4	6.83	5.92	4.34		
5	7.13	7.81			
$\overset{+}{x}$	6.26	6.04	5.02	4.69	
s	.67	1.24	.37	.41	
SEM	.17	.31	.09	.20	

APPENDIX C

OXYGEN UPTAKE IN SMALL ANIMALS AT 36, 38 and 40°C. INDIVIDUAL DATA

TABLE 5

OXYGEN UPTAKE AT 36°C. INDIVIDUAL DATA

SAMPLE	Flow ml/min	PaO_2 mmHg	PvO_2 mmHg	$PaCO_2$ mmHg	$PvCO_2$ mmHg	Tm	pH Arterial	pH Venous	Hc Wt gm	$\dot{V}O$ umoles O_2/min/100g
ANIMAL #1										
1	16.22	471	134	35	63	36	7.419	7.138	62.5	12.33
2	16.22	477	144	37	64	36	7.404	7.140	62.5	12.18
ANIMAL #2										
1	17.14	458	95	37	69	36	7.389	7.084	63.0	13.93
2*	17.14	347	60	41	88	36.	7.332	6.972	63.0	11.02
* Data not acceptable because of low PaO_2 and PvO_2										
ANIMAL #3										
1	17.14	525	163	38	65	35.7	7.391	7.137	64	13.66
2*	17.14	542	58	35	86	35.7	7.426	7.001	64	18.22
* Data not acceptable because of low PvO_2										
ANIMAL #4										
1	16.11	491	74	37	63	35.7	7.399	7.159	62	15.27
2	SAMPLING ERROR	-	-	-	-	-	-	-	-	-
ANIMAL #5										
1	17.65	581	194	32	54	36	7.432	7.217	60.5	15.91
2	17.91	550	195	36	53	36	7.405	7.225	60.5	14.90

TABLE 6

OXYGEN UPTAKE AT 38°C. INDIVIDUAL DATA

SAMPLE	Flow ml/min	PaO_2 mmHg	PvO_2 mmHg	$PaCO_2$ mmHg	$PvCO_2$ mmHg	Tm °C	pH Arterial	pH Venous	Hc Wt gm	$\dot{V}O$ umoles O_2/min/100g
ANIMAL #1										
1*	20.34	373	44	33	160	38	7.440	6.663	70	13.48
2	20.87	382	44	35	68	38	7.431	7.111	70	14.20
ANIMAL #2										
1	19.83	516	40	32	115	37.8	7.477	6.865	80	16.64
2	20.00	494	40	31	105	37.8	7.506	6.905	80	16.01
ANIMAL #3										
1	20.17	478	43	34	77	37.8	7.440	7.042	82.5	15.0
2	20.34	504	57	39	75	37.8	7.386	7.057	82.5	15.55
ANIMAL #4										
1	20.34	584	133	38	67	37.7	7.392	7.117	85.0	15.21
2	20.51	571	183	39	61	37.7	7.373	7.163	85.0	13.20
ANIMAL #5										
1	20.17	447	44	33	79	38	7.449	7.043	67	17.24
2	20.17	438	79	37	64	38	7.402	7.149	67	15.40

* Data not acceptable because of high $PvCO_2$ and low venous pH

TABLE 7

OXYGEN UPTAKE AT 40°C. INDIVIDUAL DATA

SAMPLE	Flow ml/min	PaO_2 mmHg	PvO_2 mmHg	$PaCO_2$ mmHg	$PvCO_2$ mmHg	Tm °C	pH Arterial	pH Venous	Hc Wt gm	$\dot{V}O_2$ umoles O_2/min/100g
ANIMAL #1										
1	20.0	436	50	30	61	40	7.486	7.157	67	16.24
2*	20.0	422	27	31	89	40	7.475	6.883	67	14.95
* Data not acceptable because of low PvO_2										
ANIMAL #2										
1	20.17	425	44	27	80	40	7.511	7.018	63	17.20
2	MACHINE ERROR									
ANIMAL #3										
1	20.17	498	40	32	89	40	7.462	6.976	65.5	19.17
2	20.17	462	44	33	115	40	7.450	6.889	65.5	18.30
ANIMAL #4										
1	20.34	488	105	28	59	40	7.500	7.166	67.0	16.39
2*	20.0	490	33	31	95	40	7.478	6.986	67.0	18.80
* Data not acceptable because of low PvO_2										
ANIMAL #5										
1	20.0	483	31	33	149	40	7.449	6.696	67.5	18.88
2	20.0	427	33	36	142	40	7.420	6.730	67.5	16.46

TABLE 8

SAMPLE AT 44°C. TO ILLUSTRATE EFFECTS OF LOW PaO_2

SAMPLE	Flow ml/min	PaO_2 mmHg	PvO_2 mmHg	$PaCO_2$ mmHg	$PvCO_2$ mmHg	Tm °C	pH Arterial	pH Venous	Hc Wt gm	$\dot{V}O_2$ umoles O_2/min/100g
1	17.9	318	15	25	113	44	7.559	6.837	80	-
2	16.4	349	19	29	100	44	7.515	6.902	80	-

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