THE EFFECTS OF RESPIRATORY AND METABOLIC ACIDOSIS ON THE METABOLIC RATE OF ANAESTHETIZED, PARALYZED, AND ARTIFICIALLY VENTILATED GROUND SQUIRRELS
(Spermophilus lateralis)

by

Supriti Bharma

B.Com. (Hons.), Delhi University, Delhi, 1983.
B.Sc. (Hons.), Trent University, Peterborough, 1988.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in
THE FACULTY OF GRADUATE STUDIES
Department of Zoology

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December, 1991
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Department of Zoology

The University of British Columbia
Vancouver, Canada

Date 17th JAN, 1992

DE-6 (2/88)
ABSTRACT

This study was designed to test the hypothesis that changes in arterial pH (pHa) reflecting proportional changes in arterial hydrogen ion concentration ([H⁺]a) effect a change in oxygen consumption (VO₂) or metabolic rate (MR), in euthertmic golden mantled ground squirrels (Spermophilus lateralis). Of interest were the specific effects, either local or reflex, of changing arterial pH on metabolic rate. Therefore, it was imperative that experimentally produced changes in pHa did not cause any compensatory changes in ventilation, since this would indirectly alter metabolic rate due to changes in the cost of breathing. It was also important that any changes in metabolic rate produced by the experimental manipulations did not cause any change in body temperature (Tb), since this would secondarily cause further changes in metabolic rate. As a consequence, these experiments were conducted on anaesthetized, paralyzed, and artificially ventilated animals maintained at constant Tb.

Three series of experiments were conducted, each of which altered pHa in a different fashion. In the first series of experiments, (Series I, n = 6) pHa was randomly made either alkalotic or acidotic by altering the frequency and/or volume of the respirator, to increase or decrease minute ventilation (VE), and consequently hyper- or hypo-ventilate the animals. The average pHa was varied from 7.19 ± 0.03 Units in the acidotic range, to 7.55 ± 0.02 Units in the alkalotic range with corresponding arterial partial pressures of CO₂ (PaCO₂) of 52.8 ± 3 Torr and 25.9 ± 2 Torr, respectively. In this series, VO₂ showed a significant (p < 0.001) positive correlation with pHa (r = + 0.84), decreasing 0.24 ml.min⁻¹ for every 0.1 Unit decrease in pHa.
(a 21.78% decrease in metabolic rate for a decrease of 0.36 pHa Units or a 6.04% decrease in metabolic rate for each 0.1 unit decrease in pHa).

In the second series of experiments, (Series II, n = 12), respiratory acidosis was produced by increasing the CO₂ concentration of the inspired gas (%Inspired CO₂) from 0.03% to 1 - 10% (7.07 ± 0.57% on average) in a random sequence of steps. This decreased pHa to a minimum of 7.24 ± 0.02 Units and increased Pa₉₀₂ to a maximum of 62.6 ± 3.3 Torr. The positive correlation between VO₂ and pHa (r = +0.78) was significant (p < 0.05), and similar to that obtained in Series I; VO₂ decreased by 0.27 ml.min⁻¹ for each 0.1 Unit decrease in pHa (a 28.9% decrease in metabolic rate for a 0.35 Unit decrease in pHa or an 8.38% decrease in metabolic rate for every 0.1 Unit decrease in pHa). Thus, the results of experimental series I and II clearly demonstrate that CO₂ retention leading to a respiratory acidosis will depress metabolism in these animals.

In the third series of experiments (Series III, n = 22), metabolic acidosis was produced by infusing lactic or acetic acid (n = 15) intravenously (0.08 - 0.15 mM.ml⁻¹ at 0.19 - 0.38 ml.min⁻¹) for 20 to 30 minutes. These infusions reduced pHa from 7.56 ± 0.02 Units to 7.32 ± 0.01 Units and correspondingly raised Pa₉₀₂ values from 26.9 ± 1.2 to 37.9 ± 1.9 Torr. Over this range there was a significant (p < 0.001) negative correlation between VO₂ and pHa (r = -0.65); metabolic acidosis increased VO₂ by 0.25 ml.min⁻¹ for every 0.1 Unit decrease in pHa (a 38.7% increase in metabolic rate associated with a 0.24 Unit decrease in pHa or a 16.12% increase in metabolic rate for each 0.1 Unit decrease in pHa). Similar trends were observed with the infusion of mammalian Ringer's solution (n = 7), a weakly acidic solution (pHₐ = approximately 7.3 Units) which caused a small fall in pHa. This would suggest that the infusions of lactic or
acetic acid were not increasing metabolic rate by secondarily acting as substrates for metabolism. These results are the opposite of those obtained in Series I and II.

Thus, respiratory acidosis and metabolic acidosis have rapidly reversible but opposite effects on the metabolic rate of golden-mantled ground squirrels (*Spermophilus lateralis*); respiratory acidosis inhibits aerobic metabolism whereas metabolic acidosis stimulates it. This implies that pH$_a$ is indirectly rather than directly correlated with $\dot{V}_{o_2}$ by its effect on some other factor which must be altered in different ways by respiratory and metabolic acidosis.
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HL &= \text{Heat Loss;} \\
BMR &= \text{Basal Metabolic Rate;} \\
\dot{V}_E &= \text{Minute Ventilation;} \\
\dot{V}_{E/VO_2} &= \text{Air Convection requirement;} \\
\beta_{CO_2} &= \text{CO}_2 \text{ Solubility Coefficient;} \\
C_{aCO_2} &= \text{CO}_2 \text{ Content in Arterial Blood;} \\
TG &= \text{Shivering and Non-Shivering Thermogenesis}
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ACKNOWLEDGEMENTS

I would like to thank my supervisor, Bill Milsom for his unfailing support, encouragement and patience during my years in his lab, for the exciting environment of exploration that he furthered at all times, for his uncanny "knob-twiddling" abilities with equipment that otherwise refused to work for me, and for his brow-beating ways which effectively kept me on the straight and narrow path to the completion of my thesis. To Vic, my husband, for refuelling endless cups of tea and providing me with that very special support throughout. Special thanks goes to all the "residents" of the Milsom Lab (past and present) - Greg Funk, Mark Burleson, Michael Harris, Richard Kinkaed, Julia Hunter and Pat Chan, for their willingness to help at all hours and making science fun, and to the Jones, Hochachka and Randall labs for valuable assistance with questions and lending equipment. I am deeply indebted to Tim West for analyzing my lactate samples and giving me the "right" numbers, and to Claudia Kassera for introducing me to "dilutional" acidosis and her literature collection. Thanks also goes to Sam Gopal for all the care he took of my squirrels and rats and assisting with french translations, to Alistair Blanchard for pointing out the often forgotten relevance of scatter plots, to Marianne Voldstedlemd for sharing my debut into the cannulation world, and to Sia Kaskaminidis for all her artistic efforts on behalf of my setup illustration.
INTRODUCTION

Hibernation is one of the most striking circannual rhythmicities to be seen among mammals living in temperate zones. It represents an extreme thermoregulatory adaptation for energy conservation (Walker & Berger, 1980; Heller, 1988; Trachsel et al., 1991) under the metabolically stressful conditions of food limitation and cold temperatures of the winter season. By abandoning euthermy and almost completely uncoupling the time of energy expenditure (at a hypometabolic rate) from the time of energy intake (Kenagy, 1989), mammalian hibernators such as the ground squirrel, woodchuck, brown bat, and the European hedgehog (Lyman et al., 1982a), can reduce energy expenditures by as much as 90% and survive the long winter months using only endogenous reserves or a limited supply of stored food (Wang, 1979; Storey, 1989).

Hibernation is characterized by the depression of multiple behavioral and physiological processes giving rise to bradycardia and hypometabolism (Lyman, 1948, Lyman, 1958; Lyman et al., 1982b), as well as long-term immunosuppression (Shivatcheva, 1989), hypophagia (Oeltgen et al., 1987; 1989) and markedly depressed renal function (Deavers & Musacchia, 1980). Body temperature may be lowered by more than 35° C, becoming readjusted to new, lower levels approximating ambient temperature corresponding with reductions in minute ventilation (Landau & Dawe, 1958), heart rate and metabolic rate (Lyman, 1948; 1958).

Deep hibernators such as the ground squirrel and woodchuck, unlike the bear (Nelson et al., 1973; Nelson, 1980; 1989), do not continually maintain hibernation. Hibernation bouts last for one or two weeks during which spontaneous or induced arousal to normal levels is possible.
at all times, without the addition of environmental heat (Hoffman, 1964). Some authors hypothesize that the energetic costs of periodic arousals from hibernation are incurred to receive the restorative benefits of euthermic slow-wave sleep, since during these periodic arousals, hibernators spend most of their time asleep (Trachsel et al., 1991). In the remaining time during arousal, ground squirrels always urinate, and sometimes eat (Pengalley & Fisher, 1961; Nelson, 1989). Woodchucks become uremic as hibernation progresses and arousal occurs as a result (Kristofferson, 1963; Nelson, 1989). The hibernator thus presents an interesting biological paradox, since it must be prepared for bouts of inactivity that are interrupted with short periods of intense activity (Mussachia et al., 1989).

The levels of pH, Pa\textsubscript{CO\textsubscript{2}}, and the CO\textsubscript{2} content in blood (Ca\textsubscript{CO\textsubscript{2}}) associated with these bouts of inactivity (characterized by low body temperatures and metabolic rates), have been well documented. Although pH and Pa\textsubscript{CO\textsubscript{2}} have been reported to stay relatively constant (Clausen & Ersland, 1968), decreased levels of Pa\textsubscript{CO\textsubscript{2}} (7-10 Torr), and increased levels of pH (0.05 to 0.17 Units) as well as arterial bicarbonate (8 to 15 meq.l.\textsuperscript{-1}) have been measured by several authors (Lyman & Hastings, 1951; Kent & Peirce, 1967; Goodrich & Lyman 1971; Musacchia & Volkert, 1971; Malan et al., 1973). The pH-temperature coefficient (dpH/dT) ranged between -0.0012 (Malan 1985; Bickler, 1984b) and -0.009 (McArthur et al., 1990). As a result of the increase in CO\textsubscript{2} solubility with only small decreases in Pa\textsubscript{CO\textsubscript{2}}, significantly increased Ca\textsubscript{CO\textsubscript{2}} levels have also been reported during hibernation (Howell et al., 1970).

The relative maintenance of Pa\textsubscript{CO\textsubscript{2}}, pH and [HCO\textsubscript{3}\textsuperscript{-}] with decreasing temperature, shown by hibernating mammals, is in contrast with the regulation of these acid-base variables during falling temperature by many other organisms. In some species of poikilotherms for example, for
the same decrease in body temperature (37° to 7° C), blood pH would increase by about 0.5 Units (dpH/dT = -0.0157 to -0.021 Units/°C; Reeves, 1985) in conjunction with a decrease in Paco2 and an increase in CO2 solubility.

The objective of these changes is considered to be the maintenance of a constant protein charge state in general and in particular, the maintenance of the fractional dissociation (α) of the non-bicarbonate blood buffer imidazole1, as body temperature changes (Reeves & Wilson, 1970; Reeves, 1972; Malan et al., 1976)2. The maintenance of constant charge state of key imidazole groups involved in enzyme activity confers stability of function in the form of conserved affinity constants for substrates, inhibitors, and allosteric modulators, and hence, is central to the integrity and performance of integrated multienzyme metabolic systems (Somero, 1981; Hazel et al., 1978; Hazel & Prosser, 1974).

These organisms maintain a constant charge state in several ways, including active ion pumps and ventilatory control of Paco2 (Hitzig, 1982) in both isothermal and changing temperature conditions. Ventilatory control of Paco2 (effecting a change in the CO2 titres of blood) is considered the optimum physiological regulatory mechanism employed by air breathers; by increasing relative ventilation (hyperventilation), Paco2 is decreased while the curves for blood pH3 and intracellular pH versus temperature parallel the curves for the neutral pH of water and

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1The term imidazole also includes other moieties of protein histidyl residues which have similar titration characteristics as imidazole.

2Historically, this regulation was described as a constant ratio of [H+]/[OH⁻] or relative alkalinity in plasma (Rahn, 1967; Rahn et al., 1974).

3pHa is adjusted with a dpH/dT near -0.017 U/°C, as Tb falls or rises (Reeves, 1972; 1985).
pK<sub>im</sub><sup>4</sup> versus temperature (Howell et al., 1970; Reeves, 1972; Gaillard & Malan, 1985). This offsets the increase in CO<sub>2</sub> solubility with decreasing T<sub>b</sub> and maintains a fixed pH at each temperature (Hitzig, 1982). As a result, a constant Ca<sub>CO</sub><sub>2</sub>, pH-pN difference and pH-pK<sub>im</sub> difference are maintained (Reeves, 1972). The specific temperature-controlled CO<sub>2</sub> partial pressure exists for all cellular compartments in the organism, not just for blood (Hitzig, 1982). Subsequently, even though the CO<sub>2</sub> equilibrium curves of blood proteins are very sensitive to temperature change (because of the large effect of temperature on the pK<sub>im</sub>), no titration of protein occurs (White & Somero, 1982), thus allowing optimal biochemical functioning of enzymes (Hazel et al., 1978). As a consequence of this ventilatory control of pHa, no adjustment of intracellular ion concentrations is necessitated via active ion pumps (Reeves, 1972; Malan et al., 1976; Reeves & Malan, 1976; Reeves & Rahn, 1979; Reeves, 1985) and no renal compensation is required (Reeves, 1985). A further consequence is that the plasma bicarbonate concentration and the strong ion difference (SID) or buffer-base remain preserved despite changes in metabolism and leak fluxes due to body temperature changes (Reeves, 1985). It has been emphasized, however, that these patterns of acid-base regulation may not be uniform over the whole temperature range an organism can tolerate. Instead, they may be restricted to certain temperature ranges or steady states<sup>5</sup> (Malan, 1978; Malan, 1985). Over the ranges that this control may operate, intracellular and extracellular compartments may not parallel each other in

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<sup>4</sup>The apparent pK of imidazole (pK<sub>im</sub>) varies inversely with temperature, with a dpK<sub>im</sub>/dT of approximately - 0.017 U/°C, which is similar to the change in the neutrality of water with temperature (dpN/dT) (Reeves, 1972; 1985).

<sup>5</sup>Some species such as <i>Pseudemys</i> may have large ranges over which this control may operate (Malan et al., 1976), while other species like <i>Dipsosaurus</i> are relatively circumscribed in the zone of regulation (Bickler, 1981; 1982).
the extent of regulation (Malan, 1984; Rodeau, 1984). These observations, however, indicate the existence of some mechanism for adjusting the regulated pH set-point as temperature changes (Reeves, 1985).

Since hibernating mammals do not maintain a constant $C_{a_{CO2}}$ and $pH - pK_{in}$ difference in most tissues within any temperature range characterizing the initiation of, or induction into hibernation, it follows that they allow the acid-titration of proteins (Malan, 1985). The extent of acidosis measured in blood plasma of hibernating mammals ($\frac{dpH}{dT} = -0.0016 \text{ U/°C}$) is also transmitted to the brain ($\frac{dpH}{dT} = -0.0050 \text{ U/°C}$), and skeletal muscle ($\frac{dpH}{dT} = -0.0100 \text{ U/°C}$), tissues which together represent most of the body intracellular water (Malan et al., 1981; Malan, 1982; 1985). In the liver on the other hand, and to a lesser extent in the heart, intracellular $\alpha_{in}$ is restored ($\frac{dpH}{dT} = -0.0130 \text{ U/°C}$). In other words, the intracellular pH (pHi) of these latter two tissues increases markedly during hibernation, illustrating the interplay between ventilatory and ionic control of intracellular pH (Malan, 1985). The differential extent of compensation in various tissues has been suggested to reflect the heirarchical importance of integrity and function of tissues during bouts of hibernation, the more important tissues such as the liver and heart escaping inhibition (Malan, 1985; Hochachka & Somero, 1984; Milsom, 1990).

It is well documented that as the animals enter hibernation, significant adjustments in respiratory control occur which are illustrated by profound changes in breathing pattern as well as in the relative importance of various respiratory stimuli (Landau & Dawe, 1958; McArthur & Milsom, 1991). The foremost ramification of these changes is a fall in respiratory frequency, the first indication that an animal is beginning to enter hibernation (Landau & Dawe, 1958). These
respiratory changes are followed by a decline in heart rate (Landau & Dawe, 1958; Lyman, 1958), cardiac output and mean arterial pressure (Musachia et al., 1989), and occur prior to any "test-drops" (Strumwasser, 1959a; Lyman et al., 1982b) or a linear decline in T_b and metabolic rate (Lyman & Chatfield, 1955; Lyman, 1958; Lyman & O'Brien, 1960). Metabolic rate reaches its minimum before body temperature (Lyman & Chatfield, 1955; Lyman, 1958). As shown by Snapp & Heller, (1981), the first 10 minutes of entrance into hibernation are characterized by a transitory drop in RQ values (CO_2 production : Oxygen consumption ratio) from 0.81 to 0.74. The decrease in RQ in this instance, is not indicative of shifts in metabolic substrates (carbohydrates to lipids), but instead, reflects the retention of large amounts of CO_2 due to changes in ventilation (a relative hypoventilation). Similar observations have been made by Bickler, (1984) and Nestler, (1990a). This would serve to moderately increase blood acidity by increasing PaCO_2, and consequently the CO_2 content of blood (CaCO_2), prior to the occurrence of any significant fall in T_b (Snapp & Heller, 1981). The transitory drop in RQ continues only until gas exchange equilibrium for the new ventilation level is reached, following which RQ rises to stable entrance values averaging 0.91 (Snapp & Heller, 1981). This value reflects metabolism primarily based on carbohydrate reserves during entrance, before the animals switch to lipid catabolism characteristic of deep hibernation (RQ drops to 0.78) (Snapp & Heller, 1981).

Differentiation of the two ways in which CaCO_2 may be increased during entrance into hibernation is important. An initial and moderate increase in blood acidity following hypoventilation, that occurs prior to any significant fall in T_b, would constitute a "classical" respiratory acidosis (defined by changes in the standard controlled variables pH_a and PaCO_2). This would change the pH-pK_a difference by decreasing pH directly. Subsequently, CaCO_2 would
also increase in proportion to the progressively increasing CO$_2$ solubility due to falling T$_b$\(^6\), since hibernating mammals do not hyperventilate to maintain Ca$_{co2}$ constant, as already described. The latter form of increase in Ca$_{co2}$ (with falling T$_b$) has been termed a "relative" respiratory acidosis (Milsom, 1990) as pH$_a$ remains relatively constant. The change in the pH-pK$_{in}$ difference in this instance would therefore be on account of changes in pK$_{in}$. Thus, during entrance into hibernation Ca$_{co2}$ will increase through both these mechanisms. Most authors do not make any distinction between the two types of acidosis, however, creating semantic confusion in the literature; instead, the two types of acidosis are additively referred to as "relative" acidosis (Malan et al., 1973) or simply "respiratory acidosis" (Malan et al., 1973; 1985; 1988; Hochachka & Somero, 1984 for example).

As mentioned earlier, the consequences of both types of respiratory acidosis on tissue function in hibernators will arise because of the sensitivity of all protein structure and function to proton titration. Changes in protonation of titrable groups leading to an altered charge state would confer instability of function, inevitably resulting in the depression of associated metabolic systems (See Busa & Nucitelli, 1984; Busa, 1986; and Storey, 1988 for example). This metabolic rate depression may be mediated by indirect as well as direct mechanisms (Whitwham & Storey, 1991). Indirect mechanisms include (1) changes in the Donnan equilibria across membranes associated with these proteins, and (2) changes within other substances acting as metabolites and modulators (Storey, 1987a; 1987b; 1988; Whitwham & Storey, 1991). Direct

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\(^6\)The increase in CO$_2$ solubility will decrease the amount of CO$_2$ released by the lungs. Assuming no change in CO$_2$ production occurs for a certain period, Ca$_{co2}$ will increase to the extent determined by the following equation:

Change in Ca$_{co2}$ = CO$_2$ production - CO$_2$ released by lungs
mechanisms include (1) changes in the activity state of regulatory enzymes via protein phosphorylation or dephosphorylation reactions, (2) pathway control, by altering the association or dissociation of enzymes from multienzyme complexes bound to subcellular structural elements, and (3) regulation of the use of carbohydrate reserves for anabolic purposes via fructose-2,6 bisphosphate (F2,6P2) control over 6-phosphofructokinase (PFK) (Whitwham & Storey, 1991).

In light of the teleological relevance of depressing metabolism as ambient temperature falls on the one hand, and the mechanisms known to depress metabolic systems at multiple levels of integration when protein charge states are altered, on the other, it has been suggested that hibernating mammals may be using these mechanisms as a strategy for regulating metabolic depression during any or all of the following three stages of hibernation:

1) Initiation of entrance into hibernation;

2) Induction into hibernation; and

3) Maintenance of the new steady-state of deep hibernation.

1) Initiation: The factors initiating entrance into hibernation would cause the initial fall in T_b and BMR. This initial fall would instigate the induction process, gradually leading to the minimum levels of T_b and BMR characteristic of the state of hibernation. Pioneers like Dubois, (1896), Johnson, (1930) and Kayser, (1961) speculated that increasing CO_2 concentrations in the inspiratory air due to a progressive build up of burrow gas concentrations of CO_2 might cause acidosis, consequently serving as one of the factors initiating and inducing hibernation. This conjecture was later disproven when it was shown that animals entered hibernation as easily even when CO_2 levels were not allowed to build up and that artificially increasing inspired CO_2 levels
did not by itself induce hibernation.

More recently, the transient hypoventilation which produces a rise in $P_{aCO_2}$ and a fall in pHa prior to any significant fall in $T_b$ ("classical" respiratory acidosis) has been suggested to initiate entrance into hibernation. Snapp & Heller (1981) were the first authors to explicitly consider studying the role of "classical" respiratory acidosis during entrance into hibernation. Unfortunately, they did not measure changes in pHa or blood gases but only assumed that a rise in blood acidity would occur during the transient period of hypoventilation. Making this assumption, however, Snapp & Heller, (1981) speculated that the extent of "classical" respiratory acidosis would not be sufficient to modify cellular functions that would directly reduce basal metabolism. Instead they proposed that the acidosis would contribute only indirectly to the fall in $T_b$ (induction of hibernation) by suppressing the hypothalamic threshold or "set point" for thermogenesis ($T_{set}$), resulting in a downward shift of regulated $T_b$ by shutting off thermoregulatory heat production (thermogenesis) and permitting $V_o2$ or metabolic rate to fall below the extent of heat loss (Snapp & Heller, 1981; Heller, 1988) (Fig. 1B, stage II). The latter phenomenon would be initiated by the manipulation of peripheral vasomotor tone (Wells, 1971). Several studies in non-hibernating mammals such as rats (Pepelko & Dixon, 1974), new-born guinea pigs (Farkas & Donhoffer, 1975), adult guinea pigs (Schaefer & Wüenemberg 1976), as well as hibernating mammals such as golden hamsters (Wüenemberg & Baltruschat 1982) indicate that hypercapnia does have an effect on temperature regulation. Wüenemberg & Baltruschat, (1982) further illustrated that hypercapnia depresses the firing rate of temperature-sensitive (as well as temperature insensitive) neurons of the thermosensitive and thermoregulatory preoptic area of the hypothalamus.
Some authors propose that some factor other than "classical" respiratory acidosis brings about a fall in $T_{set}$, following which the kinetics described above would occur. For example, Margules, (1979) proposed that the balance between two antagonists ($\beta$-endorphin and a hypothesized endoloxone substance) could determine the set-points for a suite of physiological functions including respiratory rates, body weight and body temperature (Fig. 1A. and 1C., stage II). Tucker, (1965) also discounted any initiating role played by "classical" respiratory acidosis. Simultaneously, however, he also discounted the possibility of any other factor actively suppressing metabolism, contending (untenably according to Lyman et al., 1982b) that if the animals did not respond to lower ambient temperatures by increasing heat production, then heat production would be less than heat loss and the animals would start to cool; the animals enter hibernation by "sliding down a temperature-metabolism function" (Fig. 1A., stage II).

In summary, there exists some concrete evidence for an indirect role of "classical" respiratory acidosis in initiating the entrance into hibernation by lowering the hypothalamic threshold for thermogenesis ($T_{set}$). A direct role of "classical" respiratory acidosis potentially leading to a reduction of basal metabolism is not considered likely although it has not been explicitly examined to date.

2) Induction: Obviously, the induction stage of entrance into hibernation follows the initiation of entrance into hibernation. It is affiliated with the additive effects of both "classical" and "relative" respiratory acidosis and is characterized by progressively falling body temperature and metabolic rate. However, metabolic rate reaches minimum values typical of deep hibernation long before $T_b$ completes its decline to minimum, as illustrated in woodchucks (Lyman, 1948;
1958), Syrian hamsters (Robertson et al., 1968), the eastern chipmunk (Wang & Hudson, 1971), and the white footed mouse (Gaertner et al., 1973) amongst others.

If metabolism in heat producing tissues were directly inhibited by "classical" respiratory acidosis during the initiation of entrance into hibernation, it would only become apparent in the induction period and only if $T_b$ fell below $T_{set}$ (Snapp & Heller, 1981). If this were to happen, both shivering and non-shivering thermogenesis would be less effective in raising the lowered $T_b$ in comparison with their effectiveness in raising $T_b$ under un-inhibited circumstances. Thus, $T_b$ would be permitted to decline, enhancing induction into hibernation (Fig. 1B. and 1C., stages II and III). Although Wünnenberg & Baltruschat, (1982) did not find evidence for the suppression of the norepinephrine mediated thermoregulatory effector system related to heat production by brown adipose tissue in the species of hamsters they studied, a significant number of other workers have provided evidence to the contrary. It has been shown that thermogenesis may be stimulated by an intracellular alkalinization that involves an activation of the Na⁺/H⁺ exchanger (Giovannini et al., 1988). Conversely, mild acidification has been well demonstrated to severely inhibit active thermogenesis by suppressing the role of oxidatively metabolizing brown adipose tissue (Chinet et al., 1978; Grav et al., 1970; Nicholls, 1976; Flatmark & Pedersen, 1975). This has been explained in terms of the increased affinity of the mitochondrial uncoupling protein (UCP - the proton channel that opens resulting in heat dissipation from the uncoupling of oxidative phosphorylation) for its inhibitor (the nucleotide GDP), thus hindering norepinephrine binding and consequently heat production (Nicholls, 1976). Malan & Mioskowski, (1988) have confirmed this phenomenon in brown adipose tissue isolated from hibernating mammals by illustrating that the facilitation of GDP binding by low pH can contribute to the reversible
inhibition of thermogenesis in hibernation. Low pH (and low temperature) also causes the active tetrameric form of the glycolytic enzyme PFK (Phospho-fructo-kinase) to dissociate into inactive or inhibited dimers (Hand & Somero, 1982; 1983; 1984; Somero and Hand, 1990). This inhibition of PFK would block shivering thermogenesis (Hochachka & Somero, 1984) initiated by increased motor activity. In summary, the inhibition of shivering and non-shivering thermogenesis could be initiated by "classical" respiratory acidosis prior to any fall in $T_b$. This could potentially facilitate the induction into hibernation by allowing $T_b$ to decline below $T_{set}$.

The foremost outcome of the continual lack of ventilatory or ionic compensation for increased blood CO$_2$ content associated with the declining $T_b$ during the induction process could be the perpetuation and enhancement of the inhibition of shivering and non-shivering thermogenesis, initiated by the "classical" respiratory acidosis. This contention is supported by studies on the interactive effects of pH and temperature on isolated brown adipose cells. At temperatures ranging from 15° to 37° C, the thermogenic response to norepinephrine is reduced up to 75% by increasing P$_{CO2}$ and reducing medium pH to values (temperature corrected) equivalent to those found in hibernation (Malan, 1977).

Heller and co-workers believe that the effects of increased CO$_2$ levels (before and after $T_b$ starts falling) during entrance into hibernation are restricted to the suppression of thermoregulatory thermogenesis, following which, reductions in general body metabolism would be initiated via the simple physiological effects of temperature on cellular functions. Induction would therefore be characterized by a continually cascading down-regulation of $T_{set}$, accompanied by decreases in MR, $T_{hy}$ (hypothalamic temperature; Heller et al., 1977; Snapp & Heller, 1981), $\dot{V}_E$ and $\dot{V}_E/\dot{V}_{O2}$ (air convection requirement), since ventilation and metabolic rate are known to
be tightly coupled with falling temperature (Osborne & Milsom, 1991) (Fig. 1B. and 1C., stage III). This cascade of down-regulations would continue until a new and lower steady state $T_{set}$ was reached (Fig. 1B. and 1C., stage IV). When $T_b$ approaches ambient temperature in deep hibernation, $T_{set}$ remains lower than $T_{hy}$ most of the time, so there is no stimulation of thermoregulatory thermogenesis (Snapp & Heller, 1981). Artificially induced hypothermia has been demonstrated to reduce the metabolic rates of hibernating squirrels to levels documented during hibernation (Osborne & Milsom, 1991), thus supporting the belief that temperature effects are sufficient to reduce BMR to levels characterizing deep hibernation, once $T_{set}$ and thermogenesis have been suppressed.

Most other authors believe that the respiratory acidosis pertaining to the induction period is not restricted merely to the inhibition of thermoregulatory processes. Instead, it could also inhibit glycolytic and respiratory enzyme activities and therefore inhibit BMR (whole animal aerobic energy metabolism) (Fig. 1B. and 1C., stage III). On going from normothermy to hibernation, the rate-limiting enzyme of glycolysis (PFK) is nearly 60% inactivated in the muscle (Malan, 1985). This would inhibit glycolysis and glycogenolysis which in turn would be accompanied by decreased contractility and suppressed substrate turnover (Malan, 1982; 1985; 1988; Busa & Nucitelli, 1984; Hand & Somero, 1982; 1983; Hochachka & Guppy, 1987; Hochachka & Somero, 1984; Storey, 1987a; 1987b). Similar inhibition levels have been reported in the brain (Malan, 1985). Hand & Somero, (1983) however, do not interpret this to result in reduced aerobic metabolism. Instead, they suggest that the inhibition of glycolytic enzymes may simply be responsible for a shift in the fuel from glucose to lipids for energy substrates during hibernation. In other words, induction into hibernation is hypothesized to be a result of
interactive pH-temperature effects on metabolism needed to alter the relative contributions of
different metabolic pathways. Bickler, (1984), however, supports the role of acid inhibition on
BMR, arguing that active inhibition during the induction process must be present to account for
the observation that simply reversing the physiologically inhibitory temperature effects by
warming hibernating squirrels does not arouse them.

In summary, evidence for a direct role of acidosis ("classical" or "relative") in reducing
BMR during entrance into hibernation is scarce despite the dense speculative literature available
on this subject (some of which has been discussed above), the correlative and repeated
observations with respect the kinetics involving changes in $\dot{V}_E$, MR, $T_b$ and pHa in these animals
(Lyman et al., 1982b), as well as the clear supportive evidence for an inhibitory role of acidic
pHi in metabolic regulation during dormancy in some other species (Barnhart, 1986a; 1986b;
1989; Barnhart & McMahon, 1987; 1988; De Laney et al., 1977; Levy & Schneiderman, 1966;
Burnett & McMahon, 1987; Seymour, 1973; Hicks & Riedesel, 1983; Bickler, 1982; Jensen &

3) Maintenance of the steady state of deep hibernation: As can be inferred from above,
two categories of hypotheses are available to date with respect to how metabolic rate is reduced
during entrance into hibernation; one set does not recognize the role of any factor other than
falling temperature in reducing metabolic rate, while the alternative set does. It follows that
opinions concerning the factors that maintain the steady state of hibernation would also be
divided: some authors believe that once the new $T_{set}$ is reached, it is quite possible that $Q_{10}$
effects (the Vant’Hoff-Arrhenius effect of temperature on biochemical reactions) alone may
maintain metabolic processes at the new steady state levels (Snapp & Heller, 1981); the majority of the postulations, however, imply that active metabolic co-inhibition of BMR and thermoregulatory thermogenesis is necessary to maintain the steady state of hibernation.

Several authors have approached this argument by comparing the metabolic rates of the two steady states of euthermia and deep hibernation respectively. They emphasize that the depression of metabolism is simply the consequence of the $Q_{10}$ effect. $Q_{10}$ values of 2 to 3 for the difference in metabolic rate between the euthermic animal at rest and the hibernating animal are noted for a number of species including golden mantled ground squirrels (Hammel et al., 1968; Heller & Colliver, 1974; Snapp & Heller, 1981), arctic ground squirrels (Hock, 1960), chipmunks (Wang & Hudson, 1971), some species of marmots (Lyman, 1958), and even the black bear (Hock, 1960). The apparent sufficiency of $Q_{10}$ effects to explain the steady state reduction of metabolism of these hibernators suggested that additional regulatory mechanisms were not necessary to alter and inhibit the relative contributions of different metabolic pathways in steady state hibernation. However, these authors do not address the high apparent $Q_{10}$ of 3.6-4.5 recorded for metabolic rate changes in some hibernators such as bats (Henshaw, 1968), Mohave ground squirrel (Bartholomew & Hudson, 1960) and European ground squirrels (Kayser, 1964). Malan (1986; 1988) reasoned that high $Q_{10}$ values were indications of the existence of a metabolic inhibition beyond the simple physiological effect of temperature. Snapp & Heller, (1981) on the other hand, suggest that these high $Q_{10}$ value measurements may be an artifact due to failure to account for changes in arousal state. They contend that hibernation is an extension of slow-wave sleep and that if comparisons are made between the metabolic rates of euthermic sleep and hibernation rather than between euthermic quiet awake and hibernation, the difference
can be explained simply by the influence of temperature on metabolism.

Bickler in 1984 demonstrated that CO₂ retention occurred only in the first two hours of entrance into hibernation, stopping well before Tₘ and MR completed their decline to minimum. Complimentary evidence was also reported by authors such as Wang & Hudson, (1970), Geiser & Kenagy, (1988), and Geiser et al., (1990). They found that Q₁₀ values were highest during entry into hibernation and that the net Q₁₀ observed was reduced as body temperature fell towards 10°C. These studies imply that the physiological effects of temperature play an increasingly dominant role as Tₘ falls, progressively replacing whatever factors cause the initial suppression of metabolism. As such they support the idea that temperature effects alone may maintain metabolism at hibernation levels. Hochachka & Somero, (1984) also concur that overall metabolic rate in the hibernator may be set by simple Q₁₀ effects on enzymes involved in oxygen consumption, but suggest that the specific reduction in metabolic systems (brown adipose tissue O₂ uptake, shivering, muscle glycogenolysis) that must be curtailed during hibernation may be largely due to pH effects. Finally, Geiser, (1988) also suggests that reduction of metabolism during hibernation is possibly the result of a large number of factors which may operate on different time scales. For example, blood ketones during hibernation may inhibit uptake of muscle glucose, or low thyroid hormones may reduce BMR during hibernation.

In summary, apart from a handful of workers such as Tucker, (1965) and Snapp & Heller, (1981), it is the belief of the majority of workers that some active inhibition of oxidative metabolic processes is necessary to maintain the steady state of deep hibernation.

Throughout these discussions, respiratory acidosis ("classical" and "relative") has been implied to be instrumental in metabolic suppression. As stated earlier, however, there appears
to be no direct evidence to support the hypothesis that "classical" respiratory acidosis directly inhibits general body metabolism below basal levels in hibernating species of mammals. The goals of this study therefore were to address the following questions:

1) Can "classical" respiratory acidosis inhibit the general body metabolism of euthermic golden mantled ground squirrels? and

2) Can metabolic acidosis similarly affect general body metabolism in euthermic golden mantled ground squirrels?

In other words, this study was designed to test the hypothesis that changes in arterial pH (pHa) reflecting a proportional change in arterial hydrogen ion concentration ([H+]a) effect a change in oxygen consumption (\(\dot{V}_{\text{O}_2}\)) or metabolic rate (MR), in euthermic golden mantled ground squirrels (Spermophilus lateralis). Of interest were the specific effects, either local or reflex, of changing arterial pH on metabolic rate. Thus, it was imperative that experimentally produced changes in pHa did not cause any compensatory changes in ventilation, since this would indirectly alter metabolic rate due to changes in the cost of breathing. It was also important that any changes in metabolic rate produced by the experimental manipulations did not cause any change in body temperature (\(T_b\)) since this would effect changes in solubility constants and/or secondarily cause further changes in metabolic rate. As a consequence, these experiments were conducted on anaesthetized, paralyzed, and artificially ventilated animals maintained at constant body temperature.
Figure 1.

Three scenarios illustrating alternative kinetics that may be occurring during entrance into hibernation.

- $T_{set}$ = Temperature Set-point;
- $T_b$ = Body Temperature;
- $HL$ = Heat Loss;
- $BMR$ = Basal Metabolic Rate;
- $V_E$ = Minute Ventilation;
- $V_{HF}/V_{O2}$ = Air Convection requirement;
- $\beta_{CO2}$ = CO$_2$ Solubility Coefficient;
- $Ca_{CO2}$ = CO$_2$ Content in Arterial Blood;
- $TG$ = Shivering and Non-Shivering Thermogenesis
Scenario 1A.

I. Steady State I.
   * T\textsubscript{b} euthermic

II. Transient State I. (Initiation)
   \begin{align*}
   \textbf{?} \quad \textup{↓} \quad \textup{↓} \\
   \text{↓ T\textsubscript{set}} \\
   \text{↓ H.L. (Decreased Conductance)} \\
   \end{align*}
   * T\textsubscript{b} not fallen

III. Transient State II. (Induction)
   \begin{align*}
   \textbf{Increasing Conductance} \quad \textup{↓} \quad \textup{↓} \\
   \text{↓ BMR, ↓ T\textsubscript{set}} \\
   \text{↓ T\textsubscript{b}} \\
   \text{↓ \hat{V}_E, ↓ \hat{V}_E/\hat{V}_CO_2} \\
   \text{↓ \beta_{CO_2}} \\
   \text{↓ Ca_{CO_2}} \\
   \end{align*}
   * T\textsubscript{b} falling

IV. Steady State II.
   \begin{align*}
   \text{MR\textsubscript{final}, T\textsubscript{set-final, T\textsubscript{b-final}} \\
   \hat{V}_E-final} \\
   \end{align*}
   * T\textsubscript{b} at minimum (Deep Hibernation)

"Classical" respiratory acidosis plays no role in initiating hibernation (Tucker) but some factor other than respiratory acidosis reduces T\textsubscript{set} (Margules).

Respiratory acidosis (from "classical" and "relative" acidosis) is not needed to play a role in the induction process (Tucker).

Respiratory acidosis is not necessary to maintain the new steady state of deep hibernation (Tucker).
I. Steady State I.

* $T_b$ euthermic

II. Transient State I. (Initiation)

* $T_b$ not fallen

1. $\beta_{CO_2}$
2. $Ca_{CO_2}$
3. $BMR$
4. $T_{set}$
5. $TG$

Respiratory acidosis ("Classical" and "Relative" collectively):

1. an indirect role in initiating hibernation by reducing $T_{set}$ (Snapp & Heller, Malan, Hochachka & Somero); or
2. no direct role in reducing BMR (Snapp & Heller); or
3. a direct role in initiating hibernation by reducing BMR (?)

III. Transient State II. (Induction)

* $T_b$ falling

When $T_b$ falls below $T_{set}$, shivering and non-shivering TG will be less effective

Respiratory acidosis ("Classical" and "Relative" collectively):

3. may not play a role in inhibiting BMR during induction into hibernation — its effects are restricted to the progressive inhibition of $T_{set}$ and TG (Snapp & Heller); or
4. must play a role in the induction process (Malan, Storey, Hochachka & Somero, Bickler, Wang & Hudson, Geiser)

IV. Steady State II.

* $T_b$ at minimum (Deep Hibernation)

Active metabolic inhibition of $T_{set}$, TG and BMR is:

* not necessary (Snapp & Heller); or
* necessary (Malan etc.) to maintain hibernation.
I. Steady State I.

* T_b euthermic

II. Transient State I. (Initiation)

* T_b not fallen

III. Transient State II. (Induction)

* T_b falling

IV. Steady State II.

* T_b at minimum (Deep Hibernation)

1. "Classical" respiratory acidosis plays a direct role in initiating hibernation by reducing BMR (?)

2. Some factor other than "classical" respiratory acidosis reduces T_set (Margules)

3. Respiratory acidosis ("Classical" and "Relative" collectively):
   - may not play a role in inhibiting BMR during induction into hibernation — its effects are restricted to the progressive inhibition of T_set and TG (Snapp & Heller); or
   - must play a role in the induction process (Malan, Storey, Hochachka & Somero, Blickler, Wang & Hudson, Geiser)

4. Active metabolic inhibition of T_set, TG and BMR is:
   - not necessary (Snapp & Heller); or
   - necessary (Malan etc.) to maintain hibernation.
METHODS

ANIMAL HOUSING AND MAINTENANCE

A total of 40 ground squirrels of both sexes and weighing 150-300 gms were used in three series of experiments. They were obtained from a commercial collector in Redding, California and housed in pairs in polycarbonate cages (45cm x 25cm x 20cm) containing wood shavings and ground corn cobbs (Sanicell) for bedding. The cages were covered with wire mesh tops. Animals were fed Purina lab rat chow and water ad libitum. Their diet was supplemented with sunflower seeds and fresh fruit. Experiments were performed throughout the year. During the hibernation season (October to May), the squirrels were housed in an environmental chamber maintained at an ambient temperature of 5 ± 1°C on a 2 hour light and 22 hour dark photoperiod (lights switched on at 10 A.M.). Approximately a week prior to an experiment, hibernating animals were removed from this cold chamber and allowed to acclimate to room temperature in a similar chamber maintained at an ambient temperature of 20 ± 1°C on a 12 hour light and 12 hour dark photoperiod (lights switched on at 6 A.M.). All animals were housed under these latter conditions during the non-hibernating period (June to October) as well.

SURGICAL AND RECORDING TECHNIQUES COMMON TO ALL 3 SERIES OF EXPERIMENTS

The experimental animal was weighed at the beginning of each experiment to facilitate the administration of weight dependent dosages of chemicals. Anaesthesia was produced by
6.5mg of sodium pentobarbital per 100gms of body weight, injected intraperitoneally (i.p.), with supplemental doses of ~6.5mg every hour or as necessary. Atropine (5 x 10^{-3} mg.ml.{-1}) was also administered (~0.3 ml.100 gms{-1} of body weight) approximately 20 minutes after the first dose of anaesthesia was given, to reduce tracheal secretions. Body temperature was monitored via an intraperitoneal needle thermistor probe attached to a digital display (Sensortek BAT-12, Clifton, N.J.) and maintained at normal levels (37°C ± 1) by an external heated water pad (Hamilton aquamatic K module), both during surgery and throughout the experiment.

The trachea was exposed via a midsagittal-cervical ventral incision and a cannula (PE 240 tubing) approximately 5 cm in length, was inserted into the trachea to enable the animal to be artificially ventilated by a piston driven (Harvard Apparatus model 665) or a pressure driven (Narco Biosystems respirator V5KG; Z4299) respiratory pump. The animals were ventilated with humidified gas mixtures produced by mixing air, 100% O_{2} and 100% CO_{2} with a series of regulated flow meters (VCC P/N FM 4333,4334 and 4335, Linde Union Carbide). Expired gas was directed from the animal through a heated (~37°C) pneumotachograph (Gould 369500-45001, Fleisch model 0000) attached to a differential strain gauge transducer (Validyne model DP103-18) connected to a transducer amplifier (Gould 13-4615-50). This produced a differential pressure record which was proportionate to the air flow rate through the pneumotachograph. This signal was in turn integrated (Gould Integrator Amplifier 13-4615-17/70), to yield the tidal volume of each breath. All recordings were made on a direct-writing ink recorder (Gould 2600) set at a speed of 100 mm/min. The expired gas was then either expelled into room air through the outlet valve on the pump or, collected in a Douglas bag (capacity: 250 mls) for subsequent analysis of the expired gas (Fig. 2.).
Figure 2.

An illustration of the general set-up used for all three series of experiments. See text for details.
A catheter was also placed in one carotid artery via the ventral incision. The tip of the arterial catheter (PE 50 tubing) was advanced into the carotid artery until it lay close to or in the ascending aorta. The opposite end of the catheter was connected to a pressure transducer (Gould P10EZ) coupled to a pressure processor (Gould 11-4113-01 or 13-4615-52), and the measured arterial blood pressure was displayed on the chart recorder. 1000 units.ml⁻¹ of heparin at a dose of 0.3 ml.100 gms⁻¹ of body weight were administered via the arterial catheter immediately upon insertion and securing of the catheter.

At least 30 minutes were allowed for the ventilatory pattern to stabilize in spontaneously breathing animals, after the completion of surgery. At the end of this stabilization period, resting blood gas measurements were made.

A sample of blood (0.3 ml) was withdrawn from a side arm of the arterial catheter into a heparinized syringe. Analysis of arterial P<sub>CO₂</sub>, P<sub>O₂</sub> and pH was performed immediately after sampling, using a micro blood gas analyzer (Radiometer model PHM 71) maintained at an operating temperature of 38° C. The electrodes were calibrated before each measurement period. The pH electrode was calibrated with standard Radiometer buffer solutions, the P<sub>CO₂</sub> electrode with humidified gases (5.3% and 10% CO₂) provided by a gas mixing pump (Radiometer GMA2 precision gas pump), and the P<sub>O₂</sub> electrode with air saturated water and deoxygenated water (Sodium borate powder was used to deoxygenate the water). After analysis, blood used for P<sub>CO₂</sub> and P<sub>O₂</sub> measurements was withdrawn from the electrode and returned to the animal, and the cannula was flushed with fresh heparinized saline. An additional sample of arterial blood (0.22 ml) was withdrawn from two animals for lactate measurements. Sample storage and analysis procedures are described in a subsequent section.
The animal was then injected with 0.3 mg Tubocurarine for every 100 gms of body weight (3 mg.ml\(^{-1}\) @ 0.1 ml.100 gms\(^{-1}\) body weight) in order to paralyze skeletal muscles. A supplementary dose of ~0.3mg was given every hour or as necessary to maintain muscular relaxation throughout the experiment. The tracheal cannula was connected to the respiratory pump that was set to ventilate the animal with air. The volume of the ventilator was set to pump 3 ml.cycle\(^{-1}\) for most animals. The frequency setting was adjusted for every animal specifically, in order to produce blood-gases and pH approximating normocapnic values. The ventilator was set to deliver a constant ratio of inspiration to expiration of 1 to 1. Once the tubocurarine had taken complete effect and the animal had become dependent on the pump for at least half an hour, another set of blood gas measurements were taken. In addition, expired air was collected for approximately 20 seconds in the Douglas bag attached to the respiratory pump. A sample of the inspired gas was also collected in a 50 cc glass syringe. Both samples were analyzed for the concentration of CO\(_2\) (%CO\(_2\)) with an infrared CO\(_2\) analyzer (Beckman, model LB2) and for the concentration of O\(_2\) (%O\(_2\)) with a polarographic O\(_2\) analyzer (Beckman, model OM11). These analyzers were frequently calibrated with gases that had been prepared by the Radiometer GMA2 precision gas pump. The volume of the expired gas collected in the Douglas bag was also carefully measured. Minute ventilation (\(\dot{V}_E\)), oxygen consumption (\(\dot{V}_{O_2}\)) and carbon dioxide production (\(\dot{V}_{CO_2}\)) were calculated at BTPS (body temperature and pressure, saturated) using the following formulae:
1. $\dot{V}_E$ (ml.min$^{-1}$)

   $\dot{V}_E = \frac{\text{Volume of gas collected in the Douglas bag (mls)}}{\text{Number of seconds taken to collect this volume (sec.)}} \times 60$

2. $\dot{V}_{O_2}$ (ml.min$^{-1}$)

   $\dot{V}_{O_2} = \dot{V}_E (\text{ml.min}^{-1}) \times (\% \text{Inspired O}_2 - \% \text{Expired O}_2)$

3. $\dot{V}_{CO_2}$ (ml.min$^{-1}$)

   $\dot{V}_{CO_2} = \dot{V}_E (\text{ml.min}^{-1}) \times (\% \text{Expired CO}_2 - \% \text{Inspired CO}_2)$

$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were temperature corrected to 37° C to account for small differences in $T_b$ between samples, assuming a $Q_{10}$ of 3.0 for this species of squirrel (Osborne, 1989) and using the formula:

$\dot{V}_{O_2}$ (or $\dot{V}_{CO_2}$) at 37° C = $\dot{V}_{O_2}$ at body temperature $T_b \times 3^{(37° - \text{body temperature})/10}$

$\dot{V}_E$ and consequently $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were not weight standardized because it was not possible to determine the squirrels’ lean body mass. The animals were growing as they moved towards the hibernation season and body weight could double over the summer, or be reduced by half over the winter. An attempt was made, however, to minimize the weight range (150 gms to 300 gms) of the squirrels used in the experiments.

PROTOCOL SPECIFIC TO EACH SERIES OF EXPERIMENTS

Series I. Hyper- Hypoventilation Experiments

A total of 6 ground squirrels were used for this series of experiments. Following the
initial surgery, period of stabilization, and measurement of expired and arterial gas concentrations, as described above, the animals were hyperventilated and hypoventilated in a random fashion. In general, animals were ventilated at 3 to 8 different levels of minute volume for 30 to 60 minutes each. Blood gas and pHa readings were made every 30 minutes and expired gas samples were measured every 15 minutes. From two animals, additional arterial blood samples (0.2 ml) were also drawn for lactate measurements. Sample storage and analysis procedures are described in a later section. Pump minute ventilation volumes were chosen to produce changes in arterial pH ranging from 6.9 to 7.8 Units. Blood pressure and body temperature were constantly monitored and $V_E$, $V_O_2$ and $V_{CO_2}$ were calculated from the data obtained at the end of each period of ventilation at each level of minute ventilation. The level of arterial plasma bicarbonate ($[HCO_3^−]$) was calculated from the $P_{CO_2}$ and $pH_a$ with the Henderson-Hasselbach equation. The values for the pK' (6.1) and solubility coefficient (0.03 mM.l.⁻¹) were taken from Reeves (1976).

Series II. CO₂ Loading Experiments

A total of 12 ground squirrels were used for this series of experiments. Following the initial period of stabilization and measurement of expired and arterial gas concentrations, the animals were pump-ventilated with varying levels of inspired CO₂ ranging from 0.04% to 10%, interspersed with periods of ventilation on air. The respiratory frequency and volume were adjusted initially to produce an arterial pH of approximately 7.4 or 7.5 Units. These pump settings were then maintained for the rest of the experiment. Inspired CO₂ levels were varied randomly by adjusting the flowmeter settings and mixing air with 100% CO₂. Each concentration was maintained for 30 or 60 minutes and blood samples were drawn and analyzed at the end of
each 30 minute period. An additional sample of arterial blood (0.2 ml) was withdrawn from two animals for the purpose of lactate measurements to be performed at a future date. Sample storage and analysis procedures are described in a later section. For every level of inspired CO$_2$, inspired and expired gas compositions were determined every 30 minutes and blood pressure and body temperature were constantly monitored. $V_E$, $V_{O_2}$, $V_{CO_2}$ and the level of arterial plasma bicarbonate ($[HCO_3]_a$) were then calculated based on these measurements.

**Series III. Acid Loading Experiments**

A total of 22 ground squirrels were used in this series of experiments. In addition to the surgical preparation described earlier, a heparinized venous catheter was also placed in the right external jugular vein for the infusion of lactic acid, acetic acid, and/or mammalian Ringer's solution. The tip of the venous catheter was advanced towards the right atrium in order to place the tip within or close to the right atrial opening. 1000 units.ml$^{-1}$ of heparin at a dose of 0.3 ml.100 gms$^{-1}$ of body weight were administered via the venous catheter, immediately upon insertion and securing of the catheter. This catheter was connected to an infusion pump. Following the surgery and initial stabilization period, expired and arterial blood gas concentrations were measured in conjunction with arterial pH. The animals were then placed on a ventilator with the respiratory frequency and volume adjusted to produce an arterial pH of approximately 7.4 or 7.5 Units.

Lactic acid (LA) at a concentration of 0.08-1.5 mM.ml$^{-1}$ (freshly prepared from a stock solution of 0.33 M diluted in mammalian Ringer's solution: pH 6.9-7.1 Units), was infused at a rate of 0.19-0.32 ml.min$^{-1}$ for a period of 20-30 minutes or until arterial pH reached a value
< 7.4. Infusion was then terminated and the venous catheter flushed with saline. A 0.1 ml.
arterial blood sample for pH analysis was withdrawn every five minutes during the infusion
period as well as during a half hour post-infusion period. Blood samples for arterial \( P_{\text{CO}_2} \) and
\( P_{\text{O}_2} \) measurements were taken less frequently (every 10-15 minutes) during the infusion as well
as post-infusion period. These samples were returned to the animal when possible. From three
animals infused with LA, blood samples were also taken every 10-15 minutes during the infusion
and post-infusion periods, and stored for the purpose of lactate measurements to be performed
at a future date. Storage and analysis procedures are described in the following section. Expired
gas was collected in the Douglas bag (for 20 seconds) following each blood sample and was
analyzed as described earlier.

A similar protocol was followed for acetic acid (AA) infusions. A concentration of 0.08-
1.5 mM.ml\(^{-1}\) of AA (prepared from a stock solution of 1M, freshly diluted with mammalian
Ringer's solution) was infused at a rate of 0.19-0.32 ml.min\(^{-1}\) for a period of 20-30 minutes or
until arterial pH reached a value < 7.4 Units.

Unacidified mammalian Ringer’s solution (MRS) was also infused following a similar
protocol. MRS served as a very weak acid (pH of 7.3 Units) which could not be used by the
animal as a metabolic substrate. It was infused at the same rate and for the same period as LA
and AA were. \( \dot{V}_E \), \( \dot{V}_{\text{O}_2} \), \( \dot{V}_{\text{CO}_2} \) and \([\text{HCO}_3^-]\) were then calculated for all three types of infusions.

LACTATE MEASUREMENTS

*Blood Sample Storage:* Using a standard centrifuge, plasma supernatant was immediately
extracted from each blood sample withdrawn from the animals for this purpose. 50 µl of the supernatant were mixed thoroughly with 75 µl of 0.3N Zinc Sulphate and 75 µl of 0.3N Barium Hydroxide and placed in ice for 3 minutes. This mixture was then centrifuged for two minutes following which a 75 µl sample of the extract was sealed and frozen for future analysis.

**Lactate Analysis:** The frozen blood samples were defrosted to room temperature. Glutamate buffer (0.15M; pH 8.9 Units), 0.1 ml of NAD (1mM.l⁻¹), 4 Units of Glutamate-Pyruvate Transaminase (GPT, SIGMA G9880), 11 Units of Lactate Dehydrogenase (LDH, SIGMA L2500) and 100 µl of the defrosted blood sample mixture were used to perform the following lactate assay using a plate spectrophotometer (Titertek Multiskan® MCC/340):

\[
\begin{align*}
NAD & \rightarrow NADH \\
LACTATE & \leftrightarrow PYRUVATE + GLUTAMATE \\
LDH & \uparrow GPT \\
ALANINE & + αKG
\end{align*}
\]

The Glutamate buffer and GPT were mixed to form a reagent mixture, 250 µl of which were added to 10 µl of each sample to obtain the first spectrometer reading. Subsequently, LDH was added to initiate the assay reaction stated above. The final reading was obtained after 10-20 minutes, the time taken for the completion of the above reaction in plasma. These readings were used to calculate arterial lactate levels.

**STATISTICAL METHODS**

All statistical computations were performed using the statistical program Systat
Linear regressions (yielding Pearson correlation coefficients) were performed on individual absolute values obtained for the independent variables ($V_E$ for Series I, %Inspired CO$_2$ for Series II, and pH$_a$ for Series III) and the dependent variables (pH$_a$, Pa$_{CO_2}$, Pa$_{O_2}$ and $V'_O_2$ for series I and II, and only the latter three variables for series III). Regressions between measured values of metabolic rate ($V'_o_2$) and blood gases as well as pH$_a$ were also performed for all three series, to correlate the trends between changes in metabolic rate and changes in the acid-base status of the animals. Because of the high intra-individual variability observed in baseline values of metabolic rate$^7$ as well as blood gases and pH$_a$$^8$, it was not considered appropriate to correlate absolute values. Instead, for each animal, absolute values were normalized with respect to baseline values, the latter being represented by 1. Following normalization, the baseline values themselves were not plotted. Bonferroni adjusted probabilities were determined in order to obtain the significance level of the Pearson correlation coefficients (yielded by regression analysis). These significance levels were consulted to validate the strength of the Pearson correlation coefficients instead of the absolute values of these coefficients. It was also elected to make multiple range statistical comparisons between absolute value data sets which were grouped firstly as a function of the variable used to manipulate animal pH$_a$'s, and secondly, as a function of pH$_a$ itself (the grouping criteria being stated below). Within each experimental series, these comparisons involved Bartlett's test of homogeneity of groups and one-way analysis of variance (ANOVA). A subsequent $a$ posteriori test (the Tukey test) for multiple comparisons (between

$^7$possibly on account of using non-weight standardized data.

$^8$possibly on account of the intra-individual differences in the effects of pump-ventilation on the acid-base status of individual animals.
mean values representing every range), was also performed. Differences were considered significant if \( p \leq 0.05 \).

**Grouping Criteria for Ranges**

The grouping of ranges was designed to emphasize the fact that though different methods (independent variables) were used to manipulate arterial pH levels (a dependent variable) in the three series of experiments, it was the study of the relationship between this dependent variable (pHa) and metabolic rate that was the focus of interest in all three series.

**Series I**

In Series I, data were sorted according to \( \dot{V}_E \) (the independent variable, used to manipulate blood gases and pHa) as well as according to pHa (the dependent variable, used to define "classical" respiratory acidosis/alkalosis).

**(i). Data Sorted According to the Independent Variable \( \dot{V}_E \):** Range 1 of data sorted according to \( \dot{V}_E \) consisted of the initial set of measurements from each animal after being pump-ventilated on air for 30 minutes. Range 2 consisted of data collected over the \( \dot{V}_E \) range of 80-119 ml.min\(^{-1}\). Range 3 consisted of data collected over the \( \dot{V}_E \) range of 120-149 ml.min\(^{-1}\) and range 4 consisted of data collected over the \( \dot{V}_E \) range of 150-189 ml.min\(^{-1}\). These ranges were meant to represent baseline measurements under normoventilated conditions, followed by hypoventilation, normoventilation and hyperventilation respectively.
(ii) Data Sorted According to pHa: Because of the individual differences in the effects of different levels of ventilation on pHa (see Fig. 3a.), data was also sorted according to pHa (a dependent variable) such that range 1 depicted the baseline measurements as described for $V_E$ sorted data, range 2 clearly represented the mean of only those individual measurements that corresponded to a pHa > 7.4 Units (respiratory alkalosis) specifically, while range 3 clearly represented the mean of all individual data exclusively corresponding to a pHa < 7.4 Units (respiratory acidosis).

Series II

In series II, data was sorted according to %Inspired CO₂ (the independent variable, used to manipulate blood gases and pHa) as well as according to pHa (the dependent variable, used to define "classical" respiratory acidosis/alkalosis).

(i) Data Sorted According to %Inspired CO₂: Range 1 of %Inspired CO₂ sorted data consisted of the initial set of measurements from each animal after being pump-ventilated on air for 30 minutes, prior to being CO₂ loaded. Range 2 consisted of data collected during the period of CO₂ loading, while range 3 consisted of data collected after the recovery period following CO₂ loading.

(ii) Data Sorted According to pHa: Again, because of the individual differences in the effects of CO₂ loading on pHa (see Fig. 3b.), data was also sorted according to pHa (dependent variable). Range 1 depicted the baseline data as described for %Inspired CO₂ sorted data. Range
Figure 3.

Changes in the absolute values of pH as $V_{E}$ (3a) was manipulated in series I, and %Inspired CO$_2$ was manipulated in series II (3b), illustrating that because of relatively high resting levels of pHa, all hypoventilated as well as CO$_2$ loaded animals did not necessarily have a pHa < 7.4 Units.
\[ \dot{V}_E \text{ (ml.min}^{-1}) \]

\[ \text{pHa} \]

Range 2 (Alkalotic)

Range 3

Range 2

Range 3

Range 4

\[ \% \text{CO}_2 \text{ (\%)} \]
2 represented the mean of all individual data corresponding to a pHa > 7.4 Units during the period of CO₂ loading while range 3 on the other hand, represented the mean of only those individual measurements during the period of CO₂ loading, that corresponded to a pHa < 7.4 Units (respiratory acidosis). Range 4 consisted of data collected after the recovery period following CO₂ loading.

Series III

Data Sorted According to the Independent Variable pHa: In series III, pHa itself was the independent variable. Consequently, it was the only variable according to which the data was sorted. Range 1 consisted of the initial set of measurements obtained from each animal after being pump-ventilated on air for 30 minutes prior to being infused with acid. Range 2 consisted of data collected during the initial stages of acid infusion while pHa was still > 7.4 Units. Range 3 consisted of data collected later in the infusion period, when pHa was < 7.4 Units (metabolic acidosis), and range 4 consisted of data collected in the recovery period following LA and AA infusions. In the case of MRS infused animals, although pHa values were lowered in the infusion period, metabolic acidosis (pHa < 7.4 Units) was absent. Data from these animals was therefore treated separately, with range 1 and range 2 sorted as in the case of LA and AA infused animals, and range 3 represented recovery data following the MRS infusion period.
RESULTS

SERIES I. HYPER- HYPOVENTILATION EXPERIMENTS

*An Individual Animal*

Increasing $\dot{V}_E$ (Fig. 4a.) by increasing levels of pump ventilation produced a fall in $P_{a\text{CO}_2}$ and a rise in $P_{a\text{O}_2}$ (Fig. 4e.) while decreasing $\dot{V}_E$ caused an increase in $P_{a\text{CO}_2}$ and a fall in $P_{a\text{O}_2}$. These changes were also accompanied by corresponding increases or decreases respectively, in pHa (Fig. 4b.). There were also changes in $V_{O_2}$ (Fig. 4c.) and $V_{CO_2}$ (Fig. 4f.) which were in the same direction as the changes in $\dot{V}_E$. A new steady state in terms of all variables was usually reached within 30 minutes of changing the level of pump ventilation. R values were inconsistently affected by the level of ventilation (Fig. 4d.) and calculated $[\text{HCO}_3^-]$a values did not show any particular trend in this animal (Fig. 4g.).

*Group Data*

(i) *Correlation Between $\dot{V}_E$ (the independent variable) and $P_{a\text{CO}_2}$, $P_{a\text{O}_2}$, pHa (Dependent Blood Gas and Acid-base Variables):* The trends illustrated for the individual animal in Fig. 4. were representative of most animals in this series of experiments. Fig. 5. shows these trends for all animals tested, with linear regression analyses for each relationship. $\dot{V}_E$ and $P_{a\text{CO}_2}$ (Fig. 5a.)
Figure 4.
Data from an individual squirrel depicting the change in pH (4b), $\dot{V}_{O_2}$ (4c), R value (4d), $P_{aO_2}$ and $P_{aCO_2}$ (4e), $V_{CO_2}$ (4f), and calculated $[HCO_3^-]$ (4g), as $V_E$ was manipulated on the respiratory pump, over time (4a).
Figure 5.

The effect of varying the levels of $\dot{V}_E$ on the blood gas variables $P_{a\text{CO}_2}$ (5a), $P_{a\text{O}_2}$ (5b) and pH$_{a}$ (5c), as well as on the level of aerobic metabolism (5d). Linear regression analysis has been performed on all relationships and equations and r values are stated for each relationship; n = 6 in all cases.
\( r = -0.85 \)
\( \text{Pa}_{\text{CO}_2} = 100.8 - 0.48 \dot{V}_E \)

\( r = +0.78 \)
\( \text{Pa}_{\text{O}_2} = -5.1 + 0.38 \dot{V}_E \)

\( r = +0.82 \)
\( \text{pHa} = 6.62 + 0.006 \dot{V}_E \)

\( r = +0.68 \)
\( \dot{V}_{\text{O}_2} = 1.09 + 0.014 \dot{V}_E \)
showed a strong negative correlation \( r = -0.85, p < 0.001 \), a change of 10 ml.min\(^{-1}\) in \( \dot{V}_E \) eliciting a change of approximately 5 Torr in \( \text{Pa}_\text{CO}_2 \) (Fig. 5a.). \( \dot{V}_E \) and \( \text{pHa} \) were also strongly correlated \( r = +0.82, p < 0.001 \) as every 10 ml.min\(^{-1}\) change in \( \dot{V}_E \) changed \( \text{pHa} \) by 0.06 Units (Fig. 5c.). A similar strong correlation between \( \text{Pa}_\text{O}_2 \) and \( \dot{V}_E \) was also prevalent \( r = +0.78, p < 0.001 \), an increase of 10 ml.min\(^{-1}\) in \( \dot{V}_E \) producing an increase of approximately 4 Torr in \( \text{Pa}_\text{O}_2 \) (Fig. 5b.). Given the strong correlations between \( \dot{V}_E \) and the acid-base variables \( \text{Pa}_\text{CO}_2 \) and \( \text{pHa} \), a predictably strong correlation was calculated between the latter two acid-base variables themselves \( r = -0.91 \). A strong inverse relationship between the two blood gases, \( \text{Pa}_\text{CO}_2 \) and \( \text{Pa}_\text{O}_2 \), was also predictable and confirmed with a regression coefficient of +0.79.

**Average Changes (\( \dot{V}_E \) Sorted Data):** Mean values obtained for the blood gas variables and \( \text{pHa} \) over specific ranges of \( \dot{V}_E \) (see Table 1. and Fig. 6.) confirmed a general success in manipulating the acid-base status of the animals by changing the levels of \( \dot{V}_E \). A mean \( \dot{V}_E \) of 141 ± 14.98 ml.min\(^{-1}\) produced a \( \text{Pa}_\text{CO}_2 \) of 28.3 ± 3.2 Torr, and a \( \text{pHa} \) of 7.54 ± 0.05 Units in animals 30 minutes after they were initially placed on the ventilator (see Range 1). These numbers indicate that the animals were, on average, moderately hyperventilated at the beginning of the experiments despite an attempt to produce neutral blood gas variables\(^9\). Over Range 2 (\( \dot{V}_E = 80-119 \text{ ml.min}^{-1} \)), the animals were hypoventilated at an average \( \dot{V}_E \) of 98.54 ± 2.61 ml.min\(^{-1}\). This produced a \( \text{pHa} \) of 7.13 ± 0.03 Units, a \( \text{Pa}_\text{CO}_2 \) of 59.2 ± 2.5 Torr, and a \( \text{Pa}_\text{O}_2 \) of 33.1 ± 1.6 Torr. These values were significantly different from those of Range 1 and 3, being

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\(^9\)A \( \text{Pa}_\text{CO}_2 \) of 34-40 Torr and a \( \text{pHa} \) of 7.35-7.4 Units is considered neutral in most mammals.
Table 1.

Values of arterial blood gases, acid-base variables and aerobic metabolism as a function of minute ventilation ($V_e$), over 4 different ranges.
Values are Mean ± S.E.; $n = 6$. 
<table>
<thead>
<tr>
<th>( \dot{V}_E ) (ml.min(^{-1}))</th>
<th>( \text{PaCO}_2 ) (Torr)</th>
<th>( \text{PaO}_2 ) (Torr)</th>
<th>pHa (Units)</th>
<th>( \dot{V}_O_2 ) (ml.min(^{-1}))</th>
<th>( \dot{V}_CO_2 ) (ml.min(^{-1}))</th>
<th>R value</th>
<th>([\text{HCO}_3^-]_a) (nM.L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td><strong>S.E.</strong></td>
<td><strong>Mean</strong></td>
<td><strong>S.E.</strong></td>
<td><strong>Mean</strong></td>
<td><strong>S.E.</strong></td>
<td><strong>Mean</strong></td>
<td><strong>S.E.</strong></td>
</tr>
</tbody>
</table>

**RANGE**

1

141 | 14.98 | 28.33 | 3.18 | 45.83 | 5.36 | 7.54 | 0.05 | 3.56 | 0.32 | 3.27 | 0.33 |

2

98.54 | 2.61 | 59.22 | 2.49 | 33.08 | 1.59 | 7.13 | 0.03 | 2.35 | 0.13 | 2.33 | 0.11 |

3

131 | 3.19 | 34.50 | 2.53 | 45.08 | 3.91 | 7.40 | 0.03 | 3.01 | 0.06 | 2.66 | 0.09 |

4

162 | 3.71 | 23.55 | 1.81 | 57.18 | 3.90 | 7.59 | 0.02 | 3.39 | 0.06 | 3.24 | 0.09 |

**RANGE 1** = First measurements from each animal after pump-ventilation on air for 30 minutes

**RANGE 2** = Data corresponding to \( \dot{V}_E \) range 80–119 (ml.min\(^{-1}\)).

**RANGE 3** = Data corresponding to \( \dot{V}_E \) range 120–149 (ml.min\(^{-1}\)).

**RANGE 4** = Data corresponding to \( \dot{V}_E \) range 150–189 (ml.min\(^{-1}\)).

* Values significantly different (p<0.05) from RANGE 1

** Values significantly different (p<0.05) from RANGE 2

*** Values significantly different (p<0.05) from RANGE 3

**** Values significantly different (p<0.05) from RANGE 4
Figure 6.

The relationship between $\dot{V}_E$ and blood gases, pH$a$, and aerobic metabolism for data sorted as a function of $\dot{V}_E$.
Values are Mean ± S.E.; $n = 6$.
Range 1 = values corresponding to the mean $\dot{V}_E$ of 141 ± 15 ml.min$^{-1}$ obtained from the first measurements from each animal after pump-ventilation on air for 30 minutes;
Range 2 = values corresponding to a mean $\dot{V}_E$ of 98.54 ± 2.61 ml.min$^{-1}$ (hypoventilation);
Range 3 = values corresponding to an intermediate level of $\dot{V}_E$ (131 ± 3.19 ml.min$^{-1}$) between range 2 and 4; and
Range 4 = values corresponding to a mean $\dot{V}_E$ of 162 ± 3.71 ml.min$^{-1}$ (hyperventilation).
** = Values significantly different ($p \leq 0.05$) from range 2.
the minimum levels of $\dot{V}_E$, pHa and PaO$_2$ and the maximum level of PaCO$_2$ obtained during this series of experiments. Measurements obtained over the $\dot{V}_E$ range of 120-149 ml.min.$^{-1}$ (Range 3, mean $\dot{V}_E = 131 \pm 3.19$ ml.min.$^{-1}$) were a mean pHa of 7.4 ± 0.03 Units and a mean PaCO$_2$ of 34.5 ± 2.5 Torr). These values were not significantly different from those of Range 1 and met the criteria for normal (neutral) acid-base balance. Finally, the maximum mean values obtained for pHa and PaO$_2$ were 7.59 ± 0.02 Units and 57.2 ± 3.9 Torr respectively, in conjunction with a minimum PaCO$_2$ of 23.5 ± 1.8 Torr over the $\dot{V}_E$ range from 150-189 ml.min.$^{-1}$ (Range 4, mean $\dot{V}_E = 162 \pm 3.71$ ml.min.$^{-1}$). These values represented the condition of hyperventilation, and were significantly different from those in Range 2 and 3. All changes in [HCO$_3^-$]a values (calculated from pHa and PaCO$_2$ for all ranges) were statistically insignificant and exhibited no particular trend with different levels of minute ventilation.

**Average Changes (pHa Sorted Data):** Table 2. and Fig. 7. illustrate data sorted by ranges of pHa rather than by ranges of $\dot{V}_E$. The three ranges represent the initial conditions (Range 1) and subsequent data collected at a pHa > 7.4 Units (Range 2) resulting from hyperventilating the animals (respiratory alkalosis) and at a pHa < 7.4 Units resulting from hypoventilating the animals (respiratory acidosis). The mean acid-base variables associated with hypoventilation were a pHa of 7.19 ± 0.03 Units and a PaCO$_2$ of 52.8 ± 3.0 Torr; the blood gas PaO$_2$ concurrently averaged 36.0 ± 2.2 Torr (Range 3). In contrast, hyperventilation produced significantly higher values for pHa (7.55 ± 0.02 Units) and PaO$_2$ (53.9 ± 3.3 Torr), and a significantly lower PaCO$_2$ (25.9 ± 1.8 Torr) (Range 2), relative to their mean values in Range 3. Again, all changes in [HCO$_3^-$]a values were statistically insignificant and exhibited no particular trend.
Table 2.

Values of $\dot{V}_E$, arterial blood gases, acid-base variables, and aerobic metabolism as a function of pHa, over 3 different ranges.
Values are Mean ± S.E.; n = 6.
<table>
<thead>
<tr>
<th>pHa (Units)</th>
<th>$\dot{V}_E$ (ml.min$^{-1}$)</th>
<th>$P_{a\text{CO}_2}$ (Torr)</th>
<th>$P_{a\text{O}_2}$ (Torr)</th>
<th>$\dot{V}_{\text{O}_2}$ (ml.min$^{-1}$)</th>
<th>$\dot{V}_{\text{CO}_2}$ (ml.min$^{-1}$)</th>
<th>R value</th>
<th>$[\text{HCO}_3^-]$a (nM.L$^{-1}$)</th>
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<tbody>
<tr>
<td>MEAN S.E. MEAN S.E. MEAN S.E. MEANS.E. MEAN S.E. MEAN S.E. MEAN S.E. MEAN S.E.</td>
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<tr>
<td><strong>RANGE</strong></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>7.54 ± 0.05</td>
<td>141.00 ± 15.04</td>
<td>28.33 ± 3.18</td>
<td>45.83 ± 5.36</td>
<td>3.56 ± 0.32</td>
<td>3.27 ± 0.33</td>
<td>0.92 ± 0.05</td>
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<tr>
<td>2</td>
<td>7.55 ± 0.02</td>
<td>149.82 ± 4.96</td>
<td>25.88 ± 1.77</td>
<td>53.94 ± 3.28</td>
<td>3.26 ± 0.06</td>
<td>2.98 ± 0.11</td>
<td>0.91 ± 0.02</td>
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</tr>
<tr>
<td>3</td>
<td>7.19 ± 0.03</td>
<td>109.58 ± 4.44</td>
<td>52.78 ± 2.95</td>
<td>35.95 ± 2.24</td>
<td>2.55 ± 0.12</td>
<td>2.48 ± 0.10</td>
<td>0.99 ± 0.02</td>
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</table>

RANGE 1 = First measurements from each animal after pump-ventilation on air for 30 minutes  
RANGE 2 = Data corresponding to: pHa range > 7.4 Units  
RANGE 3 = Data corresponding to: pHa range < 7.4 Units  

* Values significantly different (p<0.05) from RANGE 1  
** Values significantly different (p<0.05) from RANGE 2  
*** Values significantly different (p<0.05) from RANGE 3
Figure 7.

The relationship between $\dot{V}_E$ and blood gases, pHa, and aerobic metabolism for data sorted as a function of pHa.

Values are Mean ± S.E.; n = 6.
Range 1 = values obtained as the first measurements from each animal after pump-ventilation on air for 30 minutes (pHa = 7.54 ± 0.05 Units);
Range 2 = values corresponding to a pHa > 7.4 Units (respiratory alkalosis); and
Range 3 = values corresponding to a pHa < 7.4 Units (respiratory acidosis)
*** = Values significantly different ($p \leq 0.05$) from range 3.
(ii) Correlation Between $\dot{V}_E$ (the Independent Variable) and Metabolic Rate ($\dot{V}_{O2}$): With respect to metabolic rate, $\dot{V}_{O2}$ changed in the same direction as the changes in the independent variable $\dot{V}_E$ ($r = +0.68$, $p < 0.001$ respectively); an increase of every 10 ml.min.$^{-1}$ in $\dot{V}_E$ caused approximately a 0.14 ml.min.$^{-1}$ increase in $\dot{V}_{O2}$ (Fig. 5d.).

Average Changes: The average oxygen uptake of hypoventilated animals (2.35 ± 0.13 ml.min.$^{-1}$) was significantly lower than the oxygen uptake of hyperventilated animals (3.39 ± 0.06 ml.min.$^{-1}$) as demonstrated in Table 1. In this respect, $\dot{V}_{CO2}$ was also lowered significantly. R values however, did not change significantly (Table 1.). The average oxygen uptake of acidotic animals (2.55 ± 0.12 ml.min.$^{-1}$) was also significantly lower than the oxygen uptake of alkalotic animals (3.26 ± 0.06 ml.min.$^{-1}$) as shown in Table 2. Again, R values did not differ significantly (Table 2.)

(iii) Correlation Between $Pa_{CO2}$, $Pa_{O2}$, pHa (Dependent Blood Gas and Acid-base Variables) and Metabolic Rate ($\dot{V}_{O2}$): As stated in the methods section, for the regression analyses of the relationships between metabolic rate on the one hand, and acid-base as well as blood gas variables on the other, all values were normalized with respect to their baseline (= 1) measurements. A strong and significant ($p < 0.001$) direct proportionality was observed between the changes in pHa and $\dot{V}_{O2}$ as is reflected in a regression coefficient of + 0.84 (slope = 5.92 ± 0.6; Fig. 8a.). A decrease of every 0.1 pHa Unit correlated with a decrease of 0.24 ± 0.03
Figure 8a.

The relationship between pHa and $\dot{V}_{O_2}$ resulting from changes in $\dot{V}_E$. Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and the equation and $r$ value have been reported.
\[ \text{Norm.} \dot{V}_o_2 = -4.9 + 5.9 \text{Norm.} \text{pHa} \]

- \( r = +0.84 \)
- \( p < 0.001 \)
ml.min.\textsuperscript{-1} in $\dot{V}_{O_2}$. Fig. 8b. shows the negative correlation between $\dot{V}_{O_2}$ and $P_{aCO_2}$ ($r = -0.86$, slope $-0.28 \pm 0.03$, $p < 0.001$), and Fig. 8c. illustrates a moderate but significant ($p < 0.001$) change in $\dot{V}_{O_2}$ in the same direction as the change in $P_{aO_2}$ ($r = +0.60$, slope $= 0.23 \pm 0.05$).

Average Changes: Table 1. and Fig. 6. illustrate that the overall reduction of $pH$ to $7.13 \pm 0.03$ Units in hypoventilated animals, in conjunction with a decrease in $P_{aO_2}$ (to $33.1 \pm 1.6$ Torr) and increase in $P_{aCO_2}$ (to $59.2 \pm 2.5$ Torr) resulted in a general reduction of aerobic metabolism to $2.35 \text{ml.min.}^{-1}$ (Range 2.). Conversely, hyperventilation elicited an overall increase in $pH$ to $7.59 \pm 0.02$ Units in conjunction with an increase in $P_{aO_2}$ (to $57.2$ Torr) and a decrease in $P_{aCO_2}$ (to $23.5 \pm 1.8$ Torr), and resulted in a general increase in oxygen consumption to $3.39 \pm 0.06 \text{ml.min.}^{-1}$ (Range 4.). The measurements from hyperventilated and hypoventilated animals were significantly different. Table 2. and Fig. 7. illustrate that animals specifically subjected to respiratory acidosis ($pH = 7.19 \pm 0.03$ Units and $P_{aCO_2} = 52.8 \pm 3.0$ Torr) accompanied by low $P_{aO_2}$ levels ($35.9 \pm 2.2$ Torr) consumed significantly lower amounts of oxygen ($2.55 \pm 0.12 \text{ml.min.}^{-1}$) when compared with oxygen uptake values ($3.26 \pm 0.06$ to $3.56 \pm 0.32 \text{ml.min.}^{-1}$) in animals subjected to respiratory alkalosis ($pH = 7.55 \pm 0.02$ and $P_{aCO_2} = 25.9 \pm 1.8$), which in turn was accompanied by significantly higher levels of $P_{aO_2}$ ($53.9 \pm 3.3$).

(iv) Lactate Levels During Respiratory Acidosis: Table 7. shows lactate levels ($10.21$ to $14.13 \text{mM.l}^{-1}$) corresponding with the initial and the lowest $pH$ and $P_{aO_2}$ values measured in two animals subjected to respiratory acidosis by hypoventilation. Lactate levels rose with respiratory acidosis.
Figure 8b.

The relationship between $P_{aCO_2}$ and $\dot{V}_{O_2}$ resulting from changes in $\dot{V}_E$.
Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and the equation and $r$ value have been reported.
* $r = -0.86$

* $\text{Norm.} \hat{V}_o = 1.27 - 0.28 \text{ Norm.} Pa_{CO_2}$

* $p < 0.001$
Figure 8c.

The relationship between $P_{a\text{O}_2}$ and $V_{\text{E}}$ resulting from changes in $V_{\text{E}}$.
Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and the equation and $r$ value have been reported.
Normalized $\dot{V}_o_2$

Normalized $P_ao_2$

- $r = +0.60$
- $\text{Norm.} \dot{V}_o_2 = 0.62 + 0.23 \text{ Norm.} P_ao_2$
- $p < 0.001$
SERIES II. CO₂ LOADING EXPERIMENTS

An Individual Animal

With ventilation kept constant, increasing the %Inspired CO₂ (Fig. 9a) resulted in progressive hypercapnia (Fig. 9d.) and respiratory acidosis (Fig. 9b.). Both \( \dot{V}_O₂ \) (Fig. 9c.) and \( P_aO₂ \) (Fig. 9d.) decreased as the %Inspired CO₂ increased. All changes were reversible. Calculated \([HCO₃⁻]a\) did not show any particular trend (Fig. 9e.).

Group Data

(i) Correlation Between the %Inspired CO₂ (the Independent Variable) and \( P_aCO₂, P_aO₂, pH_a \) (Dependent Blood Gas and Acid-base Variables): The patterns exhibited by the individual animal in Fig. 9. were representative of the 12 animals included in this series of experiments. Fig. 10. illustrates these trends for all animals and includes linear regression analyses for each relationship. The %Inspired CO₂ and \( P_aCO₂ \) showed a significant and positive correlation \((r = +0.63, p < 0.001)\), an increase or decrease in the %Inspired CO₂ by 1 % eliciting a change of approximately 3 Torr in \( P_aCO₂ \) (Fig. 10a., Regression line slope = 3.32 ± 0.68). Changing the %Inspired CO₂ caused a similarly significant but inverse fluctuation in \( pH_a \) (Fig. 10c., \( r = -0.67, \) slope = -0.03 ± 0.006, \( p < 0.001 \)). \( P_aO₂ \) also showed a weak but significantly negative correlation with the %Inspired CO₂ (Fig. 10b., \( r = -0.54, \) slope = -1.77 ± 0.46). High intra-individual
Figure 9.

Data from an individual squirrel depicting the change in pH (9b), $\dot{V}_o_2$ (9c), $P_{ac_o_2}$ and $P_{a_o_2}$ (9d), and calculated $[HCO_3^-]_a$ (9e), as the %Inspired CO$_2$ was randomly increased and decreased over time (9a).
Figure 10.

The effect of varying levels of %Inspired CO₂ on blood gas variables $P_{a\text{CO}_2}$ (10a) and $P_{a\text{O}_2}$ (10b) as well as pH (10c) and aerobic metabolism (10d). Linear regression analysis has been performed on all relationships, and equations and r values are stated for each relationship; n = 12 in all cases.
\* r = +0.63  
* \(P_{\text{aCO}_2} = 32.3 + 3.32 \% \text{CO}_2\)

\* r = -0.54  
* \(P_{\text{aO}_2} = 63.1 - 1.77 \% \text{CO}_2\)

\* r = -0.67  
* \(p_{\text{Ha}} = 7.53 - 0.03 \% \text{CO}_2\)

\* \(\dot{V}_{\text{O}_2} = 2.22 - 0.08 \% \text{CO}_2\)
variability with respect to all these variables is evident in these figures, in conjunction with regression coefficients that are not particularly high (all values were < 0.7) despite their statistical significance.

**Average Changes (%Inspired CO₂ Sorted Data):** For animals ventilated with air prior to being CO₂ loaded, \( \text{Paco}_2 \) averaged 31.2 ± 5.1 and pHa averaged 7.59 ± 0.04 (Range 1, Table 3, Fig. 11.). These acid-base variables indicated that the animals were moderately hyperventilated at the beginning of the experiment despite an attempt to produce a neutral baseline acid-base status (pHa = 7.35 - 7.4 Units and \( \text{Paco}_2 = 34 - 40 \) Torr) prior to the commencement of the CO₂ loading period. During the CO₂ loading period (at an average %Inspired CO₂ of 6.5 ± 0.5%) the average acid-base status of hypercapnic animals was represented by a pHa of 7.3 ± 0.03 Units and a \( \text{Paco}_2 \) of 56.7 ± 3.6 Torr; corresponding \( \text{Pa}_\text{O}_2 \) values averaged to 50.1 ± 2.6 Torr (Range 2, Table 3, Fig. 11.). All these values were significantly different from those of Range 1 or baseline measurements. Recovery on air following the period of CO₂ loading resulted in an increase in pHa and \( \text{Pa}_\text{O}_2 \) to 7.5 ± 0.04 Units and 64.7 ± 2.8 Torr respectively, in conjunction with a decrease in \( \text{Paco}_2 \) to 29.2 ± 2.7 Torr (Range 3, Table 3, Fig. 11.). These values were not significantly different from those of baseline measurements obtained prior to CO₂ loading, confirming recovery from CO₂ loading. With respect to mean \([\text{HCO}_3^-]/a\) values (calculated from \( \text{Paco}_2 \) and pHa) for all these ranges, all changes were statistically insignificant and showed no trend with changes in the %Inspired CO₂.
Table 3.

Values of arterial blood gases, acid-base variables and aerobic metabolism as a function of %CO₂ in inspired gas, before, during and following CO₂ loading.
Values are Mean ± S.E.; n = 12.
<table>
<thead>
<tr>
<th></th>
<th>%CO₂ (%)</th>
<th>PaCO₂ (Torr)</th>
<th>PaO₂ (Torr)</th>
<th>pHa (Units)</th>
<th>V_o₂ (ml.min⁻¹)</th>
<th>[HCO₃⁻]a (nM.l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
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<td><strong>MEAN</strong></td>
<td><strong>S.E.</strong></td>
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<td>6.50</td>
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<tr>
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<td>0.00</td>
<td>29.18</td>
<td>2.65</td>
<td>64.73</td>
<td>2.78</td>
</tr>
</tbody>
</table>

**RANGE 1** = First measurements from each animal after pump-ventilation on air for 30 minutes

**RANGE 2** = Data corresponding to: %CO₂ range 0.04–10% (CO₂ LOADING)

**RANGE 3** = Data corresponding to: %CO₂ 0.03% (RECOVERY ON AIR)

* Values significantly different (p<0.05) from RANGE 1

** Values significantly different (p<0.05) from RANGE 2

*** Values significantly different (p<0.05) from RANGE 3
Figure 11.

The relationship between mean values of arterial acid-base variables, blood gas variables and aerobic metabolism and the %Inspired CO₂, before, during and after the CO₂ loading period. Values are Mean ± S.E.; n = 12.

Range 1 = the mean of the initial measurements obtained from each animal after pump-ventilation on air for 30 minutes, prior to CO₂ loading;

Range 2 = mean values obtained during the CO₂ loading period (%Inspired CO₂ = 6.5 ± 0.5%); and

Range 3 = mean values obtained after recovery on air, following the CO₂ loading period.

** = Values significantly different (p ≤ 0.05) from range 2.
Average Changes (pHa Sorted Data): Table 4 contains data similar to Table 3, although in this instance the data is sorted according to pHa rather than the %Inspired CO₂. The four ranges represent respectively, the initial measurements from each animal prior to CO₂ loading (Range 1), subsequent data collected during CO₂ loading but with a pHa still > 7.4 Units (Range 2), data corresponding to a pHa < 7.4 Units during the CO₂ loading period (Range 3), and finally, measurements after the recovery period following CO₂ loading (Range 4). The average values of acid-base variables for Range 3 were a pHa of 7.24 ± 0.02 Units and a Paco₂ of 62.6 ± 3.3 Torr. The corresponding PaO₂ was 45.1 ± 2.1 Torr. All these values were significantly different from baseline measurements reported in Range 1, namely, a mean pHa of 7.59 ± 0.04, a mean PaCO₂ of 31.2 ± 5.1 Torr, and a mean PaO₂ of 63.4 ± 2.5 Torr. Fig. 12 illustrates the data represented in Table 4.

(ii) Correlation Between the %Inspired CO₂ (the Independent Variable) and Metabolic Rate (VO₂): Oxygen consumption (VO₂) underwent moderate but significant changes in the reverse direction to the changes in the %Inspired CO₂ (r = -0.59, p < 0.01; Fig. 10d). An increase of 1 %Inspired CO₂ resulted in a 0.08 ± 0.02 ml.min⁻¹ decrease in VO₂.

Average Changes: As illustrated in Table 3 and Fig. 11., the average oxygen uptake was significantly lower at 1.68 ± 0.09 ml.min⁻¹ during the CO₂ loading period (corresponding to an average %Inspired CO₂ of 6.5 ± 0.5 %; Range 3) compared with the oxygen uptake measured as baseline (2.28 ± 0.17 ml.min⁻¹; Range 1). Table 4 and Fig. 12 illustrate that the average oxygen uptake (1.62 ± 0.12 ml.min⁻¹) of acidotic animals (a mean pHa of 7.24 ± 0.02 produced
Table 4.

Values of arterial blood gases, acid-base variables, %Inspired CO₂ and aerobic metabolism as a function of pHₐ, before, during and following CO₂ loading.
Values are Mean ± S.E.; n = 12.
<table>
<thead>
<tr>
<th>pHa (Units)</th>
<th>PaCO₂ (Torr)</th>
<th>PaO₂ (Torr)</th>
<th>%CO₂ (%)</th>
<th>VO₂ (ml.min⁻¹)</th>
<th>[HCO₃⁻]a (nM.l⁻¹)</th>
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<td><strong>S.E.</strong></td>
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<td>0.04</td>
<td>29.18</td>
<td>2.64</td>
<td>64.73</td>
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</tbody>
</table>

RANGE 1 = First measurements from each animal after pump-ventilation on air for 30 minutes
RANGE 2 = Data corresponding to: CO₂ LOADING but with pHa > 7.4 Units
RANGE 3 = Data corresponding to: CO₂ LOADING, with pHa < 7.4 Units
RANGE 4 = Data corresponding to: RECOVERY ON AIR

* Values significantly different (p<0.05) from RANGE 1
** Values significantly different (p<0.05) from RANGE 2
*** Values significantly different (p<0.05) from RANGE 3
**** Values significantly different (p<0.05) from RANGE 4
Figure 12.

The relationship between mean values of arterial acid-base variables, blood gas variables and aerobic metabolism and the %Inspired CO$_2$, before, during and after respiratory acidosis. Values are Mean ± S.E.; n = 12.

Range 1 = the mean of the initial measurements obtained from each animal after pump-ventilation on air for 30 minutes, prior to CO$_2$ loading (pHa = 7.59 ± 0.04 Units);

Range 2 = mean values obtained during the CO$_2$ loading period but with a pHa > 7.4 Units;

Range 3 = mean values obtained during the CO$_2$ loading period with pHa < 7.4 Units; and

Range 4 = mean values obtained after recovery on air, following the CO$_2$ loading period.

*** = Values significantly different ($p \leq 0.05$) from range 3.
by an average %Inspired CO\textsubscript{2} of 7.07 ± 0.57 %) (Range 3.) was also significantly lowered compared with baseline measurements.

(iii) Correlation Between \( P_{a\text{CO}_{2}}, P_{a\text{O}_{2}}, \text{pHa} \) (Dependent Blood Gas and Acid-base Variables) and Metabolic Rate (\( \dot{V}_{\text{O}_{2}} \)): Fig. 13 illustrates the correlations between pHa and metabolic rate on the one hand (Fig. 13a.) and blood gases and metabolic rate (Fig. 13b. and Fig. 13c.) on the other. All variables have been normalized to baseline measurements for individual animals, the latter being represented by a value of 1. The changes in \( \dot{V}_{\text{O}_{2}} \) were significantly \((p < 0.001)\) and directly correlated with the changes in pHa \((r = + 0.78, \text{slope} = 7.33 \pm 0.96; \text{Fig. 13a.})\), as was seen in the hypoventilated animals in series I; a 0.1 Unit decrease in pHa corresponded with a 0.27 ± 0.06 ml.min\textsuperscript{-1} decrease in oxygen consumption. Fig. 13b. shows a surprisingly moderate but significant \((p < 0.002)\) inverse correlation between \( \dot{V}_{\text{O}_{2}} \) and \( P_{a\text{CO}_{2}} \) \((r = -0.65, \text{slope} -0.17 \pm 0.04)\). On the other hand changes in \( \dot{V}_{\text{O}_{2}} \) were observed to be directly proportional with changes in the blood gas \( P_{a\text{O}_{2}} \) \((+ 0.71, \text{slope} = 0.87 \pm 0.14, p < 0.001)\) (Fig. 13c.).

Average Changes: In general, CO\textsubscript{2} loaded animals underwent statistically significant changes with respect to pHa and blood gases, yielding a pHa of 7.30 ± 0.03 Units, a \( P_{a\text{CO}_{2}} \) of 56.7 ± 3.6 Torr, and a \( P_{a\text{O}_{2}} \) of 50.1 ± 2.6 Torr (Range 2., Table 3., Fig. 11.). Concurrently significant changes in the aerobic metabolism \((\dot{V}_{\text{O}_{2}} = 1.68 \pm 0.09 \text{ml.min}^{-1})\) of CO\textsubscript{2} loaded animals were also evident, when compared with baseline measurements \((\dot{V}_{\text{O}_{2}} = 2.28 \pm 0.17 \text{ml.min}^{-1})\) (Range 1., Table 3., Fig. 11.) as well as measurements following recovery from CO\textsubscript{2} loading \((\dot{V}_{\text{O}_{2}} = 2.17 \pm 0.10 \text{ml.min}^{-1})\) (Range 3., Table 3., Fig. 11.). Table 4. and Fig. 12.
Figure 13a.

The relationship between pHa and $\dot{V}_{\text{O}_2}$ resulting from changes in the %Inspired CO$_2$.
Linear regression analysis has been performed on values normalized to baseline measurements;
(represented by 1) and the equation and r value have been reported.
\[ r = 0.78 \]
\[ \text{Norm.} \overline{\dot{V}O_2} = -6.3 + 7.33 \text{ Norm.pHa} \]
\[ p < 0.001 \]
Figure 13b.

The relationship between $P_{aCO_2}$ and $\dot{V}_{O_2}$ resulting from changes in the %Inspired CO$_2$. Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and the equation and $r$ value have been reported.
b.

\[ r = -0.60 \]

\[ \text{Norm.} \dot{V}_O_2 = 1.15 - 0.17 \text{Norm.} P_a_{CO_2} \]

\[ p < 0.002 \]
Figure 13c.

The relationship between $P_{aO_2}$ and $V_{o_2}$ resulting from changes in the %Inspired CO$_2$. Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and the equation and r value have been reported.
$\text{c.}$

- $r = +0.71$
- $\text{Norm. } \dot{V}O_2 = 0.09 + 0.87 \text{ Norm. } PaO_2$
- $p < 0.001$
illustrate that animals specifically subjected to respiratory acidosis (average pHa = 7.24 ± 0.02 Units and PaCO2 = 62.6 ± 2.8 Torr) consumed significantly lower amounts of oxygen (1.62 ± 0.12 ml.min⁻¹; Range 3.) compared with animals ventilated on air prior to being made acidotic (VO2 = 2.23 ± 0.17 at pHa = 7.59 ± 0.04, PaCO2 = 31.2 ± 5.1 Torr, and PaO2 of 63.4 ± 2.5 Torr (Range 1.) or after recovery from respiratory acidosis (VO2 = 2.17 ± 0.10 ml.min⁻¹ at a pHa = 7.5 ± 0.04, PaCO2 = 29.2 ± 2.6 and PaO2 = 64.7 (Range 4.).

(iv) Lactate Levels During Respiratory Acidosis: Table 7. shows lactate levels (6.49 to 13.09 mM.l⁻¹) corresponding with the initial and the lowest pHa and PaO2 values measured in two animals during respiratory acidosis on account of CO2 loading. Lactate levels rose with respiratory acidosis.

In conclusion, it is evident that both sets of experiments inducing respiratory acidosis in anaesthetized, paralyzed and artificially ventilated squirrels maintained at constant body temperature (Series I and II), produced similar results and correlations between PaCO2, PaO2, pHa and VO2: respiratory acidosis elicited a significant reduction in metabolic rate.
SERIES III. ACID LOADING EXPERIMENTS

An Individual Animal

Continuous infusion of lactic acid for 30 minutes, while maintaining $V_E$ constant by the respiratory pump, progressively lowered pHa (Fig. 14a.). pHa quickly recovered towards baseline values during the post-infusion period. $P_{aCO_2}$ and $P_{aO_2}$ increased and decreased respectively (though only to a moderate extent) during the infusion period (Fig. 14b., and 14e.). Oxygen uptake ($V_O_2$) increased during acid infusion (Fig. 14c.) as did carbon dioxide production ($V_{CO_2}$) (Fig. 14f.) and R values in this animal (Fig. 14d.). $[HCO_3^-]_a$ values decreased with acid-infusion (Fig. 14g.). With the exception of R values, all these variables changed back towards baseline values during the post-infusion period.

Fig. 15. and Fig. 16. show data from individual animals that were infused with acetic acid and mammalian Ringer's solution respectively (both solutions being relatively acidic to the neutral as well as baseline pHa of the animals). The trends exhibited by pHa, $P_{aCO_2}$, $P_{aO_2}$, $[HCO_3^-]_a$ and $V_O_2$ were similar to those seen with lactic acid infusion. R values however, did not necessarily increase during the infusion period.
Figure 14.

Data from an individual squirrel depicting the change in pHa (14a), $P_{a_{CO2}}$ (14b), $\dot{V}_{O2}$ (14c), R value (14d), $P_{a_{O2}}$ (14e), $\dot{V}_{CO2}$ (14f) and $[HCO_3^-]$a (14g), during and after the infusion of LACTIC acid.
Figure 15.

Data from an individual squirrel depicting the change in pH (15a), $P_{aCO_2}$ (15b), $\dot{V}_{O_2}$ (15c), R value (15d), $P_{aO_2}$ (15e), $\dot{V}_{CO_2}$ (15f) and $[HCO_3^-]_a$ (15g), during and after the infusion of ACETIC acid.
Figure 16.

Data from an individual squirrel depicting the change in pHa (16a), $P_{a\text{CO}_2}$ (16b), $\dot{V}_{O_2}$ (16c), R value (16d), $P_{aO_2}$ (16e), $\dot{V}_{\text{CO}_2}$ (16f) and [HC0$_3^-$]a (16g), during and after the infusion of mammalian RINGER'S SOLUTION.
Group Data

(i) Correlation Between pHa (the Independent Variable) and Pa\textsubscript{co2} and Pa\textsubscript{o2} (Dependent Variables): The trends seen for the individual animals in Figs. 14., 15. and 16. were representative of the 15 animals made acidotic in this series of experiments. Fig. 17. illustrates the changes which occurred in Pa\textsubscript{co2} (a., b.) and Pa\textsubscript{o2} (c., d.) in all these animals as pHa changed during the infusion and post-infusion periods, with linear regression analyses performed for each relationship. A negative correlation coefficient ($r = -0.77$) between pHa and Pa\textsubscript{co2} was determined during the infusion period, with a decrease of 0.1 pHa Units eliciting a change of approximately 4 Torr in Pa\textsubscript{co2} (slope = 38.1 ± 5.8) while during the post-infusion period, an increase of 0.1 pHa Units was accompanied by a fall of approximately 3 Torr. With respect to the blood gas Pa\textsubscript{o2}, the decrease in pHa during the infusion period corresponded with a moderate fall in Pa\textsubscript{o2} ($r = 0.6$, $p < 0.05$, slope = 45.04), but there was no correlation between these two variables during the post-infusion period.

Average Changes (pHa Sorted Data): The initial values measured in individual animals ventilated with air before acid infusion (Range 1., Table 5., Fig. 18.) averaged 26.9 ± 1.2 Torr for Pa\textsubscript{co2} and 7.56 ± 0.02 for pHa. Technically, these values met the criteria for classifying respiratory alkalosis. This suggested that the animals were moderately hyperventilated at the beginning of the experiment despite an attempt to produce a neutral blood acid-base status (Pa\textsubscript{co2} of 34 - 40 Torr; pHa of 7.35 - 7.4 Units). In Range 2 of the acid loading period, the mean Pa\textsubscript{co2}
Figure 17.

The effect of changing levels of pHa (metabolic acidosis), on the absolute values of $\text{Pa}_\text{O}_2$ (17a, b) $\text{Pa}_\text{CO}_2$ (17c, d) and $\dot{V}_\text{O}_2$ (17e, f) during the infusion as well as post-infusion period. Linear regression analysis has been performed on all relationships and equations and $r$ values are stated for all relationships; $n = 15$ in all cases.
Table 5.

Values of arterial blood gases, acid-base variables and aerobic metabolism as a function of pH, before, during and following lactic and acetic acid infusions.
Values are Mean ± S.E.; n = 15.
<table>
<thead>
<tr>
<th>pHa (Units)</th>
<th>PaCO₂ (Torr)</th>
<th>PaO₂ (Torr)</th>
<th>VO₂ (ml.min⁻¹)</th>
<th>VCO₂ (ml.min⁻¹)</th>
<th>R value</th>
<th>[HCO₃⁻]a (nM.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>**, ***, ****</td>
<td>**, ****</td>
<td>**, ***</td>
<td>**, ***</td>
<td>**, ***</td>
<td>1.09</td>
<td>1.04</td>
</tr>
<tr>
<td>1</td>
<td>7.56 ± 0.02</td>
<td>26.86 ± 1.18</td>
<td>70.21 ± 2.02</td>
<td>1.46 ± 0.15</td>
<td>1.55 ± 0.13</td>
<td>23.60 ± 1.05</td>
</tr>
<tr>
<td>**, ***</td>
<td>7.46 ± 0.01</td>
<td>32.00 ± 3.00</td>
<td>59.00 ± 13.00</td>
<td>2.16 ± 0.20</td>
<td>2.08 ± 0.14</td>
<td>21.68 ± 1.29</td>
</tr>
<tr>
<td>**, ***, ****</td>
<td>**, **</td>
<td>**, ****</td>
<td>**, ****</td>
<td>**, ****</td>
<td>1.01 ± 0.03</td>
<td>18.07 ± 0.83</td>
</tr>
<tr>
<td>3</td>
<td>7.32 ± 0.01</td>
<td>37.86 ± 1.87</td>
<td>58.86 ± 3.06</td>
<td>2.38 ± 0.20</td>
<td>2.31 ± 0.17</td>
<td>1.09 ± 0.04</td>
</tr>
<tr>
<td>**, ***</td>
<td>7.48 ± 0.01</td>
<td>32.73 ± 1.39</td>
<td>60.46 ± 2.82</td>
<td>1.67 ± 0.11</td>
<td>1.74 ± 0.09</td>
<td>1.09 ± 0.04</td>
</tr>
</tbody>
</table>

RANGE 1 = First measurements from each animal after pump-ventilation on air for 30 minutes
RANGE 2 = Data corresponding to: INFUSION PERIOD, but with pHa > 7.4 Units
RANGE 3 = Data corresponding to: INFUSION PERIOD, with pHa < 7.4 Units
RANGE 4 = Data corresponding to: POST-INFUSION PERIOD

* Values significantly different (p<0.05) from RANGE 1
** Values significantly different (p<0.05) from RANGE 2
*** Values significantly different (p<0.05) from RANGE 3
**** Values significantly different (p<0.05) from RANGE 4
Figure 18.

The relationship between arterial acid base variables, blood gases, and aerobic metabolism before, during, and after lactic and acetic acid infusions.
Values are Mean ± S.E.; n = 15.
Range 1 = mean of the initial set of measurements obtained from each animal after pump-ventilation on air for 30 minutes, prior to acid loading (pHa = 7.56 ± 0.02 Units);
Range 2 = mean of values obtained during early stages of infusion wherein pHa > 7.4 Units;
Range 3 = mean of values obtained during later stages of infusion wherein pHa < 7.4 Units (metabolic acidosis); and
Range 4 = mean of values obtained in the recovery period following acid infusion.
*** = Values significantly different (p ≤ 0.05) from range 3.
(32.0 ± 3.0 Torr) was higher and the mean pHa (7.46 ± 0.01 Units) was lower (though not significantly) than the baseline measurements. Despite the decline in pHa, pHa was still not low enough to be classified as metabolic acidosis. During the latter period of acid infusion however, pHa meet the criteria for metabolic acidosis. It averaged 7.32 ± 0.01 Units, in conjunction with a PaCO₂ of 37.9 ± 1.9 Torr, and a PaO₂ of 58.9 ± 3.1 Torr (Range 3.), values significantly different from those of Range 1. During the post-infusion period (Range 4.) pHa, PaCO₂ and PaO₂ returned towards baseline levels, attaining the values 7.48 ± 0.01 Units, 32.8 ± 1.4 Torr and 60.5 ± 2.8 Torr respectively. However, these were still significantly different from those in Range 1, reflecting an incomplete recovery from acid infusion in some animals. Blood bicarbonate values (calculated from PaCO₂ and pHa) did not show any significant changes between any of the above ranges. However, they did decrease as pHa was lowered in the infusion period, and returned towards baseline values during the post-infusion period.

Table 6. shows the data obtained from animals infused with mammalian Ringer's solution. Unlike lactic and acetic acid infusion (Table 5.), pHa did not drop to values < 7.4 Units during the infusion period. Although the pHa fell during infusion, the average decrease was not statistically significant and nor were the blood gases and blood bicarbonate levels significantly changed from their baseline values. However, the trends exhibited by the data were similar to those obtained during infusion of the relatively stronger acids, LA and AA.

(ii) Correlation Between pHa (the Independent Variable) and Metabolic Rate (V̇_O₂): Fig. 17e. and Fig. 17f. show the absolute values of metabolic rate (V̇_O₂) measured against various levels of the independent variable pHa, during and following the infusion period. Fig. 19a. shows the
relationship between normalized values (1 = baseline measurements) of arterial pH and aerobic metabolism ($\dot{V}_{O_2}$). Both illustrations indicate an inverse relationship between the two variables during acid infusion, a decrease in pHa of 0.1 Units resulting in a $0.25 \pm 0.10$ ml.min.$^{-1}$ increase in $\dot{V}_{O_2}$. This trend was in the opposite direction to that documented for series I and II. Although $\dot{V}_{O_2}$ correlated significantly well ($p < 0.001$) with pHa during acid infusion (Fig. 19a., $r = -0.65$, slope = -9.99 ± 1.34), there was no significant correlation between these variables during the recovery period (Fig. 19b.).

Average Changes: In terms of average values, metabolic acidosis (pHa = 7.32 ± 0.01 Units, Table 5.) was associated with a significant rise in average oxygen uptake, $\dot{V}_{O_2}$ increasing from 1.46 ± 0.15 ml.min.$^{-1}$ at baseline levels, to 2.38 ± 0.2 ml.min.$^{-1}$ during the infusion period (Table 5., Fig. 18c.). A simulataneous and significant rise was also seen in $\dot{V}_{CO_2}$ (Table 5., Fig. 18f.) in conjunction with a decrease in R values (Table 5., Fig. 18e.), the change in the latter being statistically insignificant however. Compared to LA and AA infusions, MRS infusion resulted in a moderate but statistically insignificant increase in oxygen uptake (from 1.14 ± 0.09 to 1.29 ± 0.05 ml.min.$^{-1}$) in conjunction with a similarly moderate but statistically insignificant decrease in pHa (from 7.57 ± 0.03 to 7.4 ± 0.12 Units; Table 6.).

(iii) Correlation Between $Pa_{CO_2}$, $Pa_{O_2}$ (Dependent Blood Gas Variables) and Metabolic Rate ($\dot{V}_{O_2}$): Fig. 20a. shows a significant and positive correlation between normalized values (1 = baseline measurements) of $\dot{V}_{O_2}$ and the blood gas $Pa_{CO_2}$ ($r = + 0.75$, $p < 0.001$, slope 0.64 ± 0.11) during the period of acid infusion. There was no correlation between the two variables during
Figure 19.

The relationship between pHa and \( \dot{V}_\text{O}_2 \) during the acid (lactic and acetic) infusion period as well as during the post-infusion period. Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and equations and r values have been reported for both relationships.
Infusion Period  

- $r = -0.65$
- $\text{Norm.} \dot{V}O_2 = 11.08 - 9.99 \text{ Norm.pHa}$
- $p < 0.001$

Post-Infusion Period  

- $r = -0.27$
- $\text{Norm.} \dot{V}O_2 = 4.6 - 3.5 \text{ Norm.pHa}$
- $p > 0.05$
Table 6.

Values of arterial blood gases, acid-base variables and aerobic metabolism as a function of pHa, before, during and following the infusion of mammalian Ringer’s solution. Values are Mean ± S.E.; n = 7.
<table>
<thead>
<tr>
<th>pHa (Units)</th>
<th>P\textsubscript{a}CO\textsubscript{2} (Torr)</th>
<th>P\textsubscript{a}O\textsubscript{2} (Torr)</th>
<th>(\dot{V}_{O2}) (ml.min(^{-1}))</th>
<th>(\dot{V}_{CO2}) (ml.min(^{-1}))</th>
<th>R value</th>
<th>([HCO_3^-]) a (nM.L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MEAN</strong></td>
<td><strong>S.E.</strong></td>
<td><strong>MEAN</strong></td>
<td><strong>S.E.</strong></td>
<td><strong>MEAN</strong></td>
<td><strong>S.E.</strong></td>
<td><strong>MEAN</strong></td>
</tr>
<tr>
<td><strong>RANGE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.57</td>
<td>0.03</td>
<td>27.72</td>
<td>2.25</td>
<td>69.44</td>
<td>1.94</td>
</tr>
<tr>
<td>2</td>
<td>7.40</td>
<td>0.12</td>
<td>33.44</td>
<td>4.92</td>
<td>60.86</td>
<td>6.36</td>
</tr>
<tr>
<td>3</td>
<td>7.54</td>
<td>0.02</td>
<td>27.80</td>
<td>3.31</td>
<td>68.21</td>
<td>2.03</td>
</tr>
</tbody>
</table>

RANGE 1 = First measurements from each animal after pump-ventilation on air for 30 minutes
RANGE 2 = Data corresponding to: INFUSION PERIOD, with pHa > 7.4 Units
RANGE 3 = Data corresponding to: POST-INFUSION PERIOD

* Values significantly different (p<0.05) from RANGE 1
** Values significantly different (p<0.05) from RANGE 2
*** Values significantly different (p<0.05) from RANGE 3
the recovery period however (Fig. 20b.). Fig. 21a. represents a weak but significant ($p < 0.01$) negative correlation between normalized ($1 = \text{baseline measurements}$) values of the second blood gas ($P_{aO_2}$) and $\dot{V}_{O_2}$ during the infusion period ($r = -0.55$, slope $-1.42 \pm 0.41$). Like the relationship between $P_{aCO_2}$ and $\dot{V}_{O_2}$ as well as between $pHa$ and $\dot{V}_{O_2}$, this correlation was not present during the post-infusion period (Fig. 21b.).

**Average Changes:** There was a moderate but significant increase in $P_{aCO_2}$ (from $26.9 \pm 1.2$ Torr to $37.9 \pm 1.9$ Torr) with LA and AA infusions (Table 5., Fig. 18a.) and with MRS infusion (from $27.7 \pm 2.3$ Torr to $33.4 \pm 5.0$ Torr) (Table 6.) in conjunction with decreasing $pHa$ levels (Fig. 18b.) during the infusion period. Associated with these changes in $P_{aCO_2}$ during LA and AA infusions, average oxygen uptake rose from $1.46 \pm 0.15$ ml.min.$^{-1}$ to $2.38 \pm 0.2$ ml.min.$^{-1}$ (Table 5., Fig. 18c.) and from $1.14 \pm 0.1$ to $1.29 \pm 0.05$ ml.min.$^{-1}$ with MRS infusion (Table 6.). In contrast to LA and AA, the change elicited by MRS was not statistically significant. There was a significant decrease in $P_{aO_2}$ from $70.2 \pm 2.0$ Torr to $58.9 \pm 3.0$ Torr with LA and AA infusions (Table 5., Fig. 18d.). MRS infusions on the other hand elicited an average fall from $69.4 \pm 1.9$ Torr to $60.9 \pm 6.4$ Torr, which again, was not statistically significant (Table 6.).

(iv) **Lactate Levels During Metabolic Acidosis:** Table 7. shows lactate levels ($6.89$ to $11.16$ mM.$^{-1}$) corresponding with the initial and the lowest $pHa$ and $P_{aO_2}$ values measured in three animals during metabolic acidosis induced by LA infusion. Lactate levels rose with LA infusion.
Figure 20.

The relationship between $P_a CO_2$ and $\dot{V}O_2$ during the acid (lactic and acetic) infusion period as well as during the post-infusion period. Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and equations and $r$ values have been reported for both relationships.
Infusion Period

2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.7
0.6

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5

Normalized PaCO₂

Normalised V̇O₂ =

0.45 + 0.64 NormPaCO₂

p < 0.001

Post-Infusion Period

2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.7
0.6

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5

Normalized PaCO₂

Normalised V̇O₂ =

1.1 + 0.14 Norm.PaCO₂

p > 0.05
Figure 21.

The relationship between $P_aO_2$ and $V_o2$ during the acid (lactic and acetic) infusion period as well as during the post-infusion period. Linear regression analysis has been performed on values normalized to baseline measurements (represented by 1) and equations and r values have been reported for both relationships.
Infusion Period

- \( r = -0.55 \)
- \( \text{Norm.}\dot{V}O_2 = 2.54 - 1.42 \text{ Norm.}PaO_2 \)
- \( p < 0.01 \)

Post-Infusion Period

- \( r = 0 \)
- \( \text{Norm.}\dot{V}O_2 = 1.24 + 0 \times \text{Norm.}PaO_2 \)
- \( p > 0.05 \)
Table 7.

Lactate measurements in some animals from all three series of experiments during the period of acidosis.
<table>
<thead>
<tr>
<th></th>
<th>Animal #1</th>
<th>Animal #2</th>
<th>Animal #1</th>
<th>Animal #2</th>
<th>Animal #1</th>
<th>Animal #2</th>
<th>Animal #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate (mM.l⁻¹)</td>
<td>2.38</td>
<td>10.21</td>
<td>1.86</td>
<td>14.13</td>
<td>1.61</td>
<td>14.84</td>
<td>5.68</td>
</tr>
<tr>
<td>Pao₂ (Torr)</td>
<td>60.0</td>
<td>39.0</td>
<td>59.0</td>
<td>29.0</td>
<td>55.0</td>
<td>29.0</td>
<td>51.0</td>
</tr>
<tr>
<td>Paco₂ (Torr)</td>
<td>25.0</td>
<td>66.0</td>
<td>26.0</td>
<td>58.0</td>
<td>51.0</td>
<td>87.0</td>
<td>63.0</td>
</tr>
<tr>
<td>pHₐ (Units)</td>
<td>7.58</td>
<td>7.03</td>
<td>7.57</td>
<td>7.04</td>
<td>7.33</td>
<td>7.08</td>
<td>7.38</td>
</tr>
</tbody>
</table>
COMPARISON OF THE THREE SERIES

Table 8. and Fig. 22. present a summary of major differences observed in the results from all three series of experiments. Both sets of experiments inducing respiratory acidosis in anaesthetized, paralyzed and artificially ventilated squirrels produced similar results and correlations between $\text{Pa}_{\text{CO}_2}$, $\text{pHa}$ and $\dot{V}_{\text{O}_2}$. However, the trends exhibited by $\dot{V}_{\text{O}_2}$ in relation to $\text{pHa}$ and $\text{Pa}_{\text{CO}_2}$ in Series I and II were in the opposite direction to those seen during the acid infusion experiments in Series III. In series III., instead of effecting a decrease in $\dot{V}_{\text{O}_2}$, every decrease of 0.1 pHa Units produced an increase in $\dot{V}_{\text{O}_2}$ of approximately 0.27 ml.min.$^{-1}$. Statistically significant differences were also found between the average values of blood gas variables associated with the ranges representing respiratory acidosis in series I as well as in series II (Range 3 of pHa sorted data in Table 2. and Table 4. respectively), when compared with those corresponding to metabolic acidosis (Range 3 of Table 5.) in series III.
Table 8.

Metabolic rate was altered differentially by respiratory and metabolic acidosis respectively.
<table>
<thead>
<tr>
<th>Experiment Protocol</th>
<th>Duration of Experiment</th>
<th>$\text{Pa}_{\text{CO}_2}$ range (Torr)</th>
<th>$\text{Pa}_{\text{O}_2}$ range (Torr)</th>
<th>pH range (Units)</th>
<th>$\dot{\text{V}}_{\text{O}_2}$ changes corresponding to a 0.1 Unit decrease in pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series I.</td>
<td>6 hours on average</td>
<td>Min. Max.</td>
<td>Min. Max.</td>
<td>Min. Max.</td>
<td>Decrease in $\dot{\text{V}}_{\text{O}_2}$ by $0.24$ $\pm 0.03$ ml.min$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.3 $\pm 3.2$</td>
<td>52.8 $\pm 3.0$</td>
<td>36.0 $\pm 2.2$</td>
<td>53.9 $\pm 3.3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.19 $\pm 0.03$</td>
<td>7.55 $\pm 0.02$</td>
</tr>
<tr>
<td>Series II.</td>
<td>3 hours on average</td>
<td>Min. Max.</td>
<td>Min. Max.</td>
<td>Min. Max.</td>
<td>Decrease in $\dot{\text{V}}_{\text{O}_2}$ by $0.27$ $\pm 0.06$ ml.min$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.2 $\pm 2.6$</td>
<td>62.6 $\pm 3.3$</td>
<td>45.1 $\pm 2.1$</td>
<td>64.7 $\pm 2.8$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.24 $\pm 0.02$</td>
<td>7.59 $\pm 0.04$</td>
</tr>
<tr>
<td>Series III.</td>
<td>1.5 hours on average</td>
<td>Min. Max.</td>
<td>Min. Max.</td>
<td>Min. Max.</td>
<td>Increase in $\dot{\text{V}}_{\text{O}_2}$ by $0.25$ $\pm 0.10$ ml.min$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.9 $\pm 1.2$</td>
<td>37.9 $\pm 1.9$</td>
<td>58.9 $\pm 3.1$</td>
<td>70.2 $\pm 2.0$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.32 $\pm 0.01$</td>
<td>7.56 $\pm 0.02$</td>
</tr>
</tbody>
</table>
Figure 22.

The differential effects of respiratory and metabolic acidosis on oxygen consumption.
Series #

I | II | III

Resp. Acid.

Met. Acid.

Change in $\dot{V}_{O_2}$ with a 0.1 Unit decrease in pHα

(ml.min⁻¹)
DISCUSSION

In this study respiratory acidosis was observed to inhibit whole-body aerobic metabolism in euthermic artificially ventilated golden mantled ground squirrels (Spermophilus lateralis). A decrease in pHa of 0.1 Units correlated with an approximately 0.25 ml.min⁻¹ decrease in oxygen consumption (\(\dot{V}_{O_2}\)). On the other hand, metabolic acidosis stimulated aerobic metabolism resulting in an increase of approximately 0.25 ml.min⁻¹ in \(\dot{V}_{O_2}\) for each 0.1 Unit decrease in pHa. Thus, respiratory acidosis and metabolic acidosis appeared to have opposite effects on oxygen consumption. This implies that pHa may be indirectly rather than directly correlated with \(\dot{V}_{O_2}\) by its effect on some other factor which must be altered in different ways by respiratory and metabolic acidosis.

CRITIQUE OF METHODS

Rationale for Controlling Minute Ventilation: It is well known that there is a critical link between changes in \(P_{aCO_2}/pH\) and volume loading on the one hand and respiratory drive on the other. Increased \(P_{aCO_2}\) and/or [H⁺] strongly stimulates both inspiratory and expiratory activity in mammals (Arieli & Ar, 1979; Bainton et al., 1978; Bainton, 1978; Fregosi et al., 1987; Ledlie et al., 1981; Oliven et al., 1985) as does sustained volume loading (Begle & Skatrud, 1990; Sullivan et al., 1991; Banzett et al., 1981). Furthermore, there is also a tight-coupling between ventilation and whole-animal metabolic rate (Wasserman & Whipp, 1975; Jacobi et al., 1991); on the one hand, ventilation increases to match increases in metabolic rate and on the other hand,
metabolic rate increases with increases in the cost of breathing or energy expended by respiratory muscles due to increases in respiratory activity or ventilation (Shephard, 1955; Hales & Findlay, 1968). Since the objective of this study was to test the hypothesis that changes in arterial pH elicit either local or reflex changes in aerobic metabolism in euthermic golden mantled ground squirrels, it was imperative that experimentally produced changes in \( P_{a\text{CO}_2}/P_{Ha} \) did not cause any compensatory changes in ventilation that would indirectly alter the metabolic rate. This was achieved by artificially ventilating anaesthetized and paralyzed animals.

*Effects of Anaesthesia:* Sodium Pentobarbital was the general anaesthetic used for this study. Barbiturates depress metabolism (Shackman *et al.*, 1951) and inflict a variety of side-effects with depression of ventilation being among the more ubiquitous (Hornbein, 1985). Resting \( O_2 \) consumption is known to be lowered by approximately 10% of the standard basal metabolism levels in adult mammals during anaesthesia (Nunn, 1964; 1985). This should certainly have affected calculations of absolute levels of metabolic rate in the present study but cannot account for differences in the direction of change in \( \dot{V}_{O_2} \) and thus, the overall conclusions of this study.

*Effects of d-Tubocurarine:* The use of a paralyzing agent (d-Tubocurarine) may have contributed to the barbiturate related depression of \( \dot{V}_{O_2} \). In blocking skeletal muscle contraction it is bound to reduce muscle tone and, hence, metabolic rate (Goodman & Gilman, 1970). It is also known to depress adrenal medullary cells, consequently decreasing secretion of epinephrine (Goodman & Gilman, 1970). All these effects may potentially have reduced metabolic heat
production during all series of experiments. As with anaesthesia, however, although this would affect absolute levels and degrees of change in $\dot{V}_{O2}$, it would not affect the overall conclusions of the study.

*Rationale for Maintaining Constant $T_b$:* The pervasiveness of temperature on the metabolism of organisms is well known. During this study, any change in $T_b$ could have secondarily altered metabolic rate measurements due to a) temperature effects on reaction rates, commonly known as the $Q_{10}$ effect, and/or b) temperature effects on reaction equilibria, especially those equilibria involving the formation/rupture of noncovalent ("weak") chemical bonds (Hochachka & Somero, 1984). Temperature may in fact act directly on cellular mechanisms to modify channel kinetics (Krilowitz *et al*., 1989; Eckerman *et al*., 1990) amongst other membrane mechanisms (Willis *et al*., 1981). As alluded to in an earlier section, temperature effects also include increases in $CO_2$ solubility as $T_b$ falls and vice-versa, inducing a shift in acid-base state that in turn may potentially modify properties of single-cell activity by altering the charge state of histidyl imidazole residues and conformation of proteins (Reeves, 1972; Somero & White, 1985). In light of the objectives of this study, it was critical that experimental manipulations did not initiate any direct or indirect (secondary) effects of changes in $T_b$ on metabolic rate. Experiments were therefore performed on animals maintained at a constant $T_b$ allowing only "classical" changes in the acid-base status to be induced during all the protocols described earlier.
RESPIRATORY ACIDOSIS (SERIES I AND II)

Respiratory acidosis in artificially ventilated squirrels was induced by two different methods; by hypoventilating animals and by increasing the inspired fractional concentration of CO₂. Both of these methods successfully produced a rise in $P_{aCO_2}$ and a fall in pHa. $P_{aO_2}$ also fell significantly during respiratory acidosis in both studies. This was expected in Series I due to the adverse mechanical effects of hypoventilation on gas exchange and transport (Rehder et al., 1977; Rieke et al., 1983; Epstein, 1986). The fall in Series II was much less but still significant and unexpected. It may have occurred due to a reduction in %Inspired $O_2$ as the %Inspired CO₂ levels were increased. In most mammalian species, a $P_{aO_2}$ of $< 40$ Torr is categorized as hypoxia and leads to lactate buildup in blood and tissues (Nahas et al., 1959, 1960; Epstein, 1986; Rieke et al., 1983). This was also found to be the case in the four squirrels in which lactate levels were analyzed during respiratory acidosis (Table 7.), indicating that a metabolic acidosis was superimposed over the respiratory acidosis, despite intentions to produce only a pure respiratory acidosis during Series I and II. Tissue hypoxia was not expected to serve as a complicating secondary factor at $P_{aO_2}$ levels of 35 to 45 Torr (reported during respiratory acidosis in Series I and II respectively) since euthermic ground squirrels have a left shifted equilibrium curve with a $P_{50}$ of 18.8 Torr (Maginnis et al., 1989). At the $P_{aO_2}$ levels recorded during respiratory acidosis, arterial blood should still have been $> 90\%$ saturated with oxygen. Although lactate production is not necessarily an indicator of a lack of tissue $O_2$ (Hogan et al., 1991; Brooks, 1985), what is implicit from the high lactate levels measured is that metabolic acidosis must have developed in at least some tissues during Series I and II. The additive
implications of high levels of $\text{Pa}_{\text{CO}_2}$ (since $\text{CO}_2$ has a rapid transmembrane penetrating power (Gesell et al., 1930; Winterstein, 1956; Nahas et al., 1959; 1960) and blood lactate suggest that intracellular pH must have been reduced concurrent with the respiratory acidosis produced reductions in arterial pH during Series I and II.

*Respiratory Acidosis and Metabolic Rate:* The association of $\text{CO}_2$ retention with hypometabolic states has been reported in a variety of air breathing animals including certain insects, and crustaceans (De Laney et al., 1977; Levy & Schneiderman, 1966; Burnett & McMahon, 1987), ectothermic vertebrates such as turtles (Jackson, 1988; Lutz, 1989), toads (Seymour, 1973), snakes (Hicks & Riedesel, 1983) and lizards (Bickler, 1982; 1986; Lutz, 1989), birds such as pigeons (Jensen & Bech, 1990) and mammals such as deer mice (Nestler, 1990a), ground squirrels (Malan et al., 1973; Snapp & Heller, 1981) and humans (during slow-wave sleep) (Shapiro et al., 1981). Inconsistent results have been obtained from attempts to depress metabolic rate or thermogenesis by acute exposure to hypercapnia in active animals, however. It is evident from the literature that factors such as animal size, the %Inspired $\text{CO}_2$, and physical factors such as ambient temperature and humidity contribute to differential metabolic responses to hypercapnia (Maskrey & Nicol, 1979). Although some studies have documented successful reductions in metabolic rates and/or hypothermia induction due to hypercapnic acidosis, these studies have either used extremely long (chronic) exposure to hypercapnia or extremely high levels of $\text{CO}_2$ (Giaja & Popovic, 1952 cited by Malan, 1989; Stupfel, 1960; Bullard & Crise, 1961; Szegvar & Varnai, 1962; Shelton, 1970; Pepelko & Dixon, 1974; Schaefer & Wüenemberg, 1975; Schaefer et al., 1975; Soholt et al., 1973; Lai et al., 1981). On the other hand, most acute
studies on eutherian or active animals involving moderate increases in the %Inspired CO₂ (≤ 10%) have reported no change (Arieli et al., 1977; Stupfel, 1960; Maskrey & Nicol, 1976; ) or a small increase in metabolic rate (Björck et al., 1956; Stupfel, 1960; Withers, 1977a; 1977b; Kuhnen et al., 1987; Maskrey & Nicol, 1979). The latter two observations have been attributed to the increased cost of ventilation associated with the hypercapnic ventilatory response.

To eliminate the effect of hypercapnic tachypnia and the increased work of breathing on oxygen consumption, experiments have been performed on apneically oxygenated and/or artificially ventilated animals including rabbits (Cordier et al., 1927 cited by Stupfel, 1960; 1974; Holmdahl, 1956) and dogs (Gesell et al., 1930; Holmdahl, 1956; Shires & Eyer, 1951; Spencer et al., 1956 cited by Nahas et al., 1960; Nahas et al., 1959; 1960; Ligou & Nahas, 1960; Nahas & Poyart, 1967; Cain, 1970; Usinger & Spaich, 1970). Cordier et al., (1927) and Gesell et al., (1930) were amongst the earliest workers to demonstrate the "anti-metabolic" effect of increasing PaCO₂ levels. Subsequent studies also demonstrated an unequivocal decrease in oxygen consumption when the arterial pH was lowered. Usinger & Spaich, (1970) described the relationship between pH and VO₂ by the equation:

\[ \log VO₂ = 0.265 \text{pH} - 1.165 \]

when the oxygen consumption is expressed in ml.min⁻¹.kg⁻¹. Some studies, however, have presented conflicting results. For example, Spencer et al., (1950, cited by Nahas et al., 1960) reported an initial decline of 11% in oxygen consumption following the onset of acidosis. Subsequently, aerobic metabolism remained unchanged despite a further fall in pH. On the other hand, it has been reported that aerobic metabolism is initially elevated (Shires & Eyer, 1951) or unchanged (Nahas et al., 1959), but gradually declines as CO₂ accumulation becomes
very high and pH falls to between 7.0 and 7.2 Units. Cain, (1970) reported no effect or inconsistent decreases in metabolic rate with hypercapnic acidosis. The more frequently reported trend, however, is a progressive fall in $\dot{V}_{O2}$ which becomes prominent after pHa falls to between 7.0 and 7.2 Units (Holmdahl, 1956; Nahas et al., 1959). The present study is in approximate agreement with these latter studies. It should be noted that the results reported for this study might either underestimate or overestimate the true depressant effect of respiratory acidosis on $\dot{V}_{O2}$, depending on whether the concomitant metabolic acidosis stimulated or depressed aerobic metabolism (as discussed later).

Hyperventilation of anaesthetized, paralyzed animals with a respiratory pump leading to respiratory alkalosis has also been well documented to increase oxygen consumption consistently and reproducibly (Cain, 1970; Nahas & Poyart, 1967). Results obtained during Series I of this study support these observations.

METABOLIC ACIDOSIS (SERIES III)

Acid infusion decreased blood pH and, as a result of hydrogen ions being buffered by $\text{HCO}_3^-$, increased $\text{Pa}_{CO2}$. Bicarbonate buffering in the blood must have been fairly effective since a moderate increase in $\text{Pa}_{CO2}$ of not more than 10 Torr was observed, similar to values reported by workers such as Cain, (1970) and Nahas et al., (1960) in artificially ventilated acid infused dogs. A moderate fall in $\text{Pa}_{O2}$ was also observed during metabolic acidosis.

*Metabolic Acidosis and Metabolic Rate:* In the present study, metabolic acidosis was
associated with an increase in metabolic rate unlike the results of some other studies that have attempted to analyze this relationship directly, under similarly controlled conditions. For example, Usinger & Spaich, (1970) found that oxygen consumption was depressed with acid infusion (NH₄Cl), as it was with increasing arterial CO₂ tension. It was therefore concluded that the positive correlation between pH and \( \dot{V}_o_2 \) did not depend on how arterial pH was altered. Similar conclusions were reached by authors such as Gesell et al., (1930) and Cain, (1970), as oxygen consumption was found to be depressed by metabolic acidosis. Cain, (1970) attempted to correct the rise in pHa during a respiratory alkalosis produced by the artificial hyperventilation of dogs. Restoration of the hydrogen ion content in arterial blood was associated with a complete reversal of the increased oxygen consumption associated with respiratory alkalosis. The reasons for the discrepancy between these studies (demonstrating an inhibition of metabolism with metabolic acidosis), and the present study (demonstrating the stimulation of metabolism with metabolic acidosis) are unclear. Perhaps the use of a strong acid such as HCl in these studies, in contrast with the use of weak acids such as lactic and acetic acid during the present study was partially responsible. HCl is known to elicit several effects that most weak acids do not, including changes in cardiac performance (Shirer et al., 1988).

MECHANISMS LINKING ACIDOSIS AND METABOLIC RATE

I. Effects of Changes in Arterial pH on Metabolic Rate

A. Studies at the Cellular Level: It is difficult to explain why respiratory and metabolic
acidosis had equal but opposite effects on metabolic rate. It should be remembered, however, that fundamental differences do exist between the metabolic acidosis produced by "dilution" of extracellular fluid via venous infusions of H⁺, by endogenous metabolic production of H⁺, and by respiratory acidosis resulting from CO₂ retention or loading. One difference lies in the certainty that endogenous metabolic acidosis reduces pHi. Based on the rapid transmembrane penetrating power of CO₂ (Roughton, 1964; Bidani, 1991), it may be stated with equal certainty that pHi was reduced during respiratory acidosis (Series I and II) as well. However, the same cannot be assumed for the acid infusion experiments because it is possible that the Ringer's solution (the medium in which lactic and acetic acid were infused) may have provided the animals with elevated levels of ions such as K⁺, Na⁺ and Cl⁻. This would have altered the extracellular fluid ionic composition and invalidated the assumption that the solution was isosmotic. The intracellular concentrations of K⁺, Na⁺ and Cl⁻ in turn, may have been altered and intracellular pH may have been affected as a consequence (Kowalchuk et al., 1988 and Systrom et al., 1990 for K⁺ effects; Van slyke et al., 1923 in Shires & Holman, 1948; McDowell et al., 1955; Sotos et al., 1962; Winkler et al., 1944; Wolf & McDowell, 1954 and Neely, 1959 for NaCl effects). The acid-base changes attributable to NaCl induced transcompartmental shifts of body water have been termed "dilutional" acidosis with reference to the extracellular compartment and a "contraction" alkalosis with reference to the intracellular compartment (Winters et al., 1964). It is therefore proposed that the measured decreases in arterial pH during Series III may have been caused by the additive effect of increased H⁺ loading as well as "dilution" acidosis, while intracellular pH may have become alkalotic due to "contraction" as well as a K⁺ potentiated intracellular alkalosis, for a significant part of the infusion period. It is likely,
however, that this "contraction" alkalosis was attenuated over time due to a slow intracellular penetration of the infused H⁺, thus resulting in a net effect of either a mild reduction, a mild increase or an unchanged pHi. This is in contrast to the significant decrease in pHi proposed in the case of respiratory acidosis.

The implications of differential changes in pHi could manifest themselves through differential changes in metabolic rate via several mechanisms. As reviewed by Moore, (1985), there are five alternative processes by which pHi can play the role of an intracellular regulator of metabolism:

1. By allosteric effects upon enzyme activity;
2. By effects on metabolic reactions per se;
3. By interactions with intracellular signal systems;
4. By interactions with membrane transport systems;
5. By effects on cell energetics or thermodynamics.

(1) Enzyme Activity in Response to pHi as an Allosteric Modifier: One enzyme which regulates rates of oxygen consumption is the rate-limiting glycolytic enzyme phospho-fructokinase (PFK) (Karpatkin et al., 1964). Of special interest is the paradoxical effect that pHi may have on PFK. Not only is PFK activity stimulated by increases in pHi levels (Trivedi & Danforth, 1966; Busa & Nucitelli, 1984), but it is also stimulated by mildly reduced levels of pHi (Somero & Hand, 1990). It is only with relatively high reductions in pHi that PFK activity is inhibited (Somero & Hand, 1990). Somero & Hand, (1990) hypothesize that reductions in pHi through the normal physiological pH range of muscle lead to activation of glycolysis via pH-
modulated shifts in localization of PFK. As pH\textit{i} \textit{f}\textit{i}r\textit{t} begins to decrease, PFK shifts increasingly to an actin-bound state. When actin-bound, PFK is more active because of the release of ATP inhibition. Glycolytic activity and consequently oxygen consumption is increased not only because of the activation of PFK, but also because of efficiency increases resulting from the compartmentation of PFK with other glycolytic enzymes bound to the thin filament. These authors also hypothesize a second stage wherein an acidity-induced dissociation of PFK occurs when pH\textit{i} falls to such low values that it is advantageous for the cell to curtail further acid-generating glycolytic activity. As pH\textit{i} falls towards the lower end of the physiologically permissible range, the primary effect of pH on PFK becomes an enhancement of disassembly of tetramers rather than stabilization of the PFK-actin association, and a reduction in oxygen consumption. There is relatively scattered evidence to support the first stage (Luther & Lee, 1986; Roberts & Somero, 1987; Roberts \textit{et al.}, 1988) compared with significant evidence to substantiate the second, not only with respect to PFK but other glycolytic enzymes as well (Trivedi & Danforth, 1966; Bock & Frieden, 1974; Tomoda \textit{et al.}, 1977; Gevers, 1977; Fidelman \textit{et al.}, 1982; Calguiri & Robin, 1985; Somero & Hand, 1990; \textit{cf} Masters \textit{et al.}, 1987).

The pH\textit{i} initiated molecular mechanisms of glycolytic down-regulation during hypometabolic states have received intensive study in the recent years (Hand & Somero, 1983; Lindinger \textit{et al.}, 1984; Walsh \textit{et al.}, 1988; Pörtner, 1989; Busa 1986; Barnhart & McMahon, 1988; Busa & Crowe, 1983; Fidelman \textit{et al.}, 1982; Hand & Carpenter, 1986; Hand & Gnaiger, 1988; Ellington, 1983a;b; Ellington, 1985; Walsh & Milligan, 1989) with the conservation of biochemical strategies across phylogenetic lines allowing workers to elucidate general principles (Storey & Storey, 1990). The key to the coordinated depression of metabolism in these states
is the covalent modification of regulatory enzymes via reversible protein phosphorylation (Bosca & Storey, 1991) with pH shifts providing a "metabolic context" which influences various cellular events (Busa & Nucitelli, 1984).

These findings reflect the strong possibility that whole-animal metabolism in the artificially ventilated animals subjected to respiratory acidosis in the present study was inhibited due to the allosteric modification of PFK and other enzymes in several tissues. If pH was mildly reduced or even increased during Series III as discussed earlier, the increase in metabolic rate during metabolic acidosis may be partially accounted for by these pH-enzyme interactions.

(2) Metabolic Processes in Which the Proton is Part of the Reaction: In addition to effects of pH upon enzyme activity per se (i.e., the proton acting as an allosteric modifier), changes in pH can affect the binding of effector molecules, thus, modifying the response of an enzyme to other signals (Busa & Nucitelli, 1984; Moore, 1985). For example, pH alters the ratio of free versus particle-bound glucose-6-phosphate dehydrogenase in sea urchin eggs (Aune & Epel, 1978). Through the simple law of mass action (Moore, 1985), protons also participate directly in the reaction related to enzyme dissociation from a particle-bound state or the activity of protein kinases over protein phosphatases (Storey 1987a; 1987b). Furthermore, creatine phosphate breakdown is a net proton consuming reaction (Gevers, 1977) and the hydrolysis of ATP utilizes protons either as a substrate or a product, as does the dehydrogenation of malic acid by NAD in the presence of malate dehydrogenase at different pH values (Albers, 1974; Bergmeyer & Moellering, 1966). This demonstrates that acid-base disturbances could have affected metabolism measurements obtained during this study not only via "classical" effects on
enzyme activity, but also through direct effects on chemical equilibria (Ellington, 1985; Walsh & Milligan, 1989).

(3) Possible Interactions Between pH\textsubscript{i} and Other Intracellular Signals

Interactions Between pH\textsubscript{i} and Intracellular Ca\textsuperscript{2+}: Intracellular H\textsuperscript{+} and Ca\textsuperscript{2+} "buffer" each other by Ca\textsuperscript{2+}:H\textsuperscript{+} exchange across the inner mitochondrial membrane and by competition for the same binding sites on the Ca\textsuperscript{2+}-binding protein, calmodulin (Busa & Nucitelli, 1984; Moore, 1985; Nakamori & Schwartz, 1972). These mechanisms have been speculated to account for the depression of cardiac function, for example, in the heart subjected to acidosis and hypoxia (Jackson et al., 1991). In addition to its depressant effects, there is a considerable body of evidence in support of intramitochondrial Ca\textsuperscript{2+} as a stimulatory mediator in the hormonal effects on mitochondrial oxidative metabolism (Capponi et al., 1988) through its effects on the key matrix enzymes involved in pyruvate oxidation and citrate cycle flux (Hansford & Castro, 1985; McCormack et al., 1990). Since metabolic acidosis has been demonstrated to increase intracellular or intramitochondrial Ca\textsuperscript{2+} concentration (Tullson & Goldstein, 1982; Gesser & Jorgensen, 1982), it may be inferred that increased MR due to metabolic acidosis (Series III) may have been due to the stimulation of energy requiring events by increases in intracellular Ca\textsuperscript{2+}.

Possible Interactions With cyclic AMP: Although it has been demonstrated that the effect of cAMP on some glycolytic enzymes is not pH-dependent (Reynolds & Haugaard, 1967), there is circumstantial evidence suggesting that moderate changes in pH\textsubscript{i} may regulate [cAMP]\textsubscript{i} either by inhibiting it or by decreasing its formation (Poyart & Nahas, 1968; Nahas, 1974). Thus, the cAMP-mediated stimulation of lipolysis and calorigenesis is markedly depressed by acidosis and
increased by alkalosis (Busa & Nucitelli, 1984; Moore, 1985). The possibility of cAMP regulation by pHi can therefore not be ruled out during this study.

*Interactions With GABA:* It is a well-known fact that the brain’s GABA (the most important of the inhibitory neurotransmitters) content increases in parallel with the rise in [H'] in the CSF during hypercapnia (Hoop *et al.*, 1985; Weyne *et al.*, 1977; 1978) and hypoxia (Weyne *et al.*, 1978; 1977; Kazemi & Hoop, 1991). Kneussl *et al.*, (1986a; 1986b) studied anaesthetized and paralyzed dogs and determined that GABA depresses whole-body $V_o_2$ and $V_co_2$, this reduction in metabolic function being independent of the central modulatory depressant effects of GABA on respiratory neurons (Kazemi & Hoop, 1991) and cardiovascular function (Kneussl *et al.*, 1986a; 1986b; Kazemi & Hoop, 1991). It is therefore possible that the (assumed) reductions in pHi during respiratory acidosis stimulated the release of GABA and consequently decreased aerobic metabolism.

*Interactions With Sympathetic Activity:* Hypercapnia has been reported to be a profound stimulus to catecholamine production and release (Fenn & Asano, 1956; Tenney, 1956; Ligou & Nahas, 1960; Nahas *et al.*, 1960; 1967; Cantu *et al.*, 1966; Euler & Lishajko, 1963; Nahas & Steinsland, 1968; Nahas, 1974). Although catecholamine release is usually accompanied by increased oxygen uptake (Griffith, 1951; Steinberg *et al.*, 1964), increased blood sugar and increased blood pressure, this may not always be the case (Ligou & Nahas, 1960) since the increase in enzyme activity that controls catecholamine synthesis contrasts with the inhibitory effect of [H'] on enzymes which regulate intermediary metabolism and which are activated by these catecholamines (Nahas, 1974). Acidosis (pH 7.1 to 7.2 Units) decreases catecholamine activity, and thus, catecholamines influence heart rate, blood pressure, calorigenesis and lipolysis
less at acidotic pH levels than at normal pH (Poyart & Nahas, 1966; Nahas & Poyart, 1967; Nahas et al., 1960). The normal thermogenic effect of norepinephrine on brown fat cells is also suppressed by high CO₂ levels, such as those seen during hibernation (Malan, 1988). As a consequence, the net effect of acidosis on overall sympathetic activity will be determined by the balance of these two effects. What that might have been under the conditions of the present study are not at all clear.

**Stimulation of Thyroid Hormones:** Increased thyroid hormone production or improved functional hormone receptor sensitivity to T₃ and T₄ during metabolic acidosis has been postulated (Van Hardeveld, 1986 in Hochachka et al., 1991) and may be, perhaps, another factor enhancing oxygen uptake.

**Interactions With Insulin:** There is also reason to suspect that pHi affects specific protein phosphorylation and dephosphorylation reactions which are probably involved in the amplification of many effects of insulin (Manchester, 1970; Moore, 1985) and other hormones such as epithelial growth factor (L’Allemain et al., 1984), glucocorticoids, glucagon, and parathyroid hormone (Moore, 1985). The effect of insulin in turn is upon glycolysis and is mediated by a change in pHi which is caused by activation of Na⁺:H⁺ exchange (proton extrusion) by the hormone (Moore, 1973; 1985). An increase in aerobic metabolism could therefore be expected with an increase in glycolysis and carrier-mediated activity, as elaborated below.

**(4) Membrane Transport Systems:** It is known that membrane transport systems such as the Na⁺-H⁺ antiporter (Aronson, 1985; Mahnensmith & Aronson, 1985; Fitzgerald et al., 1990; Aicken & Thomas, 1977) and Cl⁻-HCO₃⁻ transporter (Busa & Nucitelli, 1984) which maintain
ionic/acid-base homeostasis in the event of disturbances are far from passive processes. Thus, H⁺ transport is dependent on the transmembrane Na⁺ gradient established via Na⁺, K⁺-ATPase activity (Boutilier & Ferguson, 1989), a process clearly dependent on ATP consumption or energy expenditure normally provided by aerobic metabolism of glucose (Siesjö, 1985; Melton et al., 1991). Furthermore, the stimulation of these transport mechanisms may succeed in alkalinizing the cells, thus, enhancing glycolytic flux through increases in the activity of PFK (Manchester, 1970; Moore, 1973; 1985) and consequently, aerobic metabolism. This provides a potential explanation for the increased oxygen consumption observed during metabolic acidosis (Series III).

However, it is also known that pH decreases have a direct adverse effect on (Na⁺-H⁺) pump and Na⁺-K⁺-pump activating proteins or enzymes such as Na-K⁺-ATPase (Skou, 1982) if pH decreases significantly. This initiates an exponential decline of transmembrane ion flux (Balestrine & Somjen, 1988; Lutz, 1989) towards a final saturation and inhibition of these processes along with a progressively declining pH (Roos, 1965; Roos & Boron, 1981). Since lower rates of channel and pump mediated Na⁺ and H⁺ fluxes or ionic leakage in turn result in lower rates of energy metabolism (in turtle and rat brains for example) (Suarez et al., 1989; Lutz, 1989) the same may be applied to other tissues and could account for the observed decrease in $V_{O_2}$ during respiratory acidosis (Series I and II).

(5) Regulation of the Energy State of the Cell: It follows that intracellular pH can influence the energy state of the cell by affecting the free energy of hydrolysis of ATP, i.e., delta $G_{AT}$. Whether or not delta $G_{AT}$ is effected depends on how second messengers affect the other reactants and products, such as ATP, ADP, and Pi (Moore, 1985). It has been found that
intracellular acidosis in rat brains have marked effects on the creatine phosphokinase and the lactate dehydrogenase equilibria and that it also affects the cytoplasmic NADH/NAD⁺ ratio (Siesjö et al., 1974). Acute hypercapnia causes a shift in the creatine phosphokinase equilibrium, with a resultant decrease in the phosphocreatine concentration and the generation of more diprotonated phosphate or a secondary rise in inorganic P (Siesjö et al., 1974). Creatine phosphate levels have also been demonstrated to fall significantly during normoxic respiratory acidosis in turtle (Jackson et al., 1991) and rat heart (Williamson et al., 1976). In rat skeletal muscle, however, Sahlin et al., (1983) found that only after about the 95th minute of exposure to CO₂ did creatine phosphate levels fall.

**B. Studies at the Oxygen Transport Level:** Arterial pH reductions may have also inhibited metabolism at a somewhat higher level of integration by altering oxygen transport during this study. Metabolism is tightly coupled to systems responsible for oxygen transport between blood and cells and across cell membranes. Oxygen transport from the environment to the mitochondria is achieved by four linked transport mechanisms: ventilation, pulmonary gas exchange, circulation, and tissue diffusion (Lenfant, 1974) and acid-base changes are known to affect almost all of them. For example, consistent decreases in cardiac output with moderate and significant degrees of respiratory acidosis have been reported (Nahas & Cavert, 1957a; 1957b; Nahas et al., 1959; Horwitz et al., 1968; cf Richardson et al., 1961), being attributed to decreased levels of venous return and heart rate (Nahas et al., 1959). The oxygen transport function which is most unquestionably affected by acid-base changes is the oxygen-Hb equilibrium curve (Kwan et al., 1988). The effects of pH on this equilibrium may be either direct via a Bohr shift (Bohr...
et al., 1904, cited by Lenfant, 1974) or indirect via the DPG effect (Chanutin & Cornish, 1967; Benesch & Benesch, 1967, cited by Lenfant, 1974). With the acute pH changes produced during this study, only the direct effect could have played a role in affecting oxygen saturation. It is possible that a decrease in pHa may have caused the desaturation of hemoglobin or a reduction in Hb-O2 affinity and an increase in carbamino compounds in the blood (Eckert & Randall, 1984; Nahas et al., 1959; 1960; Cherniack et al., 1966; Sullivan et al., 1964; Kwan et al., 1988). This in turn may have resulted in a reduction in the oxygen content of the blood at any given level of PO2.

There is also some evidence that the mean corpuscular hemoglobin concentration (MCHC) of blood decreases with decreases in plasma pH (Granbarth et al., 1953; Bellingham et al., 1970; 1971; Gregg et al., 1989; Horstman et al., 1974; Woodsen et al., 1978; Radford et al., 1967; cf Wranne et al., 1972). This could have reduced the oxygen carrying capacity of the blood. Over the past decade, there have been considerable arguments over whether these effects limit aerobic metabolism by limiting systemic oxygen transport via convective delivery of O2 to tissues (Cain, 1977) or by limiting the driving pressure for capillary O2 diffusion to the cells (Stainsby et al., 1988; Gutierrez & Andry, 1989; Roca et al., 1989; Gutierrez et al., 1990; Hogan et al., 1990). Irrespective of the limiting mechanism, it may be inferred from this study that the low levels of PaO2 during respiratory acidosis may have affected metabolism. This inference is based on the frequent suggestion that the availability of oxygen represents a limiting factor for resting O2 consumption (Mortola & Tenney, 1986).
II. Direct Effects of CO₂ on Metabolic Rate

In addition to the indirect effect that CO₂ might have had on cellular metabolism and oxygen transport through changes in pHa and/or pHi, CO₂ may have also altered cell activity directly. High PaCO₂ could have reduced cell metabolism by inhibiting CO₂ producing reactions (Walsh et al., 1988) and the combination of CO₂ with proteins to form carbamino compounds could have altered enzyme performance (Lutz, 1989). Longmore et al., (1974) established the physiological importance of the effect of change in total CO₂ concentration at constant pH (independent of pH change) by illustrating that increased total CO₂ concentration within the physiological range regulates both carbohydrate and lipid metabolism in the isolated liver. Increasing total CO₂ concentration in certain tissues by increasing bicarbonate concentration has been shown to affect the activity of several enzymes in vitro, including glucose-6-phosphate dehydrogenase, glucose-6-phosphate phosphohydrolase, phosphoenolpyruvate carboxykinase, glyceraldehyde phosphate dehydrogenase, and aconitase (Bandurski & Lipmann, 1956; Chance & Park, 1967; Dickman & Cloutier, 1951; Dyson et al., 1969; Levy, 1963; Anderson & Nordlie, 1968; McDaniel & Longmore, 1971; Longmore et al., 1974).

It must be pointed out that these potential contributions of changes in PaCO₂ to changes in metabolic rate would be limited to affecting the magnitude of the response of metabolic rate in the present study. These changes cannot account for differences in the direction of response to respiratory and metabolic acidosis since it increased in both instances although to significantly different extents.
III. Direct Effects of Mechanical Ventilation on Metabolic Rate

In light of the frequent suggestion that the availability of O$_2$ is a limiting factor for resting O$_2$ consumption (Mortola & Tenney, 1986), it is possible that $V_{\text{O}_2}$ decreased with decreasing Pa$_{\text{O}_2}$ during hypoventilation in Series I due to an decrease in O$_2$ availability. Lung atelectasis and progressively developing pulmonary edema are known to cause arterial oxygen desaturation during prolonged periods of mechanical ventilation (Nahas et al., 1959) and could have been responsible for limiting oxygen availability. The opposite would also be true for the increase in Pa$_{\text{O}_2}$ during hyperventilation. A potential role for reductions in venous admixture (Michenfelder et al., 1966), increased cardiac output (Breivik et al., 1973; Slotman et al., 1983), alkalotic tetany and unsteady oxygen stores (Cain, 1970) in increasing aerobic metabolism during hyperventilation have also been suggested.

IV. Effects of Infusate Contents and Volume on Metabolic Rate

In addition to the effects of extra- and intracellular acid-base changes on aerobic metabolism, some effects of infusate composition and volume or extracellular fluid (ECF) expansion may have partially accounted for the increase in MR during Series III.

(1) Effect of Metabolic Substrate Availability on Metabolic Rate: An average total concentration of 2.5 mM.kg$^{-1}$ of lactate and acetate were infused into individual animals during Series III. The fate of these substrates could not be determined from this study. Acetate may
have entered the Krebs cycle within the tissues by way of acetyl Coenzyme A (Burton, 1975). The final values of arterial lactate measured in some squirrels were not more than 7mM.l⁻¹ (Table 7.) possibly reflecting the net result of several processes: 1) the rate of acid infusion, 2) distribution of lactate within the extracellular fluids, 3) transport and distribution in intracellular fluid, 4) metabolism or oxidation, 5) glycogenesis, and 6) glucogenesis (Brown & Rogers, 1983). Since \( \dot{V}_{O_2} \) increased, it is assumed that oxygen availability was not a limiting factor. Consequently, lactate measurements would not include lactate from anaerobic metabolism.

That the availability of metabolic substrates such as acetate and lactate increases oxidative metabolism is a well described phenomenon (Liang & Lowenstein, 1978; Wathen et al., 1982; Vreman et al., 1980; Mudge et al., 1949; Davidson et al., 1978; Skutches et al., 1979; Huland et al., 1974; Burton, 1975). Large increases in CO₂ output by non-exercising muscle after exercise bouts have been observed and attributed to increased rates of lactate oxidation and to CO₂ diffusion from muscle secondary to an intracellular acidification (Kowalchuk et al., 1988; Lindinger et al., 1990).

It must be noted that since infusion of plain Ringer’s solution produced qualitatively similar results to the infusion of weak acids, the difference in direction of the response produced by respiratory and metabolic acidosis cannot be explained simply by metabolic acidosis increasing substrate availability. Increasing substrate availability could, however, have contributed to the magnitude of the response.

(2) Possible Effects of Extracellular Volume Expansion on Metabolic Rate: In the present study, animals were infused with acid solutions at the rate of 0.19-0.32 ml.min⁻¹ for 20-30 minutes. As a consequence, their extracellular fluid (ECF) compartment may have increased by
6-10 ml. This would represent an increase of 24\%\textsuperscript{10}. Coordinated physiological and behavioural adjustments that mitigate the adverse hypertensive effects of volume overload and/or acute increases in sodium intake (Wang et al., 1991) are known to include the release of atrial natriuretic peptide (ANP) from the heart and from discrete areas of the central nervous system (Kawata et al., 1985; Skofitsch et al., 1985). The release of ANP, in turn may have decreased the release of arginine vasopressin (AVP) (see Samson, 1985; Samson et al., 1987). Increases in AVP have been documented to decrease oxygen uptake by a direct effect on cardiac output (Liard, 1989). As a corollary to this, an increase in ANP should consequently increase O\textsubscript{2} uptake by decreasing AVP levels following an increase in ECF.

Increased ECF may also have elicited an increase in NE (Van Huysse & Bealer, 1991). As increases in NE are well known to be associated with an increase in oxidative metabolism (Griffith, 1951), this provides yet another mechanism that may have contributed to the increase in oxygen uptake recorded during acid infusion experiments.

ARE THERE ANY POSSIBLE IMPLICATIONS OF THIS STUDY TO THE MECHANISMS OF ENTRANCE INTO AND AROUSAL FROM HibernATION?

Appropriate restraint has to be shown in transferring these findings to the natural situation of entrance into and arousal from hibernation, in light of the fact that measurements of metabolic rates were obtained from anaesthetized, paralyzed and artificially ventilated supine squirrels. The

\textsuperscript{10}ECF = \sim 17\% of body weight. Therefore, by adding approximately 8 ml. to the average body weight of 200 gms, ECF would have increased by \([8/(0.17*200)]\) or 24\%.
following extrapolations have therefore been made with caution and deserve experimental confirmation.

This study has illustrated that basal metabolism of euthermic golden mantled ground squirrels can be inhibited by respiratory acidosis. In light of the various scenarios constructed to illustrate the alternative kinetics of entrance into hibernation, this observation may be applied to infer that in addition to the inhibition of $T_{\text{e}}$, "classical" respiratory acidosis may contribute to the initiation of hibernation by directly inhibiting basal metabolism, prior to any significant fall in $T_v$.

This study also illustrated that basal metabolism of euthermic golden mantled ground squirrels can be stimulated or enhanced by "classical" respiratory alkalosis. If applicable to natural arousal where hyperventilation has been suggested to precede increases in $T_v$, this finding may be inferred to emphasize the possibility that hyperventilation does not merely remove the inhibition over thermogenesis brought about by increased CO$_2$ concentrations during entrance into hibernation, but also enhances cellular function.

On first impression, the results from acid infusion experiments illustrating that metabolic acidosis increased MR may seem to partially explain the increased MR seen during arousal since arousal is typically accompanied by metabolic acidosis. However, it is quite evident that the increased MR in arousing squirrels largely arises from non-shivering thermogenesis which in turn is responsible for the accompanying metabolic acidosis (Ambid & Agid, 1975; Malan et al., 1988; Willis, 1982a; 1982b). It follows that metabolic acidosis is not the cause of increased MR but a result of it. Since endogenous metabolic acidosis will produce a fall in pH$i$, if the arguments outlined above prove valid, this would inhibit rather than increase metabolic rate.
CONCLUSIONS

Based on the data, it may be concluded that the general response to extracellular acidosis depends not only on the pH of the solution but also on the type of acidosis imposed. Presumably, these differences depend on the severity of cellular acidification by the various acids. It is suggested that the change in pH and not just the pH of the solution appears to be the critical factor determining the direction and extent of change in MR. It is hypothesized that significant differences in PaO\textsubscript{2} and PaCO\textsubscript{2} during the two types of acidosis produced in this study, combined with the well documented differential rates of transmembrane penetration of H\textsuperscript{+} and CO\textsubscript{2} respectively, as well as the effects of volume loading, contributed to a significantly reduced pH during respiratory acidosis, on the one hand, and a mildly reduced, unchanged, or even elevated pH during acid infusion, on the other.

As with the factors that regulate tissue respiration (See Hogan et al., 1991), the mechanisms responsible for regulating total body MR appear to interact with extracellular acidosis in a complex fashion. It appears that changes in pH, P\textsubscript{CO\textsubscript{2}} and [HCO\textsubscript{3}\textsuperscript{-}] each may potentially influence metabolic reactions. Different factors influence different mechanisms which may stimulate or inhibit aerobic metabolism. In some cases the same factor may both stimulate and inhibit metabolic rate depending on the level of reduction in pH. Furthermore, some of the differential effects of metabolic and respiratory acidosis on aerobic metabolism may be due to differences in the net balance of the various inhibitory and stimulatory influences of acid-base changes that may be occurring simultaneously. Finally it should be kept in mind that the mechanisms responsible for altering \( \dot{V}_{O2} \) when pH falls below normal may not necessarily be
the reverse of those responsible for altering $\dot{V}_{O_2}$ when pH rises above normal levels.

Mechanisms aside, in the present study, respiratory acidosis and metabolic acidosis have rapidly reversible but opposite effects on the metabolic rate of golden-mantled ground squirrels ($Spermophilus$ *lateralis*); respiratory acidosis inhibits aerobic metabolism whereas metabolic acidosis stimulates it. This implies that pHa is indirectly rather than directly correlated with $\dot{V}_{O_2}$ by its effect on some other factor which must be altered in different ways by respiratory and metabolic acidosis.
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