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UNIVERSITY OF ALBERTA

PROLONGED SEVERE HYPOTHERMIA IN THE LABORATORY RAT

BY

MICHAEL L. JOURDAN

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

DEPARTMENT OF ZOOLOGY

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Facility of Graduate Studies
and Research
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Dr. Robert Hoo-Paris

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Dr. Raymond Rajotte

Facility of Graduate Studies
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University of Alberta
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Corinne Moreau-Hamsany

Facility of Graduate Studies
and Research
University of Alberta
Edmonton, Alberta

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Dr. Lawrence C.H. Wang

M. Dawn McArthur

Facility of Graduate Studies
and Research
University of Alberta
Edmonton, Alberta

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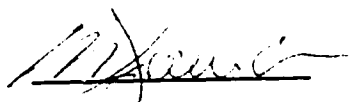

Michael L. Jourdan
7112 - 105A St.
Edmonton, Alberta
Canada T6E 4H9

October 10, 1991

Faculty of Graduate Studies
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University of Alberta
Edmonton, Alberta

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Professor Lawrence C.H. Wang

Professor M.T. Clandinin

Professor R.J. Christopherson

Professor X.J. Musacchia

Assistant Professor W.J. Gallin

September 24, 1991

ABSTRACT

A technique has been developed with which rats can be made hypothermic to a body temperature (T_b) of 19.0±0.3°C for periods up to 24h and then rewarmed to normothermia without any apparent ill effects. Similar results are obtainable with ground squirrels at a T_b near 7°C for 72h. Repeated exposure to hypothermia with this method does not appear to affect the animal's tolerance or response to hypothermia. Since animals can be maintained for a relatively long time at a very stable T_b this model has proven to be a useful tool for comparative studies on the pharmacological and physiological aspects of hypothermia.

At a stable body temperature (T_b) of 19°C, hypothermic rats are capable of homeostatic control over water and ionic balance for up to 20 hours. Hematocrit increases seem to be associated with red blood cell (RBC) swelling rather than increased numbers of RBCs. Decreased turnover and oxidation of glucose in conjunction with decreased turnover and plasma levels of FFA suggest that the hypothermic rat may be in an energy deficient state. This is further substantiated by the increasing arterial lactate and decreasing P_aCO_2 levels, which when viewed in light of the depressed glucose oxidation, suggest tissue hypoperfusion and poor oxygenation. This apparent energy deficiency is a progressive problem as shown by a decrease in sensitivity to insulin, and glucagon and in the turnover of glucose and FFA with the progression of hypothermia. Further, there is a time dependent decrease in metabolic resistance as measured by the hypothermic rat's efforts to resist cooling. Thus, as energy availability decreases, so does metabolic resistance until a point is reached when energy shortage becomes critical. At that point, irreversible perturbation of the system occurs and survival is no longer possible. These observations, in conjunction with previously reported evidence of cardiac

insufficiency during hypothermia, suggest that prolonged hypothermia in the rat produces an energy deficient state. Therefore, remedial measures taken toward improving the energy status of the animal via improved circulatory sufficiency, tissue perfusion and oxygenation, and the efficiency of substrate utilization, should prove both rewarding and enlightening.

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INTRODUCTION

Under conditions which promote rapid heat loss, failure to match the rate of heat loss with heat production results in progressive reduction of body temperature (T_b). The resultant hypothermia is predictably fatal. Without aid, such as the application of exogenous heat, hypothermic animals are typically unable to restore normal T_b. Survival then depends on the innate cellular cold tolerance of the animal until critical failures occur and death becomes inevitable.

When homeotherms are cooled a general pattern of responses can be characterized. During the initial cooling there is a general increase in many physiological processes (O_2 consumption, cardiac output, respiration and kidney function). The second phase of progressive cooling is characterized by a continuous reduction in physiological processes paralleling the decrease in T_b. Finally, as profound hypothermia sets in (T_b<28°C depending on species) a state of general depression is apparent which changes many of the physiological functions and in which physiological disruptions are observed.

Insufficient knowledge of the fundamental physiological changes created by hypothermia, combined with a large variability of experimental conditions (the use of a wide range of T_b's, a variety of species and differences in the length of hypothermic exposure) contribute to the difficulty in interpretating existing hypothermia data. Considering the diverse approaches to hypothermia research it is not surprising that death, as a consequence of hypothermia, has been attributed to failure of respiratory and/or cardiovascular functions (13,20), renal function (39), acid-base regulation (37), ion regulatory mechanisms (26,27,28) and substrate mobilization and utilization dysfunctions (9,21,25). To date, however, there is no consensus as to what physiological changes occur during prolonged severe hypothermia or on the quantitative importance of the

various observed changes to hypothermic survival. Establishing uniformity in experimental approaches could be the first important step towards answering this important question.

Failure of the respiratory system is most probably secondary to other failures and is quite likely associated with a decrease in the efficacy of the circulatory system. However, the ventilatory rate and perhaps also tidal volume are decreased, while both airway and physiologic dead space are increased. Pulmonary edema not associated with cardiac failure is common in human hypothermic victims, possibly due to a reduction of ciliary function in the bronchial epithelium (16,23).

The mechanical efficiency and work output of the heart is reduced on cooling (12,30,18). At the same time total peripheral resistance is increased, probably due to an increase in blood viscosity as well as a decrease in the flexibility of vessel walls. Haematocrit usually increases (36,32,11). Overall it appears that circulatory function is only adequate to support survival in deep hypothermia for short times. Sooner or later the distribution of blood is disrupted, probably due to scattered microcirculatory obstructions and cardiac arrhythmias, resulting in death. Obstruction of the microcirculation is suggested by Swan (35) to be the fundamental physiologic failure in prolonged hypothermia. Among the reported factors affecting microcirculation in hypothermia are increased haematocrit and fluid shifts (10), aggregation of erythrocytes (4,22) and the agglutination of platelets (17).

Though cardiac output is decreased, renal blood flow appears to increase during cooling, only decreasing when kidney temperature is quite low (6). A similar pattern is seen in urine flow which first increases then decreases only during profound hypothermia (15). However, during progressive cooling other processes of the kidney are affected in dissimilar ways, some processes are

decreased [glomerular filtration (19), reabsorption of sodium and water (33) and bicarbonate (6,14)] while others are increased [isosmotic reabsorption (40) and urine volume]. In profound hypothermia all renal processes are decreased or even abolished. Most effects of hypothermia appear to be protective and reversible upon rewarming, if the hypothermic exposure is not extended for too long, a fact which is used extensively in kidney preservation for transplantation. This protective and reversible action on the kidney may however, have deleterious effects on other systems (acid-base and water balance and ion regulation) in hypothermia.

There appears to be no classical acid-base regulation existing in hypothermia. Extracellular pH normally depends on the complex interaction of respiratory rate, gas mixture, T_b, efficiency of metabolism and internal temperature gradients. Many investigators suggest that both respiratory and metabolic acidosis occur at some point in hypothermia (3,8,24,38). During cooling without assisted respiration, respiratory acidosis develops rapidly as the efficiency of ventilation decreases, unless hyperventilation is present (26,35). At the same time metabolic activity tends to be maximized with the concomitant production of CO_2 and lactic acid resulting in respiratory and metabolic acidosis. However, respiratory acidosis tends to be buffered by the increased solubility of CO_2 with falling temperature leading to increased plasma bicarbonate concentration. On the other hand, the metabolic acidosis may be augmented by a decrease in the utilization of lactic acid by both the liver and heart. This results in a predominant metabolic acidosis during hypothermia. How detrimental this acidosis is to survival in hypothermia is still an open question and bears further investigation.

Bristow (7) suggests that acute hypothermia per se probably does not cause any significant changes in most ions. However, in light of the changes observed in kidney function and acid-base status some change in the ion profile

of the blood can be expected and therefore, may affect other functions such as membrane excitability and enzyme activity. Hypothermia induced changes in electrolyte metabolism have often been reported, but the overall picture is still quite obscure. For example, serum potassium in hypothermia is reported to increase (34), decrease (14) or remain unchanged (5). Considering the wide-ranging effect of potassium on excitability any one of these changes could have profound implications.

The most frequently stated limitation to survival in hypothermia is failure to meet metabolic requirements. This admittedly covers a rather broad range but the least common denominator would appear to be substrate supply and utilization. Popovic (26) characterised the onset of hypothermia in the rat as a period of transient hyperglycaemia followed by progressive hypoglycaemia. In the hamster, depletion of carbohydrate reserves has been correlated with survival time (29) and further substantiated by the demonstration that infusion of glucose during hypothermia improved survival 3-4 fold (31). In the rat, administration of glucose does not appear to improve survival (28; personal observation). However, significant reduction in glucose transport across cell membranes (1,25) may explain why exogenous glucose is of no benefit to hypothermic rats. This impaired glucose transport may be a consequence of high free fatty acid (FFA) levels (21). In a normothermic animal actively producing heat to maintain T_b, high levels of FFA are required, but in a hypothermic animal struggling to maintain basic homeostasis, they may be a detriment, especially if they interfere with the transport of the primary metabolite for regulatory centres in the brain. For example, several investigators have observed that respiratory failure occurs prior to cessation of heart activity (1,20,personal observation) and an impairment in glucose uptake by the respiratory centre has been suggested as the cause. Further investigation into the role of substrate in hypothermic survival should prove both enlightening and useful in therapeutic attempts.

This project was undertaken to establish a standardised animal model for hypothermic induction and maintenance with the hope that it will provide a means by which the intricacies of hypothermia physiology may be systematically evaluated. The laboratory white rat (*Rattus norvegicus*) was selected as the animal because its size allowes serial blood sampling and the continous monitoring of vital signs as profound hypothermia progresses.

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I. METHOD DEVELOPMENT [1]

INTRODUCTION

Literature concerning hypothermia is often confusing and contradictory. Much of this confusion arises from the multitude of approaches to the study of hypothermia. Conclusions have been based on data collected from: 1) animals entering hypothermia [during the period of declining body temperature (T_b)], 2) short periods (usually sublethal) of low T_b, 3) during rewarming from low T_b, and 4) from human victims with a wide variety of T_b's of unknown duration.

In order to understand the physiology of survival under profound, long term hypothermia a suitable animal model is required. This model should include a maximum duration of steady state at depressed T_b with a minimum of pharmacological interference, so that the effects of temperature only on physiological systems can be evaluated over time. Further, the animal must be capable of surviving this manipulation with full recovery within a prescribed and/or predictable period. This will allow the testing of treatments aimed at improving hypothermic survival.

Early methods of hypothermia induction often involved the use of CO_2 narcosis (5-8) or long-acting narcotic anaesthetics (e.g. barbiturates), both are known to interfere with central thermoregulatory functions, blood gas and pH regulation and therefore, affect survival in hypothermia (9). To circumvent this problem, Fischer and Musacchia (2) developed the helium-oxygen (HeO_2) method of hypothermia induction which involves exposing the animal (Syrian

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1. A version of this chapter has been published. Jourdan, Michael L. and Lawrence C.H. Wang. 1987. J. Therm. Biol. 12(2):175-178.

hamsters) to low ambient temperature (T_a) and the high thermal conductivity of helium (He). This method was later demonstrated to be effective on rats as well (3). With this method, however, resistive metabolic efforts seriously deplete the metabolic reserves of the animal prior to becoming hypothermic thereby minimizing survival in hypothermia. Volkert and Musacchia (10) demonstrated an inverse relationship between induction time and duration of survival in hypothermia when the induction time was altered with a volatile anaesthetic, halothane. The use of halothane, which significantly shortened the induction time, by decreasing or eliminating altogether the resistive metabolic activity, increased survival time 2-5 fold in hypothermic hamsters. Thus, in addition to maximum duration of survival the model should include an induction strategy which is relatively quick with minimal stress to the animal. Further, the induction should not include any lasting pharmacological interference which might alter the "normal" physiological condition of the animal at the specific hypothermic T_b (ie. carry over effects of the induction should be minimized).

While the series of studies by Musacchia and co-workers (1-4,10) introduced valuable and effective techniques for induction of hypothermia, no attempt was made to precisely regulate T_b during hypothermia so that a relationship between T_b during hypothermia and survival could be established. This is an important requirement in evaluating the role of metabolic regulation under depressed T_b and in identifying possible mechanisms which may limit survival in hypothermia. Therefore, the new model should be capable of regulating T_b within 0.5°C of the desired temperature.

In view of the above, it was apparent that a new model had to be developed to meet the requirements of the present study on long term hypothermia survival. This new model, which incorporated modifications of previous methods of hypothermia induction with new computer technology for hypothermia maintenance and recovery, has provided heretofore unattained

precision in control of T_b during hypothermia and in achieving maximum duration of hypothermic survival. To compare species differences in capacity of tolerance to long term hypothermia, performance of a nonhibernating species, the rat, was compared to that of a hibernating species, the ground squirrel.

METHODS

Male Sprague Dawley rats (300-400gms) and golden-mantled ground squirrels (*Spermophilus lateralis*; approximately 200gms) were housed individually, in shoe box cages, under 12L:12D photoperiod at 23°C with ad libitum water and food (rat chow and a rat chow dog chow mix supplemented with sunflower seeds for rats and squirrels, respectively). Animals were initially anaesthetized with halothane (5%) in helium-oxygen (79% He, 21% O_2; HeO_2) at 1.5 l/min.. When the animal was unconscious the halothane was reduced (to 3% or 1.5% for squirrels or rats, respectively) and the animal was transferred to a surgical board where rectal thermocouple (copper-constantan, placed approximately 5cm deep) and stainless steel needle ECG electrodes (attached to the right fore limb and left hind limb) were attached. The animal was then placed in a water jacketed plexiglas chamber (a horizontal tube, 29.0cm long by 10.0cm inside diameter; Fig. I-1), where T_a was controlled by a circulating water bath (NESLAB, Endocal RTE-9DD OR 8DD) at 0°C. Cooling was effected under a HeO_2-halothane (1.5%) atmosphere to a predetermined T_b, usually 2 to 3°C above the desired final T_b. During this initial rapid cooling, the amount of halothane in the open flow system was reduced by about 0.1% per 1°C drop in T_b down to a T_b of 28°C where for rats it was discontinued (Fig. I-2). For squirrels the halothane was maintained at 0.5% from T_b = 28°C to 20°C and discontinued at T_b = 20°C. The subsequent period of slower cooling allowed the halothane to slowly wash out of the animal's system as T_b dropped and cold narcosis took over. When T_b reached the predetermined level (20-22°C for rats and 11-12°C for squirrels) T_a was increased, to 15 or 7°C for rats and squirrels

Figure I.1: Diagrammatic view of the hypothermia work station. Letters refer to the major components of the system; (A) digital telethermometer (Sensortek, Bat-12); (B) computer-controllable 4-channel thermocouple switch (Sensortek, Channelizer); (C) IBM-PC with ATOD and DTOA interfaces for reading and controlling various components of the system; (D) refrigerated circulating water bath with analog temperature control unit (Neslab, endocal models); (E) water-jacketed plexiglas animal chamber; (F) air flow control and gas selector; (G) ECG amplifier (Technical Services, University of Alberta); (H) oscilloscope; (I) printer; (J) video monitor. During maintenance of hypothermia, T_b measured by the telethermometer via the channelizer is transmitted to the computer and digitized by an analog to digital converter. A custom program then displays and stores the T_b and compares it to a predetermined set temperature. The error value is then used to modulate the output of the water bath thereby controlling the T_a of the chamber. The precision of regulation is within $\pm$ 0.2°C of the desired level over a relatively broad range of T_b. The computer also monitors the physiological parameters (T_b,T_a,ECG,$\dot{V}O_2$ etc.) during induction and rewarming phases of hypothermia.

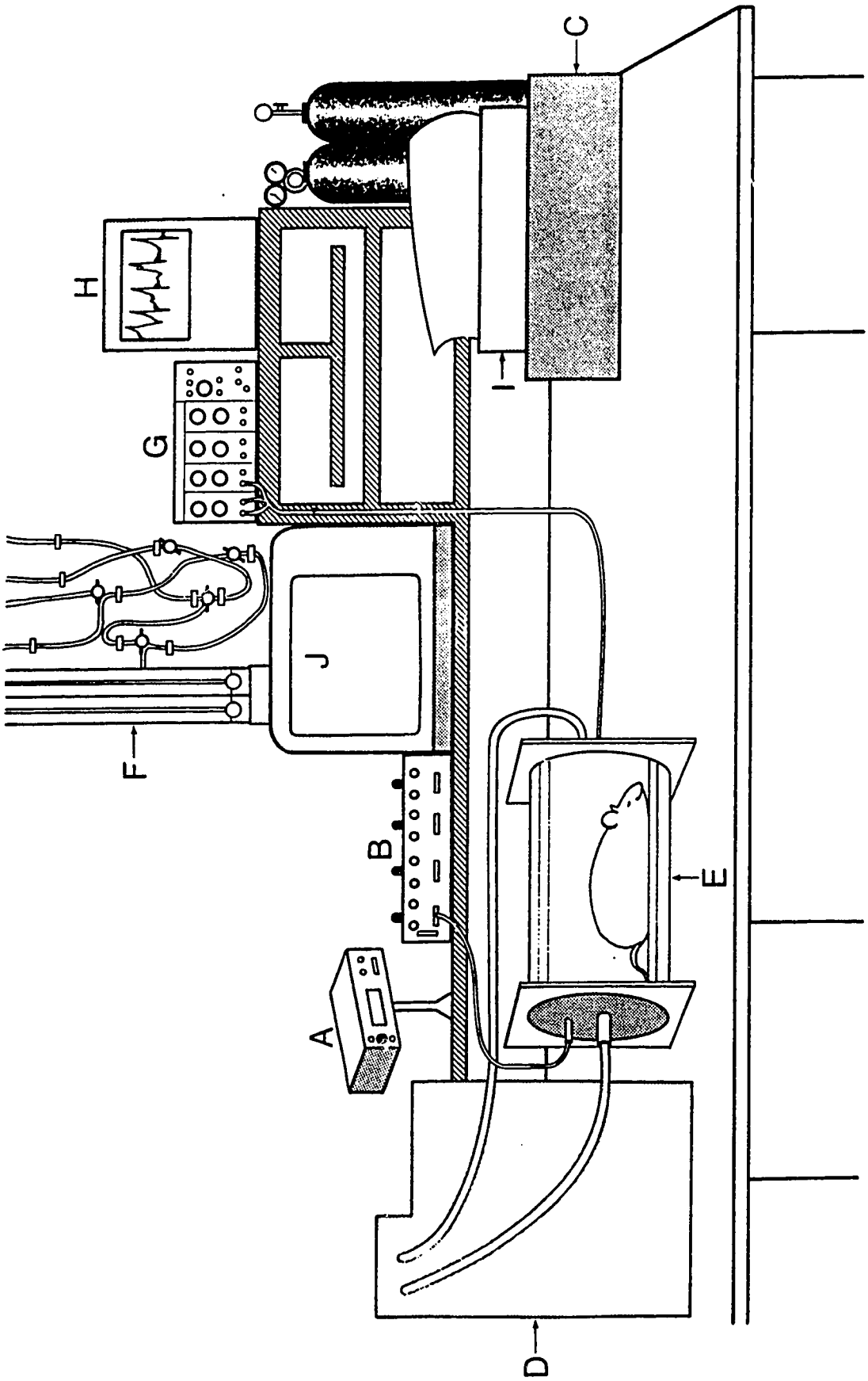
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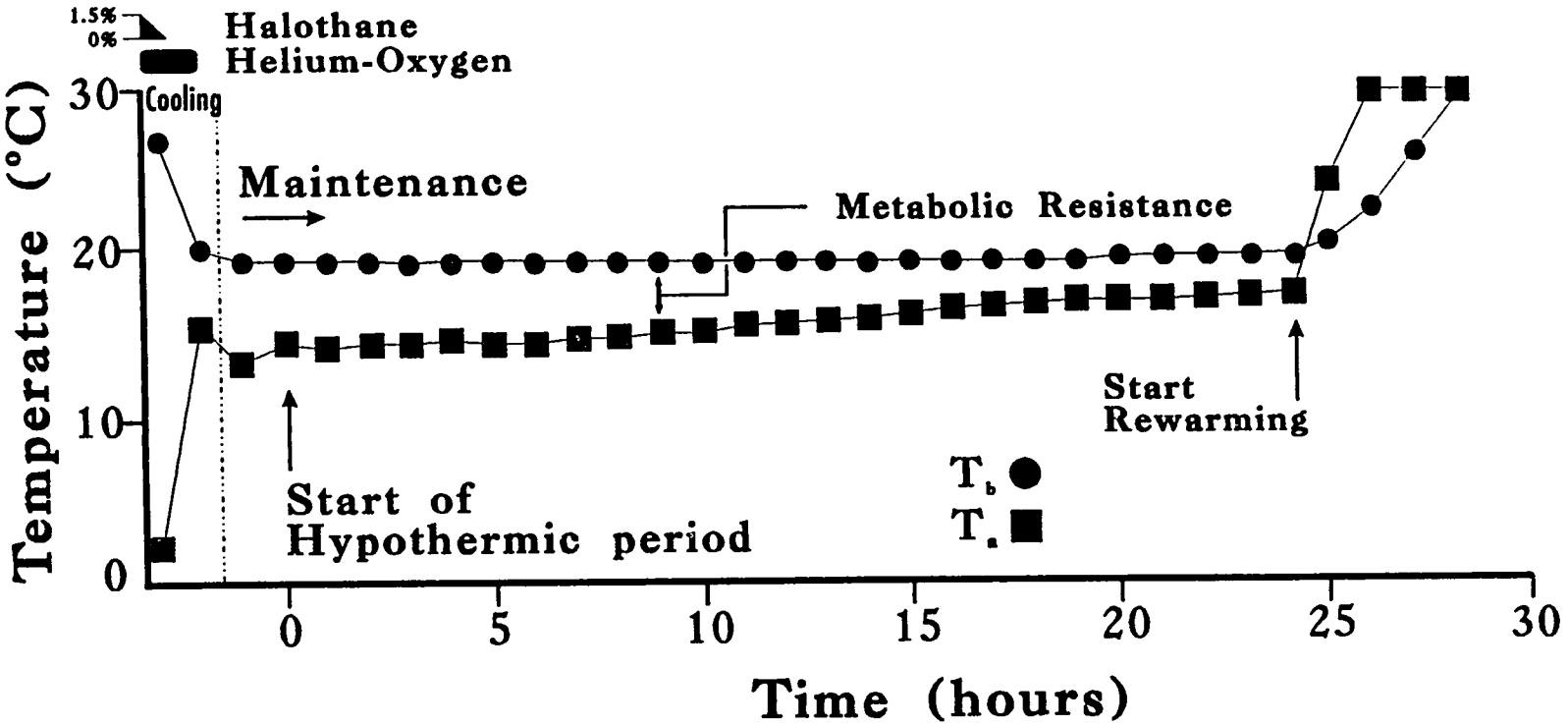
Figure I.2: Protocol and changes of body (T_b) and ambient (T_a) temperatures during the induction, maintenance (24 h) and rewarming from hypothermia in rats. Shown are the periods of transient exposure to halothane the concentration of which in the atmosphere decreased from 1.5% to 0 and the duration of helium-oxygen during the hypothermia cooling period. Time 0 is the time at which the animal's body temperature reached 19°C. The difference between T_b and T_a is taken as a measure of the rat's metabolic effort.


1.5%
0%
Halothane
Helium-Oxygen
Cooling
Maintenance
Metabolic Resistance
Start
Rewarming
Start of
Hypothermic period
T_b
T_a
Temperature (°C)
30
20
10
0
0
5
10
15
20
25
30
Time (hours)

respectively, and the HeO_2 was replaced with pre-cooled room air to allow the animal to cool slowly to the desired T_b (19°C and 7°C for rats and squirrels respectively). T_b was maintained by feed-back control, using the animal's T_b to adjust the temperature of the circulating water bath which perfused the animal chamber, via a computer algorithm. At the end of the hypothermic bout, generally 24 hours for rat and 72 hours for ground squirrels, T_a was programmed to increase to 30°C and the animal allowed to rewarm to euthermia.

RESULTS

A typical hypothermic bout for ground squirrels is shown in Figure I-3. It can be seen that the animal entered hypothermia relatively quickly and smoothly, it's T_b was then held within 0.2°C of the desired level until rewarming was initiated (after 72 h of hypothermic exposure) and the animal returned to euthermia. Repeated exposure, at weekly intervals, in the rat (Fig.. I-4) did not appear to affect the animal's tolerance or response to hypothermia. Survival, based on the ability of the rat to rewarm to euthermia and display a normal activity pattern, ie. feeding, drinking and grooming, following the hypothermic bout, was evaluated in white rats maintained at various T_b's during hypothermia. From figure I-5, 19±0.3°C appears to be the lowest temperature a rat can survive for 24 hours using this method. To date the survival time at different T_b's has not been determined in hypothermic squirrels (due to the long survival time), however, Columbian and Richardson's ground squirrels consistently survive for over 72 h at a T_b of 7°C and golden-mantled ground squirrels regularly survive repeated exposure to 48 h at a T_b of 7°C.

DISCUSSION

The study of the physiological and pharmacological manifestations of hypothermia often requires that specific duration and temperature conditions be maintained. Investigation in to the factors which might limit long term

Figure I.3: Body temperature and heart rate of a golden-mantled ground squirrel during induction, maintenance and rewarming from a hypothermic bout of 72 h duration at T_b=7°C.

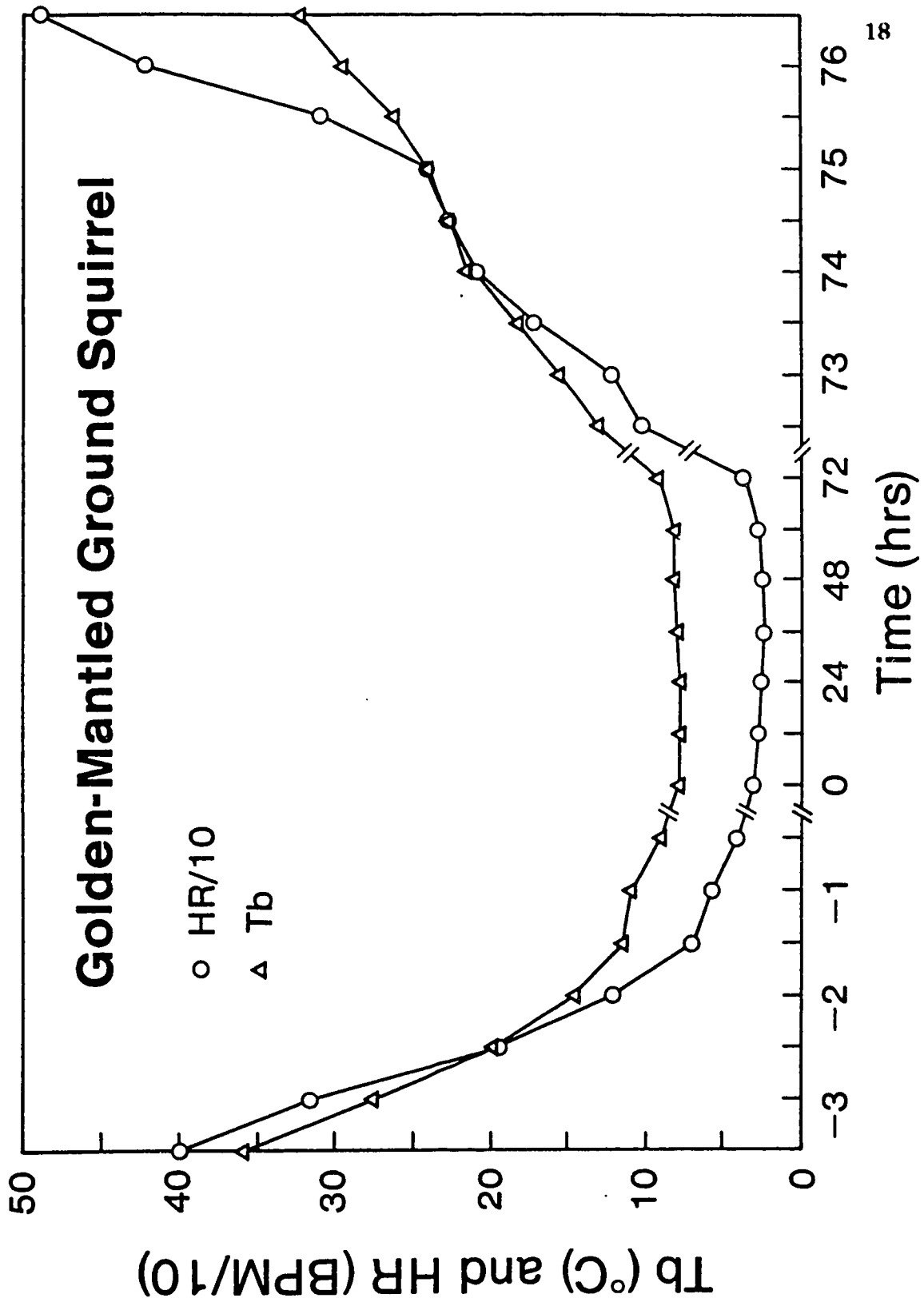

Golden-Mantled Ground Squirrel
HR/10
Tb
Tb (°C) and HR (BPM/10)
Time (hrs)
50
40
30
20
10
0
−3
−2
−1
0
24
48
72
73
74
75
76

Figure I.4: Body temperature and heart rate of a Sqrague-Dawley rat during 2 successive 24 h hypothermic bouts at T_b=19°C.

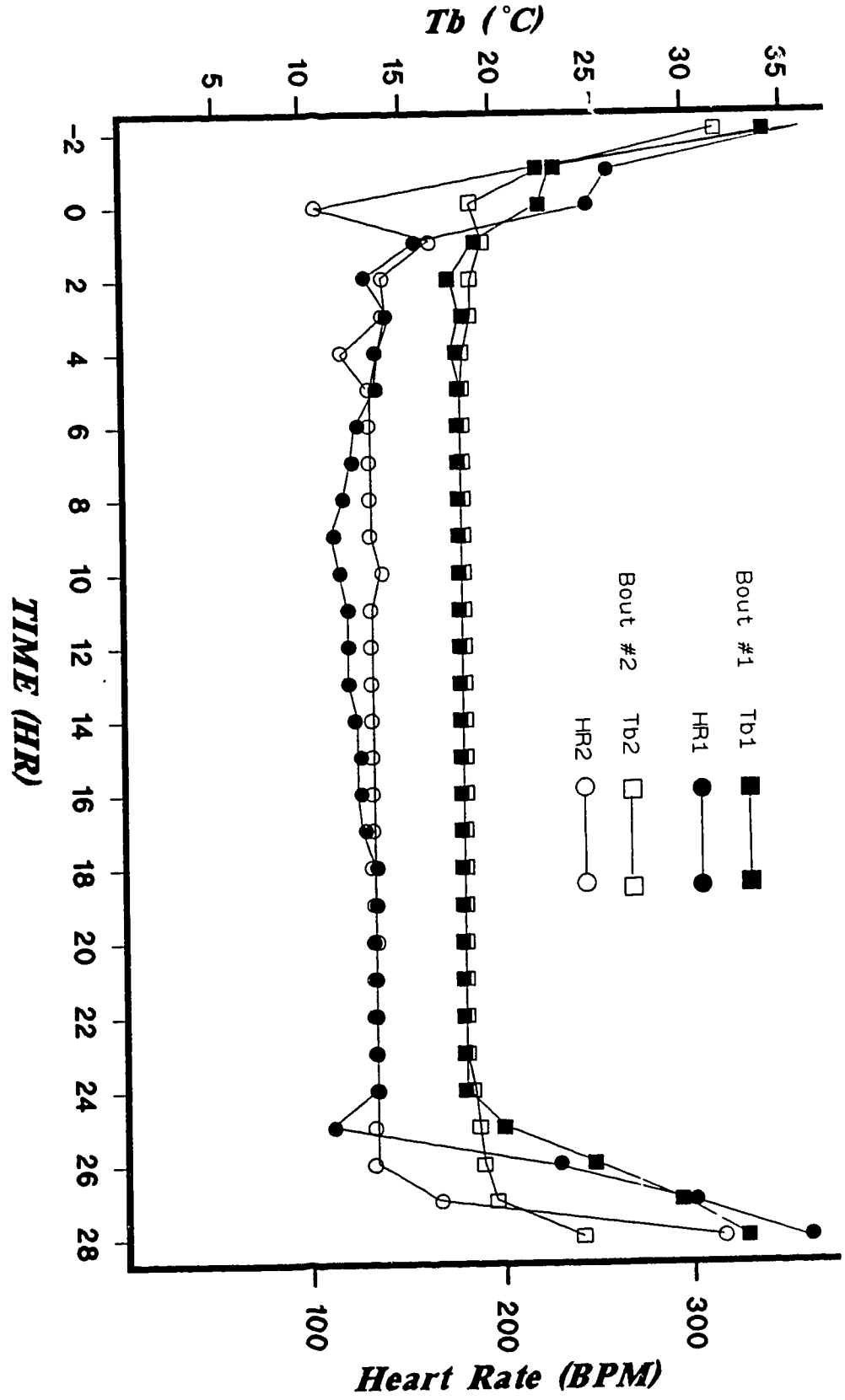
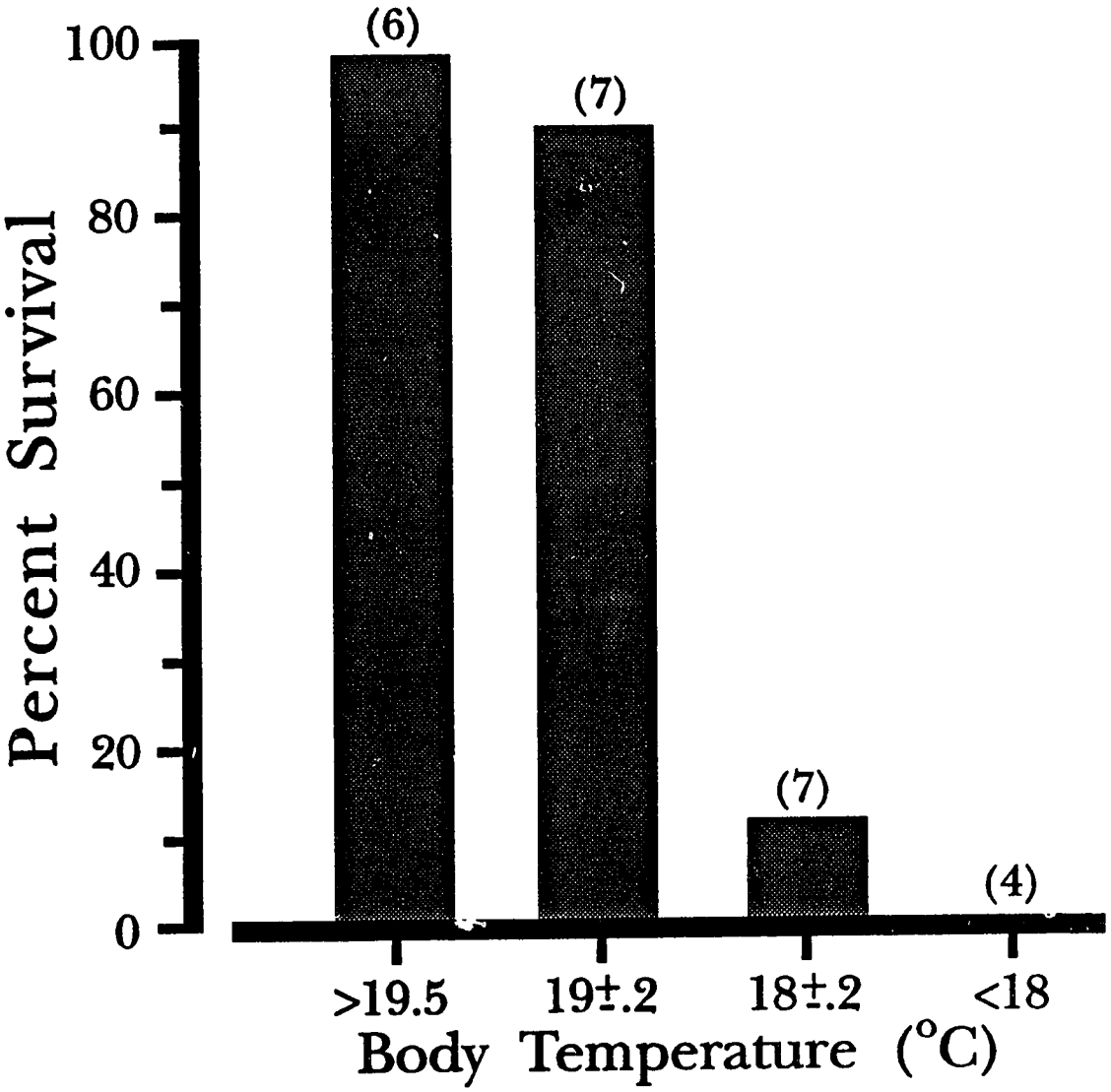

Tb (°C)
5
10
15
20
25
30
35
TIME (HR)
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Heart Rate (BPM)
100
200
300
Bout #1
Tb1
HR1
Bout #2
Tb2
HR2

Figure I.5: Percent survival of rats maintained 24 h in hypothermia at different body temperatures.


100
80
60
40
20
0
Percent Survival
(6)
(7)
(7)
(4)
>19.5
19±.2
18±.2
<18
Body Temperature (°C)

survival in hypothermia, and the comparative aspects of survival in hibernation vs hypothermia for example, require hypothermic durations longer than were previously attainable using existing techniques. Our model was developed to meet this need; it provides precise control of T_b during hypothermia and allows for survival for a maximum length of time in both rats and ground squirrels. To our knowledge, the 24 h survival at 19°C for rats and 72 h for squirrels at 7°C are the longest reported to date for hypothermia without any supportive measures (i.e. infusion of substrate, metabolic stimulators, buffer solutions, etc). The present results confirm the beneficial contribution of halothane to hypothermia induction as it allows for a rapid, reproducible reduction in T_b, and produces an energy sparing effect. These results also demonstrate the advantage of precise T_b control in hypothermia since differences in T_b of ± 0.5°C make a significant difference in survival (Fig. I-5). Further, earlier attempts at inducing hypothermia in ground squirrels which were unsuccessful due to the squirrels' high thermogenic capabilities for spontaneous rewarming from very low T_b (10) have also been overcome through the use of computerized negative feed-back control over T_b. Repeated exposure to hypothermia does not appear to improve the animal's tolerance or response to subsequent hypothermia, indicating that exposure to low T_b does not evoke an acclimatory response.

Since animals can be maintained for relatively long time at very stable T_b's, this hypothermia model is a useful tool for comparative studies of hibernation and hypothermia where T_b-dependent changes vs. T_b-independent changes can be separated and evaluated, ie. changes in some physiological function noted during hibernation but not during hypothermia in the same species at the same T_b would suggest that the change is T_b-independent and specific to hibernation rather than to the low T_b. Further, the use of a computerized feed-back loop to control T_b via a circulating water bath provides a measure of metabolic resistance to hypothermia. This measure, the difference between T_b and T_a,

indicates that there is a time-dependent decrease in metabolic resistance as hypothermia progresses. The approach of a null temperature difference is generally coincident with the death of the animal. Therefore, using the time-dependent change in (T_b-T_a), this model is also useful in elucidating the limiting factors on metabolic heat production during hypothermia and the effectiveness of various remedial pharmacological treatments which may enhance metabolism and improve long term survival in hypothermia.

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II. PHYSIOLOGICAL CHANGES IN CATIONS AND WATER BALANCE DURING STABLE LONG TERM HYPOTHERMIA IN RATS. [2]

INTRODUCTION

Hypothermia, a state of abnormally low body temperature, occurs, in mammals, when the rate of heat loss exceeds thermogenic capacity. Once hypothermic an animal's most pressing problem is the maintenance of homeostasis. To date, there is no consensus as to what physiological changes occur during prolonged severe hypothermia or on the quantitative importance of the various observed changes to hypothermic survival.

Diverse approaches to the study of hypothermia have often generated contradictory and inconclusive results with respect to changes in plasma sodium (Na^+), potassium (K^+) and calcium (Ca^{++}) concentrations. Because of the relatively large quantities of these ions, especially Na^+ and K^+, they are considered an important factor influencing the distribution of body water. Further, they can have profound effects on membrane excitability (K^+) and enzyme activity (K^+, Ca^{++}). Observations of general tissue edema tend to support reports of loss of ion regulation in hypothermia. Indeed, Brendel (16) suggests that cold-induced swelling of the brain is one of the primary factors limiting survival in hypothermia. However, reported changes in plasma cations during hypothermia include increases (18), decreases (2,3), or no change (5,11) for Na^+, K^+ and Ca^{++}. The disparity of results may be due to the methods used for cooling as discussed by Swan (19) or possibly to the timing of

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2. A version of this chapter has been published. Jourdan, Michael L., Debbie L. McAllister and L.C.H. Wang. 1989. In "Thermoregulation: Research and Clinical Applications", P. Lomax and E. Schonbaum eds., pp.157-161.

observations. For example, observations made during the cooling or warming phases of hypothermia most likely reflect the perturbation of the system by changing temperature. However, observations during the stable phase of hypothermia should reflect the regulatory activity, or lack of activity, at that temperature.

Recently, we have developed a system (10, Chap.I) in which rats can successfully recover from deep hypothermia after being maintained at a stable low T_b (19.0°C) for 24h. This has enabled us to re-evaluate the effects of hypothermia on metabolic homeostasis over time. We therefore, investigated cation concentrations and the occurrence of tissue edema in hypothermia as a function of time in hypothermia.

METHODS

Male Sprague-Dawley rats were housed individually under 12L:12D photoperiod at 23°C. The rats were fed a ration, designed to maintain weight constant at 400g, and provided with water ad libitum.

Animals were subdivided into two groups: 1) Euthermic: cannulated rats which had fully recovered from anaesthesia and were free-moving were maintained at a T_a of 22°C without food, for 24 h; and 2) Hypothermic: cannulated rats which were made hypothermic and maintained at a T_b of 19°C for 20-24 h. Hypothermia was induced by the method of Jourdan and Wang (10, Chap.I). Briefly, animals were anaesthetized with halothane (5%) in Helox (21% O_2; 79% He: HeO_2) at 1.5 litres/min. Rectal temperature (T_b) was measured with a thermocouple (Copper-Constantan) positioned 5-7cm deep and secured to the tail with tape. ECG was measured with electrodes attached to the left fore-leg and right hind-leg. The animal was then cooled in a water-jacketed plexiglas chamber at 0°C under halothane (1.5%) and Helox. During the initial rapid cooling halothane was decreased by approximately 0.5%/3°C

drop in T_b. When T_b reached 28°C, halothane was discontinued and cooling was continued under Helox to the desired T_b (19°C). Stabilization at 19°C from the onset of cooling generally required 60 min. Hypothermia was maintained via a feedback loop using T_b as the reference for the adjustment of the circulating water bath temperature.

Blood sampling was via an arterial cannula (Micro-renathane, .040" O.D X .025" I.D., Braintree Scientific, Inc.) implanted 3 cm into the left carotid artery under halothane anaesthesia just prior to hypothermia induction. Blood samples (0.5ml with replacement) were taken immediately (initial) after surgery and at intervals over the hypothermia period. An aliquot was used for haematocrit determination and the rest centrifuged at 10,000 RPM's for 3 min at 2°C the plasma was then frozen (-70°C) for later analysis of Na^+ and K^+ (Flame Photometry) and Ca^{++} (Atomic Absorption Spectrophotometry).

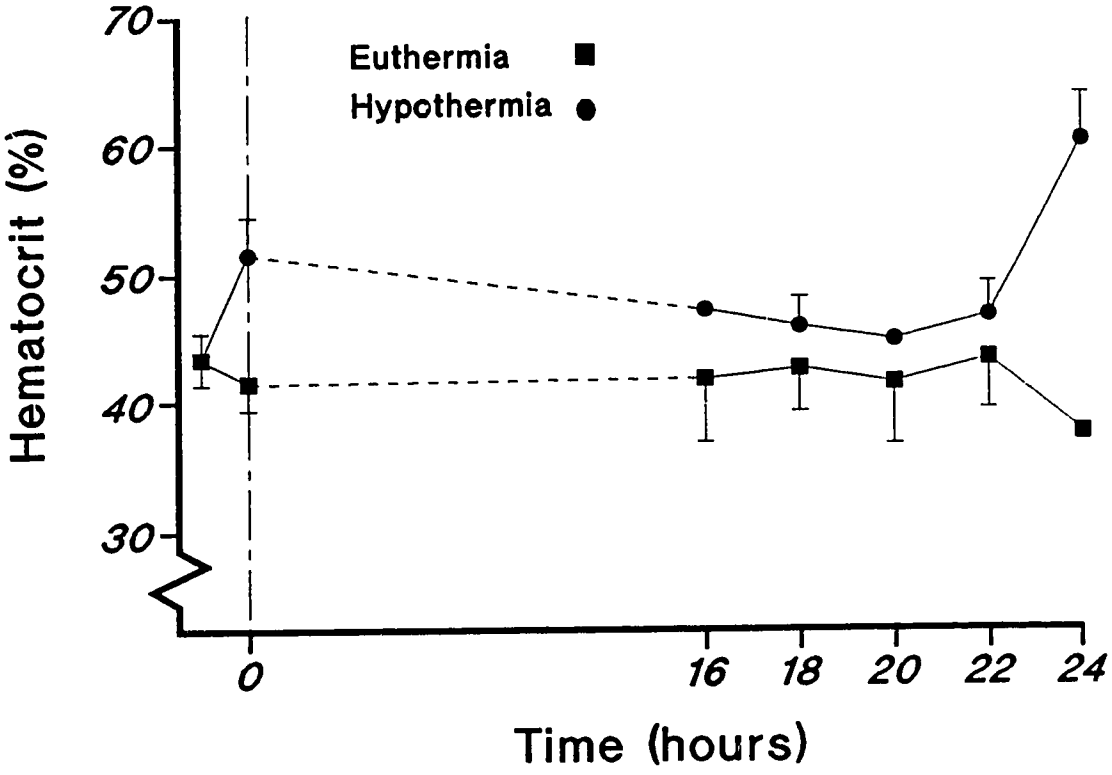
Tissue samples for evaluation of tissue edema were collected following decapitation of animals which had spent 2, 6, 16, 20 and 24 hours in hypothermia at 19°C. Tissues were minced, weighed, and placed in a drying oven at 100°C for 48h. Following drying the samples were placed in a vacuum desiccator to allow cooling before weighing.

Statistical analysis included analysis of variance for time-dependent changes and unpaired t-tests for differences between times for each variable. Analysis was done on a IBM-PC using a statistical graphics package (Statgraphics, Statistical Graphics Corp. or SPSS-PC, SPSS INC.).

RESULTS

In euthermic animals, haematocrit was relatively constant at 42% over the entire sampling period (24 h)(Fig. II.1). In hypothermia animals haematocrit values increased from 42% to 51.5% immediately after T_b reached 19°C. By 16

Figure II.1: Changes in hematocrit ratio of euthermic (T_b=37°C) and hypothermic (T_b=19°C) rats with time. Means±sem; N=5 for all times except 24 h.


70
60
50
40
30
Hematocrit (%)
Euthermia
Hypothermia
0
16
18
20
22
24
Time (hours)

hours it had returned to initial levels and remained relatively constant to about 22-23hrs; after that, it increased sharply to 60% (Fig. II.1). Both hypothermic and euthermic Na^+ levels were relatively constant throughout the sampling period, no significant difference was observed between them at any time. The final Na^+ level for hypothermic animals however, was significantly lower than the initial level (136.1±2.15 vs 141.1±1.0 mmol/l: Fig. II.2). Hypothermic and euthermic K^+ levels were again very stable. Hypothermic K^+ however, was significantly lower than both the initial values and euthermic values at 2 hours and remained significantly lower throughout the hypothermic period (Fig. II.2). Hypothermic but not euthermic Ca^{++} levels fluctuated significantly about the initial level. At 6h the Ca^{++} level was 6.26±0.69 mg/dl (1.35 mg/dl lower than the initial levels) and at 10h it was 8.95±0.77 mg/dl (1.34 mg/dl higher than the initial level) however, by 15h hypothermic Ca^{++} was not significantly different from the initial level (Fig. II.2).

The water content of euthermic kidney, heart, lung and brain was about 76%, while that for liver averaged slightly lower at about 70%. Prolonged exposure to hypothermia at 19°C had a significant effect on the water content of all tissues examined except liver. The tendency in all tissues is toward water loss after 6h in hypothermia. Kidney water content increased significantly by 2h and then decreased relatively steadily; at 24h of hypothermia, there was no significant difference between it and the euthermic control. All other tissues either decreased in water content (brain) or didn't significantly change in water content at 2h (Fig. II.3).

DISCUSSION

Popovic (15,17) observed a continuous increase in haematocrit ratio from 42 to 70 % in rats cooled to 15°C. Our haematocrit ratio increased significantly as well initially however, it then returned to control level and remained

Figure II.2: Plasma concentrations of Na^+, K^+ and Ca^{++} in euthermic (T_b=37°C) and hypothermic (T_b=19°C) rats. Means±sem; N=8.

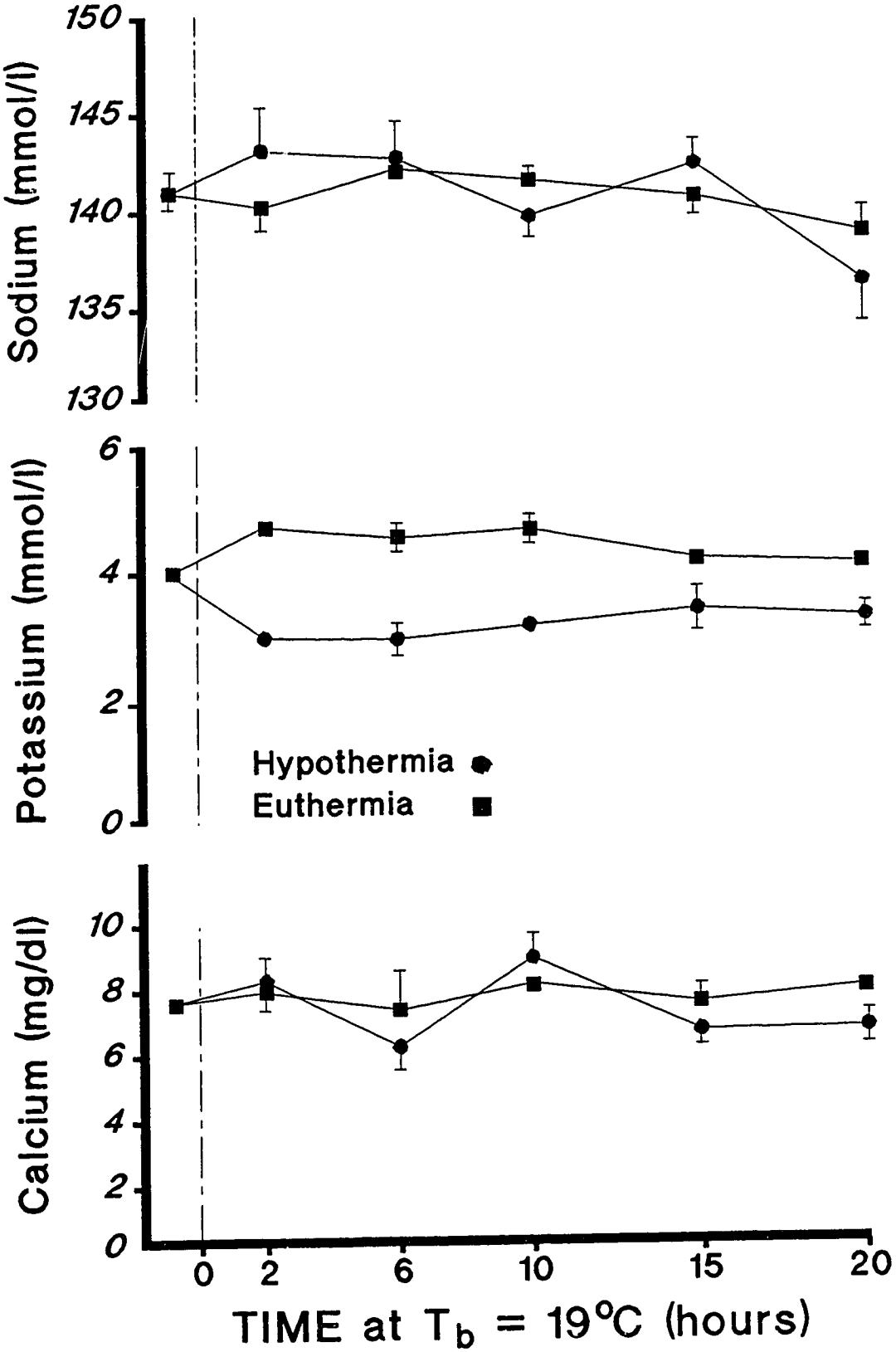
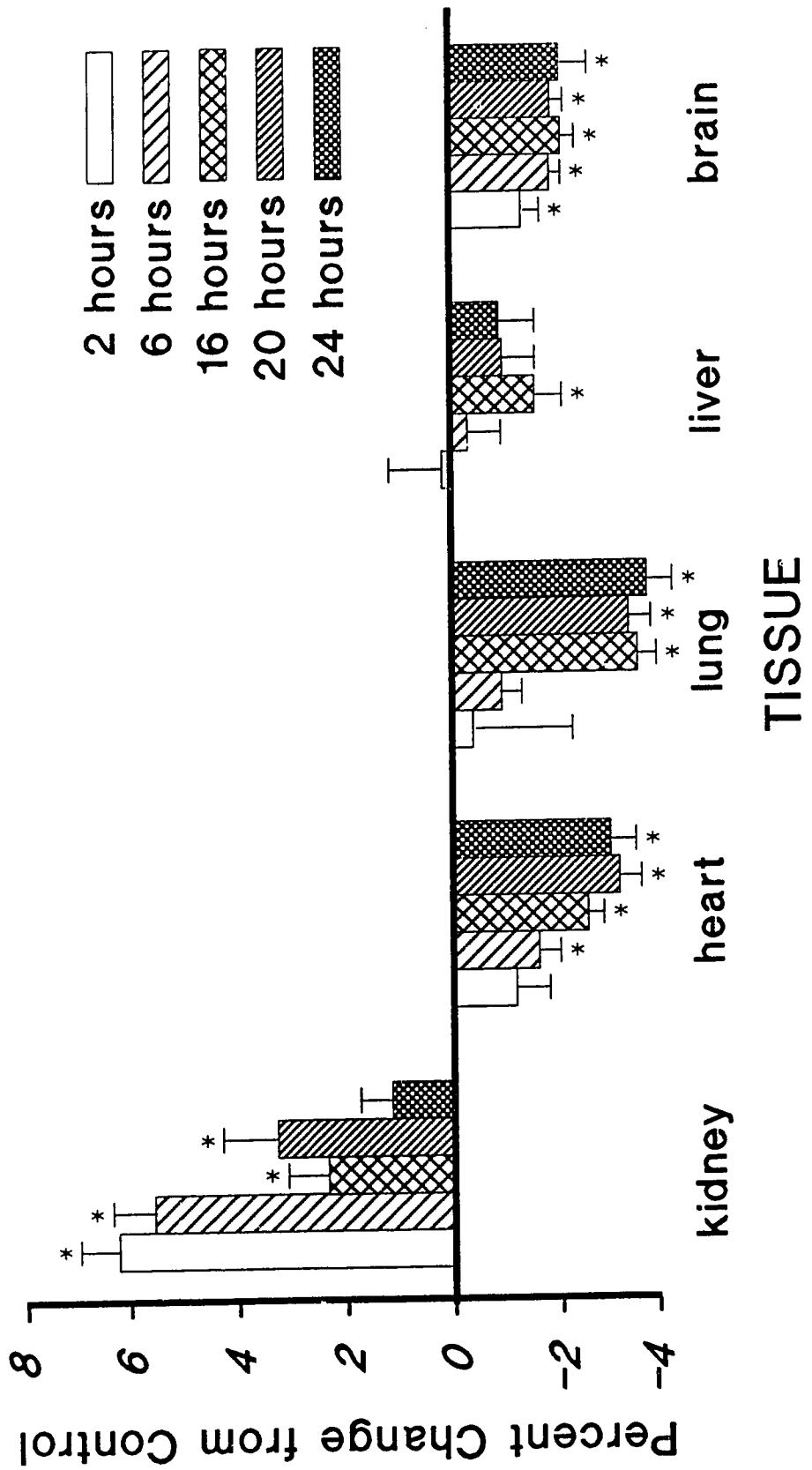

Sodium (mmol/l)
150
145
140
135
130
Potassium (mmol/l)
6
4
2
0
Hypothermia
Euthermia
Calcium (mg/dl)
10
8
6
4
2
0
0
2
6
10
15
20
TIME at T_b = 19°C (hours)

Figure II.3: Tissue water content of hypothermic (T_b-19°C) rats at different times after hypothermic induction. Means±sem; N=8. Asterisks (*) signify significant differences from euthermic control (0%).


2 hours
6 hours
16 hours
20 hours
24 hours
Percent Change from Control
8
6
4
2
0
-2
-4
kidney
heart
lung
liver
brain
TISSUE

relatively constant until late in the hypothermic bout. Since haemoconcentration has also been observed in normothermic cold exposed animals (1), this suggests that the initial rise seen in our rats may be a typical cold shock response. Alternatively, the increase in haematocrit could be due to a decrease in plasma water content, a possibility which is suggested by the loss of water in other tissues examined (Fig. II.3) and by the diuresis observed throughout the hypothermic period (0.55±0.2ml/h at 2h). The rather dramatic increase in haematocrit to 60% by 24h comes at a time when remedial measures aimed at reviving the animal are relatively ineffective, and may be one manifestation of a general failure of the regulatory system in prolonged hypothermia (4,13,14).

The maintenance of regulatory control in hypothermia is evidenced by the regulation of the plasma K^+. There was an early and significant decrease in K^+. However, following this initial change, the K^+ level was maintained relatively constant for the duration of the hypothermic bout. Since glucose administration tends to decrease K^+ levels in hypothermic animals (16), the initial decrease in K^+ may be secondary to the observed increase in plasma glucose in hypothermic rats (9,8,7). Further, this decrease may have the beneficial effect of reducing the tendency for ventricular fibrillation in hypothermia (16) by reducing the excitability of the cardiac membranes. No significant difference was noted in the trend of Na^+ or Ca^{++} in hypothermia as compared to euthermic animals, indicating again that ionic regulatory functions are operable even at reduced T_b. The increase in fluctuation over time of Ca^{++} suggests some instability in the control of Ca^{++} as compared to euthermic animals; however, by 15h in hypothermia stability was reestablished.

On the surface the observed decrease in water content of the tissues examined is somewhat contradictory to reports of edema in hypothermic animals. However, since we did not examine all tissues it is possible that water

we observed leaving one tissue was accumulated in another. For example, at 2h the kidney's water content had increased by 6% whereas that of the heart and brain had decrease by 1-2% over the same time period. Further, evidence of this redistribution of water has been noted in the gastrointestinal tract, which normally excludes water, but tends to accumulate fluid in the hypothermic animal (unpublished observation). The reason for this redistribution of water is not well understood at this time but may be related to differential changes in membrane permeability in different tissues (6,12). If this is the case then there could be extracellular dehydration as a result of intracellular hydration with no apparent overall net change in total tissue water content. Evidence supporting cell hydration in hypothermia is seen in the red blood cell whose fragility increased during hypothermia and that the observed increase in haematocrit during hypothermia is not due to increased red cell numbers (unpublished observation).

Taken together, these data indicate that hypothermia is not the uncontrolled state typically depicted in the literature. Under our protocol, which maintains a constant stable T_b ($\pm 0.3^\circ C$) for long periods of time, the plasma concentration of key ionic species and water balance appear to be quite stable and well regulated. This indicates that the hypothermic rat does not suffer irreparable damage to its homeostatic mechanisms governing ion regulation up to about 20-22 h. However, based on our indirect measure of metabolic resistance, i.e. the difference between T_b and T_a, there is a progressive weakening in the hypothermic animal. The approach of a null temperature difference between T_b and T_a is generally coincident with the loss of regulatory control and the death of the animal. Why metabolic resistance deteriorates with time in hypothermia is currently unknown, but it must be closely coupled with energy metabolism in hypothermia. It therefore, appears that an understanding of the substrate profile and its regulation in hypothermia is a required first step in understanding energy metabolism in hypothermia.

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III. GLUCOSE AND FREE FATTY ACID HOMEOSTASIS: THE ROLE OF INSULIN IN HYPOTHERMIA [3]

INTRODUCTION

Hypothermia is a state of sub-normal body temperature (Tb) which occurs when heat loss exceeds heat production. Unlike hibernating animals, which can regain euthermia spontaneously, hypothermic animals require the support of exogenous heat to restore euthermia. If external heat is not received in time, death is inevitable.

The limitation to survival during deep hypothermia is unknown, but is likely to involve many factors. One of these may be the impairment of substrate mobilization and utilization under depressed T_b. For instance, glucose intolerance has been observed during accidental hypothermia in humans (24) and in experimental hypothermia of mammals (4). This intolerance has been associated with an inhibition of insulin secretion (2, 5, 8, 13, 17) due in part to the direct effect of low temperatures on the pancreas (9, 14, 20) and to alpha-adrenergic hyperactivity (3). However, most of the observations of inhibited insulin secretion by hypothermia have been obtained using either in vitro measurements alone or in animals submitted to moderate or short term hypothermia. Lacking is the resolution of time-dependent changes which might affect substrate mobilization and utilization in prolonged hypothermia. Without this demonstration, the role of substrate metabolism in limiting survival in hypothermia is difficult to evaluate.

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3. A version of this chapter has been published. Hoo-Paris, R., Michael L. Jourdan, Lawrence C.H. Wang and Raymond Rajotte. 1988. Am. J. Physiol. 255 (Regulatory Integrative Comp. Physiol. 24): R1035-R1040.

Recently, an improved method for inducing hypothermia and prolonging survival in hypothermia has been developed in our laboratory for several species (16). By transiently depressing heat production with a low level of halothane anaesthesia and increasing heat loss by exposure to cold and helium oxygen (79% He, 21% O_2) (1, 19, 21, 27, 29), an animal's Tb can be quickly lowered to the desired level. Once in hypothermia, the animal's Tb can be maintained by computerized auto-feedback regulation of ambient temperature (water bath temperature; Ta) while exposed to pre-cooled normal air. Using this technique, rats survive a Tb of 19°C for 20-30 hours (16 and unpublished observations M.L. Jourdan). When T_a is increased all animals can fully recover from hypothermia and suffer no apparent adverse effects (16).

Because of the capacity for recovery inherent in this hypothermia model it appears to be ideally suited for the investigation of factors which limit survival in hypothermia. The present study was undertaken with two major objectives: (1) to examine time-dependent changes in substrate profiles and their hormonal regulation in hypothermia with particular emphasis on insulin concentration and tissue sensitivity to insulin; and (2) to test the hypothesis that the suppression of insulin secretion during hypothermia is a limiting factor for survival in prolonged hypothermia.

METHODS

Male Sprague Dawley rats (300g-400g) were housed individually at 22°C with food and water ad libitum. All experiments utilizing these animals followed the "Guiding Principles in the Care and Use of Animals" published by the Canadian Council on Animal Care and were approved by the University of Alberta animal use committee. The left jugular vein was cannulated under halothane anaesthesia (1.5%). Animals were subdivided into two groups: 1) Euthermic: cannulated rats which had fully recovered from anaesthesia and were free-moving were maintained at a T_a of 22°C without food, for 24 h; and 2) Hypothermic: cannulated rats which

were made hypothermic and maintained at a T_b of 19°C for 20-24 h; hypothermia was induced immediately following cannulation, by the method of Jourdan and Wang (16). Briefly, a rectal thermocouple (copper-constantan) was inserted 5cm beyond the anus and electrocardiographic electrodes were attached. The animal was then transferred from the surgical board to a water-jacketed plexiglas chamber at 0°C under a HeO_2 (79% He, 21% O_2) atmosphere containing 1.5% halothane in an open flow system. The concentration of halothane was progressively reduced by about 0.1% for every 1°C drop in T_b and discontinued when T_b reached 28°C. When T_b reached 23°C, the HeO_2 was replaced by pre-cooled normal air and Ta was increased in order to slowly cool the animal to 19°C. To maintain T_b at 19°C, Ta was continuously adjusted via a computer algorithm which used rectal temperature and a reference temperature (19°C in the present study) to alter the temperature of the circulating water bath which perfused the animal chamber.

Blood samples were taken, from the jugular vein, at intervals (-1, 0, 2, 6, 10, 15 and 20 h) over the experimental period for glucose, free fatty acids (FFA) and insulin assays. The amount of each blood sample was never more than 0.4 ml which resulted in all cases to less than a 10% decrease in estimated total blood volume (8% of body weight or 24-32 ml) over 20 h. Blood was rapidly centrifuged at 5°C and the plasma frozen at -70°C until assayed.

Insulin (.1, .5 and 1 U/kg) was injected i.v. 2 or 16 h after the start of hypothermia induction. By 2 h the animal's T_b had stabilized at 19°C and varied less than 0.2°C. In the euthermic animals injections were made 2 h after surgery. In order to track the effect of insulin injection on glucose, FFA and insulin concentrations, blood samples (0.4 ml) were taken at 0, 10, 20 and 40 min. or 0, 60, 120 and 240 min after injection, from the euthermic and hypothermic animals, respectively. Blood was rapidly centrifuged at 5°C and the plasma frozen at -70°C until assayed for glucose, FFA and insulin. Control animals were injected i.v. with saline (NaCl, 0.9%; 1ml/kg).

To test the effect of insulin on survival in prolonged hypothermia, a separate group of rats was injected i.p. with either saline (as above) or insulin (.1 and 1 U/kg). Injections were done 2 h after the start of hypothermia and the animals were then not disturbed for the duration of hypothermia survival (up to 30 h).

Blood glucose was measured in 20 µl of plasma using the glucose-oxidase method (Sigma Kit #510). Free fatty acids were measured by the colorimetric method (11) using a Technicon Auto-Analyzer. Insulin was measured by radio-immuno-assay (RIA: Pharmacia, Insulin RIA100, Uppsala, Sweden) using a double antibody, guinea pig human insulin anti-serum, and rat insulin as the standard.

Pancreatic islets were isolated from euthermic rats by collagenase digestion (18). Rats were decapitated without anaesthesia in order to minimize stress-related sympathetic activation, since sympathetic inhibition of insulin secretion in isolated pancreas is well known (7). Fifty islet cells were perifused in a perifusion chamber (volume: 0.5 ml) at a flow rate of 1 ml/min. The perifusion solution was Krebs-Ringer bicarbonate, pH 7.4, supplemented with 0.5% (w/v) dialysed albumin, gassed with O_2-CO_2 (95-5%), and containing glucose (150 mg/dl or 400 mg/dl). The perifusate was periodically collected and kept at -20°C until assayed for insulin. The influence of cooling on insulin release was investigated using islets perifused at decreasing temperatures from 37 to 19°C. The rate of temperature decrease was 1°C every 3 min in order to approximate the rate of T_b decline in the in vivo experiment.

All results are expressed as means ± standard error of the mean. Statistical evaluation was performed using One-Way Analysis of Variance followed by post-hoc analysis using Student-Newman-Keuls (SNK) and Student's t-test for paired or unpaired values wherever appropriate.

RESULTS

The decrease of T_b was rapid and steady; in about 60 min the T_b approached 20°C and stabilized at 19°C (Fig. III.1). During the first few hours of hypothermia, the Ta required to maintain T_b at 19°C was about 14°C, it then gradually increased until it was only 1-2°C less than the T_b at the end of the hypothermic period.

Immediately following cannulation, plasma insulin of euthermic rats (control) was 100±32 μU/ml, it decreased to 35±9 μU/ml ($p<.05$ versus the preceding value) 2 h later, this relatively high initial value and the subsequent significant decrease are probably the result of halothane exposure during the cannulation period. The insulin concentration decreased steadily after 2 h to 15 μU/ml at the end of the fasting period of 20 hours (Fig. III.1). Plasma glucose and free fatty acids were 190±8 mg/dl and 814±105 μEq/l, respectively, immediately after cannulation and decreased slightly during the 20 hour period, reaching 154±11 mg/dl and 634±57 μEq/l, respectively at the end (Fig. III.1).

Experimental rats were slightly hypothermic (35°C), following cannulation, due to the combined effect of halothane anaesthesia and the HeO_2 used in preparation for hypothermia. At this T_b, plasma insulin concentration was 22±5 μU/ml; it decreased to 9±2 μU/ml 2 hours later ($p <.05$ versus the preceding value) at T_b=19°C and remained low until the end of the 20 hour hypothermia period. Insulin concentrations measured at 10, 15 and 20 hours in hypothermic animals (T_b=19°C) were not statistically different from the corresponding values measured in euthermic rats. Plasma glucose concentration was 178 mg/dl at the beginning of hypothermia (T_b=35°C) then increased dramatically reaching very high values (304±49 mg/dl, range 117 and 593 mg/dl) by the 15th hour. All the values measured in hypothermic rats (T_b=19°C) were significantly higher than corresponding values in euthermic animals ($p<.05$). Free fatty acid concentrations in hypothermic rats were not significantly different from the concentrations in euthermic animals ($p >.05$). However, at the end of the hypothermic period, the

Figure III.1: Plasma insulin, glucose and FFA concentrations in euthermic (●) and in hypothermic rats (■). * $p < .05$ between groups; the number of animals in each group was 6. Time -1 indicates sample taken immediately following cannulation, time 0 is when the hypothermic animals reached 19°C (approximately 1 h after cannulation) all other times indicate time in hypothermia.

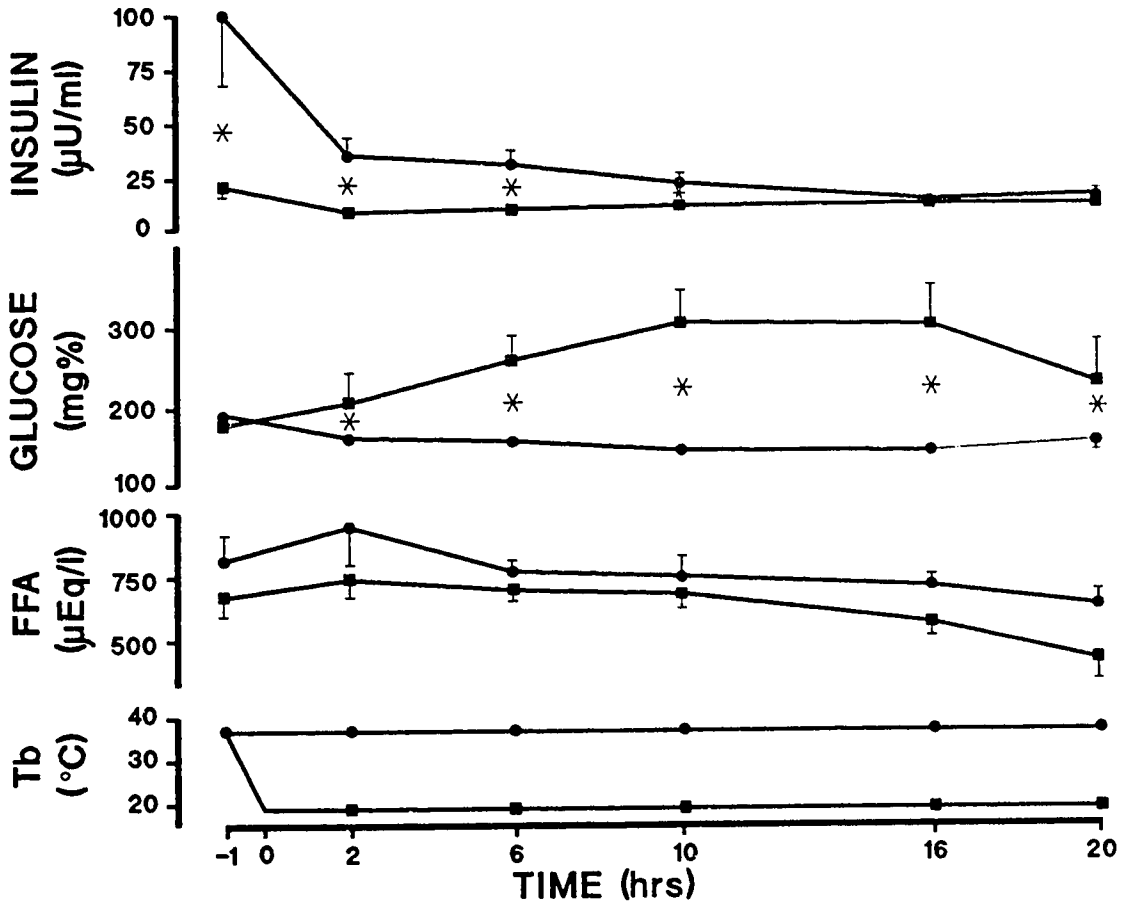
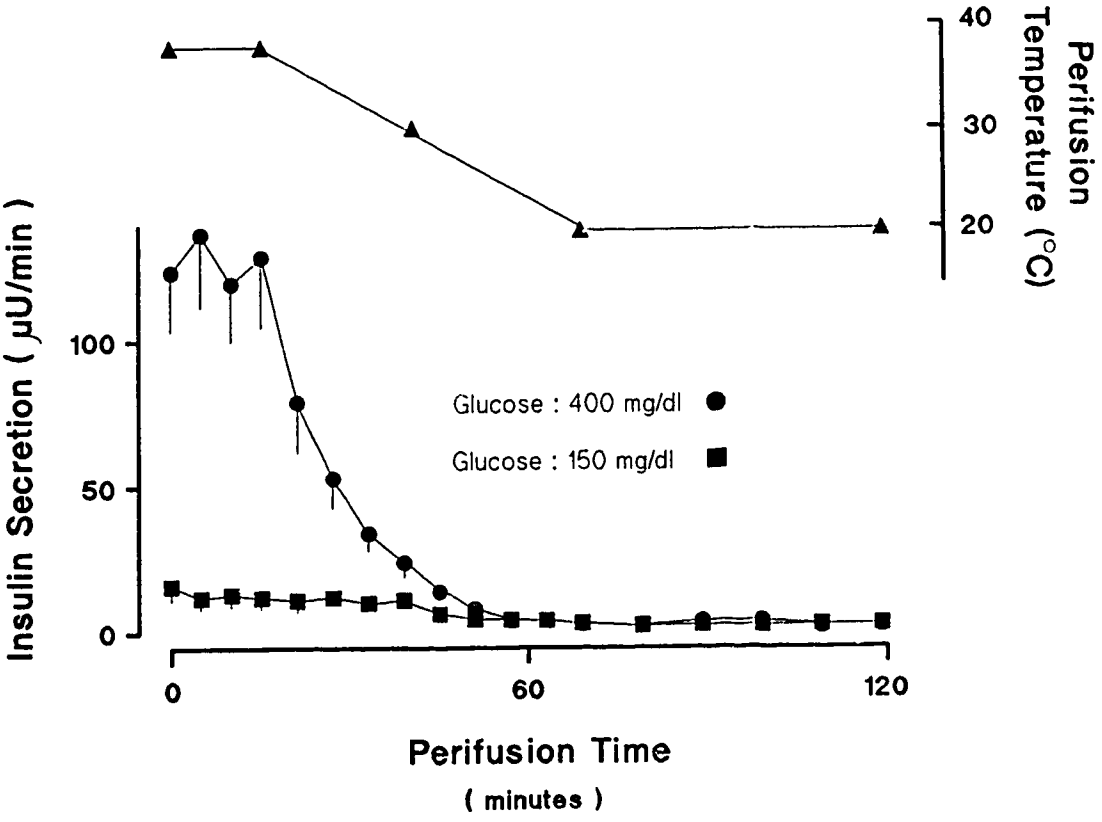

INSULIN (μU/ml)
100
75
50
25
0
GLUCOSE (mg%)
300
200
100
FFA (μEq/l)
1000
750
500
Tb (°C)
40
30
20
-1
0
2
6
10
16
20
TIME (hrs)

Figure III.2: Insulin secretion from 50 rat islets of Langerhans during the in vitro cooling from 37 to 19°C. The perifusion medium contained either 150 (n=7) or 400 mg/dl (n=8) of glucose.


Insulin Secretion (μU/min)
100
50
0
Glucose : 400 mg/dl
Glucose : 150 mg/dl
0
60
120
Perifusion Time
(minutes)
40
30
20
Perifusion
Temperature (°C)

decrease in FFA concentration was more pronounced (62% from the initial concentration) than in euthermic animals (78%).

In islets isolated from euthermic rats and perifused with a medium containing 150 mg/dl glucose for 30 min at 37°C, insulin secretion plateaued at about 12 μU/ml (Fig. III.2). When the temperature of the perifusion chamber was linearly decreased from 37 to 19°C, insulin secretion decreased steadily to 3±0.4 μU/ml at 19°C and remained near this value for the remaining 60 min (Fig. III.2). With medium containing 400 mg/dl glucose, insulin secretion was approximately 130 μU/ml at 37°C; it decreased sharply during cooling, reaching 2.7±.6 μU/ml at 19°C and remained at this value during the ensuing 60 min (Fig. III.2). These results indicate that at 19°C insulin secretion induced by glucose was inhibited completely.

Injection of insulin (.1 U/kg, i.v.) in euthermic rats resulted in a significant rise in plasma insulin level which returned to the basal values between 10 and 20 min (half life was about 5 min)(Fig. III.3). In the hypothermic animals, insulin injection (.1U/kg, i.v.) induced a 100 fold increase in plasma insulin level which slowly returned to the basal values by 240 min . Following the injection of 1U/kg insulin in the hypothermic rats, plasma insulin showed a corresponding 10 fold further increase beyond that after .1 U/kg (Fig. III.3). The rate of disappearance of insulin following injection was however, similar for the two doses (Fig. III.3).

Injection of insulin (.1 U/kg, i.v.) in euthermic animals induced a transient, significant decrease ($p<.05$) in plasma glucose with a nadir at 10 min (65±4% of the pre-injection level) and a sustained decrease in plasma FFA (Fig. III.4). After 2 hours of hypothermia at 19°C, injection of 0.1 U/kg insulin induced only a slight decrease in plasma glucose (nadir at 120 min, 89±3% of the pre-injection value), indicating a reduction of peripheral sensitivity to insulin in hypothermia (Fig. III.4). Higher doses of insulin 0.5 and 1 U/kg induced marked decreases in plasma

Figure III.3: Plasma insulin after i.v. injection of insulin in euthermic rats (.1 U/kg) and in hypothermic rats (.1 or 1 U/kg). Empty symbols denote: $p < .05$ versus initial value at time 0 using unpaired t-test. The scale of insulin concentrations is logarithmic. n = number of animals.

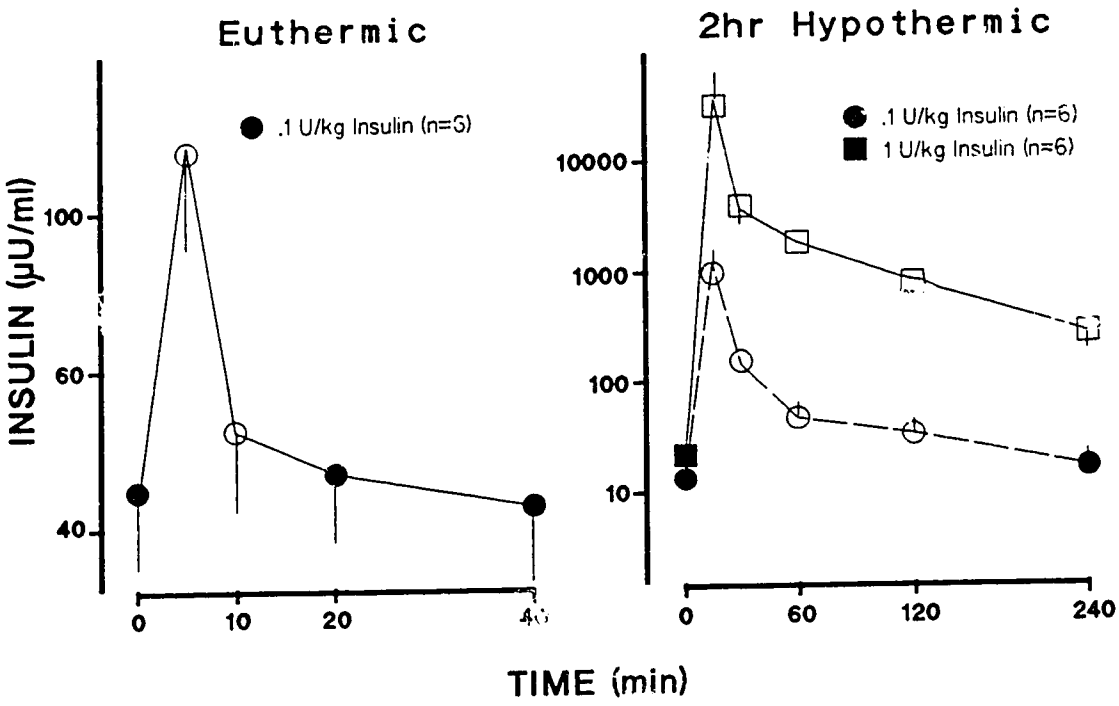

Euthermic
2hr Hypothermic
.1 U/kg Insulin (n=5)
.1 U/kg Insulin (n=6)
1 U/kg Insulin (n=6)
INSULIN (μU/ml)
100
60
40
10000
1000
100
10
0
10
20
0
60
120
240
TIME (min)

Figure III.4: Effect of insulin injections on plasma glucose and FFA concentrations in euthermic rats (.1U/kg of insulin:■) and in hypothermic rats at 2 hours (.1 (■),.5 (◆) and 1 (▲) U/kg of insulin) or 16 hours (1 (▲)U/kg of insulin). Open symbols denote: $p < .05$ versus control (saline;●) values for each condition using unpaired t-test; n= number of animals.

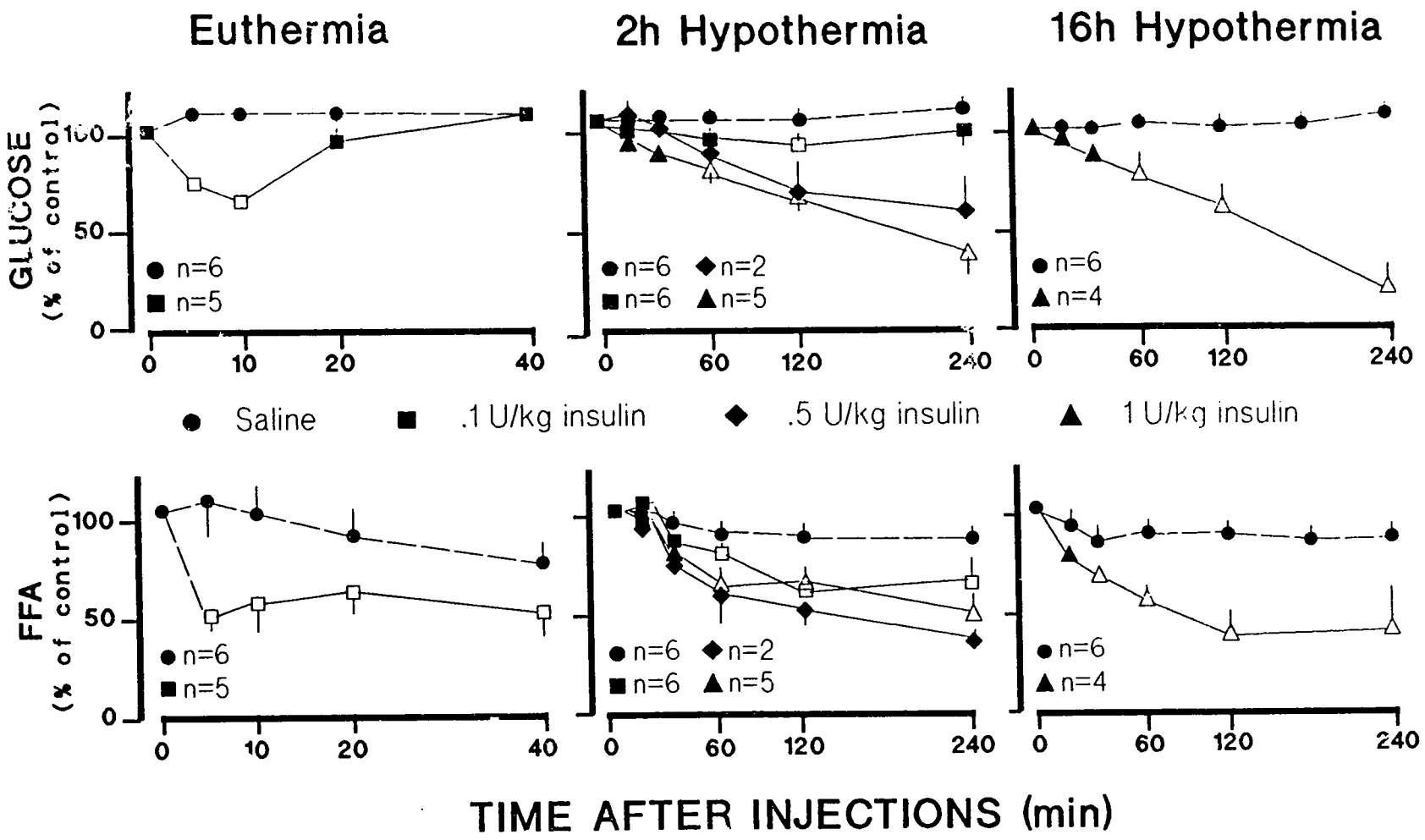
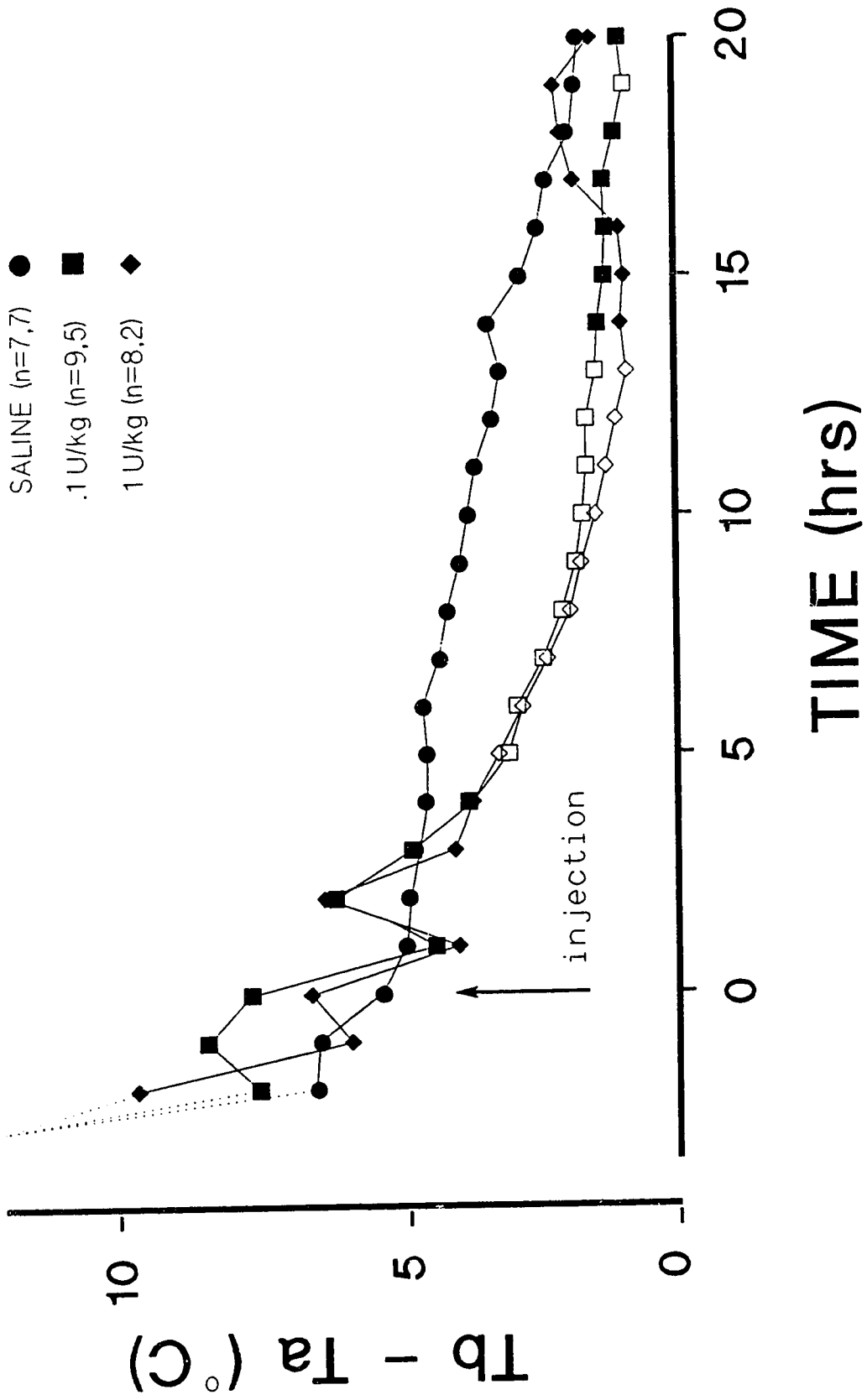

Euthermia
2h Hypothermia
16h Hypothermia
GLUCOSE (% of control)
100
50
0
● n=6
■ n=5
0
10
20
40
● n=6 ◆ n=2
■ n=6 ▲ n=5
0
60
120
240
● n=6
▲ n=4
0
60
120
240
● Saline
■ .1 U/kg insulin
◆ .5 U/kg insulin
▲ 1 U/kg insulin
FFA (% of control)
100
50
0
● n=6
■ n=5
0
10
20
40
● n=6 ◆ n=2
■ n=6 ▲ n=5
0
60
120
240
● n=6
▲ n=4
0
60
120
240
TIME AFTER INJECTIONS (min)

Figure III.5: Effect of intraperitoneal saline (●) or insulin (.1 (■) or 1 (◆) U/kg) on the difference between T_b and T_a required to maintain T_b at 19°C. Empty symbols denote: $p < .05$ versus saline value at the same time using unpaired t-test. (n,n) = number of animals tested, and number of rats surviving at 20 h. Time 0 indicates the time of injection (approximately 2hr after T_b first reached 19°C). Values are means of the hourly means for each animal in the group, standard error bars are omitted for clarity.


SALINE (n=7,7)
.1 U/kg (n=9,5)
1 U/kg (n=8,2)
injection
Tb - Ta (°C)
TIME (hrs)
0
5
10
15
20

glucose (at 240 min, plasma glucose was 59±17% and 39±3%, respectively, of pre-injection values) (Fig. III.4). A significant decrease in the plasma FFA concentration ($p < .05$) was also observed after administration of insulin in hypothermic rats: at 240 min post injection of .1, .5 and 1 U/kg, FFA concentrations were 65±13, 37±7 and 50±9% of the pre-injection values (Fig. III.4). Injection of 1 U/kg insulin at 16 hours of hypothermia, resulted in a similar decrease in plasma glucose and FFA as that observed at 2 hours of hypothermia with the same dose of insulin. Thus there appears to be no further reduction of peripheral sensitivity to insulin despite the prolongation of hypothermia.

Indirect measure of heat production in hypothermic rats as reflected by the level of Ta required to maintain the T_b at 19°C, indicated a transient but non-significant enhancement by insulin (.1 and 1 U/kg; Fig. III.5), which peaked 2 h after injection. This enhancement, however, was short-lived; by 3 h post injection, heat production was diminished, as compared to saline controls and remained significantly ($p < .05$) lower for approximately 10 h. After about 14 h hypothermia, there was no significant difference in heat production between the saline controls and the insulin treated rats (Fig. III.5). Treatment with insulin at hour 2 of hypothermia, however, had an apparent negative effect on survival in the hypothermic rats. Only 58 and 25% of the insulin-injected rats (.1 and 1 U/kg respectively: Table 1) survived to 20 h whereas 100% of the saline controls survived at least 20 h of hypothermia.

DISCUSSION

Our improved technique for the induction and maintenance of hypothermia allowed the study of time-dependent changes of physiological variables which may limit survival at depressed T_b. The use of halothane during the initial cooling phase offers the advantage of suppressing shivering thermogenesis, resulting in

Table III.1 Survival of rats at time after intraperitoneal injection of saline or insulin.

	Time in Hypothermia (h)			
Treatment	0	8	16	20
Saline	7	7	7	7
Insulin				
0.1U/kg	9	9	7	5
1.0U/kg	8	8	4	2

Time 0 represents time of injection, and the number at Time 0 indicates the total number of rats tested. With the progression of hypothermia (i.e., time = 8, 16 & 20) equals the number of rats still surviving at that time.

shorter induction time for hypothermia than that generally observed without anaesthesia. Since it has previously been demonstrated that the induction time is inversely correlated with the survival time in deep hypothermia (21, 25, 29), perhaps this is one of the main reasons for the particularly long time of survival (24hr) observed with this technique (16). Another possibility may be due to the precision feedback control of T_b in hypothermia, which never varied more than 0.2°C around the predetermined level 19°C.

Evidence that deep hypothermia results in a progressive decrease in heat production is shown in Fig. I.2 (Chap. I). The level of T_a needed to maintain the T_b at 19°C increased throughout the hypothermic period, indicating a diminishing metabolic effort for heat production from the rat. This progressive decrease in metabolic heat production may result from a shortage of fuel, specifically glucose in insulin-dependant tissues. Previous studies have suggested that the availability and the use of energetic stores can be a limiting factor to survival in hypothermia. Indeed, a significant correlation has been found between survival time, plasma glucose concentration and liver glycogen reserve in hypothermic hamsters (25) and infusion of glucose prolongs survival in the hypothermic hamsters (26). Furthermore, glucocorticoid pretreatment which aims to enhance endogenous gluconeogenesis improves muscle tone and the ability of the hypothermic hamster to spontaneously rewarm to euthermia (9). Similar pretreatment, however, does not enhance the capacity for survival or spontaneous rewarming in the hypothermic rat (28). Furthermore, hyperglycaemia rather than hypoglycaemia is found in the hypothermic rat (Fig. III.1) in contrast to that seen in the hamster. It is therefore, unlikely that the progressively reduced heat production in our hypothermic rat is due to hypoglycaemia but it may be related to the transport and utilization of glucose in target tissues.

Another possibility for the decrease in heat production in the hypothermic rat may be the result of reduced cellular oxidation of fuel. This is evident by the

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Another possibility for the decrease in heat production in the hypothermic rat may be the result of reduced cellular oxidation of fuel. This is evident by the

increase in plasma glucose concentration with prolonged hypothermia. Glucose intolerance and insulin resistance have been observed during hypothermia in other mammals (6, 8, 13, 17) and during natural hibernation in the hedgehog (15). Therefore, the hyperglycaemia seen in our hypothermic rats is probably due to the low plasma insulin level found during hypothermia (Fig. III.1). In fact, this seems to be the case. The plasma insulin level was significantly reduced to below 10 μU/ml after two hours in hypothermia (Fig. III.1). This in vivo observation is corroborated by our in vitro demonstration that insulin secretion was significantly inhibited when pancreatic islets were cooled from 37 to 19°C (Fig. III.2). Furthermore, the extended disappearance time of injected insulin shown in Fig. III.3 also suggests a marked inhibition of insulin catabolism in the hypothermic rat. Taken together, it can be argued that at 19°C, the low plasma insulin concentration is due to a strong and perhaps complete inhibition of insulin secretion. This low insulin level may in turn hamper tissue utilization of plasma glucose for heat production.

In addition to the reduced insulin secretion and catabolism, tissues of hypothermic rats were less sensitive to injected insulin since only high doses of insulin induced a delayed but extended decrease in plasma glucose and FFA concentrations (Fig. III.4). This reduced insulin sensitivity, however, was not time dependent, as the sensitivity was not modified after 16 h in hypothermia (Fig. III.4). The delayed response to insulin is probably due to the redistribution of regional blood flow during hypothermia (13) and to the direct effect of cold on insulin-dependent tissues (6, 12). The extended duration of the insulin action is probably the result of its slow catabolism during hypothermia (Fig. III.4). Although a high insulin dose (0.5 U/kg, i.v.) was required to counter the reduced tissue insulin sensitivity due to hypothermia, it did offer the possibility that exogenous insulin could improve the transport and utilization of glucose in insulin-sensitive tissues during hypothermia and this could in turn improve energy utilization in hypothermia. To test this possibility, exogenous insulin was given to

the hypothermic rat at a T_b of 19°C when secretion of endogenous insulin was completely inhibited. Although this treatment transiently enhanced the thermogenic efforts of the hypothermic rat, this was followed by a significant depression of thermogenesis a few hours later (Fig. III.5). It is possible that a transient influx of glucose to metabolizing tissues induced by insulin could cause the initial stimulation. However, since insulin is anti-lipolytic as attested to by the decreased FFA following insulin injection (Fig. III.4), and since lipid utilization in hypothermia has been indicated to be more important than glucose (23), it is possible that the detrimental effects of reduced FFA outweighs the metabolic benefit of an increased glucose flux into the tissues following insulin. Perhaps for this reason, our insulin treatment (Table 1) not only did not produce a sustained beneficial effect for hypothermia survival, but in fact was deleterious. Further experiments involving the stimulation of lipolysis at low T_b in conjunction with the stimulation of glucose utilization should be interesting in regard to whether long term survival in hypothermia may be improved.

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IV. GLUCOSE AND FREE FATTY ACID HOMEOSTASIS: THE ROLE OF GLUCAGON IN HYPOTHERMIA [4]

INTRODUCTION

Accidental hypothermia occurs in homeotherms when the rate of heat loss exceeds the maximum rate of heat production. Survival in hypothermia depends to a large extent, on appropriate distribution of metabolic fuels to vital tissues. A significant positive correlation has been found between survival time and plasma glucose concentration in hypothermic hamsters at a body temperature (T_b) of 5°C (22). In hamsters, both survival and the ability to rewarm from hypothermia are enhanced following either glucose infusion during hypothermia (26) or after pretreatment with glucocorticoids (6). However, comparable treatments in the rat appear to have no beneficial effect (26; Jourdan and Wang, unpublished). Whether this is due to species differences or the ability to hibernate (hamster) is presently unknown.

Death in hypothermia has previously been attributed to failure of cardiac muscle function (12), which preferentially utilizes FFA (2, 4, 18). Since it has been shown in dogs (20) and rats (24) that a sustained increase in plasma glucose concentration inhibits FFA metabolism, it is possible that the ineffectiveness of using glucose to improve hypothermic survival in rats could be due to a disturbed FFA metabolism in hypothermia. Due to its modulation of both glucose and lipid metabolism, glucagon is considered an important hormone for metabolic homeostasis under stress (27). It seems plausible therefore, that changes in

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glucagon secretion and/or tissue sensitivity and responsiveness to glucagon at depressed T_b could lead to inadequate maintenance of metabolic substrate mobilization and utilization during hypothermia.

Recently it was shown that glucagon secretion is increased and normal liver sensitivity to glucagon is maintained in hypothermic rats. These surprising results were obtained in anaesthetized rats at a T_b of 25°C (9) and are in agreement with previous *in vitro* studies which showed that glucagon secretion was independent of temperature in the range of 28-37°C (17). However, no study to date has examined glucagon homeostasis under conditions of either long term (ie. $\geq$ 20h), or severe (ie. at T_b < 20°C) hypothermia.

Recently, an improved method for maintaining prolonged hypothermia and extending survival has been developed (13). It uses the rapid induction method of helium oxygen (HeO_2) plus halothane (13,28) which results in minimal stress to the animal, and produces a stable T_b (19±0.3°C) for up to 24 h during hypothermia, with successful rewarming. This technique provides an ideal model for the study of time-dependent changes in physiological function and for identifying possible limiting factors for survival in hypothermia. Using this technique, we have undertaken to examine substrate profiles and their hormonal regulation at a constant low T_b (19°C) over a 20h period. Of particular interest are the time-dependent changes in secretion and tissue sensitivity to glucagon and the interrelationship between glucagon, glucose and FFA in deep hypothermia.

METHODS

Male Sprague Dawley rats (400g), housed individually at 22°C with water ad libitum, and food rationed to maintain body weight at about 400g, were cannulated in the left jugular vein under halothane anaesthesia (1.5%). The animals were subdivided into two groups: 1) euthermic; cannulated rats

maintained at 22°C ambient temperature (T_a), T_b remained near 37°C, without food for 24 h. 2) hypothermic; cannulated rats rendered hypothermic to a T_b of 19°C by the method of Jourdan and Wang (13). Briefly, hypothermia induction involved placement of a thermocouple (copper-constantan) approximately 5 cm into the rectum and the attachment of electrocardiographic electrodes. The animal was then transferred from the surgical board to a water-jacketed plexiglas chamber at 0°C under a helium-oxygen (HeO_2; 79% helium, 21% oxygen) atmosphere containing 1.5% Halothane in an open flow system. The amount of Halothane, in the HeO_2 atmosphere, was progressively reduced by about 0.1% with every 1°C drop in T_b, and was discontinued when T_b reached 28°C. When T_b reached 23°C, the HeO_2 was replaced by air and T_a was increased in order to slowly cool the animal to 19°C. T_b was maintained at 19°C for 20h by continuously adjusting T_a via a computer algorithm which used the difference between rectal temperature and a reference temperature (19°C in the present study) to adjust the temperature of the water bath which perfused the animal chamber.

All experimental manipulation of the rats followed the "Guiding Principles in the Care and Use of Animals" published by the Canadian Council on Animal Care and were approved by the University of Alberta animal use committee.

Blood samples were taken from the jugular vein at various intervals: 0 (immediately after cannulation), 2, 6, 10, 15 and 20 h over the experimental period. The amount of each blood sample was never more than 0.4 ml, this 2.0 ml total sample volume over the 24 h hypothermic period was less than 10% of the total blood volume of a 400g rat based on 8% of body mass (23). Blood was rapidly centrifuged at 5°C and the plasma frozen at -70°C until assayed for glucose, FFA and glucagon concentrations.

Glucagon at a concentration of 20 µg/ml of saline was injected (20 µg/kg) i.v. 2, 8 or 15 h after the hypothermia induction; by 2 h the animal's T_b had stabilized

at 19°C and varied by less than 0.3°C over the rest of the experimental period. In euthermic animals, injections were made 2 h after surgery when the animal had fully recovered from the halothane and T_b was around 37°C. Following glucagon injection blood samples were taken at 0, 5, 10 and 20 min. in euthermic and 0, 15, 30, 60, 120 and 240 min. in hypothermic rats, respectively.

Blood glucose was measured in 20 µl plasma using the glucose-oxidase method (Sigma Kit 510). Free fatty acids were measured by the colorimetric method (7) using a Technicon Auto-Analyzer. Glucagon was measured by double anti-body RIA (University of Alberta hospital).

The surgical technique for cannulation of the pancreas was as described by Penhos et al. (21). The pancreas was perfused with Krebs-Ringer bicarbonate medium, supplemented with 0.25% bovine crystallised albumin (Sigma) and continuously gassed with a mixture of O_2 and CO_2 (95:5), pH 7.4, with a peristaltic pump at a flow rate of 1 ml/min. Perfusion pressure was recorded and was found to vary between 20 and 40 mm Hg between preparations. The perfusate was periodically collected (1 ml fractions) and kept at -20°C until assayed for glucagon. Two protocols were used: 1) the influence of cooling on glucagon secretion was investigated using pancreas perfused at temperatures decreasing from 37 to 19°C at a rate of 1°C/3 min in order to approximate the rate of T_b decline in the in vivo experiment. 2) the kinetics of arginine- (a potent stimulator of pancreatic α cells) induced glucagon secretion was assessed using pancreas perfused at two constant temperatures (37 and 19°C).

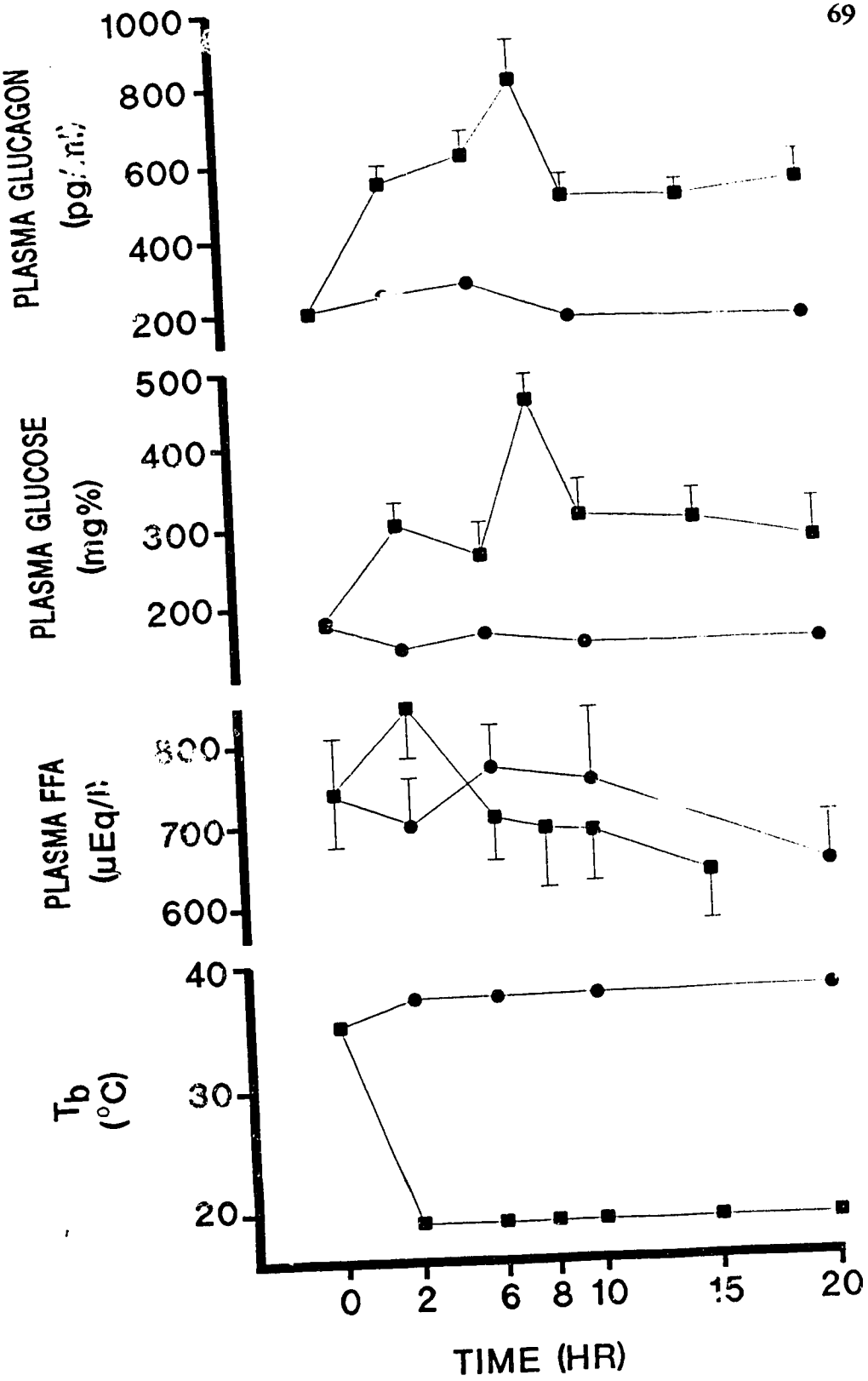
All results were expressed as means ± standard error of the mean. Statistical evaluation was performed using one-way analyses of variance followed by post-hoc analysis using SNK and Student's t-test for paired or unpaired values wherever appropriate.

RESULTS

Following jugular cannulation (time 0), plasma glucagon, glucose and FFA concentrations were 187±18 pg/ml, 183±10 mg% and 739±68 μEq/l (n=13), respectively. In the control group (euthermic rats), plasma glucagon increased during the first 6 h, reaching 269±23 pg/ml then decreased to 152±13 pg/ml by 20 h after cannulation. Plasma glucose decreased to 144±7 mg% by 2 h ($p<.05$ versus the first value) and then remained the same throughout the experimental period (Fig. IV.1). Plasma FFA concentrations did not vary significantly throughout the 20 h period (Fig. IV.1).

Plasma glucagon concentration was high during the entire 20 h period in hypothermic rats: 2 h after the start of hypothermia induction (T_b = 19°C), the glucagon concentration was 536±55 pg/ml ($p<.01$ versus the value at time 0), by 8 h it reached a peak of about 810±103 pg/ml ($p<.05$ versus the preceding level). Glucagon concentration then decreased and remained near 500 pg/ml ($p<.05$ between 8 h and measurements at 10, 15 and 20 h) from 10 h to the end of the experimental period (Fig. IV.1). The levels of plasma glucagon in hypothermic rats were significantly higher ($p < 0.05$) than levels in euthermic rats at all times except time 0. Plasma glucose concentration followed a similar pattern: it was very high at 2 h (304±26 mg%, $p<.01$ versus level measured at time 0), and reached 451±33 mg% ($p<.05$ versus the preceding value) by 8 h, it then decreased and remained near 300 mg% ($p<.05$ between the levels measured at 10, 15 and 20 h and the 8 h value) for the last 10 h of the hypothermic period (Fig. IV.1). Plasma FFA concentration was 844±81 μEq/l at 2 h ($p>.05$ versus the value measured at time 0) it then gradually decreased during the hypothermic period reaching 496±64 μEq/l by 20 h ($p<.05$ versus levels measured at 2 and 6 h) (Fig. IV.1). Plasma FFA levels in the hypothermic rats were only significantly different from euthermic rats at 2 h.

Figure IV.1 : Plasma glucagon, glucose and FFA concentrations in euthermic (●) and in hypothermic rats (■). The number of blood samples per mean is in parenthesis.


PLASMA GLUCAGON
1000
800
600
400
200
PLASMA GLUCOSE
(mg%)
500
400
300
200
PLASMA FFA
700
600
T_b
(°C)
40
30
20
0
2
6
8
10
15
20
TIME (HR)

In hypothermic rats, considering the change in plasma glucagon and glucose concentrations (Fig. IV.1) and the responses to injected glucagon (Fig. IV.3), two periods can be distinguished: the first included the concentrations of plasma glucagon and glucose measured at times 2, 6 and 8 h and the second included those concentrations measured at times 10, 15 and 20 h. During the first period, a positive correlation between plasma glucagon and glucose concentration ($r = .49$, 23 values, $p < .05$, Fig. IV.2a) and a negative but non-significant correlation between plasma glucagon and FFA concentration ($r = -.30$, 24 values, $p > .10$, Fig. IV.2b) was found. During the second period, there was no significant correlation between glucagon and glucose or FFA concentrations ($r = -.02$, 17 values, Fig. IV.2a and $r = -.10$, 17 values, Fig. IV.2b). No statistically significant correlations between glucagon, glucose and FFA concentrations were found in the euthermic animals.

In euthermic rats injection of glucagon (20 µg/Kg, i.v.) 2 h after cannulation (Fig. IV.3a), produced a rise in plasma glucagon concentrations followed by a rapid decrease. The half-life of exogenous glucagon was around 5 min. In hypothermic animals, glucagon injections performed at times 2, 8 or 15 h (Fig. IV.3b, c and d) were followed by a dramatic increase in plasma glucagon concentrations which slowly decreased and stabilized at an elevated level. The half-life estimated between min 30 and 120 following glucagon injection was around 100 min in all 3 cases.

The effect of glucagon upon the concentration of plasma metabolites was calculated as the change post-glucagon injection compared to the baseline value obtained immediately prior to glucagon injection. Glucagon (20 µg/kg, i.v.) in euthermic animals (Fig. IV.3a) induced a transient increase in plasma glucose ($+35\pm7$ mg% and $+36\pm10$ mg% from the pre-injection level at 5 and 10 min respectively), it then returned to the basal concentration by 20 min; the integrated (average) glucose response was 25 ± 8 mg%/min. FFA concentration increased

Figure IV.2: Correlations between (a) plasma glucagon and glucose and (b) glucagon and FFA concentrations during the first period in hypothermia (from 2 to 8 h;●) or during the second period in hypothermia (from 10 to 20 h;○). The only correlation that was significant was between glucagon and glucose during the first period: r=.49, n=23, p<.05.

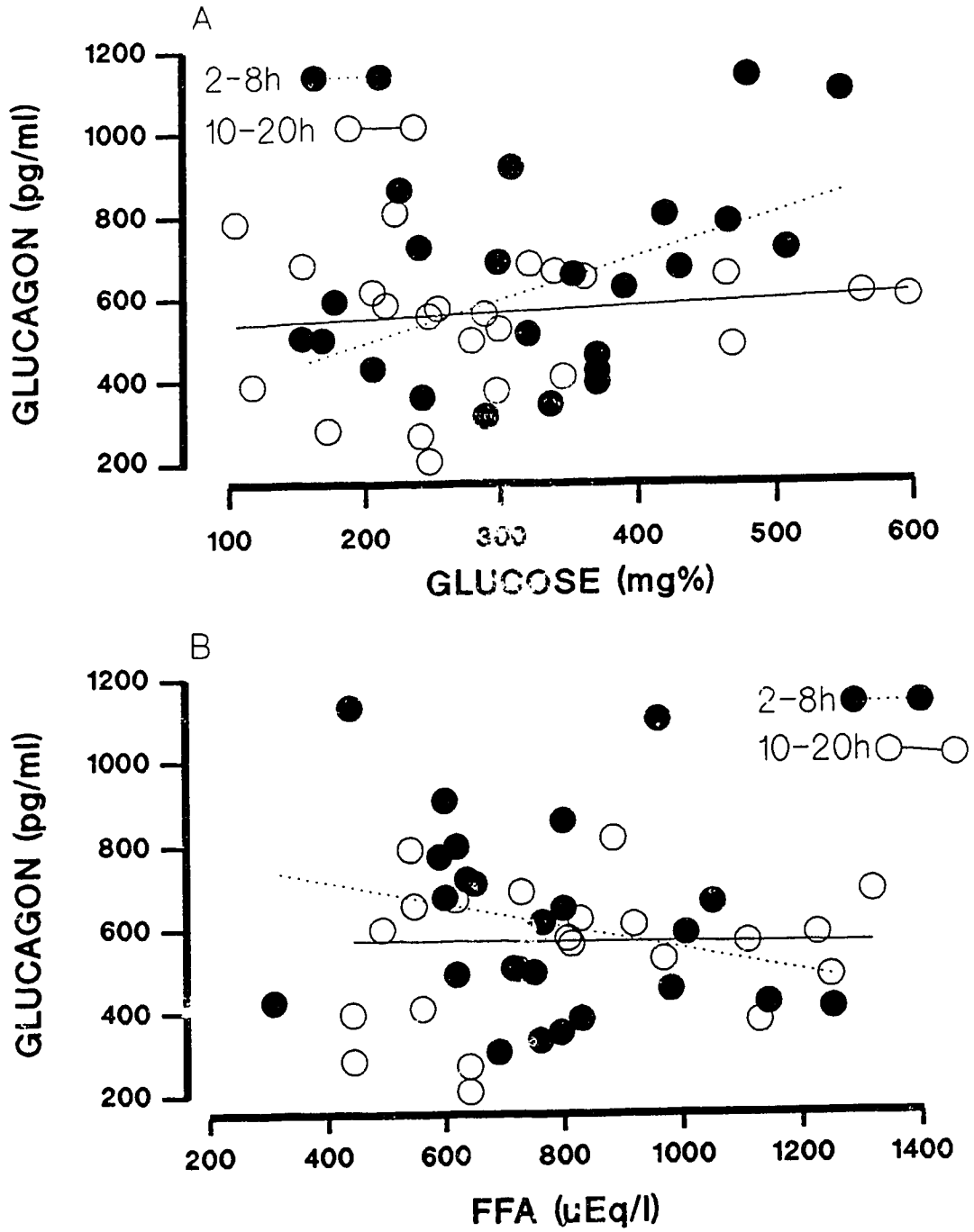
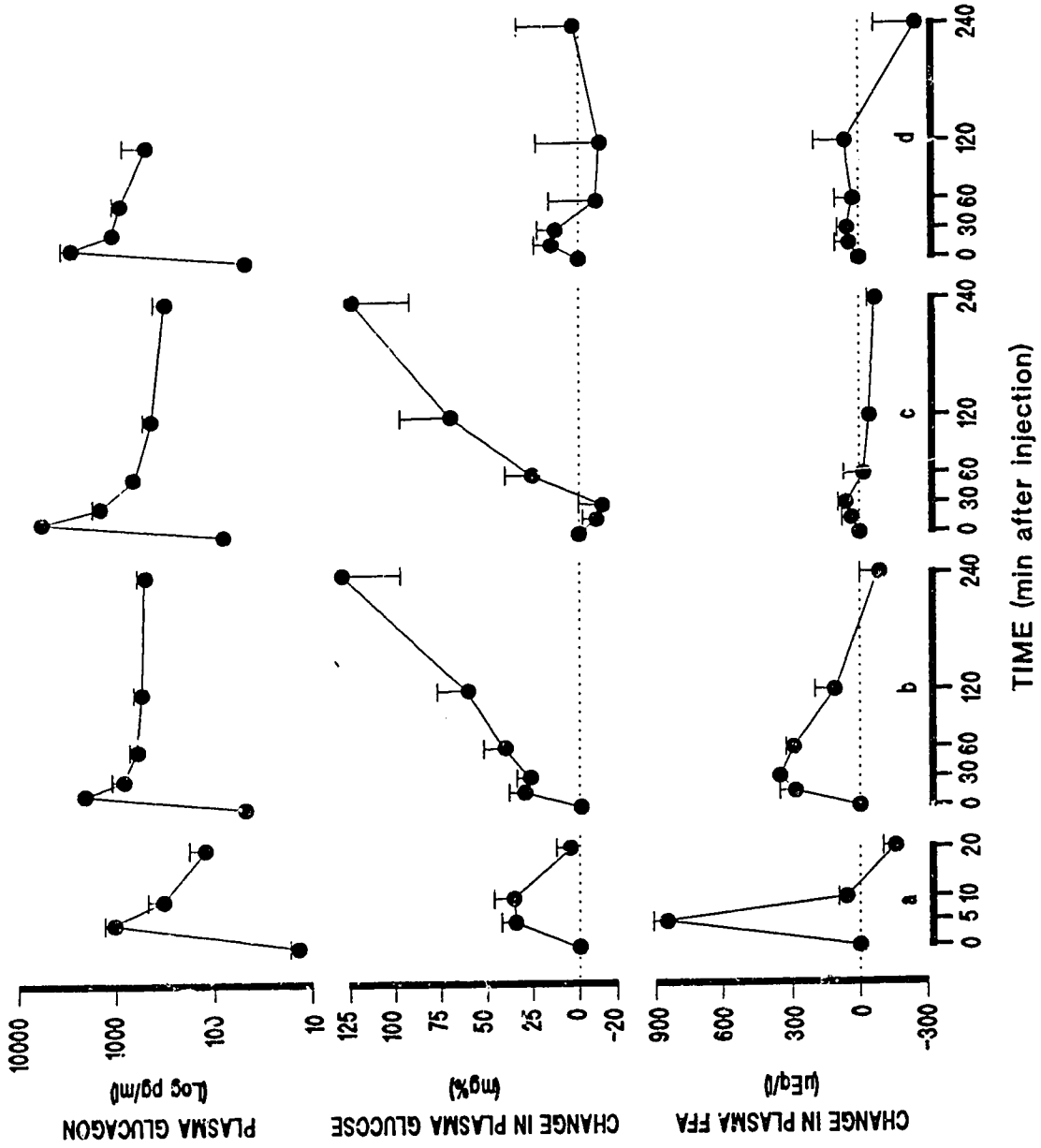

A
1200
1000
800
600
400
200
GLUCAGON (pg/ml)
2-8h
10-20h
100
200
300
400
500
600
GLUCOSE (mg%)
B
1200
1000
800
600
400
200
GLUCAGON (pg/ml)
2-8h
10-20h
200
400
600
800
1000
1200
1400
FFA (µEq/l)

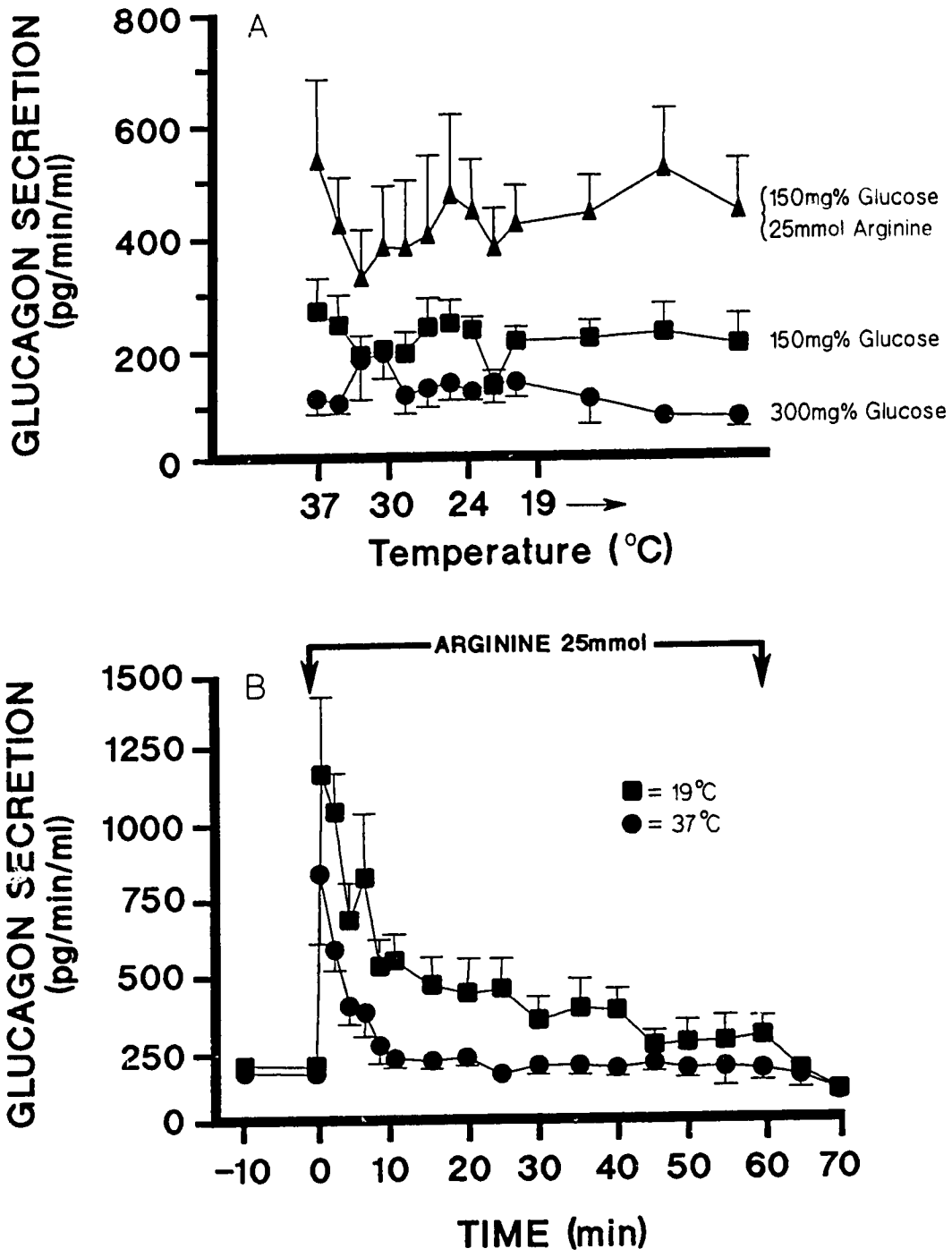
Figure IV.3: Plasma glucagon concentrations and metabolic effects of injection of glucagon (20 µg/Kg) in euthermic (a) and hypothermic rats 2(b), 8(c) or 15h (d) after hypothermic induction. The scale of glucagon concentrations is logarithmic. The effects of glucagon injections on plasma glucose or FFA concentrations were calculated as the change after injection compared to the baseline concentrations measured immediately prior injection. Mean±sem; The number of animals studied is in parenthesis.


PLASMA GLUCAGON
(Log pg/ml)
10000
1000
100
10
CHANGE IN PLASMA GLUCOSE
(mg%)
125
100
75
50
25
0
-20
CHANGE IN PLASMA FFA
(μEq/l)
900
600
300
0
-300
0 5 10 20
0 30 60 120 240
a
b
c
d
TIME (min after injection)

dramatically 5 min after glucagon injection (+852±54 µEq/l from the pre-injection level) it then returned to the basal concentrations by 10 and 20 min post-injection; the integrated FFA response was 580±40 µEq/l/min. Two hours after hypothermia induction (Fig. IV.3b), glucagon administration induced a progressive increase in plasma glucose concentration which reached +129±31 mg% from the pre-injection level after 240 min and a rapid and transient increase in FFA concentrations (maximum at 30 min, +351±8 µEq/l from the pre-injection level) which returned to the basal concentration between 120 and 240 min. The integrated glucose and FFA responses to glucagon were 60±16 mg%/min and 190±59 µEq/l/min, respectively. Glucagon injected at 8 h of hypothermia (Fig. IV.3c), resulted in an increase (+124±32 mg%), after a delay of 1 h, in plasma glucose which peaked at 240 min; the integrated glucose response was 58±23 mg%/min. The changes in plasma FFA concentrations following an injection of glucagon at 8 h were not statistically significant. No statistical variation of plasma glucose or FFA concentrations were found when glucagon was injected at 15 h in hypothermia (Fig. IV.3d).

Perfusing the pancreas with medium containing 150 mg% glucose (a concentration approximately equal to that found in the euthermic rats) for 30 min at 37°C (Fig. IV.4a) resulted in glucagon secretion of around 250 pg/ml/min. When the temperature of the medium was decreased from 37 to 19°C, glucagon secretion stabilized at about 200 pg/ml/min and did not vary statistically during cooling or at 19°C. With medium containing 300 mg% of glucose (a concentration approximately equal to the mean for hypothermic rats), glucagon secretion was around 120 pg/ml/min at 37°C and again did not vary statistically during the cooling period. With medium containing 150 mg% of glucose and 25 mmol of arginine, the level of glucagon secretion was around 400 pg/ml/min and no significant variation was observed either at 37°C or during the cooling period. These results suggest that hypothermia did not inhibit glucagon secretion. To further support this suggestion, perifusion of the pancreas was performed at 37°C

Figure IV.4: (A) Glucagon secretion from perfused rat pancreas during *in vitro* cooling from 37 to 19°C. The perfusion medium contained either 150 (■) or 300 (●) mg% of glucose, or 150 mg% of glucose and 25 mmol of arginine (▲). The number of perifusions is in parenthesis. (B) Glucagon secretion from perfused rat pancreas at 37°C (●) or 19°C (■). Glucose concentration of the perfusion medium was 150 mg%; Arginine (25mmol) was added at time 0 and continued to 60 min.


A
800
600
400
200
0
GLUCAGON SECRETION
(pg/min/ml)
150mg% Glucose
25mmol Arginine
150mg% Glucose
300mg% Glucose
37 30 24 19 →
Temperature (°C)
B
ARGININE 25mmol
1500
1250
1000
750
500
250
0
GLUCAGON SECRETION
(pg/min/ml)
■ = 19°C
● = 37°C
-10 0 10 20 30 40 50 60 70
TIME (min)

or 19°C by adding arginine (25 mmol) abruptly, after a 1 h washing period with medium containing 150 mg% of glucose (Fig. IV.4b). At 37°C, before arginine stimulation (time = -5 min), glucagon secretion was 187±29 pg/ml/min. When arginine was added at time 0, glucagon secretion increased rapidly, reaching 940±230 pg/ml/min 2 min after the introduction of arginine, it then decreased and stabilized at about 200 pg/ml/min. Removal of arginine at 60 min produced a significant decrease in glucagon secretion (120±22 pg/ml/min). At 19°C, before arginine introduction (time = -5 min), glucagon secretion was 210±32 pg/ml/min, not significantly different from the level at 37°C. Glucagon secretion reached 1160±260 pg/ml/min 2 min after the addition of arginine then decreased and stabilized at about 300 pg/ml/min. When arginine was removed, glucagon secretion decreased rapidly, reaching 123±12 pg/ml/min 15 min later. Glucagon secretion was significantly higher at 19°C than at 37°C at all times from 2 to 40 min.

DISCUSSION

Deep hypothermia in rats results in a marked modification of plasma glucose concentration. Although endogenous glucose production is decreased by 2.5 times in hypothermic rats as compared to euthermic rats (Jourdan et al, unpublished), marked hyperglycaemia (mean 300 mg%),lasting up to 20 h, was detected as early as 2 h after the beginning of hypothermia induction (Fig. IV.1). Hyperglycaemic tendency associated with glucose intolerance has been frequently observed in hypothermic neonates (5, 10, 14, 19), juvenile (26) and adult animals (29) but never have such high concentrations been measured. An imbalance between endogenous production and removal of glucose (utilization by various tissues) occurs in our animals during the rapid induction of hypothermia; this imbalance is probably the result of the marked suppression of glucose metabolism by low T_b and/or shifts in the balance of hormones. Indeed, an increase in plasma catecholamines (9), glucagon concentrations (Fig. IV.1), and a dramatic decrease in plasma insulin concentration (11) have been observed in hypothermic rats.

The parallel increases (Fig. IV.1) and the significant ($p < .05$) positive correlation (Fig. IV.2a) between endogenous glucagon and plasma glucose concentrations measured at hours 2 and 8 in hypothermia suggest that glucagon contributes to the development of hyperglycaemia in hypothermic rats. Since no glucose recycling from lactate has been found during hypothermia (unpublished results), glucagon probably increases the hepatic glucose production via glycogenolysis rather than gluconeogenesis .

Unlike changes seen for glucose, no significant difference was found between plasma FFA concentration measured before and 2 h after the induction of hypothermia, in spite of an estimated 3.1 times decrease in FFA turnover in 2 h hypothermic rats (Chap. VI) and the hormonal imbalance mentioned above. However, plasma FFA significantly and regularly decreased during the 20 h hypothermia period. This suggests that lipolysis maybe depressed despite the persisting imbalance between plasma insulin (11) and glucagon (Fig. IV.1) concentrations (Insulin/glucagon molar ratio calculated with concentrations measured on the same blood samples was about 4 in euthermic rats and averaged .4 in hypothermic rats) which should favour an increase lipolysis and therefore in plasma FFA.

The absence of correlation between plasma glucagon and FFA during the first period in hypothermia is in strong contrast to the positive correlation observed between plasma glucagon and glucose (Fig. IV.2a). This suggests that endogenous glucagon does not contribute to the regulation of lipolysis in hypothermic animals. This is further supported by the depressed response of plasma FFA to injection of exogenous glucagon at 2 h (Fig. IV.3b), suggesting that low T_b may be responsible for the depressed response of plasma FFA to glucagon. That FFA levels are responsive to exogenous glucagon until 15 h after induction of hypothermia (see below), however, suggests that this temperature dependent depression of the FFA

response to glucagon maybe due to the effect of temperature on FFA kinetics rather than on the glucagon adipose tissue interaction.

In vitro glucagon secretion from the perfused pancreas of rat appears to be completely independent of the temperature between 37 and 19°C. Indeed, secretion at 37°C, induced by low (150 mg%) or high (300 mg%) glucose concentration or by arginine (25 mmol) remained stable during the *in vitro* cooling (Fig. IV.4a). Insulin release however, was dramatically inhibited during the cooling period (11, Chap. III), in agreement with previous results obtained with isolated islets of rat pancreas (11). At 19°C, the pancreas was more responsive to arginine (no delay in response and very high secretion especially during the initial period of secretion) than it was at 37°C. *In vivo*, the hyperglycaemia observed in hypothermic rats should be an important inhibiting factor of glucagon secretion; further, based on the *in vitro* results glucagon secretion in the hypothermic rats should be about one half that of euthermic rats (Fig. IV.4a). However, the plasma glucagon concentration was on the average 3 times higher in hypothermic rats than in euthermic rats. This may be due to an initial dramatic increase in glucagon secretion during hypothermia induction followed by a decrease in the plasma clearance of glucagon during hypothermia. The nearly 10 times longer half-life of clearance of exogenous glucagon in hypothermic than in euthermic animals (Fig. IV.3) is consistent with this interpretation.

Glucagon is known to increase substrate production through stimulatory effects on hepatic glycogenolysis (15), gluconeogenesis (8) and on adipose tissue lipolysis (16). Glucagon injections in euthermic rats (Fig. IV.3a) resulted in a rapid but transient increase in both glucose and FFA concentrations despite persistently high glucagon concentrations. This apparent "down" regulation of responsiveness has been previously observed after glucagon injection and found to be not entirely attributable to the glucagon-induced rise in plasma insulin, as it also occurs in insulin-dependent diabetes (3). Exogenous glucagon induced a delayed and

sustained rise in plasma glucose concentration after 2 h of hypothermia, (Fig. IV.3b), this metabolic action was still present after 8 h, but disappeared after 15 h (Fig. IV.3c). Interestingly, this disappearance of glucagon's metabolic activity coincides with an increase in mortality in 19°C rats. Previous studies (11,13) have shown that 100% of the rats run under this protocol will survive for 15 - 18 h, after which the percent surviving gradually declines to about 90 - 95% at 24 h.

Long term exposure to hypothermia (10 to 20 h) resulted in the disappearance of the metabolic effects of exogenous glucagon (Fig. IV.3d) and no correlation between plasma glucagon, glucose or FFA (Fig. IV.2b) at a time when animals begin to die in hypothermia. This could possibly be attributed to a decrease in mobilizable energy stores in the liver but probably not to depletion of adipose tissue triglyceride stores which are too large in most animals to be used up in 18 h. Thus, this and the persistently high glucose concentrations suggest that both liver and adipose tissue become insensitive to glucagon when hypothermia is prolonged. Perhaps the long term exposure of tissues to the high glucagon concentrations observed during hypothermia provokes a loss of hormonal action, as has been shown in euthermic men perfused with exogenous glucagon (3). This chronic exposure may induce down-regulation of the membrane receptor-hormone binding kinetics or in post-receptor processes (for example, activation of protein kinases or enzyme phosphorylation). If this is the case then the lack of FFA response to exogenous glucagon at 15 h suggests a further depression of the already depressed FFA turnover in the hypothermic rat at 19°C. Thus, a lack of sufficient FFA as an energetic substrate maybe responsible for death in hypothermic rats.

On the other hand, results presented here and in Hoo-Paris et al. (11) show that a great similarity exists between the metabolic state in hypothermic rats and diabetic animals. In diabetes mellitus, in which hypoinsulinemia and hyperglucagonemia are major characteristics (27), the lack of glucose homeostasis

due to accelerated glucose production by glucagon unopposed by insulin, results in extreme degrees of hyperglycaemia and the development of the hyperglycaemic dehydration syndrome (review in 30). In diabetic subjects, or in animals submitted to a persistent glucose infusion (1), tissue oedema, specially in the brain, is a frequent cause of death, following rapid reduction of the hyperglycaemic (hyperosmolar) condition. It is possible that in hypothermic rats hyperglycaemia is responsible for the observed decrease in tissue water (chap. II). Further, the rapid decrease in the glycaemic level following insulin treatment (chap. III) may result in oedema (1,30) and therefore, explain why treatment with insulin does not yield any inprovement in hypothermic survival. Thus, the insulin-glucagon imbalance, and the subsequent dehydration due to hyperosmolarity could be one reason for the limited survival in deep hypothermia.

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V. PLASMA METABOLITES AND HYPOTHERMIC SURVIVAL [5]

INTRODUCTION

Hypothermia is a state of low body temperature (T_b), which occurs when heat loss exceeds heat production and from which animals cannot rewarm without aid in the form of exogenous heating. The hypothermic animal survives by virtue of its innate cellular cold tolerance until critical failures occur, then death is inevitable. The limitations for survival in hypothermia are still unclear, but appear to include perturbations of cardiovascular and respiratory function, tissue perfusion and O_2 delivery and substrate mobilization and utilization (4,7). During rewarming, rising T_b may cause further imbalances, due to increased metabolic demand, that jeopardize maintenance of homeostasis (4). In particular, the availability of glucose has often been cited as a primary factor in hypothermic survival (4,7,9).

The development of a method that provides rapid induction and 24 hours of survival at T_b=19°C in rats, has allowed the study of factors that control and may limit survival during prolonged deep hypothermia and/or subsequent rewarming (7,3,6). Previous studies have indicated that animals that do not survive rewarming from prolonged hypothermia (18-24h) generally succumb when T_b reaches 22-25°C. This suggests that critical failures occur in this T_b range, although the primary disruption may have occurred earlier in the bout. In order to assess some of the differences between survivors and non-survivors, we have compared the

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5. A version of this chapter has been submitted. McArthur, M. Dawn, Michael L. Jourdan and Lawrence C.H. Wang. Pharmacology of Thermoregulation: Eight International Symposium, August 26-30, 1991.

metabolite profiles of these two groups of rats during induction, maintenance and rewarming from prolonged deep hypothermia.

METHODS

Male Sprague-Dawley rats, housed individually at an ambient temperature (T_a) of 22±1°C under 12L:12D, were ration to maintain mass near 400 gm and given water *ad libitum*. The rationing regime resulted in hypothermic induction occurring approximately 18 h after the animals last feeding.

Cannulation (right jugular vein and left common carotid artery) and induction of hypothermia were as previously described (2,3,5,6). Briefly, following surgery, the halothane anaesthetized rat was transferred to a water-jacketed Plexiglas chamber at T_a=0°C under He-O_2 in an open-flow system. The halothane concentration (1.0% at start of induction) was decreased by ~0.1% per 1°C drop in T_b: the anaesthetic was removed when T_b reached 28°C. When T_b reached 23°C, the He-O_2 was replace by room air. In hypothermia, T_b was maintained at 19°C by a computerized feedback loop using rectal temperature as the reference for adjustment of T_a. Rewarming was initiated by raising T_a to 30°C after approximately 18 hours at T_b=19°C. Animals were grouped as either Group A (surviving to T_b>26°C) or Group B (surviving to T_b=22-25°C) (Table V.1).

Blood samples for metabolites were taken from the arterial cannula before and after cooling, at several times during hypothermia and rewarming (Table V.1). A 250µl aliquot of the blood samples (0.4 ml) was centrifuged immediately and the plasma frozen at -20°C until assayed for glucose and FFA concentrations. Plasma glucose was measured using the glucose-oxidase method (Sigma Kit 510) and plasma FFA by the colorimetric method (1). A 150µl aliquot of whole blood was deproteinized in 300µl of 0.4% perchloric acid, centrifuged and lactate measured spectrophotometrically using LDH-linked reduction of NAD^+.

RESULTS

All rats survived induction and maintenance of hypothermia. During rewarming, all animals survived until T_b reached 22°C, but only half survived to T_b>26°C. On average, Group B rats rewarmed somewhat more quickly than did Group A rats (Table V.1).

As can be seen in Figure V.1, the most striking differences in the metabolite profiles of Group A and B rats were indicated by the changes in plasma glucose and lactate. Plasma glucose did not differ between the survivors and non-survivors before cooling (12.3±1.9mM vs 10.7±0.9mM). During hypothermia and rewarming, Group A rats maintained a plasma glucose that was approximately twice that of Group B rats (~20mM vs ~12mM) (Fig. V.1). Plasma lactate was very low in both groups initially, and increase over 18 h at T_b=19°C only in Group B rats. During rewarming, lactate rose more rapidly in non-survivors, so that the final lactate concentration of Group B rats was significantly greater than that of Group A rats (Fig. V.1). Plasma FFA was slightly higher in Group A during induction and early hypothermia, but decreased by 18% over 18 h to the level of Group B. Plasma FFA was similar in the two groups at the first rewarming sample, but rose substantially higher in Group A at T_b ~26°C, before falling at T_b ~30°C to the final level of Group B (516±144 μEq/l vs 545±38 μEq/l) (Fig. V.1).

DISCUSSION

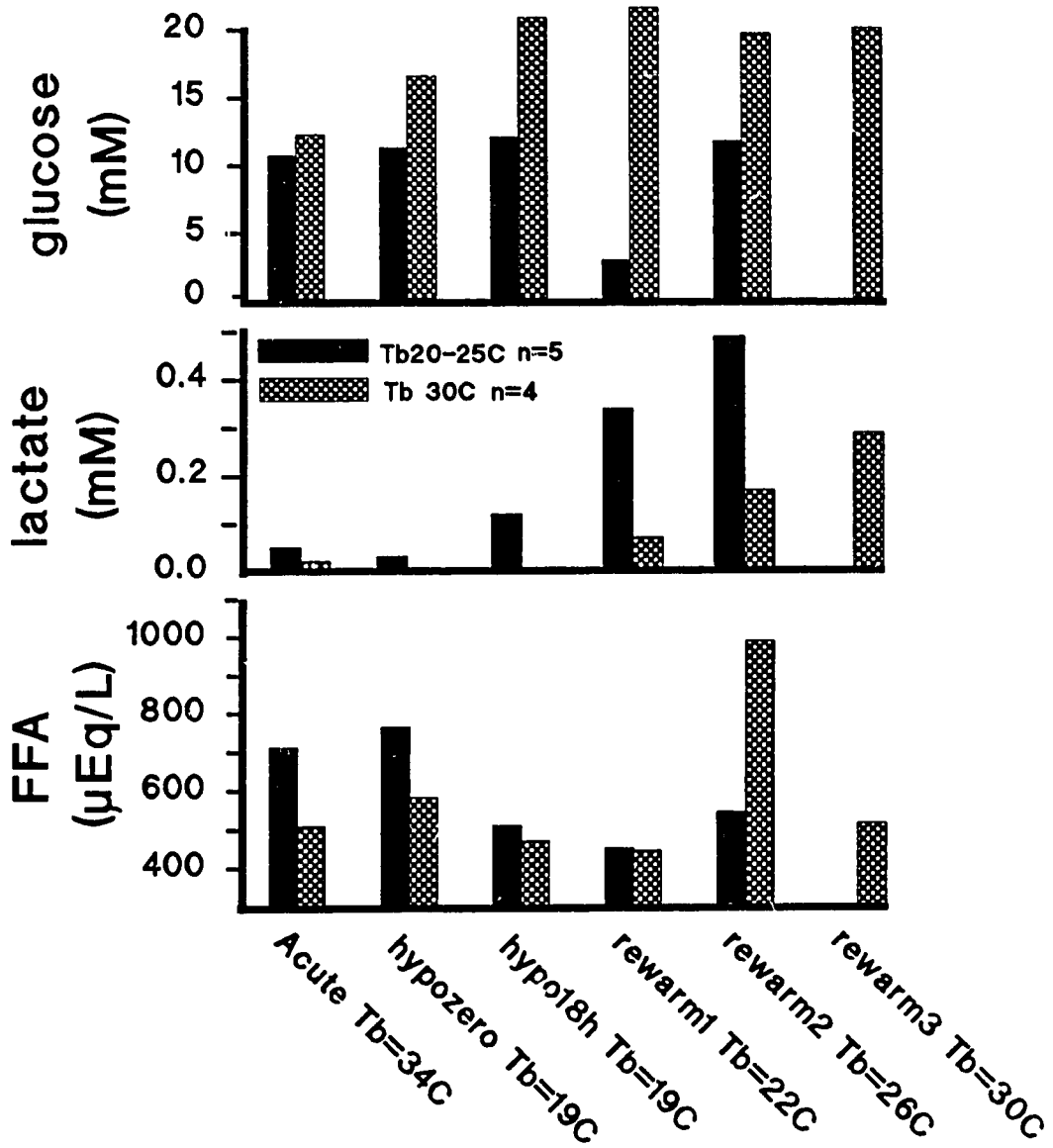
Compared to survivors, rats that do not survive rewarming have lower plasma glucose and FFA concentrations during hypothermia, as well as lower glucose and higher lactate concentrations during rewarming (Fig. V.1). The potential capability of the hypothermic animal to rewarm successfully may be related to its responsiveness to cooling during induction of hypothermia. Increases in glucose and FFA are attributed to both stress and cold-exposure responses, which include decreased insulin and increased catecholamine, glucagon and corticosterone levels

Table V.1 Body temperatures (T_b) and time-course data for group A and B rats.

Sample	Group	N	T_b (°C)	Time (h)
Post-surgery	A	4	34.4±0.3	-3.3±1.0
	B	5	34.3±0.9	-4.3±1.2
Initial Hypothermia	A	4	19.7±0.4	0
	B	5	19.4±0.2	0
Final Hypothermia	A	5	19.3±0.3	17.6±0.5
	B	5	19.2±0.2	16.3±0.4
Rewarm (T_b=22°C)	A	4	22.7±0.5	18.4±2.2(0.8)
	B	5	22.5±0.4	17.6±0.2(1.3)
Rewarm (T_b=26°C)	A	5	25.8±0.6	19.6±2.2(2.0)
	B	3	26.1±0.3	18.7±0.1(2.4)
Rewarm (T_b=30°C)	A	5	30.6±1.0	22.2±3.1(4.6)
	B	0	—	—

Group A = animals surviving to T_b > 26°C during rewarming;
Group B = animals surviving to T_b = 22-25°C during rewarming;
Time in parentheses indicate mean time from onset of rewarming;
Values are mean±sem; no significant differences were found between groups.

Figure V.1: Plasma concentrations of glucose, FFA and lactate during induction, maintenance and rewarming from hypothermia in rats. Group A) animals surviving rewarming to T_b>26°C; Group B: animals surviving rewarming to T_b=22-25°C. Times, actual T_b and N are shown in Table V.I. Means±sem; * Significantly different ($P<0.05$, ANOVA) between groups A and B.


glucose (mM)
20
15
10
5
0
lactate (mM)
0.4
0.2
0.0
Tb20-25C n=5
Tb 30C n=4
FFA (µEq/L)
1000
800
600
400
Acute Tb=34C
hypozero Tb=19C
hypo18h Tb=19C
rewarm1 Tb=22C
rewarm2 Tb=26C
rewarm3 Tb=30C

(4,3,8). This leads to mobilization of glucose via glycogenolysis in heart, liver and muscle, and of fat via lipolysis in adipose tissue. Rats in Group B showed a less vigorous response to lowered T_b than did rats in Group A, and thus began the hypothermic bout with lower glucose and FFA concentrations (Fig. V.1). It should be noted, however, that the glucose and FFA levels of even non-survivors are similar to those of euthermic animals, and theoretically should be adequate to sustain the reduced metabolic demand of hypothermia.

The differences in FFA profiles of survivors and non-survivors are less obvious than those of glucose or lactate profiles. The decrease in FFA observed in both Group A and B rats (Fig. V.1) suggests that FFA are an important substrate during hypothermia, as is true of cold-exposed animals and hypothermic humans and rabbits (8). The initial rise in FFA in Group A rats between T_b ~22°C and ~26°C suggests that survivors retain the capacity to respond to the increased demand for metabolic fuel during rewarming, whereas non-survivors do not.

In hamsters, hypothermic survival at T_b=5°C is positively correlated with glucose concentration, and survival, both in hypothermia and during rewarming is augmented by glucose administration or pretreatment with glucocorticoids (4,7). This does not appear true of rats, since similar treatments have no beneficial effect (10, personal observation). Hypothermic survival in rats appears instead to be more dependent on the ability of tissues to use substrates (4,9,2,3). The striking difference between Group A and B rats suggests a correlation between survival and glucose concentration in the rat, which may seem at odds with these observations. However, unlike hypothermic hamsters the rats in Group B were not hypoglycaemic compared to euthermic rats (Table V.2). In contrast, Group A rats were severely hyperglycaemic during hypothermia (Fig. V.1). This hyperglycaemia may have a protective effect in hypothermia. Indeed it has been suggested that glucose administration can reduce the hyperexcitability of the hypothermic heart

Table V.2 Plasma glucose, FFA and lactate values for euthermic rats maintained at T_a=23°C.

Sample (time following surgery)	Glucose (mM)	FFA (μEq/l)	Lactate (mM)
Immediately	12.1±1.6	588±67	1.0±0.3[1]
	(6)	(6)	(5)
60 min	9.5±0.4	677±82	0.3±0.1
	(6)	(6)	(5)
24 hours	9.5±1.2	429±18	0.1±0.03
	(6)	(5)	(5)

Values are mean±sem (N);
[1]Significantly greater than 60 min or 24 hours ($P<0.05$,ANOVA)

to normal levels. Further, hyperglycaemia may play a role in balancing osmotic stress (4) in hypothermia and during rewarming.

Despite availability of substrate, glucose and FFA oxidation may be decreased by an overall depression of aerobic metabolism. Plasma lactate increased during rewarming in both survivors and non-survivors, but the rise occurred earlier and to a greater extent in non-survivors than in survivors (Fig. V.1). In Group B rats, this increase in anaerobic metabolism is concomitant with decreased cardiovascular/respiratory function, as these animals also had higher haematocrit ratios (55-60% vs 45-50 in Group A rats), lower arterial P_{O_2}, higher arterial P_{CO_2} and lower arterial pH than did the survivors (6). This pattern suggests that low tissue blood flow, hence decrease tissue oxygen and substrate delivery, rather than general substrate availability in the plasma *per se*, may be the primary causative factor in the suppression of oxidative metabolism in hypothermia and rewarming. Thus, investigation into substrate turnover and oxidation rates as well as blood gases and acid-base balance may prove enlightening. Further, remedial measures directed towards maintenance of cardiovascular function and peripheral blood flow may provide more benefits than augmentation of substrate concentrations in improving the survival of hypothermic rat.

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VI. GLUCOSE AND FREE FATTY ACID TURNOVER AND OXIDATION IN RATS DURING STABLE SEVERE HYPOTHERMIA

INTRODUCTION

The state of experimental or accidental hypothermia in mammals is characterized by low body temperature (T_b) and depressed metabolism. If hypothermia is not rectified by the application of external heat, death is a predictable outcome. Variability in sensitivity of tissues and cells to cold, regional disturbances in tissue micro-circulation and the ability to utilize substrates for energy at low T_b are some of the general considerations which could lead to the disturbance of physiological homeostasis at low T_b. An example is seen in blood glucose regulation: hyperglycaemia and glucose intolerance have been observed during hypothermia in nonhibernating species such as humans (20), rabbits (3) and rats (12), indicating an inability to use glucose at low T_b. In contrast in the hypothermic hibernator the golden hamster, which can survive hypothermia at 5°C much longer than rats, exhibits a significant correlation between plasma glucose concentration and survival time, indicating its ability to use glucose at low T_b (21). This drastic species difference is further demonstrated by the observation that infusion of glucose prolongs survival in hypothermic hamsters (22) but not in rats (27).

Extensive documentation exists showing serious inhibition of carbohydrate metabolism during hypothermia (3, 13, 14, 21, 22, 27, 28), and respiratory quotient (RQ) is generally observed to decrease during hypothermia, suggesting that there is preferential lipid utilization during hypothermia. Somewhat

paradoxically, the brain, which is solely dependent on glucose metabolism, fares much better in hypothermia than does the heart, which mainly depends on lipid metabolism (4,7). Data on lipid metabolism during hypothermia, however, is practically nonexistent. It seems reasonable to suspect that alterations in the availability and/or the ability to utilize metabolic substrates such as glucose and FFA's at low T_b could be one of the main reasons why survival of hypothermia is limited in nonhibernators.

Techniques for the measurement of substrate turnover require that steady-state conditions prevail during the experimental period (17). Due to the essentially transient nature of most approaches to experimental hypothermia, previous attempts to evaluate the effects of low T_b on substrate metabolism have not included direct measurement of substrate turnover. With the development of a new model for stable and prolonged hypothermia (15) it is now feasible to conduct substrate turnover studies at low T_b (19°C) under the required steady-state conditions. To evaluate the possibility that perturbation of substrate availability in hypothermia could be a limitation to hypothermic survival, the turnover rates of glucose and free fatty acids (FFA) were studied in hypothermic rats.

METHODS

Male Sprague Dawley rats housed individually at 22°C under a 12L:12D light cycle were rationed to maintain weight at 400g and provided with water ad libitum. Rats were cannulated, in the left jugular vein and right carotid artery, under halothane anaesthesia (1.5%) and subdivided into three groups: 1) euthermic; rats acutely cannulated immediately prior to experimentation and maintained at 25°C ambient temperature (T_a) without food or water throughout the experimental period, 2) hypothermic 2h; rats acutely cannulated immediately prior to experimentation and maintained at a T_b of 19°C for approximately 2h prior to the start of tracer infusion, and 3) hypothermic 18h; rats treated as the 2h group except they were maintained at a T_b of 19°C for approximately 18h prior

to the start of tracer infusion. Hypothermia was induced immediately after cannulation by the method of Jourdan and Wang (15). Briefly, a thermocouple (copper-constantan) was inserted approximately 5 cm into the rectum, and electrocardiographic electrodes attached. The animal was then transferred from the surgical board to a water-jacketed plexiglass chamber at 0°C under a HeO_2 atmosphere containing 1.5% halothane in an open flow system. The concentration of halothane was progressively reduced by about 0.1% with every 1°C drop in T_b and was discontinued at T_b=28°C. When T_b reached 23°C, the HeO_2 was replaced by air and the T_a increased to near 15°C in order to slowly cool the animal to 19°C. The T_b was maintained at 19°C by automatically adjusting the temperature of the water bath perfusing the animal chamber using a computer algorithm which generates a signal based on the difference between actual T_b and 19°C.

Tracer injections, followed by continuous infusion of tracer, were made 2 h after cannulation in euthermic rats or 2 or 18 h after the beginning of stable hypothermia. Blood samples were collected at time 0 (immediately prior to tracer injection) and at 30, 60, 70, 80, 90, 100 and 120 min after the start of tracer infusion. Injection and infusion were via the jugular cannula. Blood sampling was via the carotid cannula, the volume of each blood sample was never more than 0.3 ml. An aliquot of whole blood was taken for haematocrit determination and the remaining blood was centrifuged at room temperature and the plasma frozen at -20°C until assayed.

For glucose turnover, a mixture containing 30 μCi of U-^{14}C-glucose and 30 μCi of 2-^{3}H-glucose per ml of saline was injected in a bolus of .1 ml, followed by a constant infusion of ≈12 μl/min (≈0.30-40μCi/h, total) for 90-100 min. Both 2-^{3}H and U-^{14}C glucose were used to differentiate between irreversible loss of glucose and the recycling of glucose carbon (17). Glucose uniformly labelled with ^{14}C is taken up by cells and can be stored as glycogen, oxidized to CO_2 or metabolized

to labelled lactate which can potentially be recycled to glucose. Therefore, the turnover of ^{14}C glucose alone does not accurately represent the uptake of glucose by cells. 2-^{3}H-labelled Glucose, labelled in the 2-H position, follows similar uptake pathways; however, its metabolic fate is different as catabolism of the labelled substrate yields labelled water rather than labelled gluconeogenic precursors (17). The turnover of 2-^{3}H-glucose then, represents peripheral glycolysis or the irreversible loss of glucose. By combining ^{14}C and ^{3}H labelled glucose in the evaluation of turnover, the percentage of glucose recycling in the system can be estimated.

Plasma (30-50µl) was chromatographed with a double exchange column containing DOWEX (1X2-200 anion and 50X1-100 cation) resins (Sigma) with water elution. Following evaporation of the water, ^{3}H and ^{14}C glucose radioactivity was measured simultaneously using a LKB liquid scintillation counter. Plasma glucose concentration was determined in 20 µl of plasma deproteinized by adding equal volumes of 0.3N $ZnSO_4$ and 0.3N BaOH, vortexing and rapidly centrifuging to precipitate the protein, and an aliquot of the resuspended eluate from the ion exchange columns using the glucose oxidase method (Sigma). The ratio of eluate glucose concentration to plasma glucose concentration was used to adjust tracer recovered from the columns to the plasma glucose concentration.

U-^{14}C-Na-Palmitate was prepared from U-^{14}C-palmitic acid (New England Nuclear) by the method of Moryia (personal communication). Briefly, the U-^{14}C-palmitic acid-benzene solution was evaporated to dryness under nitrogen and resuspended in ethanol. To the U-^{14}C-palmitic acid-ethanol solution, NaOH was added at 18 to 20 times the molar concentration of palmitate and the resultant solution was heated to 60 to 80°C. The solution was then evaporated to dryness and the U-^{14}C-Na-palmitate resuspended to a concentration of approximately 25 µCi/ml in saline. Labelled Na-palmitate was bound to albumin immediately prior to the start of the experiment by warming the palmitate solution to 40°C and

mixing with an equal volume of 6% albumin solution (pH 7.4). The albumin bound palmitate solution was injected as a bolus (1.5-2.5μCi in .1-.15 ml) followed by a continuous infusion of 26μl/min (≈0.53μCi/h) for 90-100 min.

The FFA concentration and radioactivity were determined in duplicate 40μl aliquots of plasma extracted with 2 ml isopropyl ether containing 50μl isopropyl alcohol. After 15 min vigorous shaking, the ether phase was transferred to another tube and evaporated to dryness. The FFA's were then resuspended in 1.5 ml chloroform and an aliquot (.25 ml) was transferred to a counting vial and evaporated to dryness. Five ml of liquid scintillation cocktail was added and the activity was measured with a LKB liquid scintillation counter. FFA concentration was determined in a 1 ml aliquot of the chloroform with a Technicon Auto-Analyzer using the method of Duncombe (7).

Turnover of glucose and FFA's was based on the calculations of Katz et.al. (19) as follows:

$$Replacement\ Rate = \frac{I.R.}{S.A._{(T)}}$$

Where $I.R.$ = Rate of tracer infusion (dpm/min)
$S.A._{(T)}$ = Plasma SA at time (T)

Under steady state conditions it is assumed that input and output are equal and that output is equal to the ratio of infusion rate to plasma SA. This assumption is based on the fact that at a constant substrate concentration any given ratio of input to infusion rate will yield a specific equilibrium plasma SA over time.

Since turnover measurements do not give any indication of the fate of the substrate after it leaves the plasma, the specific activity (SA) of respiratory CO_2 was measured to estimate substrate oxidation. Fifteen min before injection the animal chamber was connect to an infrared CO_2 analyzer (Applied Electrochemistry CD-2

Analyzer). To collect the measured fraction of expired CO_2, a CO_2 trap containing 7 ml of a solution of 27% hyaminehydroxide, 27% methanol, 46% toluene and 4 g/l Omniflour (New England Nuclear) was connected to the exhaust of the CO_2 analyzer. Preliminary test showed that 100% of the CO_2 in a 15 min period was trapped using this solution. Instantaneous and total $\dot{V}CO_2$ were monitored continuously using a computerized data acquisition system (Issac 91, Cyberg corp.) connected to an IBM-PC. The trapping solution was changed every 15 min for 135 min and placed directly into scintillation vials for counting using a LKB liquid scintillation counter.

Oxidation of FFA in euthermic and hypothermic rats and glucose in euthermic rats was estimated from the plasma SA and respiratory $^{14}CO_2$ at times when the respiratory release of $^{14}CO_2$ was essentially stable. Glucose oxidation in hypothermic rats was estimated by averaging CO_2 SA over 4 time points, as glucose SA in hypothermic rats showed a continuous increase over the entire experimental period. Oxidation rate was calculated as:

$$Oxidation\ (mg/\text{min}) = \%P \times R$$

Where %P = Ratio of CO_2 dpm's to Plasma Pool dpm's
Plasma Pool dpm's= [0.04 x body weight (kg)] x dpm/ml
R = Replacement rate of glucose or FFA

All results were expressed as mean ± standard error of the mean. Statistical evaluation was performed using one-way analysis of variance followed by post-hoc analysis using SNK (Student-Newman-Keuls) and Scheffe procedures and Student's t-test for unpaired values wherever appropriate. P was set at 0.05 unless other-wise stated.

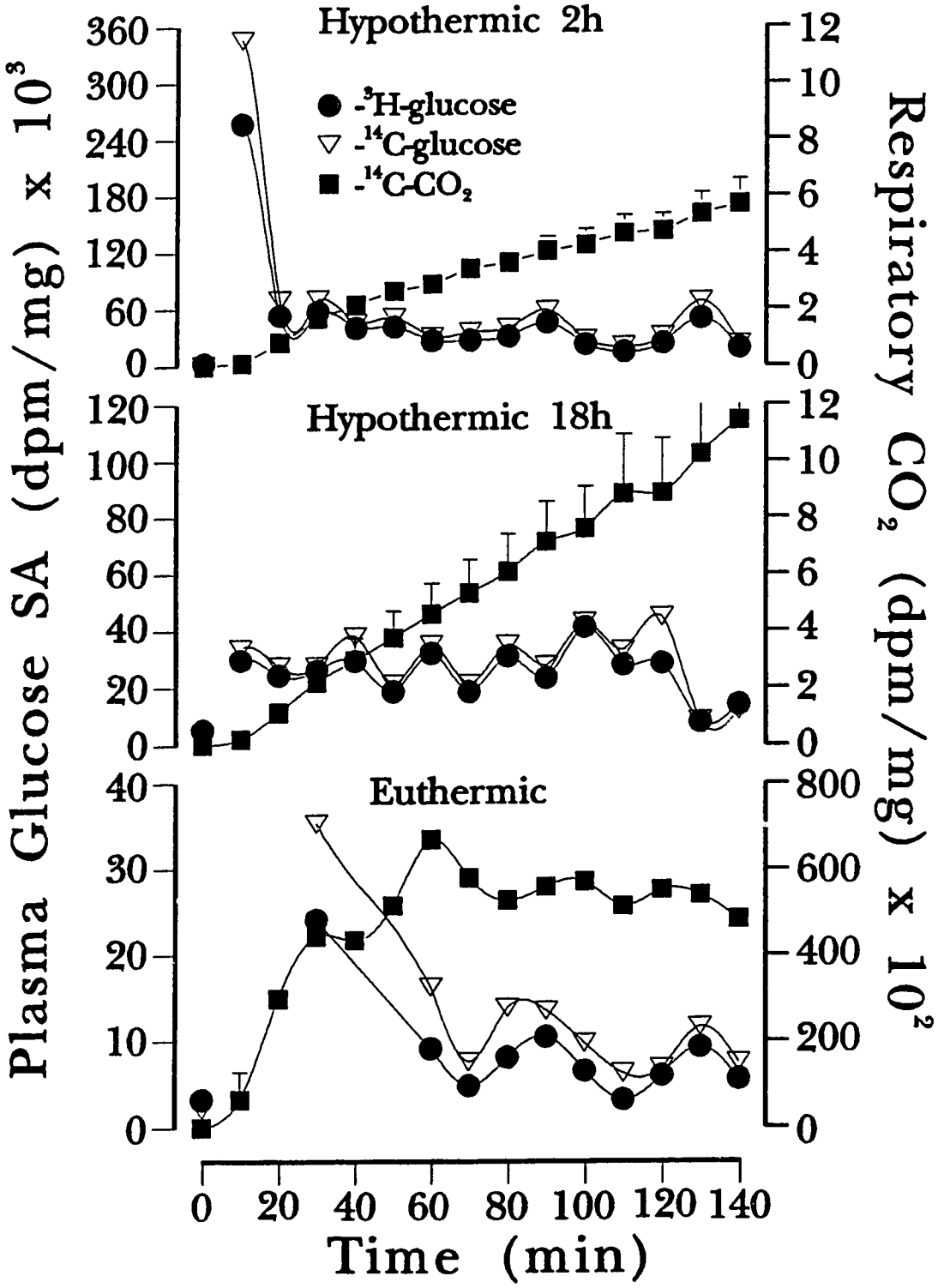
RESULTS

The mean plasma glucose concentration in euthermic rats over the infusion period was 140.8±62.9 mg/dl. Mean plasma glucose concentrations, in

hypothermic animals, were significantly greater than euthermic animals, 236.5±105.8 and 242.8±108.6 mg/dl for group 2 (2h) and group 3 (18h) respectively, but were not significantly different from each other (Table VI.1). Previous studies have confirmed that most of the increase in plasma glucose in hypothermic rats develops during cooling (13,14). The specific activities of plasma 2-^{3}H and U-^{14}C-glucose for all three groups of rats are shown in fig. VI.1. All groups showed the same basic pattern to infused tracer, however, the hypothermic rats demonstrated, in addition, a pattern of random non-significant fluctuations over the sampling period particulary in the group 3 rats (Fig VI.1), therefore, the mean of levels at 60-90 min were used for replacement calculations (Fig. VI.1). Glucose replacement rates in euthermic rats were 39.1±2.1 and 72.4±6.8 mg/kg/h for 2-^{3}H and U-^{14}C-glucose respectively, indicating an apparent recycling rate of ≈45.9±2.7% (Table VI.1). In group 2 hypothermic rats replacement rates for 2-^{3}H and U-^{14}C-glucose were significantly lower (11.5±2.0 and 23.5±5.8 mg/kg/h for 2-^{3}H and U-^{14}C-glucose respectively; Table VI.1) than the euthermic rats. The recycling rate ≈51.9±3.0 % however, was not significantly different. The irreversible replacement rate (based on 2-^{3}H-glucose) in group 3 rats (16.2±4.2 and 34.8±19.9 mg/kg/h, for 2-^{3}H and U-^{14}C-glucose respectively; Table VI.1) was also significantly lower than euthermic rats and although higher than group 2 rats not significantly different. The apparent recycling of glucose (53.6±1.2 %) in group 3 rats was however, significantly higher than the euthermic rats.

Respiratory CO_2 production was significantly lower in hypothermic rats than in euthermic rats (0.14±0.01 and 0.09±0.01 cc/g/h for group 2 and group 3 rats respectively vs .61±0.02 cc/g/h for euthermic rats; Table VI.1). The SA of respiratory CO_2 increased rapidly and significantly in euthermic rats (Fig.VI.1), indicating a rapid incorporation of tracer atoms into the CO_2 pool through

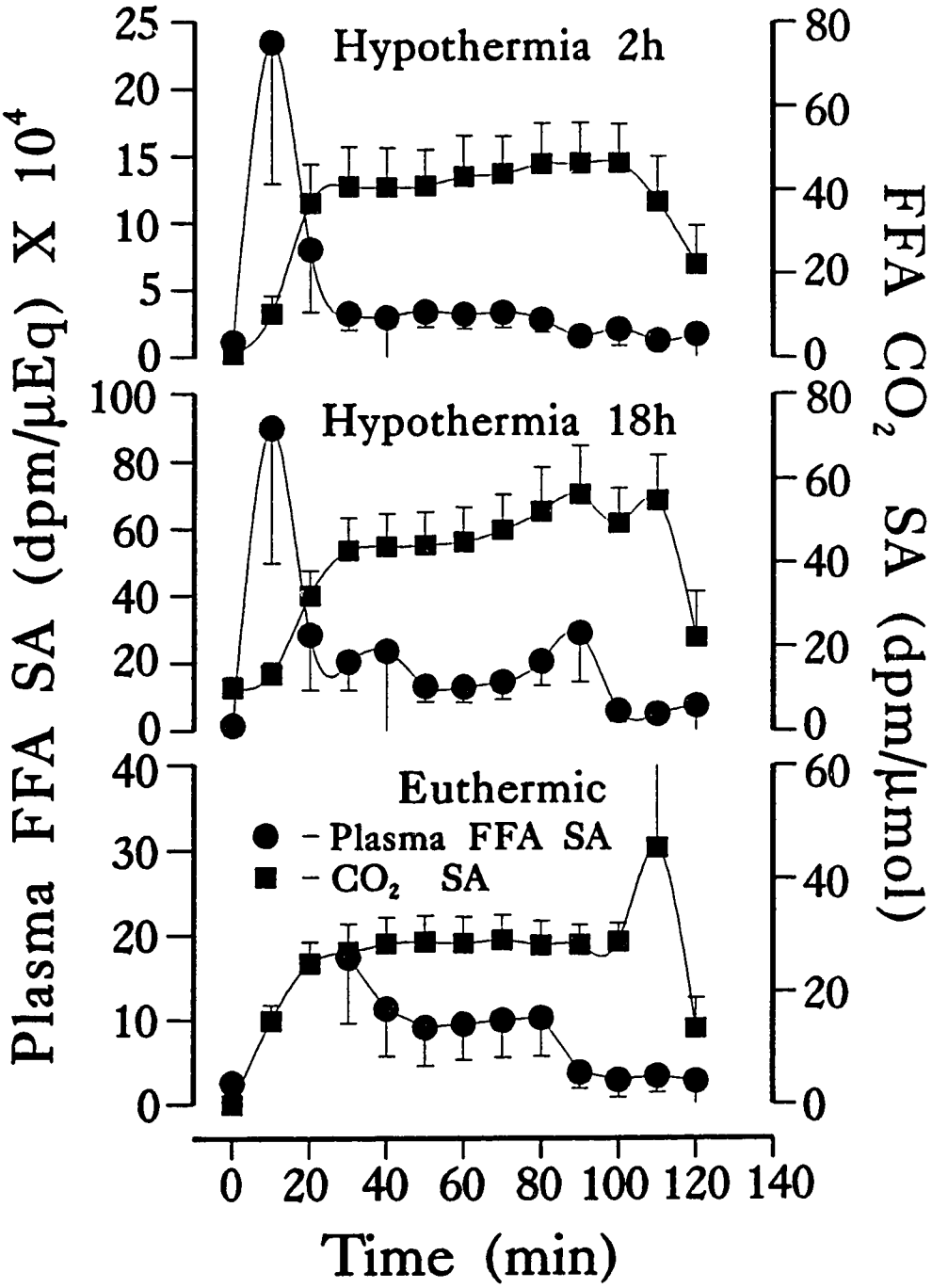
Figure VI.1: Specific Activity of plasma ^{3}H- (●) and ^{14}C-glucose (▼) and $^{14}CO_2$ (■) in euthermic, 2 h and 18 h hypothermic rats. Plasma glucose SA is divided by 10^3 and respiratory CO_2 is divided by 10^2. Time is minutes after the start of constant infusion of 2-^{3}H- and U-^{14}C-glucose. Means, error bars for plasma SA are omitted for clarity; N=4 (euthermic), 6 (hypothermic).


Hypothermic 2h
-³H-glucose
-¹⁴C-glucose
-¹⁴C-CO₂
Hypothermic 18h
Euthermic
Plasma Glucose SA (dpm/mg) x 10³
Respiratory CO₂ (dpm/mg) x 10²
Time (min)
0 20 40 60 80 100 120 140
360 300 240 180 120 60 0
120 100 80 60 40 20 0
40 30 20 10 0
12 10 8 6 4 2 0
800 600 400 200 0

oxidation of the labelled glucose. After 60 min there was no significant difference between successive samples. In hypothermic rats, there was a constant increase in CO_2 SA throughout the infusion period for both group 2 and 3 rats (Fig. VI.1), even when the infusion was continued for over 3 h. This suggests that although plasma glucose SA is relatively stable total plasma activity (dpm/ml) is increasing due to an increasing incorporation of label into the substrate pool via partial break down products (ie. lactate and perhaps lesser amounts of octopine, succinate, propionate). Therefore, the ratio of label to glucose substrate continously increases such that there is an increased probability of label being oxidized and thus, a constantly increasing $^{14}CO_2$ SA. Estimates of oxidation based on the ratio of mean CO_2 dpm vs mean plasma pool dpm's over the period 60-90 min indicate that there is a significant decrease in glucose oxidation in both hypothermic groups (34.7±2.7%, 0.6±.06% and 0.7±0.05%, representing approximately 13312.2, 61.1 and 114.2 μg/kg/h for euthermic, group 2 and group 3 hypothermic rats, respectively). This probably represents an over-estimation of glucose oxidation, due to the above stated increase in plasma label, and as such suggests that glucose oxidation is severely limited in hypothermia.

The mean Plasma FFA concentration (928.5±144.9 μEq/l) in euthermic rats was significantly higher than group 3 hypothermic rats (663.8±65.2 μEq/l) and significantly lower than group 2 hypothermic rats (1213.0±54.4 μEq/l; Table VI.2). As shown in figure VI.2 the pattern of FFA SA was also similar in all groups. FFA SA plateaued over the first 30-50 min and then remained relatively constant over the rest of the infusion period. While the SA for plasma FFA was not absolutely stable over the period used for calculation of replacement rate (Fig. VI.2) there was no significant difference between individual values, at 60-90 min, or between the replacement rate calculated using Nesbakken's (19) equation for changing SA and those calculated using the standard formula shown above over the same period. Therefore, the standard formula was used to maintain consistency with

Figure VI.2: Specific Activity of plasma ^{14}C-FFA (▼) and respiratory $^{14}CO_2$ (■) in euthermic, 2 h and 18 h hypothermic rats. Time is minutes after the start of the constant infusion of U-^{14}C-palmitic acid. Means, error bars for plasma SA are omitted for clarity; N=5 (euthermic), 6 (hypothermic).


Hypothermia 2h
Hypothermia 18h
Euthermic
● - Plasma FFA SA
■ - CO_2 SA
Plasma FFA SA (dpm/μEq) X 10^4
FFA CO_2 SA (dpm/μmol)
Time (min)
0 20 40 60 80 100 120 140
25 20 15 10 5 0
100 80 60 40 20 0
40 30 20 10 0
80 60 40 20 0
60 40 20 0

glucose calculations. The replacement rate of FFA in euthermic rats (814.5±175.9 μEq/kg/h) was significantly higher than group 2 (258.6±55.1 μEq/kg/h) hypothermic rats. Replacement in the group 3 hypothermic rats (44.3±10.8 μEq/kg/h) was significantly lower than both euthermic and group 2 hypothermic rats.

Respiratory CO_2 production during the FFA infusion was again significantly different in all three groups (0.53±0.07, 0.18±0.01 and 0.10±0.01 cc/g/h for euthermic, group 2 and 3 hypothermic rats, respectively). In contrast to the CO_2 SA in glucose infused rats the CO_2 SA of FFA infused rats increase to a plateau within the first 20-30 min and then remain relatively stable for the remainder of the infusion period (fig. VI.2). Based on the ratio of mean CO_2 SA to mean plasma pool SA between 60 and 90 min and the calculated replacement rate there was a significant difference in the percent of CO_2 derived from FFA oxidation between all three groups (57.5, 13.8 and 5.6 % for euthermic group 2 and group 3 hypothermic rats, respectively; Table VI.2). Due to the differences in replacement rates between the three groups there was also a significant difference in oxidation rates (50.5, 2.03 and .1 μEq/kg/h for euthermic, group 2 and group 3 rats, respectively; Table VI.2).

DISCUSSION

In the present study, stable long-term hypothermia was induced in rats using a technique developed by Jourdan and Wang (15, Chap. I). This method incorporates short induction times, using light anaesthesia with halothane, and precise control of T_b during hypothermia by a computerized feed-back loop. Consequently, the hypothermic animal could be maintained at a T_b of 19°C for up to 24 hours with less than ±0.3°C variation. During induction into hypothermia at a T_b of 19°C, heart rate and metabolic activity (as measured by $\dot{V}CO_2$) were

Table VI.1

Influence of hypothermia on the biokenetics of glucose in rats.

Parameter	Euthermic	Hypothermic 2h	Hypothermic 18h
No. of Rats	4	6	6
Weight (g)	397±3	401±5	393±3
T_b (°C)	37	19	19
$\dot{V}CO_2$ (cc/g/min)	0.61±0.02	0.14±0.01	0.09±0.01
Plasma Glucose (mg/dl)	140.8±62.8	236.5±116.1	242.8±79.9
Glucose Pool (mg/kg)	79.6±2.9	43.3±3.6	53.9±31.3
Turnover Rate ^{3}H-(mg/kg/h)	39.1±2.1	11.5±2.0	16.1±4.2
Turnover Rate ^{14}C-(mg/kg/h)	72.4±6.8	23.5±5.8	34.8±5.5
Recycling (%)	45.9±2.7	51.1±3.0	53.6±1.2
Oxidation Rate (µg/kg/h)	13312.6±2.1	61.1±28.1	114.2±23.9
Ratio Oxidation to Turnover (%)	34.7±2.7	0.55±0.06	0.70±0.05
Respiratory CO_2 from glucose(%)	8.08	4.20	4.12

observed to decrease by approximately 60 (120 vs 300 bpm) and 65% (≈.15 vs ≈.59 cc/g/h) respectively. However, once T_b stabilized both heart rate and metabolic rate also stabilized and remained relatively constant throughout the experimental period. This new method then enabled us to evaluate metabolic adjustments during hypothermia under steady state conditions.

During cooling the plasma glucose concentration of hypothermic rats increased dramatically from 140.8±62.9 to about 236.5±105.8 mg/dl and then remained stable until the end of the hypothermic period (up to 20 h) (13). This rise in blood glucose suggests a transient imbalance between hepatic production and plasma clearance of glucose during cooling, probably due to the differential sensitivity of pancreatic A and B cell function to low temperature (13,14, Chap. IV & V). The observed increase in plasma catecholamine (18) and glucagon concentrations (14) during cooling stimulate glucose mobilization whereas the dramatic inhibition of insulin secretion and the subsequent cessation of glucose utilization by the insulin-dependent tissues at low T_b (13) could explain the marked increase in plasma glucose during hypothermia.

The steady-state, albeit elevated level of plasma glucose during hypothermia allowed us to investigate glucose turnover using the constant infusion method. Our observed tissue uptake and endogenous production (replacement or turnover rate) of glucose, of euthermic rats, based on U-^{14}C-glucose was 72.4±6.8 mg/kg/h. Approximately 45.9% of this apparent glucogenesis came from recycling of glucose since, the values based on 2-^{3}H-glucose were 39.1±2.1 mg/kg/h. In 2h hypothermic rats, the observed replacement rate of ^{3}H glucose (11.5±2.0 mg/kg/h) represents an approximately 70% decrease in glucose turnover. The apparent recycling (51.1%) however, was not different at this time. At 18h in hypothermia there was a slight rebound in glucose replacement and a significant increase in recycling. Turnover, based on 2-^{3}H-glucose in the 18h group (16.1±4.2 mg/kg/h) however, still represents

Table VI.2

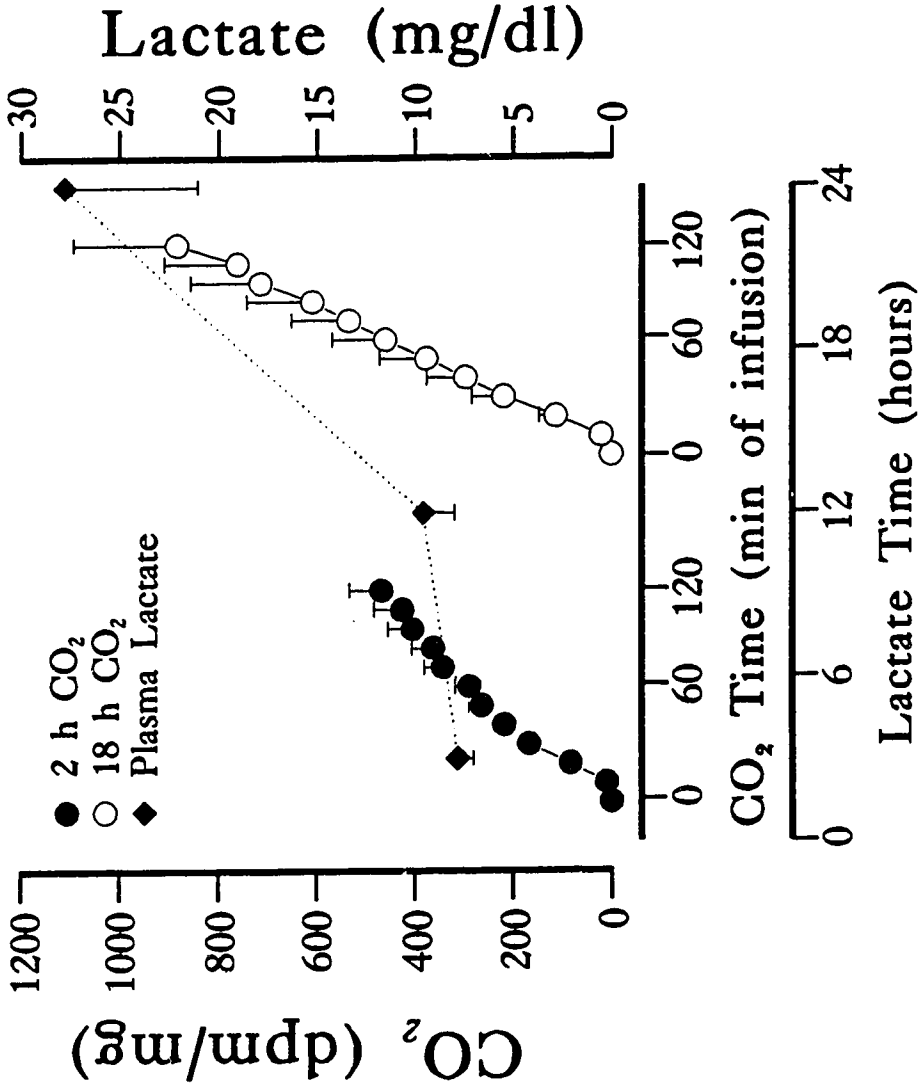
Influence of hypothermia on the biokenetics of FFA in rats.

Parameter	Euthermic	Hypothermic 2h	Hypothermic 18h
No. of Rats	5	8	8
Weight (g)	400±5	394±3	395±4
T_b (°C)	37	19	19
$\dot{V}CO_2$ (cc/kg/min)	0.53±0.07	0.18±0.01	0.10±0.01
Plasma FFA (μEq/l)	928.5±144.9	1213.0±54.4	663.8±65.2
Plasma Pool (μEq/kg)	28.5±0.6	49.4±1.3	24.9±1.5
Turnover (μEq/kg/h)	814.5±175.9	258.6±55.1	44.3±10.8
Oxidation Rate (μEq/kg/h)	50.5±2.8	2.03±0.12	0.10±0.01
Ratio Oxidation to Turnover (%)	6.2±1.6	0.78±0.22	0.23±0.10
Respiratory CO_2 from FFA (%)	57.5	13.8	5.6

a 59% decrease from that of euthermic animals; recycling, on the other hand, increased further to 53.6%, and was significantly higher than the euthermic group. An interesting observation in relation to the plasma SA is the apparent exaggerated cyclic pattern. This seems to suggest that there was circulatory insufficiency which could result in exaggerated pulsatile flow and/or periodic interruption of flow. This could be producing or resulting in localized areas of poor blood flow (pooling of blood) leading to the build up of partial glucose oxidation products such as lactate. This interpretation is supported by reports of poor cardiac performance (2,6), sludging of the blood and cell aggregation (5,16). Further, hypothermic rats are observed to periodically display a whole body contraction (shiver) which would help to mix, displace and distribute blood pooled in various places in the periphery and thus periodically increase the concentration of label in the sampling pool and enhance the cyclic appearence of the plasma SA. Therefore, the increase in turnover and recycling in the 18h group could represent either a further breakdown of the glycolysis - TCA cycle pathways or an inefficient circulation such that there is reduction in the complete oxidation of glucose and an increase in partial oxidation products, such as lactate (see chap. III), and thus an increase in recycling of glucose carbon. On the other hand, it could be a last effort of the hypothermic rat to maintain energetic balance since it occurs at a time when FFA concentrations, turnover and oxidation are significantly decreased (see below).

No direct connection can easily be made between respiratory CO_2 production and substrate oxidation, particularly due to the somewhat peculiar CO_2 SA responses of the hypothermic rats (fig. VI.1). This constantly increasing $^{14}CO_2$ SA could be due to an increasing release of labeled partial oxidation products of glucose (eg. lactate) from tissues into the plasma pool. Thus, while plasma glucose SA is relatively stable there is an extra labelled partial oxidation product contributing to the $^{14}CO_2$ SA. This is supported by the correlation between the rate of increase in CO_2 SA and plasma lactate concentrations (Fig. VI.3 and

Figure VI.3: Relationship between plasma lactate (◆) and respiratory $^{14}CO_2$ SA in 2 h and 18 h hypothermic rats. Time for lactate is hours after the start of hypothermic induction (Chapter VII). Time for CO_2 is minutes after the start of the constant infusion of 2-^{3}H- and U-^{14}C-glucose. Means±sem; N=6.


Lactate (mg/dl)
30
25
20
15
10
5
0
CO_2 (dpm/mg)
1200
1000
800
600
400
200
0
2 h CO_2
18 h CO_2
Plasma Lactate
0
60
120
0
60
120
CO_2 Time (min of infusion)
0
6
12
18
24
Lactate Time (hours)

Chap. III). Therefore, calculation of oxidation based on CO_2 is probably an over-estimation of prevailing glucose oxidation but more accurately a representition of overall carbohydrate oxidation. The decrease in respiratory CO_2 in conjunction with a significant decrease in pool size (79.8±2.9, 43.3±3.6 and 53.9±31.3 mg/kg for euthermic, group 2 and 3 hypothermic rats, respectively; Table VI.1) suggests that there is significantly less oxidition in both groups of hypothermic rats. Indeed, estimates based on the ratio of CO_2 SA to plasma pool SA suggest that glucose oxidation in hypothermic rats is .45 and .85%, for group 2 (61.1±28.1 µg/kg/h) and 3 (114.2±23.9 µg/kg/h) respectively, of that in euthermic rats (13312.6±2.1 µg/kg/h). This apparent severe reduction in glucose oxidation in conjunction with the recycling mentioned above and increasing lactate concentration (Fig. VI.3 and Chap. III) suggest an increase in anaerobic glycolysis and dependence on partial oxidation products (eg. lactate) with time in hypothermia and further, suggests that tissue oxygenation may be insufficient (or perhaps intermittent).

Typically the rate of removal of FFA from the plasma is closely correlated with the concentration of FFA in the plasma in both euthermic and mildly hypothermic humans, rabbits and dogs (1,15). Based on the data presented here this does not appear to be strictly the case in severely hypothermic rats. Although plasma FFA concentration increased significantly (1213.0±54.4 vs 928.5±144.9 µEq/l) in the group 2 rats the replacement rate was significantly depressed (258.6±55.1 vs 814.5±175.9 µEq/kg/h). Processes involved in FFA turnover such as activation, carnitine-mediated transfer, storage, or oxidation are all enzymatically mediated (26). These steps are therefore temperature-dependent and may be partially responsible for the reduced FFA turnover observed in group 2 hypothermic rats. As hypothermia progressed FFA concentration decreased significantly (663.8±65.2 µEq/l) as did the rate of removal (69.9±14.6 µEq/kg/h). Since hyperglycaemia is known to inhibit lipolysis (19) via enhanced FFA re-esterification in adipose tissue,

it is possible that the hyperglycaemia observed in our rats is depressing lipolysis resulting in the decline in FFA concentration in spite of the reduced FFA removal in 2 and 18 h hypothermic rats. However, it seems more likely that at low T_b (19°C) there is a decrease in the volume of distribution for FFA's which limits the distribution of FFA's to some tissues. This in conjunction with decreased heart rate (ie. reduced tissue flow) yields a decrease in FFA turnover.

As shown in fig. VI.2, CO_2 SA suggests that the proportional contribution of FFA oxidation to $\dot{V}CO_2$ during hypothermia is increased over that seen in euthermia. Indeed, the SA of hypothermic rats (44.1±14.7 and 49.1±17.4 dpm/mmol for group 2 and 3 rats, respectively) is 1.5 and 1.7 times that of the euthermic rats (28.6±12.8 dpm/mmol) (Fig. VI.2) and suggests that a greater proportion of the plasma FFA is contributed to $\dot{V}CO_2$ via FFA oxidation during hypothermia. Since overall metabolic activity is reduced (0.18 and 0.10 cc CO_2/g/h vs 0.53 cc CO_2/g/h for hypothermic and euthermic rats, respectively; Table VI.2) a significant decrease in the estimated FFA oxidation (2.0, 0.1 vs 50.5 µEq/kg/h), in hypothermic rats is expected. However, it is obvious that the decrease in FFA oxidation is much greater that would be expected from $\dot{V}CO_2$ alone (34 and 18% for CO_2 vs 4 and 0.19% for oxidation between euthermic and group 2 and 3 hypothermic rats, respectively). It is not clear at this time why the CO_2 SA is higher in the hypothermic rats. Perhaps the extra label appearing in the CO_2 of hypothermic rats arises from an enhanced ketone body production due to the accumulation of acetylCoA in arising from the depressed glucose oxidation. No information is currently available on ketone production or utilization in hypothermia, however, hibernating rodents appear to rely on ketones during hibernation bouts (11). While the absolute oxidation of FFA's is depressed in hypothermia, the proportion of FFA contributing to $\dot{V}CO_2$ is greater than glucose (1:6.18, 1:4.22 and 1:1.51 for euthermic, 2h and 18h hypothermic rats, respectively) in all groups. What this seems to suggest is that while the hypothermic rat would prefer to utilize FFA it

is unable to maintain sufficient plasma concentrations to support even depressed oxidative activity. This is probably due to reduced heart rate and tissue distribution and possibly also to the inhibition of lipolysis, due to hyperglycaemia, and depressed oxidative activity at low T_b.

In hypothermic rats, relatively high glycogenolysis leads to a rapid exhaustion of hepatic glycogen (27,28), and results in hyperglycaemia. This leads to a urinary loss of glucose and possibly osmotic disorders but not to an increased utilization of glucose. Further, there appears to be a preferential utilization of FFA in hypothermic rats but as hypothermia progresses this is hampered by depressed FFA mobilization. In hibernators, the plasma glucose concentration is generally low (27) as is glucose turnover (11), while gluconeogensis is relatively active (6,9). Further, there is preferential utilization of lipids (30). These results suggest that glucose is spared during hibernation. This major difference in glucose metabolism could in part explain why survival in hypothermia of non-hibernating mammals is generally limited to less than 24 h vs days or weeks in hibernators and why survival in hypothermia can be prolonged in some species (e.g. hamsters) when glucose metabolism, during hypothermia, is maintained (22). Thus, it would appear that remedial measures taken to control hyperglycaemia and enhance glucose and FFA metabolism in hypothermic animal may contribute to the enhancement of hypothermic survival. Further, this data suggests circulatory insufficiency and poor tissue oxygenation may in part be responsible for the observed decrease in glucose and FFA oxidation.

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VII. PROLONGED STABLE HYPOTHERMIA: EFFECT ON BLOOD GASES AND PH IN RATS [7]

INTRODUCTION

Hypothermia is a non-facultative state that results when heat loss surpasses metabolic heat production. Unlike hibernating animals, which can return to euthermia spontaneously using endogenously produced heat, hypothermic animals require the application of exogenous heat to regain the euthermic body temperature (T_b). In the absence of an exogenous heat source, the hypothermic animal survives by virtue of its innate cellular cold tolerance until critical failures occur and death becomes inevitable. The limitations for survival in the hypothermic animal are not clear, but appear to include impairment of cardiovascular function and peripheral blood flow, O_2 uptake and delivery to tissues, and substrate mobilization and utilization at low T_b (5, 14, 21, 24).

It has been known for some time that hibernators, such as hamsters and ground squirrels, are able to survive experimentally-induced hypothermia for a longer time than are non-hibernators, such as rats and dogs. Generally, the longer survival time of the hibernator is assumed to be due to its higher intrinsic cellular cold tolerance, leading to maintenance of nervous, cardiovascular and respiratory system function, tissue O_2 delivery, and substrate homeostasis during hypothermia (14, 21). There have been, however, few systematic studies of the effects of prolonged hypothermia on regulation of blood gases and pH in non-hibernators. In part, this is because studies of physiological homeostasis in hypothermic animals, whether hibernators or non-hibernators, have been hindered by the

7. A version of this chapter has been submitted for publication to the American Journal of Physiology.

instability of the hypothermic preparation and the resulting short-term survival of the hypothermic animal. Clarification of the innate ability of the hypothermic animal to regulate blood gases and pH is complicated further in some studies by the use of high inspired CO_2/low inspired O_2 (Giaja technique) to aid induction of hypothermia (23-25), and by mechanical ventilation with or without high O_2 (9, 20).

The development of an experimental model (16 and Chapter I) that enables the induction and maintenance of stable hypothermia for prolonged periods has overcome many of these obstacles. By this method, heat production is depressed transiently by light halothane anesthesia (1.5-2%) and heat loss is increased by exposure to cold and HeO_2 (79% helium, 21% Oxygen), providing rapid lowering of T_b with minimal stress to the animal. During hypothermia, the animal is exposed to cooled room air, and the desired low T_b is maintained within ±0.2°C by a computerized feedback loop regulating the ambient temperature (T_a). Apart from the control of T_b, the hypothermic animal is given free reign over maintenance of physiological homeostasis, allowing elucidation of the factors governing, and perhaps limiting, survival during prolonged hypothermia. In this model a non-hibernator, such as the rat, is able to survive hypothermia at a T_b of 19°C for 20-24 hours with full recovery.

The role of changes in regulation of blood gases and pH in determining hypothermic survival in small mammals has been examined in few previous studies. Musacchia and coworkers (1, 26, 29) concluded that the primary cause of death in both hypothermic rats (T_b=15°C) and hamsters (T_b=7°C) was respiratory failure due to glucose depletion, especially during prolonged hypothermia. Uchida et al. (28), however, felt that during acute hypothermia at T_b=18°C respiratory depression (and acidosis) caused death in rats while carbohydrate depletion caused death in hamsters. The aspect of carbohydrate metabolism in prolonged

hypothermia has been addressed extensively, in our lab (11, 13 and Chap. 3-6) and by others (1, 21, 23, 24, 26, 28). To our knowledge, however, there is little information available concerning homeostasis of blood gases and pH during prolonged hypothermia in small rodent non-hibernators. Ventilatory control appears depressed in hypothermic rodents, and spontaneous respiration usually ceases before cardiac activity does (1, 21, 28, 31). Changes in respiratory function may, therefore, be an important indicator of survival at low T_b.

The present study concentrates on aspects of blood gas and pH homeostasis in prolonged stable hypothermia, at the T_b yielding maximal survival, in a non-hibernator, the rat. The primary objective was to evaluate the ability of the animal to maintain stable blood oxygenation and acid-base status during prolonged stable hypothermia and whether these parameters may be involved in determining hypothermic survival in this species.

METHODS

Male Sprague-Dawley rats ≈400 g were used in this study. All animals were housed individually in shoe box cages at a T_a of 22±1°C under 12L:12D or natural photoperiod. Rats were given rations of Purina lab chow to maintain mass near 400 g and had free access to water. Experiments were performed in the fall (September to December).

The left common carotid artery and right jugular vein were cannulated (PE50) under halothane anesthesia (1-1.5%), as previously described (11, 15). The tips of the cannulae were positioned about 3 cm into the vessels; that of the venous cannula was situated at the junction of the anterior and posterior vena cavae, at the entrance to the right atrium. The carotid cannula was placed at the junction

of the carotid artery and the aorta. The cannulae were exteriorized and flushed with heparinized isotonic saline (50 U/ml) to maintain patency between samples.

Hypothermia was induced immediately following cannulation by the method of Jourdan and Wang (16 and Chap. I). Briefly, a rectal thermocouple (copper-constantan) was inserted 5 cm beyond the anus and taped to the tail. Stainless steel needle ECG electrodes were attached subcutaneously to the right foreleg and left hind leg. The animal was transferred to a water-jacketed Plexiglas chamber at T_a=0°C under HeO_2 (79% He-21% O_2) atmosphere containing 1.5% halothane in an open-flow system. The concentration of halothane was decreased gradually by ≈0.1% per 1°C drop in T_b, and was discontinued when the T_b of rats reached 28°C (Fig. VII.1). At a T_b of 23°C the HeO_2 was replaced by precooled room air and T_a was raised to cool the animal more slowly to 19°C (Fig. VII.1). This period of slow reduction of T_b facilitates halothane washout from the hypothermic animal. To maintain T_b at the desired level, T_a was continuously adjusted by a computer-assisted negative feedback loop using the difference between the animals T_b and the desired set-temperature (i.e. 19°C) as the error signal to alter the temperature of the circulating water bath that perfused the animal chamber. In this way, T_b could be controlled to within ±0.2°C of 19°C for as long as was required by the experimental protocol. Further, the difference between T_b and T_a gives an estimate of the "metabolic resistance" of the hypothermic animal (Fig. I.2, Chap. I), because it reflects the degree of exogenous cooling required to counter the endogenous thermogenic efforts of the animal. The T_b used was optimal for survival of prolonged stable hypothermia. At lower T_b, survival times are shortened, and at higher T_b, it is difficult to maintain a stable T_b due to the thermogenic capacity of the animal.

Blood samples were taken from both cannulae prior to induction of hypothermia (*time -2 h*) and at intervals during the course of hypothermia (*time*

4, 12, 24 h). The P_{O_2}, P_{CO_2} and pH were measured from each sample (0.2 ml) immediately after sampling using a Radiometer BMS3 Mk2 Blood Gas Analyzer set at 25°C. The blood gas analyzer was calibrated before each measurement using two levels of CO_2 produced by a Radiometer GMA2 Precision Gas Supply and two phosphate buffers (Radiometer pH 7.383 and 6.841 Precision buffer solutions at 25°C using the temperature conversion provided with the buffers). Blood gases and pH were measured at constant temperature (25°C) to facilitate direct comparison of data from *in vivo, in vitro* studies and from animals at different T_bs, for example, during rewarming from hypothermia (19, 27; see Discussion for further explanation and Table VII.II for values converted to 19 and 37°C). Consequently, in most cases, the values reported here do not represent the actual *in vivo* values at a given T_b. Arterial and venous variables at 25°C are indicated as $^{25}PaO_2$, $^{25}PaCO_2$ and ^{25}pHa and $^{25}PvO_2$, $^{25}PvCO_2$, and ^{25}pHv, respectively, to distinguish them from values either measured at or corrected to actual T_b. Blood gas and pH values given in Table VII.2 were corrected to T_b or 37°C by the methods of Ashwood (2).

Lactate concentration was determined from duplicate 20 µl samples of arterial plasma by LDH-catalyzed oxidation of lactate and NADH to pyruvate and NAD^+. The production of NAD^+ was determined spectrophotometrically at 339 nm; all chemicals and reagents were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). The hematocrit of arterial blood was measured using 75 µl heparinized capillary tubes centrifuged at 10,000 rpm for 5 minutes.

All results are expressed as mean±SE. Time-dependent comparisons were performed using one-way analysis of variance, with post hoc multiple comparisons using Student-Newman Keuls or Scheffe's procedures, where appropriate.

RESULTS

The changes in hematocrit during hypothermia are shown in Fig. VII.1. Hematocrit did not change significantly during induction of hypothermia. After 4 h of hypothermia, however, hematocrit increased steadily and significantly with time at T_b=19°C, reaching 60% by 24 h (Fig. VII.1).

Immediately following surgery and prior to induction of hypothermia (time -2 h), $^{25}Pa_{O_2}$ and $^{25}pH_a$ were lower and $^{25}Pa_{CO_2}$ was higher than in unanesthetized euthermic rats (Table VII.1) probably due to the depressive effect of anaesthesia on ventilation and T_b, which fell a few degrees, during surgery.

During induction, arterial and venous $^{25}P_{O_2}$ and $^{25}P_{CO_2}$ were increased, and ^{25}pH decreased, compared to pre-induction values (Fig. VII.2). The arterial $^{25}P_{O_2}$ value of the first hypothermic sample (77.1 torr) and all following samples were significantly higher than that of the pre-induction sample (18.9 torr), but not significantly different from each other. Venous $^{25}P_{O_2}$ at 4 h was also significantly higher (28.0 torr) than pre-induction values (8.4 torr), however, it then decreased over the rest of the hypothermic period to a level (3.6 torr) at 24 h which was significantly ($P < 0.001$) lower then that at 4 h but not significantly different than the pre-induction level (Fig. VII.2).

Arterial and venous $^{25}P_{CO_2}$ values were also significantly higher (49.9 and 54.1 torr respectively) than pre-induction values (Fig. VII.2). Venous $^{25}P_{CO_2}$ values were essentially constant during hypothermia, while $^{25}Pa_{CO_2}$ showed a significant decline (Fig. VII.2) at 24 h.

Both $^{25}pH_a$ and $^{25}pH_v$ decreased significantly during induction (7.4 to around 7.2) and continued to decrease during the rest of the hypothermic bout

Table VII.1. Effect of halothane on arterial blood gases and pH in euthermic rats.

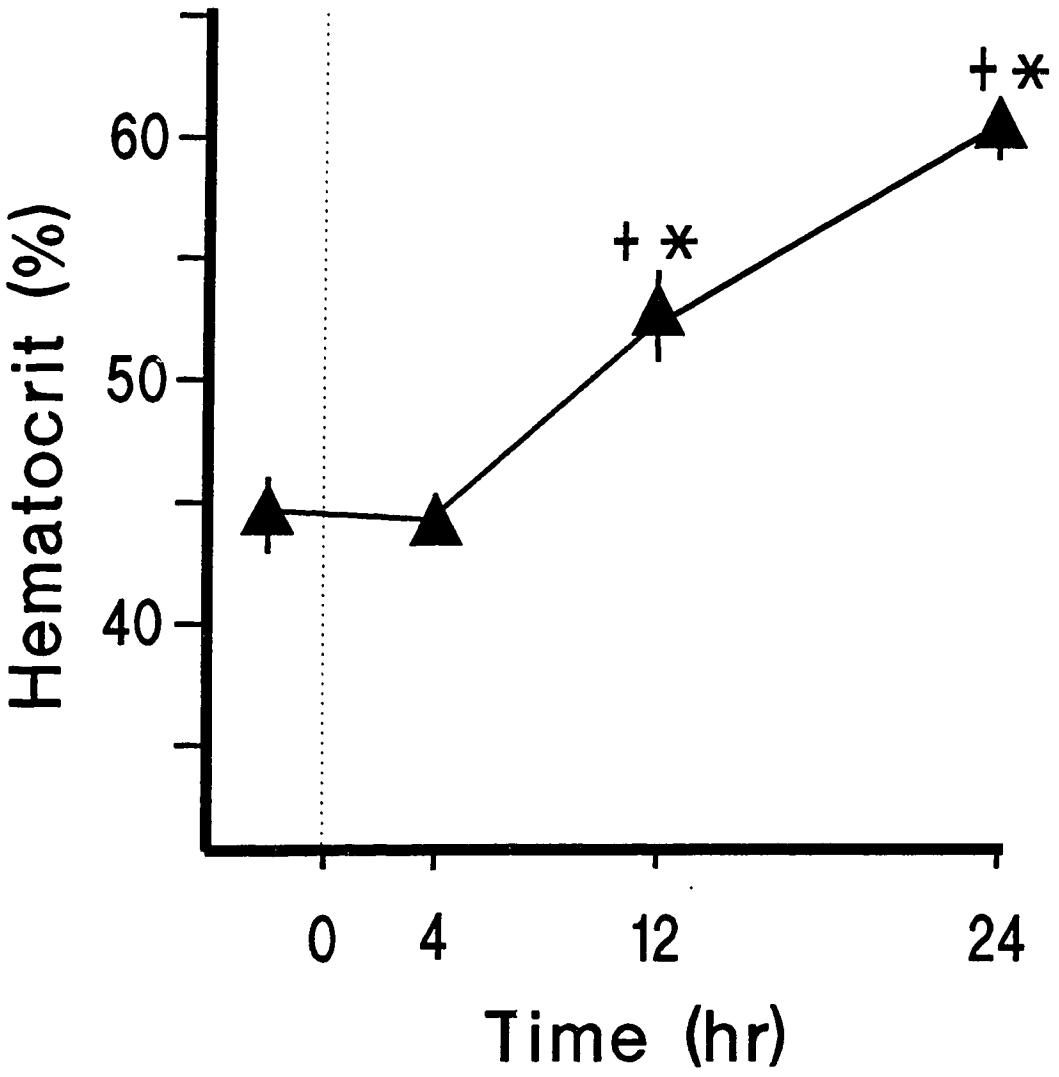
	T_b	N	$^{25}pH_a$	$^{25}P_{aCO_2}$ (torr)	$^{25}P_{aO_2}$ (torr)
unanesthetized	37	6	7.668	19.5	38.0
			±0.01	±0.4	±1.0
halothane	33.7	14	7.468*	27.2*	18.9*
anesthetized	±0.3		±0.01	±0.9	±1.7

Values are means ±SE of variables measured at 25°C. N = number of animals; T_b = body temperature. Halothane exposure (1.5-2%) corresponds to that prior to induction of hypothermia. * Significantly different from unanesthetized by *t*-test, $P < 0.05$.

Table VII.2 Blood gases and pH of hypothermic rats: comparison of mean values measured at 25°C and corrected to 19 and 37°C.

T_m	Sample (time)	T_b (°C)	pH_a	P_{aCO_2} (torr)	P_{aO_2} (torr)	pH_v	P_{vCO_2} (torr)	P_{vO_2} (torr)
25°C	-2h	33.8	7.47	27.4	18.9	7.45	29.3	8.4
	4h	19.5	7.19	49.9	77.1	7.17	54.1	28.0
	12h	19.1	7.13	47.8	85.1	7.11	59.2	18.5
	24h	19.1	7.13	37.8	83.8	7.08	59.6	3.6
19°C	-2h	33.8	7.33	41.8	35.3	7.32	44.7	16.0
	4h	19.5	7.27	37.8	53.6	7.26	41.2	19.0
	12h	19.1	7.23	35.5	58.2	7.24	44.0	12.1
	24h	19.1	7.23	27.9	57.2	7.17	44.6	2.4
37°C	-2h	33.8	7.29	47.9	44.3	7.28	51.0	19.7
	4h	19.5	7.02	84.2	170.8	7.00	90.7	65.6
	12h	19.1	6.96	80.7	184.1	6.98	98.7	43.4
	24h	19.1	6.95	64.5	182.1	6.91	99.1	8.4

Figure VII.1: Hematocrit changes in hypothermic rats. Values are mean±sem for 8-16 rats. * Significantly different ($P < 0.05$) compared to -2 h and 4 h. *Time -2 h*, sample taken immediately after cannulation; *time 4 h*, sample taken at stable hypothermic T_b (≈3-4 h after the start of hypothermic induction); all other times indicate time in hypothermia.


Hematocrit (%)
60
50
40
+ *
+ *
0
4
12
24
Time (hr)

(Fig. VII.2). However, only $^{25}pH_V$ was significantly lower at 24 h than the value at 4 h (7.07 vs 7.17, Fig. VII.2).

The changes in arterial plasma lactate concentration are shown in Fig. VII.3. Prior to induction of hypothermia, the rats had slightly elevated plasma lactate level (1.8±0.2 mM) compared to the first hypothermia sample (4 h, 1.7±0.2 mM). During hypothermia, there was a pronounced increase in plasma lactate to a peak at 24 h of 5.5±1.6 mM.

DISCUSSION

The different temperatures at which blood gas and acid-base variables are reported in various hypothermia studies impedes direct comparison of results, since interpretation of values reported at T_b differs from that of values reported at constant temperature (2, 8, 19, 27, 32). In most cases, comparison of blood acid-base variables measured at (or corrected to) T_b indicates no change or a mild respiratory alkalosis in hypothermia. Comparison of variables measured at (or corrected to) a constant temperature indicates instead an apparent respiratory acidosis in hypothermia.

In previous studies of experimental hypothermia in small mammals blood gases and pH were measured at T_b of the hypothermic animal, leading to the conclusion that hypothermic animals were able to regulate ventilation and acid-base state within normothermic limits (17, 28, 29, 31). In fact, when compared at constant temperature, the blood gas and pH values reported in hamsters and rats indicate acidification during hypothermia. For example, Volkert and Musacchia (29) state that "ventilation was sufficient to maintain a rather constant acid-base balance" in hypothermic hamsters, on the basis of pH and P_{aCO_2} measured at T_b (7.348 and 56 torr at T_b=38°C; 7.455 and 29.3 at T_b=10°C). Correction of the pH

Figure VII.2: Blood gases and Ph during hypothermia in rats at T_b=19°C. Values are mean±sem for 8-16 rats. $^{+}$ significantly different ($P < 0.05$) compared to initial value (*time -2 h*); * significantly different ($P < 0.05$) compared to first hypothermic sample (*time 4 h*).

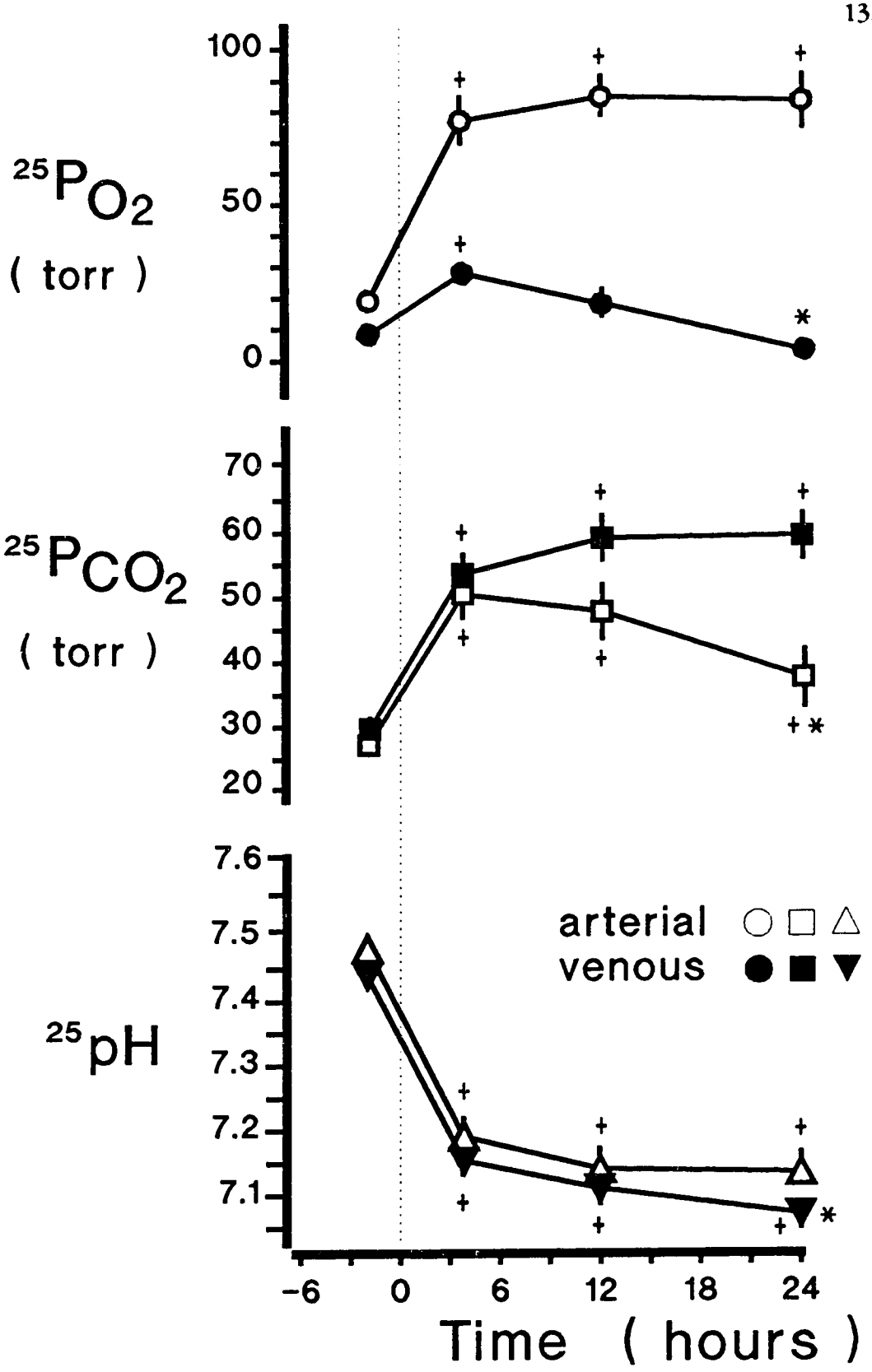
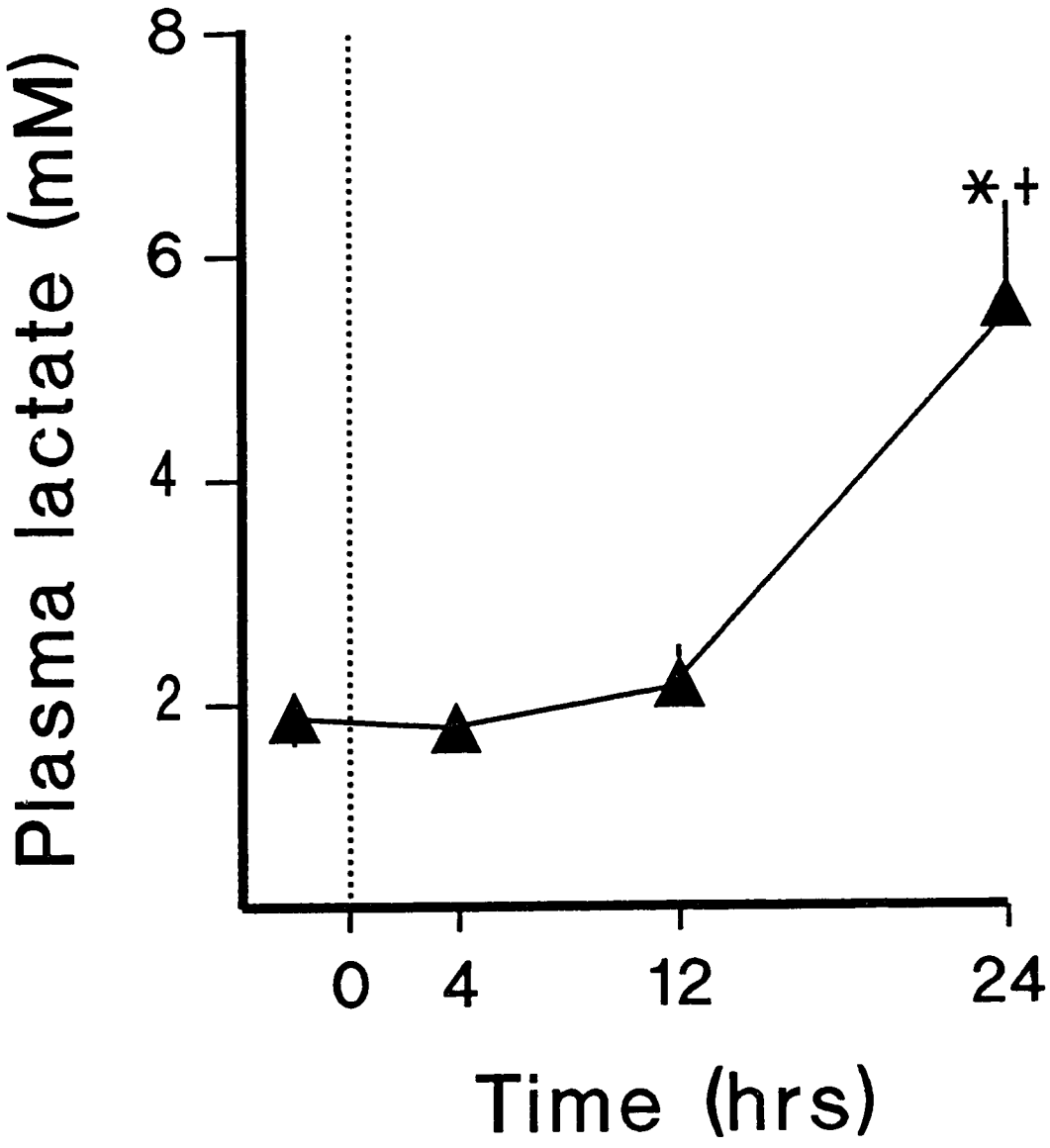

$^{25}P_{O_2}$
(torr)
100
50
0
$^{25}P_{CO_2}$
(torr)
70
60
50
40
30
20
^{25}pH
7.6
7.5
7.4
7.3
7.2
7.1
arterial ○ □ △
venous ● ■ ▼
-6
0
6
12
18
24
Time (hours)

Figure VII.3. Plasma lactate concentration in hypothermic rats. Values are mean±SE for 7-14 rats. * significantly different ($P < 0.05$) compared to values at *times -2, 4, 12 h.*


Plasma lactate (mM)
8
6
4
2
*+
0
4
12
24
Time (hrs)

and Pa_{CO_2} of hamsters at T_b=10°C to 38°C gives values of 7.045 and 112 torr, respectively, which are quite acidified compared to the values of euthermic hamsters at T_b=38°C.

The concept of measuring blood gases and pH at constant temperature during T_b transients stems from the temperature-dependence of the definitions of neutrality and of acid-base state, so that comparison of variables measured at different T_b is confounded by the additional influence of T_b itself (2, 19, 32). For comparison of species or states having different T_b, one choice is to compare temperature-independent variables (e.g. CO_2 content or the dissociation ratio of imidazole (alpha imidazole)); animals are considered to have maintained a constant acid-base state if these variables are constant despite alterations of T_b (19, 32). An alternative is to compare temperature-dependent variables (e.g. pH, P_{CO_2}) at a constant temperature, and to interpret the data as reflecting the overall acid-base state of the animal rather than the actual *in vivo* values (2, 19, 27). In this case, a constant acid-base state exists if pH and P_{CO_2} follow the predicted temperature-dependence under closed system conditions, and so are the same at any given constant temperature regardless of T_b. A parallel analysis can be made for the P_{O_2} and saturation state of the sample, assuming constant O_2 content in a closed system (2).

Malan (19, 27) has suggested that blood gases and pH be measured at a constant temperature when T_b is varied experimentally in non-clinical settings, particularly in comparative studies. He has proposed that 25°C be used, as it is a standard temperature for dissociation constants and is in the mid-range of T_b usually manifested by diverse groups of endothermic and ectothermic vertebrates, so that correction errors will be shared between high and low T_b (15, 19).

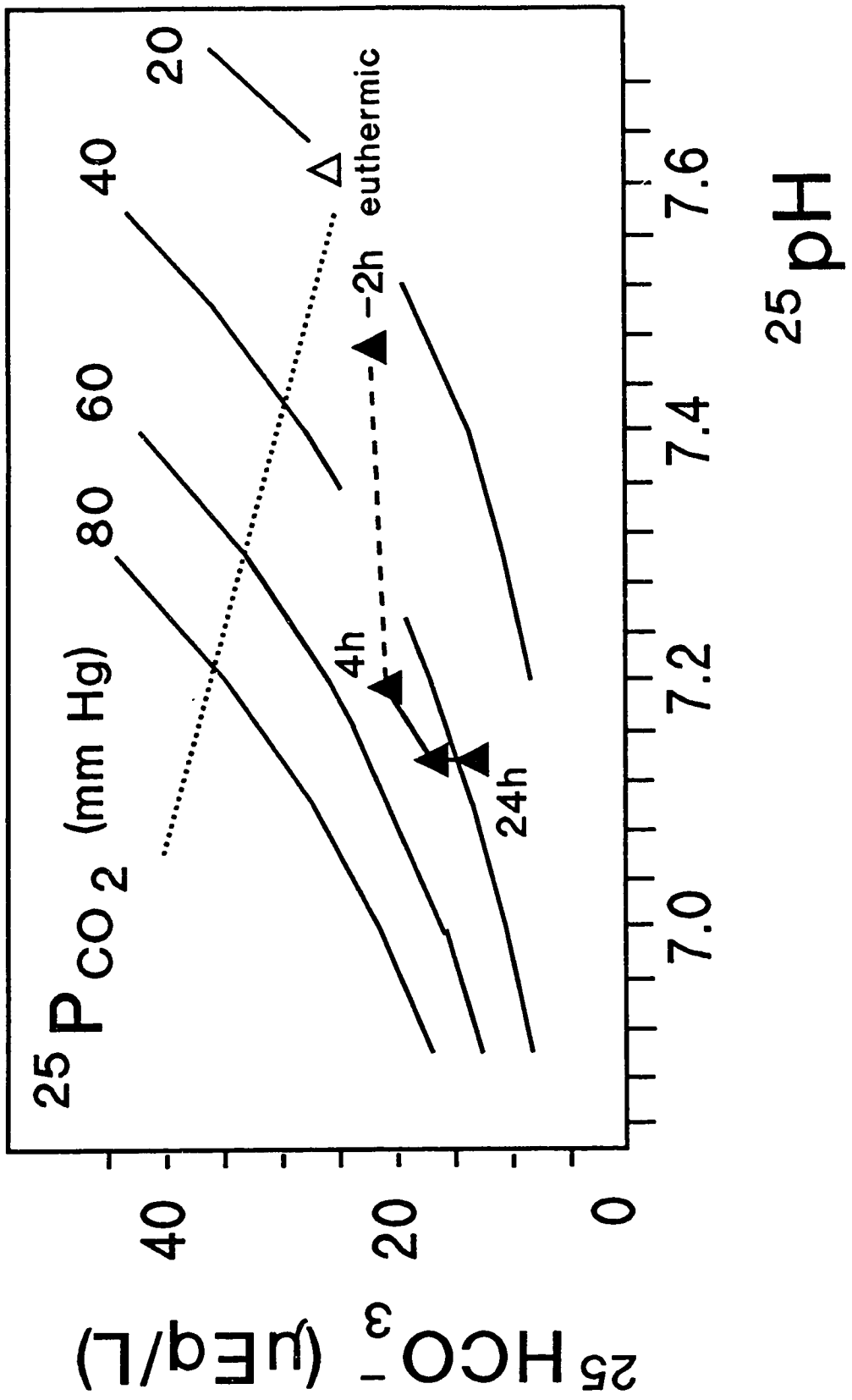
In the present study, we were interested in the patterns of change of blood gases and pH during prolonged hypothermia. We wanted to assess whether the

animal could maintain a constant acid-base status during prolonged hypothermia, as well as the changes in blood gases and pH that might indicate deterioration of homeostasis as hypothermia progressed. Furthermore, these data were intended to be compared eventually to blood gas and pH data collected at variable T_b during rewarming from hypothermia. For this reason, we chose to follow the method of Malan (19, 27) and to measure blood gases and pH in hypothermic rats at 25°C. However, for completeness in presentation, the mean blood gas and pH data for hypothermic rats measured at 25°C have also been corrected to actual T_b and 37°C [Table VII.2;(2)].

The overall pattern of changes in arterial blood acid-base state during hypothermia in rats is shown in Fig. VII.4. When acid-base variables are presented as 25°C values, the reduction of T_b leads to an acidification characterized by increased $^{25}P_{aCO_2}$ and decreased $^{25}pH_a$ with little change in calculated $^{25}HCO_3^-$. Maintenance of stable hypothermia leads to the progressive development of metabolic acidosis. (Fig. VII.4).

Metabolic acidosis often occurs during accidental hypothermia in man, especially during rewarming (5). In experimental hypothermia it depends on species, on the techniques used to induce and maintain hypothermia (eg. anesthesia, muscle relaxants, artificial ventilation), and on the depth and duration of hypothermia (24). Consequently, varied responses are reported, particularly in studies of acute hypothermia. In non-hibernators without artificial ventilation, metabolic acidosis occurs due to increased plasma lactate (14, 24), but this does not appear true of golden hamsters (1, 29). The dramatic increase in plasma lactate between 12 h and 24 h in hypothermic rats probably includes accumulated lactate produced in hypoperfused tissues earlier in the bout that is washed out slowly and not metabolized by liver and heart at low T_b (24).

Figure VII.4. $^{25}HCO_3^-$ vs. ^{25}pH diagram for arterial blood, summarizing changes in relative acid-base state during induction and maintenance of hypothermia in rats. Values are shown for samples from hypothermic rats (n=8-16) at all times during the hypothermic bout (closed symbols). *-2 h* corresponds to the sample taken prior to induction, *4 h* to the first hypothermic sample, and *24 h* to the final hypothermic sample. Values for samples from euthermic, unanesthetized rats (n=7) are shown for comparison (open symbols). Arterial $^{25}HCO_3^-$ was calculated from the Henderson-Hasselbalch equation, using measured $^{25}PaCO_2$ and $^{25}pH_a$ values and the apparent pK'= 6.152 and CO_2 solubility = 0.0401 mmol/L/torr at 25°C (15, 23). The dotted line indicates the estimated in vitro buffer line for true plasma, calculated at 25°C using the buffer value of 30.8 mmol/L/pH unit given by Rodeau and Malan (23).


$^{25}HCO_3^-$ (μEq/L)
0
20
40
^{25}pH
7.0
7.2
7.4
7.6
$^{25}P_{CO_2}$ (mm Hg)
20
40
60
80
euthermic
-2h
4h
24h

In the terminal stages of hypothermia, the rats showed a partial respiratory compensation for the metabolic acidosis, reducing $^{25}PaCO_2$ to 31 torr by 24 h, and maintaining constant $^{25}pH_a$ despite the rise in plasma lactate at this time (Fig. VII.4). The partial compensation by hypothermic rats may be caused directly by the increased $[H^+]$, indicating retention of chemosensitivity of respiratory centers. It also may be an indirect consequence of hyperventilation due to either; 1) a mismatch between ventilation and depressed CO_2 production resulting from hypoperfusion, or 2) ventilatory stimulation (gasping) resulting from severe brain hypoxia in the late stages of hypothermia. Moreover, hypothermic rats are hyperglycemic at death (11,12,13 and Chap. III-VI), so that respiratory failure due to glucose depletion, as is suggested to occur in hypothermic hamsters (1, 21, 26, 29), appears unlikely.

Despite lower overall ventilation, pulmonary O_2 uptake is not reduced in short-term hypothermia, as normal or slightly elevated PaO_2 values (at T_b) were noted in spontaneously-breathing humans (5), rats (6, 28, 31), and hamsters (28, 29). Our data show that this is also true of rats during prolonged hypothermia at T_b=19°C.

The PaO_2 values of hypothermic animals are expected to saturate arterial blood fully due to the left-shift of the oxygen dissociation curve (O_2 half-saturation pressure, P_{50}, decreased) at low T_b (5, 14, 29). Normal PaO_2 and O_2 saturation do not necessarily reflect adequate tissue oxygenation, however, since in hypothermic animals cardiac output falls, peripheral resistance rises and tissue blood flow is decreased (5, 6, 20, 25). Conversely, if capillary beds remain open, increased erythrocyte transit time may enhance O_2 delivery, giving high or normal O_2 extraction ratios at low T_b (5, 9, 14, 20, 24).

The observed decrease in venous $^{25}PO_2$ with time at low T_b is consistent with previous reports of acute or prolonged hypothermia in humans (8), dogs (9,

20), rats (28), hamsters (28, 29) and woodchucks (7). If mixed venous P_{O_2} is considered an estimate of overall tissue P_{O_2} these data indicate a progressive decline in the oxygenation of at least some tissues with time in hypothermia. In our experiments, the decrease in $^{25}P_{vO_2}$ was, in fact, a reasonably good indicator of the declining condition of the hypothermic animals. At the limit of hypothermic survival, the $^{25}P_{vO_2}$ values of rats were very low (3.6 torr at 24 h). These values are also comparable to the terminal P_{vO_2} of 3.2 torr (corrected to T_b; $^{25}P_{vO_2}$=2.2 torr) reported by Gutierrez et al. (9) in dogs after 24 h of hypothermia at T_b=30.5°C, and are near the predicted critical P_{O_2} (6.9±1.4 torr at T_b=30.5°C; $^{25}P_{vO_2}$=4.7 torr) below which tissue O_2 uptake becomes limited by O_2 supply (6). This is supported by the observation that metabolic resistance and $\dot{V}O_2$, which increase initially and then decline gradually with time, are very low in the terminal stages of the hypothermic bout in both rats and ground squirrels (30; unpublished results).

Consistent with previous studies is the marked increase in hematocrit in hypothermic rats at 12 h and beyond (Fig. VII.1). Haematocrits of 50-70% are reported in most non-hibernating species in hypothermia (5, 13, 23, 24, 28) and also were observed in acute hypothermia in golden hamsters (28). Hemoconcentration in hypothermic animals appears to be due to one or more of the following: (i) a stress-induced splenic release of erythrocytes and/or cold-induced diuresis during induction of hypothermia (5, 14, 24); (ii) fluid shifts to interstitial compartments and the gastrointestinal tract during prolonged hypothermia (15); (iii) swelling of erythrocytes themselves. Hemoconcentration does not occur during induction in our rats, since hematocrit did not rise until 12 h (Fig. VII.1). Furthermore, preliminary data indicate that erythrocyte number does not increase during induction or the first 4 h of hypothermia in rats (D. Belke, unpublished observations); however, there is some indication of increased

erythrocyte fragility in blood from hypothermic rats, which suggests swelling of the rat erythrocytes (personal observations).

During hypothermia, high hematocrit and increased blood viscosity, concomitant with low cardiac output and intense peripheral vasoconstriction, result in aggregation of blood in capillary beds and poor tissue perfusion (5, 14, 24). In particular, hemoconcentration can reduce cerebral blood flow and compound the depressant effects of hypothermia on central regulation of cardiovascular and respiratory function (6, 9). Although the increased hematocrit of hypothermic rats probably is not the primary cause of decreased tissue blood flow, it would exacerbate other deleterious cardiovascular changes.

Hypothermic survival probably is not limited by a single component of the biological system, but rather by a cascade of failures of the control system, organ and tissue functions. Our results support the contention (6, 9, 20, 25) that decreased tissue blood flow and O_2 delivery are important factors limiting survival in hypothermia. The data suggest that nonhibernators experience a critical reduction of tissue oxygenation during prolonged hypothermia, indicated by the decrease in $^{25}P_{vO_2}$ and the increase in plasma lactate concentration, despite consistently high arterial $^{25}PO_2$ (Fig. VII.2 & 3). These observations suggest a time-dependent deterioration of the regulation of physiological homeostasis during prolonged hypothermia.

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GENERAL DISCUSSION AND CONCLUSIONS [8]

The clinical features and physiological changes in the function of different systems and organs of the body during cold stress and hypothermia have been well reviewed (18,23,42,53,55,69,70). Even though the physiological changes occurring during hypothermia have been extensively studied in the last twenty five years, data accumulated from actual human experimentation has been restricted to the measurement of various changes occurring only during the mildest levels of hypothermia (body temperature [T_b] > 34°C). From such a state, the subject usually has no problem rewarming and the physiological response to profound hypothermia is not fully activated. Much of the information on hypothermia has been gleaned from data collected from victims of accidental hypothermia. However, this compilation is complicated by the urgent and uncontrolled nature of the situation and often the presence of complicating factors, such as disease, drug or alcohol abuse, or age. Another clinical strategy for the study of hypothermia has been the use of patients undergoing cardiac or neurosurgical procedures at reduced T_b. Under the controlled conditions of the operating room, hypothermia to T_b's as low as 7°C has indicated that acute hypothermia probably does not significantly affect electrolyte balance, blood sugar or renal function (14).

Clinically, hypothermia is defined as a deep T_b (e.g. rectal) ≤35°C (2°C below its normal 37°C). Depending on the depth to which T_b is lowered (14,22), hypothermia can be characterized as mild (T_b = 32 to 35°C), moderate (T_b = 28

8. A version of this chapter has been published. Michael L. Jourdan, T.F. Lee and Lawrence C.H. Wang. 1991. Clinical Applications of Cryobiology, Brian W.W. Grout and Barry J. Fuller eds., CRC Press, chap. 2.

to 32°C) or severe (T_b < 28°C). Survival in man is usually associated with core temperatures above 25°C, however, survival below this T_b is possible providing appropriate treatment is employed (14). The clinical signs and functional changes that can be observed in hypothermic man depend on the extent to which T_b is lowered. One of the earliest signs of hypothermia is a change in personality. A person may become uncooperative and show signs of incoordination (23). Loss of consciousness is highly variable and may occur at T_b's as high as 33°C or as low as 27°C (69). Similarly shivering also has a variable threshold, it has been recorded in some victims with T_b's as low as 24°C while other victims never shiver (61), but generally shivering ceases at 30°C (75).

Mild levels of hypothermia are associated with intense sympathetic stimulation and concomitant increase in left ventricular work and cardiac index (14). These changes are associated with peripheral vasoconstriction resulting in increased systemic vascular resistance and hypertension (14,58). As hypothermia deepens there is a progressive fall in both left ventricular work and cardiac index to about 30% of the normothermic level at 25°C (37). Blood pressure, which would be expected to decrease as well, tends to stabilize below 30°C possibly due to continued peripheral vasoconstriction. At T_b's less than 25°C there is danger of fatal ventricular fibrillation unless rewarming and defibrillation procedures are initiated (69,58).

Hemoconcentration involving an increase in haemoglobin and hematocrit by more than 30% occur as T_b falls below 30°C (14). The mechanism of this fluid shift is not well understood but likely involves movement of water from the intra to extravascular space due to intense vasoconstriction and low flow rate. This in turn results in sludging of blood in the capillary beds and consequent poor tissue perfusion.

A progressive decrease in respiratory rate and tidal volume is observed during cooling to 30°C, however, the partial pressure of O_2 (P_aO_2) remains essentially normal (14). Initial intense shivering significantly elevates CO_2 production but as T_b falls CO_2 production is diminished. This in conjunction with increased solubility of CO_2 in blood at the lower T_b, tends to decrease P_aCO_2 despite diminished respiratory rate and tidal volume (14). The net effect of these changes is a mild respiratory alkalosis at the initial stage of acute hypothermia (14). The alkalosis is, however, gradually replaced by metabolic acidosis as cooling continues, possibly due to poor tissue perfusion and oxygenation as noted above.

From what has been described above regarding diminished O_2 delivery it might be assumed that cellular survival is jeopardized primarily due to diminished O_2 delivery with falling T_b. This may not be the case with acute hypothermia however, as cellular O_2 demand is also decreased with falling T_b. In fact diminished O_2 demand, in particular that of the brain, is one of the most important protective aspects of clinical hypothermia (28). However, despite the fact that hypothermia protects the brain from the effects of anoxia (20), prolonged survival in hypothermia is almost totally dependent on having sufficient cardiovascular function for adequate perfusion of important organ systems for the supply of substrates as well as O_2 (58).

Even though the final T_b of the subject may drop to the same level, the survival rate from clinically induced hypothermia is much higher than that of accidental hypothermia. It would be beneficial, therefore, to systemically compare the similarities and differences of these two different kinds of hypothermia for clues to improve hypothermic survival.

In the case of accidental hypothermia, homeotherms respond to severe cold with immediate activation of heat production as well as inhibition of heat loss. There are of course species as well as sex, age, and degree of fitness variations in the

timing and intensity of the thermogenic response as well as the duration over which this effort can be maintained. When maximum thermogenesis fails to balance minimum heat loss, a heat deficit occurs and hypothermia results. With the progression of hypothermia, cold narcosis of the central nervous system supervenes and respiratory efforts decline. The heart which may continue to beat for sometime, depending on the rate of cooling, slows as T_b declines. When T_b reaches a specific level, 15-25°C, the heart either stops or enters ventricular fibrillation. In the fight to maintain T_b the animal has exhausted itself.

In contrast to the scenario presented above clinically induced hypothermia is a sedate, relatively non-stressful and intensively monitored condition. The subject is initially anaesthetized and rapidly cooled with little or no metabolic resistance. Further, the subject is continuously monitored throughout the hypothermic bout so that complications which may present themselves can be immediately corrected or rewarming instituted before they become lethal.

It seems plausible that the difference in duration of hypothermic survival at a given T_b between accidental and clinical hypothermia could be attributed to variations in metabolic resistance prior to onset of hypothermia. There have been many case-reports of survival from phenomenally low T_b of individuals severely inebriated prior to hypothermia. It has been suggested that alcohol may have some beneficial effect on the cardiovascular system in such instances (83,94); however, the data can also be interpreted that the subject entered hypothermia in a clinical manner and therefore was energetically less exhausted. Further, several studies have shown that glucose availability or utilization is severely depressed at low T_b (1,27,65) and hypothermic survival has been shown to improve in hamster with exogenous supply of glucose (63) and pretreatment with glucocorticoid (65). It seems likely therefore, that death as a result of accidental hypothermia could be due an insufficiency in meeting the specific metabolic demands of specific cell types (e.g. brain) in maintaining functional integrity. However, in view of the very

limited information available on this aspect from human studies, the development of an animal model was required to gain more insight on improving survival in hypothermia.

Problems of applicability notwithstanding, animal studies have provided much detailed information on the physiological changes occurring during various stages of hypothermia. Of particular interest are comparative studies of hypothermia and hibernation. Research into the physiology and biochemistry of hibernation has provided much insight into how the hibernator survives at low T_b (24,38,44,52,59,60,61,78,91,90). Specific aspects of hibernation have been intensively investigated and reviewed, for example neural (5,6,35), endocrinological (43,92), ionic (97), and membrane aspects (2,3,96). Even though recent evidence indicates that some of the changes observed in hibernation are due to the effect of temperature on the system, many of the physiological and biochemical changes are specific adaptations for functional integrity at low T_b (89). These specific adaptations in the hibernator may shed light on understanding and improving survival in hypothermia.

In order to study the physiological aspects of survival under profound and prolonged hypothermia we have developed an animal model (47). This model, which incorporates modifications of previous methods of hypothermia induction (63,64) and computer technology has provided heretofore unattained precision in control of T_b during hypothermia and maximum duration of hypothermic survival and recovery in rats and ground squirrels. Further, all of this is accomplished with a minimum of pharmacological interference. Briefly, our model has the following characteristics: a) induction into hypothermia is relatively quick with minimal stress to the animal; b) deep T_b in hypothermia is regulatable within 0.2-0.3°C of the desired level; c) the animal is readily accessible for experimental manipulation (i.e. blood sampling, drug administration, etc.); d) the physiological condition of the subject can be made to reflect as nearly as possible the physiology

of either accidental or clinical hypothermia; and e) the metabolic resistance of the subject can be monitored for time- and/or state-dependent changes.

This model has been applied to study of hypothermia physiology in rats (a nonhibernating species) at a T_b of 19°C for 24-30 hours and in ground squirrels (*S. richardsonii, S. columbianus* and *S. lateralis*; hibernating species) at a T_b of 7°C for over 96 hours. Since animals can be maintained for relatively long time at very stable T_b's, this allows the elucidation of limiting factors governing hypothermic survival and the effectiveness of various remedial pharmacological treatments for improving long term survival in hypothermia. It is also a useful model for comparative studies of the physiological aspects of hibernation and hypothermia in the same species.

Diverse approaches to hypothermia research have often generated contradictory and inconclusive results with respect to changes in volume and composition of body fluids. Popovic (72) observed an increase in hematocrit values from 42% to 70% in rats cooled to 15°C. We found an initial, significant increase in hematocrit from 45 to 51% in the first two hours of hypothermia, however, it then returned to control level (44%) and remained relatively constant until late (22 hr) in the hypothermic bout (Chapter II, Fig. II.1). An increase in hemoconcentration has also been observed in euthermic, cold-exposed animals (4), this suggests that the initial rise seen in our rats may be a typical stress response to cold exposure and not related directly to the fall in T_b. By 24 hr, however, the hematocrit in our hypothermic rats increases to 60%. This rather dramatic increase comes at a time when remedial measures aimed at reviving the animal are relatively ineffective. This may be one manifestation of a general failure of the regulatory system or vascular membrane break down allowing water to escape from the circulation. Previous observations of general tissue edema tend to support the latter. For example, in hypothermic man, hematocrit also rises, partly due to cold diuresis and partly to subcutaneous edema (69). Brendel (13) also suggested that cold-induced sweiling

of the brain is one of the primary factors limiting survival in hypothermia. Whether this is universal is currently uncertain since gross tissue edema has not been observed in the brain, liver or heart of our hypothermic rats although it is seen in the gastrointestinal tract late in the hypothermic bout (49, Chapter II). Further, the increased haematocrit in our rats could be due to swelling of RBC alone because no increase in number of RBC's was observed (unpublish observations, D. Belke).

The ions, Na^+ and K^+, are considered important factors influencing the distribution of body water. Further, they can have profound effects on membrane excitability and enzyme activity. There is an early and significant decrease in K^+ in our rats (49 & Chapt. II). However, the K^+ levels are maintained relatively constant at this lower level for the duration of the hypothermic bout. Since glucose administration tends to decrease K^+ levels in hypothermic animals (74), the decrease in K^+ observed in our hypothermic rats may be secondary to the observed increase in plasma glucose (41). Further, this decrease may have the beneficial effect of reducing the tendency for ventricular fibrillation in hypothermia (74). No significant difference was noted in the trend of Na^+ or Ca^{++} ions in hypothermia as compared to euthermic animals (49 & Chapt. II), indicating that regulatory functions are operable at this reduced T_b and that the differences noted in the literature are probably the result of different approaches to, or degrees of hypothermia (12,54,84).

Blood O_2, CO_2 and acid-base balance during hypothermia is complex and poorly understood. In hypothermia the solubility of O_2 and CO_2 in plasma is increased. In prolonged severe hypothermia, arterio-venous (AV) O_2 difference tends to increase. In rats kept at a Tb of 15°C for 9 h the AV O_2 difference increased from 5 to 15 volume percent (73). This increased O_2 extraction could be due to an increase in the mean transit time and/or an increase in tissue PCO_2 or local acid build up (see below) facilitating O_2 unloading.

Arterial PO_2 in our hypothermic animals is comparable to previously documented values (88,15) although much more variable than euthermic values. Other than the expected exponential decrease in O_2 consumption with temperature (Q_{10} effect), the variation in P_aO_2 may be due to insufficient cardiac output. Burlington (16) found no cardiac output in isolated rat hearts at 17°C. They suggested that below 20°C the rat heart may be faced with insufficient coronary flow, depressed Ca^{++} transport in the sarcoplasmic reticulum (SR), reduced FFA oxidation and a loss of phosphate potential in the cardiac cell. Recently, Belke (9) demonstrated a 30% reduction from euthermic controls in SR Ca^{++} uptake measured at 19°C in hearts of hypothermic rats after 24 h exposure to a T_b of 19°C. Whether this would cause a disturbance in intracellular Ca^{++} concentration and thus cardiac contractility is unknown. Reduced cardiac output, increased peripheral vasoconstriction, and increased viscosity and sludging of the blood, may serve to occlude capillary beds resulting in impaired O_2 delivery to tissues without adequate reflection on measured P_aO_2 (15,87).

Simultaneous measurement of P_vCO_2, lactate and pH in our rats, indicated that although the low P_vCO_2 would suggest respiratory alkalosis, concomitant high plasma lactate concentrations indicate that the low P_vCO_2 is more likely due to depressed oxidative metabolism resulting in metabolic acidosis (pH 6.9; corrected to 37°C, Chap. VII). It is likely that increased plasma lactate is a direct consequence of hypoperfusion of peripheral tissues (14) and decreased utilization of lactate by the cold liver and heart (82). Because of the complexity of the cause and effect relationships of blood acid-base balance it is difficult to postulate what this 0.5 pH unit shift means to the animal especially since it is only 0.2 pH unit at the prevailing T_b. It is, however, apparent that this shift itself does not reflect a major life threatening disturbance since the pH is already low by 2 h, yet the animal is capable of surviving for an additional 20 some hours in our experiments.

Taken together, it would seem that although arterial O_2 appears to be sufficient to maintain metabolic activity at 19°C, other physiological indicators, i.e. high plasma lactate and low P_vO_2 suggest that peripheral O_2 delivery is depressed. This is further substantiated by the findings of Kameya and his associates (50) that at 20°C O_2 uptake is significantly affected by flow rate. Moreover, Bond (11) demonstrated the aggregation of red cells in the mesenteries of dogs cooled to 28°C by perfusion with the pressure and rate held constant. The aggregation increased with time and lower temperature with red cell velocity decreasing until capillary flow ceased entirely. The significant increase in both P_aO_2 and lactate in our hypothermic rats towards the end of the hypothermic period tend to support this failure of tissue perfusion.

The most frequently stated limitation to survival in hypothermia is failure to meet metabolic requirements. For instance, glucose intolerance has been observed during accidental hypothermia in humans (68) and in experimental hypothermia of mammals (10,41,86). During the cooling of our rats to 19°C the plasma glucose concentration increased dramatically from 9 to 18 mmol/l, then remained stable for up to 20 hours. This rise in blood glucose suggests a transient imbalance between hepatic production and plasma clearance of glucose during cooling which is probably due to the differential sensitivity of pancreatic α and ß cell function to temperature (85,97). The observed increase in plasma catecholamines (54) and glucagon concentrations (40) during cooling tend to stimulate glucose mobilization, whereas, the dramatic inhibition of insulin secretion and subsequent prevention of glucose utilization by insulin-dependent tissues (41) could explain the depression of plasma glucose clearance and thus hyperglycaemia during hypothermia.

Fuhrman and Fuhrman (27) using a bolus injection of radio-labelled glucose found no measurable glucose clearance over a 2 1/2 hour period in rats (T_b=15°C). Recent evidence (Chapter VI) indicates that glucose turnover in

hypothermic (T_b=19°C) rats is indeed depressed but not to the extent suggested by Fuhrman and Fuhrman (27). Using the constant infusion technique and doubly labelled glucose, the cellular uptake of glucose is approximately 59-70% of that of euthermic rats. However, glucose oxidation is depressed to 0.45-0.85% of that in the euthermic rat (chap. VI). This observation in conjunction with the elevated lactate levels noted earlier again suggest that tissue perfusion may be impaired during prolonged hypothermia to such an extent that anaerobic glycolysis predominates.

Free fatty acids (FFA), the primary substrate for thermogenesis in cold-exposed euthermic animals have not been extensively studied in hypothermic animals possibly due to the over-shadowing effect of the glucose response. Contrary to the effect on glucose, the concentration of plasma FFA is generally maintained during the cooling period. In spite of the increased secretion of lipolytic hormones, i.e. glucagon and catecholamines (101) a decrease in FFA turnover was observed in hypothermic rats 2 h after induction (Chap. VI). However, plasma FFA levels significantly and progressively decrease throughout the hypothermic period. Respiratory CO_2 specific activity derived from FFA oxidation during hypothermia suggests that a greater proportion of the CO_2 production in hypothermia is derived from FFA oxidation than glucose oxidation (Chap. VI). Thus, it seems that the decrease in FFA turnover is the result of a depression of oxidation by low temperature as well as the hyperglycaemia, which has been shown to inhibit lipolysis (Chap. VI, 66) and depress FFA mobilization, resulting in an eventual shortage of FFA oxidation and energy supply in prolonged hypothermia.

Interestingly, metabolic rate in hypothermic rats is not as depressed as the oxidation of glucose and FFA would suggest. For example, glucose and FFA oxidation in 2 hour hypothermic rats only accounts for approximately 18% of the $\dot{V}CO_2$. This suggest that hypothermic rats are utilizing other substrates for the majority of their energy metabolism. What substrate is been utilized is not

currently known however, there is some correlation between observed changes in glucose oxidation (Chap. VI) and plasma lactate concentrations (Chap. VII). Even the lowest level plasma lactate in hypothermia appears to represent sufficient carbon to account for the difference in $\dot{V}CO_2$. This presents a somewhat paradoxical problem as the increase in lactate is assumed to be due to poor tissue oxygenation however, for the lactate to be oxydized to CO_2 there must be oxygen present. This paradox may be partially explained by the apparent cyclic nature of the plasma glucose SA, suggesting that there is exaggerated pulsatile blood flow resulting in periodic periods of low blood flow and poor oxygenation followed by periodic flushing with oxygen rich blood. This would allow for the buildup of lactate followed by either movement of the lactate to more oxygenated tissues or to oxygenation of the local tissues.

Taken all together it appears that at a stable 19°C T_b, hypothermic rats are capable of homeostatic control over water and ionic balance for up to 20 h. The decreased turnover and oxidation of glucose and FFA in conjunction with decreased plasma levels of FFA suggest that the hypothermic rat may be in an energy deficient state. This is further substantiated by the increasing arterial lactate, increasing P_vCO_2 and decreasing P_vO_2 levels which when viewed in light of the depressed glucose oxidation suggest tissue hypoperfusion (perhaps increased oxygen transit time). Further, our use of a computerized feed-back loop to control Tb via a circulating water bath provides a measure of metabolic resistance of the animal to hypothermia. This measure, the difference between T_b and T_a, indicates that there is a time dependent decrease in metabolic resistance with the prolongation of hypothermia. The approach of a null temperature difference is generally coincident with the death of the animal. Thus as energy deficit increases metabolic resistance decreases until a point, yet to be determined, is reached when the energy deficit becomes critical. At that point irreversible perturbation of the system occurs or possibly increases to such a level that survival is no longer a possibility.

Although reductions in O_2 consumption, respiratory rate, heart rate and T_b are observed in both induced hypothermia and hibernation, the hibernators overcome the harshness and survive much better, even when subjected to deep hypothermia during their nonhibernating phase. Differences in physiological and biochemical performance observed between hibernators and nonhibernators under prolonged hypothermia may therefore provide suggestions as to remedial measures which might improve hypothermic survival.

Cellular death from cold is related to the effects of extra- and intracellular ice formation. However, at temperatures well above the phase transition temperature of water, energy transformation may become progressively inadequate as the rates of enzymic reactions slow down leading to partial or total blockade of vital pathways of intermediate metabolism. Possibly due to an insufficiency of specific substrates, ATP production in the mitochondria is severely depressed and cellular functions deteriorate. Death from hypothermia in nonhibernators is probably characterized by this progressive energy exhaustion (87). In contrast, the hibernators are capable of maintaining energy balance by enhancing fat utilization as well as maintaining a normal glycemic level. For example, the muscle mitochondria of hibernating bats preferentially oxidize fatty acids over pyruvate and physiological concentrations of palmityl-carnitine inhibit pyruvate oxidation (101). This preferential utilization of fat results in an increased plasma ketone body concentration in hibernation in ground squirrels (57,79). Further, it has been suggested that oxidation of ketone bodies supplies 43, 21, and 31% of the energy requirements of the heart, pectoralis and diaphragm muscles, respectively, during hibernation (57). The result of this preferential fat utilization is glucose sparing which is enhanced by a greater gluconeogenic capacity during hibernation (30,71,95). Thus it is plausible that the differences in survival between induced hypothermia and hibernation could be attributed to the ability (or inability) to meet specific metabolic demands. However, it seems unlikely that substrate availability alone can be the limiting factor for hypothermic survival since: 1)

hyperglycaemia is observed in many hypothermic victims and experimental animals (see Chapters III, IV, V, VI); 2) no difference in survival time has been observed between well-fed and 24-h fasted rats in prolonged hypothermia (86,42); increasing glucose availability by pretreating the rats with glucocorticoids fails to prolong their survival time in deep hypothermia (85). These observations suggest that glucose utilization, not its supply, is impaired during hypothermia in nonhibernators. On the other hand, it has been shown that hypothermic survival can be improved by infusing exogenous glucose in the hamster, a hibernator which is apparently capable of utilizing glucose at low T_b (81). In view of this major difference in glucose utilization at low T_b between hibernators and nonhiberators, how to improve glucose utilization as well as the utilization of other substrates (see Chapter VI) at low T_b may be a key issue in improving prolonged survival in hypothermia.

Either directly or indirectly related to their inability in maintaining energy sufficiency, the nonhibernators also cannot maintain cardiovascular functions for perfusion of vital organs at low T_b. Decreases in a wide variety of functional parameters have been reported: heart rate (77), left ventricular power and mechanical efficiency (25), systemic arterial pressure (34), coronary blood flow (17) and renal blood flow (45). Cardiac output decreases continuously while total peripheral resistance, primarily associated with increased blood viscosity, increases. Peripheral microcirculation is further inhibited by aggregation of red blood cells which tends to block capillary flow entirely (67).

In contrast, the hibernators are capable of maintaining cardiac force to overcome the increased peripheral resistance at low T_b. This appears to be achieved by adaptive changes in intracellular Ca^{2+} regulation of hibernators cardiac SR. Three primary adjustments appear to take place in the hibernating Richardsons ground squirrel; a) the rate of SR Ca^{2+} uptake is 1.7-2.7 times greater in the hibernating phase (8), providing the capacity for rapid restoration of low resting intracellular

Ca^{2+} after each depolarization; b) significant increases in Ca^{2+} ATPase activity and coupling ratio of SR Ca^{2+} transport (i.e. Ca^{2+}/ATP) are seen in the hibernating state, accounting for the increased rate of SR Ca^{2+} transport during hibernation (7); c) the maximum capacity for the accumulation of Ca^{2+} by SR is significantly greater by two to three fold during hibernation, indicating greater amount of stored SR Ca^{2+} available for release during depolarization (7). These adaptive changes in intracellular Ca^{2+} regulation which resulted in enhanced cardiac contractile force in the hibernating state may be essential for maintaining adequate circulation under a very slow heart rate. Hibernators are also capable of preserving intracellular ionic homeostasis at low temperature by preventing passive leakage of K^+ as well as maintaining active transport of Na^+ (26,31,32,56,98,99,100). Thus they avoid problems associated with the peripheral circulation such as cell swelling, increased haemoconcentration and increase viscosity and ceil aggregation. Since hibernators can maintain their physiological functions at low temperature with the above adaptive cardiovascular changes, it is possible that hypothermic survival can be prolonged in nonhibernators by mimicking these adaptive changes with various treatments. Therefore, remedial measures aimed at improving the energy status of the animal which lead to improved circulatory sufficiency, tissue perfusion and oxygenation, and efficiency of substrate utilization, could prove to be useful in enhancing hypothermia survival.

Several treatments have been shown to improve peripheral circulation, of these low-molecular-weight dextran, which is effective by virtue of hemodilution and increased blood volume (80), and heparin (19) or Pluronic-F68 (93), which reduce cell aggregation, are the most effective. Further, hibernators not only maintain an efficiently regulated microcirculation but also an efficient pump. Therefore, measures taken to enhance cardiac function and to decrease peripheral resistance may enhance hypothermic survival. The decrease in cardiac performance in hypothermia could be due to an intracellular Ca^{++} overload, since recent *in vitro* studies have shown that the application of calcium channel blockers diltiazem

(29,62), verapamil (76) and nifedipine (21,62) produce significant improvements in: a) cardiac contractility (verapamil, diltiazem), b) stroke volume, coronary blood flow and myocardial O_2 consumption (nifedipine) and c) reduced incidence of severe arrhythmias (diltiazem) under a variety of conditions. Since these are the same types of improvements observed in the hibernating animal, they may have a beneficial effect on the hypothermic victim as well. The only reservation for these treatments is that usually there is a marked decrease in blood pressure after treating the animal *in vivo* with calcium channel blockers (33,39). Such a hypotensive state may result in further reduction of the already inadequate perfusion pressure against high peripheral resistance in hypothermia. Further studies are thus required to investigate the possibility of using calcium channel blocker to improve hypothermic survival by combining its use with other drugs to compensate its hypotensive effect or developing more specific calcium agonists or antagonists which will improve the cardiac contractility and dilate peripheral vessels at the same time.

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Vita

MICHAEL LEIGH JOURDAN

October 8, 1991

ADDRESS: Department of Zoology
University of Alberta
Edmonton, Alberta,
Canada T6G 2E9
Office - (403) 492-1277
Home - (403) 433-5798

BIRTH DATE: July 9, 1949

CITIZENSHIP: UNITED STATES

MARITAL STATUS: Married to Anne Wilson, June 14, 1975 (3 children)

EDUCATION: B.Sc. University of Alaska, Fairbanks, Alaska 1980

M.Sc. University of Alberta, Edmonton, Alberta 1983

Ph.D. University of Alberta, Edmonton, Alberta 1991

SPECIAL AWARDS:

Alberta Heritage Foundation for Medical Research

Studentship 1981-1986.

PROFESSIONAL EXPERIENCE:

Teaching and Research Assistant, 1980-1989, Univ. of Alberta

PUBLICATIONS:

Holleman, D.F., R.G. White, K. Frisby, M.L. Jourdan, P. Henrechsen and P.G. Tallas. 1984. Food passage rates in captive muskoxen as measured with non-absorbed radiolabeled markers. IN "Proceedings of the First International Muskox Symposium." D.R. Klein, R.G. White and S. Keller eds. Biol. Pap. Univ. Alaska Spec. Rep. No.4:188-192.

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