TIME COURSE OF AMMONIA ACCUMULATION DURING EXERCISE

by

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ABSTRACT

The time course of accumulation of lactate and ammonia was assessed in tissues following two exercise protocols in female Wistar rats. Blood ammonia and lactate were measured during a progressive treadmill run to exhaustion. Ammonia and lactate were also measured in tissues from animals who ran at a constant workrate of 45 $m.min^{-1}$ (sub-maximal exercise) for 2, 4, 6, 8, and 10 minutes of exercise, and following 5 minutes recovery after exercise.

Progressively increasing work to exhaustion resulted in an exponential increase in both ammonia and lactate in blood. During submaximal exercise, ammonia and lactate reached peak concentrations during the 6th to 8th minute, then decreased during the 10th minute of exercise and during recovery in blood and skeletal muscles. In liver and heart tissue, the time course of lactate accumulation was similar to that in blood and muscle, whereas in heart, ammonia increased gradually throughout the entire exercise period, and in liver, ammonia concentration remained unchanged as a result of the exercise protocol. Brain ammonia increased significantly only in the cerebellum during the recovery period.

It was concluded that:

1. the accumulation of ammonia and lactate is affected by exercise intensity.
2. progressively increasing workrate to exhaustion results in an exponential increase in both ammonia and lactate in blood.

3. during submaximal exercise, the time course of ammonia and lactate accumulation are similar in blood and skeletal muscles.
4. during submaximal exercise, ammonia accumulation is more gradual in heart tissue than in blood or in skeletal muscle.
5. submaximal exercise does not produce marked changes in brain ammonia concentrations.

DEDICATION

To my Mother, for her continuous support and understanding,

and to many others
for their Love, Trust, and Integrity.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. Introduction

Interest in ammonia metabolism, its involvement and possible detrimental effects during intense physical exercise has grown primarily from observations which have linked increased ammonia concentrations in blood to a number of severe metabolic and neurological disorders. (In this context, ammonia refers to the sum of the ammonium ion (NH_4^+) and ammonia gas (NH_3), unless otherwise specified).

Certain disorders of nervous function, including lethargy, convulsions, ataxia, and coma, which have been reported in conjunction with liver disease (Lockwood et al., 1979) or inborn errors of metabolism (IEM's) (Guroff, 1979), are associated with high concentrations of blood ammonia (Iles and Jack, 1980). Frequently, the intensity of the neurological disturbances has been related to the degree of excess blood ammonia.

Of particular relevance to this study are experiments which have demonstrated that intense physical exercise is accompanied by increased blood ammonia concentrations to levels which are similar to those which have been reported in clinical disorders. In man, blood ammonia concentrations up to 400% of resting values have been measured at the termination of intense exercise (Wilkerson, et al., 1977; Dawson, 1978; Banister, et al., 1983). Several investigators have made oblique reference to the possibility that ammonia may be associated with, or may ultimately precipitate the fatique of intense physical activity

(Laborit, et al., 1957 and 1958; Allen and Conn, 1960; Barnes, et al., 1964; Golding, 1972; Brodan, et al., 1974; Wilkerson, et al., 1975 and 1977).

This study has investigated the appearance and the time course of accumulation of ammonia and lactate in various tissues during exercise. The relationship between changes in ammonia concentration and work rate during exercise and between ammonia and lactate accumulation was examined. Previous reports of the time course of accumulation of ammonia during exercise do not consider the relationship of accumulation of ammonia between various tissue. This was of interest in relation to ammonia production resulting from exercise, since the net accumulation of ammonia is dependent on the interaction of production and buffering of ammonia in several different tissues.

Specifically, the objectives of the study were:

i)to establish the time course of ammonia accumulation in tissue during exercise in rats, particularly in blood, muscle, heart, liver, and brain.

ii) to compare the time course of blood ammonia accumulation to that of lactic acidduring exercise, and to determine, if possible, whether the onset of hyperammonaemia during exercise preceded or followed the onset of lactacidemia.

The exact functional effect of ammonia upon exercising animals with respect to its production, transport, uptake, and metabolic function remains equivocal. However, if

elevated ammonia precipitates physiological disturbances, then it seems reasonable to suppose that the rapid accumulation of ammonia in blood during intense exercise may interfere with normal metabolic functions, and as such, may affect total exercise performance.

2.0 Review of Literature

2.1 Ammonia Production During Exercise

Ammonia was first linked to the development of fatigue by Tashiro (1922) when he questioned whether or not there could be any relationship between ammonia production and fatigue. Subsequent to this, frequent observations of elevated blood ammonia following exercise have been reported (Laborit, et al., 1957 and 1958; Schwartz, Lawrence and Roberts, 1958; Allen and Conn, 1960; Salvatore and Bocchini, 1961; Barnes, et al., 1964; Ahlborg, et al., 1968; Golding, 1972; Brodan, et al., 1974; Wilkerson, et al., 1975 and 1977; Meyer, Dudley, and Terjung, 1980; Banister, et al., 1983; Dudley, et al., 1983).

Wilkerson, et al., (1977) established a significant correlation between the concentration of peripheral venous ammonia and oxygen uptake ($\dot{V}O_2$) at rest, during exercise, and in post-exercise recovery in man. The relationship proposed by the Wilkerson group was:

$$\log_{10} \text{ammonia} = 0.0036(\%\dot{V}O_2 \text{ max}) + 0.6303 \quad (1)$$

Relationships between blood concentrations of ammonia and glucose, lactate, and pyruvate were also established by Wilkerson, et al., (1977). The correlations were significant, however, only at oxygen uptake values greater that 70 percent of $\dot{V}O_2$ max. A similar correlation between blood ammonia and blood

lactate levels was reported in rats after treadmill running (Meyer, Dudley, and Terjung, 1980). Again, the relationship was considered to be significant only at high intensity exercise, above 80 percent of $\dot{V}O_2$ max of the rats.

2.2 Sources of Ammonia During Exercise

The major metabolic reactions involving ammonia in the body have been comprehensively reviewed by Goldstein (1976) and Tannen (1978). Under ordinary circumstances, most blood ammonia is of dietary origin. Normal digestive processes generate ammonia from ingested protein, while bacteria in the gastrointestinal tract generate ammonia by metabololizing protein, products of dietary protein digestion, and urea. Other mechanisms for significant ammonia production (in the presence or absence of food) exist in liver, muscle, intestine, nerve tissue, brain, kidney, and red blood cells (Onstad and Zieve, 1979). Kidney, muscle, and brain tissue are minor sources of ammonia production in normal, resting metabolism (Lockwood, et al., 1979; Onstad and Zieve, 1979).

Exercise affects the rate of production of ammonia. In normal resting skeletal muscle, the arteriovenous concentration difference for ammonia is close to zero (Lockwood, et al., 1979). After mild exercise, no change is observed in blood ammonia concentrations in normal subjects. However, severe exercise is accompanied by increases in blood ammonia up to 400% of resting values (Allen and Conn, 1960; Dawson, 1978; Banister,

et al., 1983). During exercise, ammonia production by muscle seems to be proportional to the work done (Parnas, 1929a and 1929b) and has been related to the oxygen uptake before, during, and after exercise (Wilkerson, et al., 1977).

It has become accepted that exercise triggers ammonia production in skeletal muscle primarily via the purine nucleotide cycle (PNC) (Lowenstein, 1972; Goodman and Lowenstein, 1977). Lowenstein and Tornheim first proposed the existence of the PNC (Lowenstein and Tornheim, 1971; Lowenstein, 1972), the net sum of which is the deamination of aspartate to yield ammonia. The PNC consists of the following sequence of reactions:

1) AMP + H_2O ⟶ IMP + NH_3 (2)

2) IMP + aspartate + GTP ⟶ adenylosuccinate + GDP + Pi (3)

3) adenylosuccinate ⟶ AMP + fumarate (4)

Overall:

4) aspartate + GTP + H_2O ⟶ fumarate + NH_3 + GDP + Pi

Evidence that the PNC is the major pathway of ammonia synthesis in muscle stems from observations that in skeletal muscle, adenylate deaminase activity is high, whereas the activities of glutamate dehydrogenase and glutaminase, important enzymes in ammonia production in the liver and kidney, are negligible (Lowenstein, 1972).

The physiological function of the purine nucleotide cycle has been summarized by Lowenstein (1972) as:

i) to provide a pathway for the liberation of ammonia from amino

acids;

ii) to provide a pathway for adjustment in the levels of TCA cycle intermediates;

iii) to provide a pathway for amino acids to be used as a source of carbon for energy production;

iv) to provide a pathway for the regulation of the relative levels of adenine nucleotides, ATP, ADP, and AMP;

v) to provide a pathway aiding in the control of glycolysis by stimulation of phosphofructokinase activity.

Other metabolic pathways exist which may be involved in the production of ammonia during exercise. In the catabolism of many amino acids, the α-amino group in enzymatically removed by oxidative deamination, producing the corresponding α-keto acid and ammonia. Deamination of catecholamines may also be a source of ammonia during intense activity. In response to exercise, there is an increase in catecholamines in plasma (Banister and Griffiths, 1972; Ostman and Sjostrand, 1975), and a changed vascular sensitivity to catecholamines has been reported to accompany physical training (Ostman, 1975). A quantitative estimate of these reactions as a source of ammonia production has not been made, and they are generally considered to be of minimal importance relative to the PNC.

AMP breakdown may occur, however, by two main pathways, both of which are regulated by ATP (Burger and Lowenstein, 1967; Setlow, Burger, and Lowenstein, 1966). The first pathway is by deamination of AMP (ATP activated). This is followed by

dephosphorylation of IMP to inosine. Overall, the reaction is considered to be the "direct" pathway of AMP breakdown. A second, or "indirect" pathway involves dephosphorylation of AMP (ATP-inhibited) followed by deamination of adenosine (Wegelin, Manzoli, and Pane, 1978). The relationship between the indirect and direct pathways of AMP breakdown are illustrated in Figure 1.

FIGURE 1

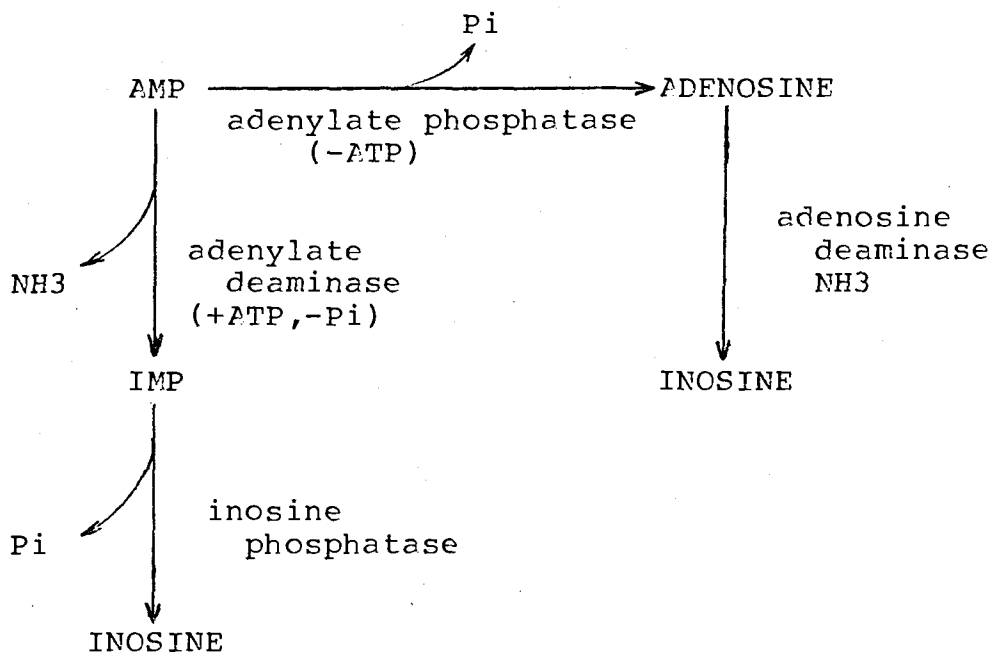


Figure 1: Proposed pathways of AMP breakdown leading to synthesis of ammonia. - indicates inhibitory influence, + indicates stimulatory influence.

2.3 Ammonia Production and Muscle Fibre Type

Fundamental differences in ammonia and adenylate metabolism exist between contracting fast glycolytic (FG), fast oxidative glycolytic (FOG), and slow oxidative (SO) fibres in mammalian muscle (Meyer and Terjung, 1979; Meyer, Dudley, and Terjung, 1980; Dudley, et al., 1983). Different muscle fibre types have different levels of AMP deaminase activity, a principal enzyme of the purine nucleotide cycle catalyzing the deamination of AMP to IMP (Gerez and Kirsten, 1965; Paggi, et al., 1969; Winder, et al., 1974; Meyer and Terjung, 1979). Muscles poor in mitochondria are reported to produce more ammonia during exercise than muscles which are rich in mitochondria (Gerez and Kirsten, 1965; Paggi, et al., 1969).The relevance of these fibre-type differences in ammonia metabolism during exercise will be discussed below.

Meyer, Dudley and Terjung (1980) examined ammonia and IMP contents in skeletal muscle fibres of rats after exercise. Exercise consisting of 4 minutes of treadmill running either at 45 or at 60 m. min $^{-1}$ in untrained animals resulted in ammonia concentrations which were greatest in FG, intermediate in FOG, and lowest in SO muscle. These changes in ammonia paralleled increases in IMP content of the same muscle type. IMP is considered to be a good indicator of PNC activity since IMP does not diffuse out of muscle tissue (Meyer, Dudley, and Terjung, 1980). These investigators reaffirmed that fast glycolytic

muscle is a more important source of blood ammonia during exercise than FOG or SO fibres. This interpretation, however, is complicated by two aspects of ammonia metabolism. First, even under resting conditions there should be more ammonia in muscle than in blood due to differences in pH values in these tissues at rest (Meyer, Dudley and Terjung, 1980). Secondly, muscle is a major source of ammonia uptake when blood ammonia concentrations are increased (Allen and Conn, 1960). Up to 50 percent of circulating ammonia is taken up by skeletal muscle in normal subjects at rest (Lockwood, et al., 1979), although the response of exercising skeletal muscle to a sudden blood ammonia load has not been investigated.

Much of the variability reported in blood ammonia concentrations in exercising humans may be related to the wide range of fibre-type compositions among individuals, and by the order of recruitment of fibre types during exercise. Slow-twitch fibres are predominantly recruited at low workrates, whereas during intense exercise, recruitment of fast-twitch fibres is increased and ammonia production increases exponentially with work rate (Meyer, Dudley and Terjung, 1980). This recruitment of a higher proportion of fast-twich fibres during intense activity may help to explain the accumulation of ammonia at high workrates. Dudley, et al., (1983) demonstrated that in humans, the proportion of ST fibres was inversely related to the increase in blood ammonia with exhaustive exercise. It was suggested that since ST fibres have a higher respiratory

capacity than FT fibres, then they may be able to resynthesize ATP at a greater rate, and would avoid an accumulation of ADP and AMP, which could upset the energy charge ratio. In addition, FT fibres have a greater level of AMP deaminase activity, and theoretically therefore have a greater capacity for ammonia production via the PNC (Meyer and Terjung, 1979).

Gerez and Kirsten (1965) suggested a possible relationship between ammonia production and glycolytic (anaerobic) metabolism within muscle, and Tornheim and Lowenstein (1973) demonstrated that the PNC is closely linked to the glycolytic process. Ammonia increases the activity of phosphofructokinase, and therefore may directly influence the rate of glycolysis in muscle and other tissues (Lowenstein, 1972). (This and other aspects of the effect of ammonia on metabolism are discussed under the heading of Metabolic Effects of Ammonia.) Thus, ammonia production from muscles during exercise may ultimately be regarded as an "anaerobic" or "fast-glycolytic" phenomenon.

2.4 Reducing Blood Ammonia Delays Fatigue

Experimental evidence that a reduction in blood ammonia can increase the exercise time required to exhaust an individual has provided support for the hypothesis that ammonia precipitates exercise-induced fatigue. Observations that the onset of fatigue may be delayed by reducing blood ammonia have been reported in both animal and human experiments. A reduction in blood ammonia levels during exercise has been accomplished by training (rats:

Barnes, et al., 1964), by administration of ammonium carbonate (rats: Barnes, et al., 1964), by administration of aspartic acid salts (rats: Laborit, et al., 1957; Barnes, et al., 1964; man: Ahlborg, et al., 1968; Golding, 1972), and by administration of sodium glutamate (man: Brodan, et al., 1974). The improvement in exercise performance, determined by delaying fatigue, was attributed to more efficient elimination of ammonia by increased urea cycle capacity in experiments involving training, ammonium carbonate, and aspartic acid salts. The effect of sodium glutamate on improving exercise performance was attributed to an increase in the glutamate-glutamine buffering of the ammonia. By increasing the availability of glutamate, more glutamate could react with endogenous ammonia to produce glutamine, which is non-toxic to metabolic systems.

Contrary evidence was presented by Consolazio, et al. (1964), who could not demonstrate a positive relationship between aspartic acid therapy or training and the delay of fatigue. However, in the Consolazio study, training was not strenuous (30 min treadmill walking 5 days per week for 5 weeks), and no prior fitness tests were performed so that no change in the fitness levels, if they were present, could be determined as a result of their training programme. No blood ammonia concentrations were reported either prior to, or resulting from exercise, so that it is not possible to determine in a quantitative manner whether or not their experimental protocol affected ammonia metabolism.

2.5 Movement of Ammonia and Lactate Between Tissue Compartments

Ammonia and lactate are produced primarily in muscle tissue as a result of physical exertion (Hultman and Sahlin, 1980; Tesch, 1980; Lowenstein, 1972) However, in many studies, both convenience and practicality substitute estimation of ammonia and lactate production in muscle for their respective blood concentrations. It is important therefore to substantiate this substitution by considering the mode of movement of both ammonia and lactate from muscle to blood.

The accumulation of ammonia in blood and other tissues is determined by a balance between its production, its buffering (i.e. its removal by other metabolic reactions), and its transport or diffusion out of the producing tissues. As a weak base, the predominant physiological form of ammonia is NH_4^+. Cell membranes are generally more permeable to neutral molecules than to ions . (Roos and Boron, 1981). Ammonia may move between tissue compartments by diffusion (Stabenau, Warren and Pall, 1959; Cooper, et al., 1979) or by active transport (Fazekas,et al., 1956; Hindfelt and Siesjo, 1971). The pH gradient between tissues also influences the direction of movement of ammonia. It is retained in tissues with a low pH.

Blood is a common pool for tissue produced ammonia and the acid-base changes in tissue resulting from intense exercise could markedly affect the distributon pattern of ammonia between tissue compartments and the blood. During exercise in rats,

blood pH may fall from 7.4 (rest) to 7.2 (exercise) and muscle pH may decrease from 7.2 (rest) to 6.5 (exercise) (Meyer, Dudley, and Terjung, 1980). Thus, intense activity might result in ammonia retention in muscle relative to blood, as a consequence of the adverse pH gradient (Roos and Boron, 1981). An additional complicating factor in identifying muscle as the overall major contributor to rising blood ammonia concentration is the fact that muscle may also be a major site of ammonia uptake whenever blood ammonia levels are increased (Rosada, et al., 1962; Lockwood, et al., 1979).

2.6 Removal of Ammonia From Tissue

The removal of ammonia from tissue is affected in four principal ways. First, by the formation of glutamate by the glutamate dehydrogenase reaction; second, by glutamine synthesis catalyzed by the enzyme glutamine synthetase; third, by general transamination reactions to other amino acids; and fourth, by diffusion of ammonia from the tissue compartment to blood. Blood ammonia may then be transported to other tissues for removal, in particular to liver for conversion to urea (White, Handler, and Smith, 1973).

Glutamine synthesis is particularly relevant to this study. The formation of glutamine by glutamine synthetase (EC 6.3.1.2) requires ammonia, glutamate, and ATP. Provision of ammonia for this reaction has been suggested to be rate limiting for glutamine synthesis in skeletal muscle (Garber, Karl, and

Kipnis, 1976 a and b).

Another pathway to be considered in the analysis of ammonia metabolism is that catalyzed by glutamate dehydrogenase (GlDH). The net reaction is:

$$\text{Glutamate} + \text{NAD+} + \text{2-oxoglutarate} \xrightarrow{\text{GlDH}} \alpha\text{-ketoglutarate} + \text{aspartate} + \text{NH3} \qquad (8)$$

As written, this reaction fulfills the function of producing the liberation of ammonia from glutamate, following the latter's formation from transamination of various amino acids (Braunstein and Bychkov, 1939). However, the free energy change of the glutamate dehydrogenase reaction as written above is approximately +6.5 kcal at pH=7. Thus, the direction of this reaction towards reductive amination of α-ketoglutarate, rather than towards oxidative deamination of glutamate (Lowenstein, 1972) acts as a potential buffer system for the removal of tissue and circulating ammonia. The several tissue buffer systems for ammonia outlined above indicate that net ammonia concentration may not in fact reflect its gross tissue production.

In Table 1, a comparison of adenylate deaminase and glutamate dehydrogenase activity in rat tissue are shown. Tissues with high AMP deaminase activity have greater potential for ammonia production via this pathway, whereas high glutamate dehydrogenase activity may indicate a greater capacity for buffering of ammonia to form a less toxic metabolite. Liver and

heart display high glutamate dehydrogenase activity, which may account partially for the substantially lower ammonia levels found in these tissues (Table 1).

Table 1: Adenylate deaminase and glutamate dehydrogenase activity of various rat tissues. The source of leg muscle was unspecified. (Modified from Lowenstein, 1972).

Tissue	Adenylate Deaminase Activity (μmoles per g fresh weight per minute)	Glutamate Dehydrogenase Activity (μmoles per g fresh weight per minute)
Liver	1.1 - 2.0	248
Brain	14.7	32
Heart	1.6 - 4.7	11
Leg Muscle	127	1.3

2.7 Lactate: Movement from Muscle to Blood

Considerably more research has been done on lactate metabolism during exercise (for review, see Tesch, 1980) than on ammonia metabolism. In 1937, Sachs and Sachs reported that the concentration of lactate within the working muscle, even at steady state, was higher than that in the blood (Hultman and Sahlin, 1980). It has since been demonstrated that the increased release of lactate from muscle occurring with increased exercise intensity is related to the accumulation of lactate within the muscle (Hultman and Sahlin, 1980). Jorfeldt, Juhlin-Dannfelt and Karlsson (1978) using femoral arterial-venous cannulation, found that the release of lactate increased linearly with muscle lactate content during bicycle exercise up to 4-5 $mmol \cdot min^{-1} \cdot leg^{-1}$ when muscle lactate content was about 4 $mmol \cdot kg^{-1}$ wet weight. This concentration of muscle lactate would represent moderate intensity exercise in humans. Further increases in muscle lactate did not increase the rate of efflux from muscle. This, and the relatively constant tissue/blood lactate concentrations were interpreted as being due to the lactate translocation process reaching saturation point (Jorfeldt, Juhlin-Dannfelt, and Karlsson, 1978).

Benade and Heisler (1978) found a much more rapid efflux of hydrogen ions than of lactate from stimulated isolated rat diaphram and frog sartorius muscle. However, the significance of these *in vitro* findings was not made clear. Hultman and Sahlin

(1980) have suggested that with the exception of the early phase of recovery after exhaustive exercise, lactate and hydrogen ions pass across the muscle cell membrane at approximately the same rate. Early recovery from exhaustive exercise has been associated with a greater rate of efflux of hydrogen ion than lactate from muscle.

Lactate efflux from muscle is also affected by pH gradient (Hirche, et al., 1975; Mainwood and Worseley-Brown, 1975), by lactate gradient (Steinhagen, et al., 1976), and by local glycogen stores (Essen, et al., 1973). The movement of lactate out of muscle cells is believed to occur both by passive diffusion, and by a carrier mediated mechanism in passive transport (Hultman and Sahlin, 1980). The saturation of lactate efflux from muscle could be explained either by saturaton of the carrier-mediated transport mechanism, or by restriction of lactate diffusion as a result of increased water content in muscles causing local muscle swelling.

2.8 Ammonia Distribution in Tissue Compartments

2.8.1 pH Dependence

Blood ammonia concentration reflects the net flux of ammonia from tissue compartments into the blood, and only indirectly reflects tissue ammonia. Ammonia, a weak base (pK = 9.3) exists predominantly as ammonium ion (NH_4^+) at physiological pH. Less than 5% of the total ammonia is carried

in physical solution as NH_3. Non-ionized ammonia is more than 1200 times more soluble than CO_2 in water, and is 14 times more soluble than CO_2 in lipid (Lawrence, et al., 1946; Dutton and Berkman, 1978). Cellular membranes, however, are relatively impermeable to NH_4^+, and thus to the major portion of circulating ammonia under normal conditions (Hindfelt, 1975).

The pH gradient between tissues determines their relative ammonia concentrations, and the direction of movement of ammonia between intra- and extra-cellular tissue compartments (Cnstad and Zieve, 1979). Thus, a greater proportion of the ammonium ion would be trapped in tissues with a lower pH than plasma (Visek, 1968). As discussed above, this occurs since only the non-protonated form of ammonia is freely diffusible across cell membranes. Under resting conditions, there would be a 2.5 times greater concentration of ammonia in muscle than in plasma, assuming pH values of 7.0 and 7.4 respectively in these tissues (Meyer, Dudley and Terjung, 1980). During exercise, when the pH difference between muscle and blood is even greater, an increasing proportion of the ammonia should be retained by muscle.

2.8.2 Active Transport

A completely pH-driven ammonia distribution does not, however, appear to be fully supported by experimental evidence. Support for an active transport system between blood, cerebrospinal fluid (CSF), and brain has grown from observations

that the intracellular ammonia content of the brain is greater than that which may be predicted solely from CSF ammonia concentrations and the pH gradients between the two compartments (Roos, 1965; Hindfelt, 1975). Two possible mechanisms of active transport suggested are the existence of a cell membrane pump moving ammonia from extracellular fluid into the brain, and the resting membrane potential of neurons and glia (-70 to -90 mV) promoting an accumulation of ammonia inside the cell relative to the outside . Hindfelt (1975) suggested that the CSF/plasma ammonia ratio may provide a significant measure of the degree of cerebral dysfunction in states of hyperammonaemia.

The question arises as to how the concentrations of ammonia in muscle, blood, and brain are affected by the respective changes in pH or in active transport mechanisms in these tissues during exercise. While both muscle and blood become more acidic during intense exercise, muscle pH can drop to a pH value of 6.6 in association with a blood pH value of 7.2 (Sahlin, et al., 1976). This indicates that intense activity will result in more ammonia being retained in muscle relative to blood. Blood ammonia concentrations are elevated with exercise, however, and although the pH of CSF and brain during exercise are not known, evidence that elevated blood ammonia concentrations are accompanied by elevated brain ammonia (Cooper, et al., 1979; Lockwood, et al., 1979) would suggest that brain ammonia concentration will also be increased with increased exercise intensity. If an increase in brain and CSF ammonia during

exercise can be demonstrated, then the toxicity and potential role of ammonia as a fatiguing agent could be related to both its central and peripheral actions.

2.9 Effects of Ammonia on Neural Activity

The action of ammonia on neural activity has been ascribed to four major effects: changes in intracellular pH, shifts in electrolytes between intracellular and extracellular compartments, interference with biochemical reactions, and depression of hyperpolarizing inhibition in both motoneurons and cortical neurons (Iles and Jack, 1980). It also appears that ammonia may interfere directly with neuromuscular function, initially by potentiating and then depressing twitch tension (Heald, 1975). A gradual loss of twitch tension in muscle preparations exposed to high ammonia concentrations is attributed to a loss of membrane excitability and a progressive decrease in the electrical excitability of individual muscle fibres.

Ammonia can also act as a powerful ventilatory stimulant, and induces hyperventilation by intravenous or intraventricular infusion (Wichser and Kazemi, 1974). Respiratory stimulation has been related to the ammonia concentration in cerebrospinal fluid, however, and not to blood ammonia, and it has been suggested that ammonia may be acting as a respiratory stimulant at an intracranial site (Wicher and Kazemi, 1974). Although this question was not investigated in this study, it is pertinent to

ask whether or not the observed increase in ammonia as a result of intensive exercise may be related to the concommitant exercise hyperpnea.

2.10 Metabolic Effects of Ammonia on Metabolism

Ammonia has a number of diverse effects on biochemical reactions. Ammonia increases the rate of flux of metabolites through glycolysis by directly increasing the activity of phosphofructokinase (PFK), one of the rate limiting enzymes of glycolysis (Lowenstein, 1972). Ammonia can stimulate PFK activity without a change in pH (Lowenstein, 1972). McKann and Tower (1961) reported that the addition of ammonia to incubated cortex slices resulted in a 2-fold decrease in the rate of glycolysis, a rise in lactate production, along with decreased pyruvate utilization and decreased oxygen consumption. This was attributed to a direct interference by ammonia with oxidative decarboxylation of pyruvate and α-ketoglutarate. Katanuma, Okada, and Nishii (1966) also reported inhibition of oxidation of pyruvate, citrate, and/or isocitrate by ammonia.

Pyruvate carboxylation, which is the first reaction of gluconeogenesis, is also inhibited in the presence of ammonia (Bryla and Niedzwiecka, 1979). This could be responsible for suppression by ammonia of gluconeogenesis from pyruvate, which has been observed by Zaleske and Bryla (1977). The overall functioning of the TCA cycle also appears to be affected by ammonia. At least two separate steps in the cycle are involved.

Isocitrate dehydrogenase, which catalyzes the conversion of citrate to α-ketoglutarate, is specifically inhibited by ammonia (Katanuma, Okada and Nishii, 1966). Additionally, it has been observed that mitochondrial respiration is inhibited by ammonia (Worcel and Erecinska, 1962). Pyruvate dehydrogenase is a mitochondrial enzyme which catalyzes oxidative decarboxylation of pyruvate to acetyl CoA for entry into the TCA cycle. As stated above, ammonia inhibits mitochondrial respiration, which suggests that this reaction will be inhibited.

As a result of the specific actions of ammonia on a number of metabolic reactions which result in an increased availability of pyruvate (ie. stimulation of glycolysis, inhibition of TCA cycle and mitochondrial oxidation, and inhibition of gluconeogenesis from pyruvate), an increased proportion of the pyruvate may be available for conversion to lactate by the enzyme lactate dehydrogenase. A drive to lactate production could itself aid in maintaining an elevated glycolytic rate, by providing re-oxidized NAD^+ (nicotinamide adenine dinucleotide) which is necessary for continuation of glycolysis. This is supported by experiments which indicate that the rise in pyruvate concentration in the presence of ammonia is accompanied by an even greater proportional rise in lactate (Schenker, et al., 1967; Hindfelt and Siesjo, 1970) and thus an increase in the lactate/pyruvate ratio (Dutton and Berkman, 1978). Other investigators have suggested that changes in glycolytic and TCA cycle metabolism which result from increased ammonia

concentrations may be related to the accumulation of lactic acid (Koyuncuoglu, et al., 1978). Thus, ammonia accumulation may accelerate lactate production, to the point where lactate and its associated hydrogen ion interfere with continued exercise performance.

During exercise, pH decreases in blood and muscle (for a review see Tesch, 1980). This observation, associated with lactic acid accumulation, is considered to be of fundamental importance in producing muscular fatigue specifically, and exercise fatigue in general. However such an increased production and subsequent accumulation of lactic acid may in fact be secondary to ammonia accumulation.

2.11 A Possible Role for Ammonia Production During Exercise

Ammonia accumulation in tissue during intense muscular activity may have an important role in control of energy production, and indirectly, in the protection of cells from the acid load imposed by exercise. The Purine Nucleotide Cycle, reviewed by Lowenstein (1972), is generally considered as the major source of ammonia production during exercise by deamination of AMP by the enzyme AMP-deaminase. The physiological significance of the PNC may be that :

1. it provides a pathway for amino acid deamination.
2. it provides a pathway for adjustment in TCA cycle intermediates.
3. it helps to maintain the ratios of ATP to ADP and AMP, the

energy charge ratio proposed by Atkinson (1968) as a major regulatory factor between energy-yeilding and energy-demanding or energy-storing processes.

4. it aids in the control of glycolysis by ammonia's stimulation of phosphofructokinase activity. It is through control of glycolysis that ammonia production may be considered a cellular protective mechanism. Tissues must by protected from excessive acidification, to avoid destruction of acid-labile cellular components. The interaction of AMP-deaminase activity, ammonia, hydrogen ion production, and PFK activity may provide fine-tuning in the balance between energy production and excessive acidification during intense activity.

3.0 Methodology

3.1 Animal Selection and Care

Female Wistar rats (Charles River, Montreal) were obtained at 8 weeks of age at an average body weight of approximately 147.7 ± 16.3 grams. Animals were individually housed, fed rat chow and water ad libitum, and maintained on a 7 am: 7 pm dark:light cycle.

3.2 Animal Training

Following one week acclimatization during which animals were handled daily, animals were given preliminary exercise bouts to become acquainted with running on the treadmill (Quinton model 42-15, Quinton Instruments, Seattle). All animals were exercised four times per week, for approximately 10 min. each session, to allow an habituation to running. This period of habituation required seven to eight weeks to ensure consistent running during experiments. At the end of the period of habituation, the maximum amount of daily work was 12 minutes of exercise, including 7 minutes at 35 $m.min^{-1}$, and 5 minutes at 40 $m.min^{-1}$, respectively. A typical programme for endurance training consists of 5 to 6 weeks of habituation to running as described above, followed by exercise up to an intensity of 1 hour per day, 5 days per week at a speed of 28 to 30 $m.min^{-1}$ on

a 15% grade for several weeks (Harpur, 1980; Patch and Brooks, 1980). A typical programme for sprint training would consist of 6 to 8 weeks of interval training up to 50 repetitions of 10 seconds work: 30 seconds rest at 80 $m.min^{-1}$ on a 15% grade, after habituation training (Hickson, et al., 1976; Harpur, 1980). The animals in this study were considered to acclimated to running, but not highly trained.

Following the period of habituation in this experiment, animals were randomly assigned to two experimental groups, designated as either Experiment 1 - Catheter/Run Protocol, or Experiment 2 Tissue-Time Course Protocol.

3.3 Experiment 1 - Catheter/Run Protocol

Habituated animals were accustomed to running on the modified one-animal treadmill (detailed in Appendix 1) wearing a nose cone assembly for gas collection and gas analyzing procedures (described in Appendix 3). This required eight to ten sessions of exposure to running while wearing the nose cone, at a frequency of three times per week. The maximum oxygen uptake of each animal was measured, using a flow-through mask to measure oxygen consumption (Fussel, Campagna, and Wenger, 1980; Gleeson and Baldwin, 1981).

$\dot{V}O_2$ max was measured to assess the aerobic capacity of different animals, and to express more accurately any subsequent exercise work rates as a percentage of each animal's individual work capacity. The usual method of doing this has been to use

treadmill velocity as an indication of the intensity of exercise which an animal undertakes, however this does not take into account the individual running ability of each animal.

After $\dot{V}O_2$ max estimation, each animal had an indwelling catheter inserted via the right jugular vein into the right atrium of the heart, according to the surgical procedure described in Appendix 2. During two days of post-operative recovery, animals were exercised on the treadmill for 10 minutes each day at 15 $m.min^{-1}$ (representing easy exercise). On the third post-operative day, exercising animals (n=6) performed a progressive treadmill run on a small animal treadmill, initially at 15.0 $m.min^{-1}$, 0% grade, and increasing thereafter every 2 minutes by 5.0 $m.min^{-1}$ until exhaustion. This represented an exercise duration of 14 to 20 minutes. Blood was sampled serially at rest, and every two minutes throughout exercise and recovery, during the last 30 seconds of each time interval. This was accomplished without any apparent detrimental effect on performance. An equivalent volume of whole blood was reinfused after withdrawl of each sample from donor blood maintained at $4^{\circ}C$ taken from a freshly killed rat of the same strain. Control animals (n=7) were sampled and reinfused without exercise according to the same time schedule as the exercised rats for a period of 20 minutes. The blood samples were processed and stored for enzymatic analysis of ammonia and lactate, according to the procedures outlined in Appendix 4.

3.4 Experiment 2 - Tissue-Time Course Protocol

This study was conducted to investigate ammonia and lactate changes in blood, muscle, heart, liver and brain at fixed time points during exercise. Animals were treated and habituated to exercise as described previously. On the day of each experiment, rats were exercised at 45 $m.min^{-1}$, 0% grade during which groups of animals were sacrificed at rest, and at six time points during the exercise conditions. The time points were at 2, 4, 6, 8, and 10 minutes of exercise, and after 10 minutes exercise and 5 minutes recovery. A group of control animals (n=4), who were of the same strain and age as the exercised rats but had never been exposed to treadmill running, were sacrificed to determine if there were changes in resting values attributable to the training procedure. Animals were lightly anesthetized by ether and killed by exsanguination. The abdominal cavity was opened, blood was withdrawn from the abdominal aorta and immediately treated for subseqent analysis as detailed in Appendix 4. Liver, heart, and brain were excised and immediately frozen in liquid nitrogen at -30° C. Hindlimb muscles (specifically soleus, plantaris, and vastus lateralis) were then exposed, excised, and immediately frozen in liquid nitrogen.

Two investigators performed the dissections to minimize the time expended between anaesthesia and freezing of the tissue. Tissue sampling and processing techniques are detailed further in Appendix 4.

The water content of muscle of eighteen animals selected from different exercise groups was determined by drying a small section of fresh medial gastrocmenius tissue (100 - 250 mg) to a constant weight at 60° C.

Blood, heart, liver, muscle and brain tissue were assayed for ammonia (Kun and Kearney, 1974; Sigma, 1980). Blood, heart, liver and muscle were assayed for lactate (Gutmann and Wahlefeld, 1974; Boehringer Mannheim Diagnostica, Chemical assays are described in detail in Appendix 5.

The overall time course of the experiments is outlined in Table 2.

Table 2: Timetable of Experimental Procedures
Showing the program of training and experimental procedures.

Week #	Procedure
1	-Received animals, 8 weeks of age -Handled daily
2-7	-Habituation to running
8	-Tissue-Time Course Experiment
8-9	-Habituation to small treadmill and gas analyzing equipment
10	-$\dot{V}O_2$max testing
10-12	-Catheterization -Post-operative recovery -Catheter-Run Protocol

The total study involved 70 animals. In Experiment 1, 13 animals were used with 6 animals as an exercise group, and 7 as unexercised controls.

The second experiment, which required eight groups representing the time-points covered during a period from rest,

through exercise, and recovery, used 57 animals in total.

3.5 Statistical Considerations

Group means and standard error of the mean (SEM) were calculated for blood lactate and ammonia concentrations, body weights, and oxygen uptake measurements. Oneway analysis of variance (SPSS ANOVA) was performed between experimental groups, for body weights, and ammonia and lactate concentration changes with respect to exercise time, and to tissues. Post hoc Scheffe analysis was used to test for significance ($p<0.05$). When tests for homogeneity of variances indicated significant differences in homogeneity of groups (Bartlett-Box test, $p<0.05$), a multiple comparison procedure was used, based on Cochran and Cox's test for the equality of two means and Bonferroni's test for inequalities.

Non-linear regression analysis (BMDP-3R) was performed to obtain the best-fit exponential equation for the changes in blood ammonia and lactate concentrations with respect to time in Experiment 1. Relationships between ammonia and lactate concentrations in blood and tissue were determined by linear regression analysis (MIDAS). Group differences in oxygen uptake values were determined by One-Sample t-Test (BMDP-3D), where $p<0.05$ was used as a measure of significant difference between groups.

4.0 Results: General

4.1 Animal Body Weights

The group means obtained for body weights of each experimental group are shown in Table 3 for Experiment 1, and Table 4 for Experiment 2. In Experiment 1 (Table 3), both the control and exercised animals showed a significant growth during the period of the experiment ($p<0.05$). There were no other significant differences in body weight between Control and Experimental animals with respect to their pre- or post- surgery weight, the percent weight loss as a result of the surgery, or their weight on the day of their experiment.

Table 3: Animal body weights in Experiment 1 (control n=7, run n=6). Showing weight on arrival, pre- and post-surgery, respectively. Weight loss as a result of surgery, and weight on the day of the experiment are also shown. Values are means ± SEM.

Group	Control (Catheterised-Resting)	Run (Catheterised-Exercised)
Arrival	175.5 ± 8.3	177.0 ± 5.5
Presurgery	* 314.5 ± 6.9	* 285.3 ± 16.1
Postsurgery	* 301.2 ± 6.3	* 274.5 ± 14.9
Percent Weight Loss	4.2 ± 0.9	3.7 ± 0.6
Experimental Weight	* 299.2 ± 4.9	* 273.3 ± 14.2

* indicates significant increases above arrival weight, $p<0.05$. No significant differences were detected in body weight between Control and Run animals with respect to group.

Groups of animals in Experiment 2 also showed significant growth during the period of the experiment ($p<0.05$) (Table 4) There were no other significant differences between groups of animals with respect to either their weight at the time of their arrival, or on the day of their experimental run.

Table 4: Animal body weights in Experiment 2. Showing differences between arrival weight and weight at the time of sacrifice. (Values are Means ± SEM).

Table 4

GROUP PUN TIME (minutes)	WEIGHT ON ARRIVAL (gm)	EXPERIMENTAL WEIGHT (gm)
CONTROL (n=4)	142.8 ± 10.4	* 265.7 ± 15.8
ZERO (n=7)	127.0 ± 6.7	* 239.2 ± 10.9
TWO (n=9)	144.8 ± 10.2	* 243.9 ± 9.3
FOUR (n=7)	148.1 ± 11.4	* 251.9 ± 16.5
SIX (n=8)	142.6 ± 8.5	* 258.9 ± 8.7
EIGHT (n=8)	131.8 ± 7.9	* 253.6 ± 10.2
TEN (n=8)	145.1 ± 9.4	* 247.1 ± 8.2
RECOVERY (n=6)	141.8 ± 13.8	* 258.5 ± 14.1

* indicates significant increase from arrival weight, ($p<0.05$). No significant differences in body weight were detected between groups with respect to weight on arrival or experimental weight.

4.2 Results - Experiment 1

4.2.1 Oxygen Uptake Measurements

Oxygen uptake measured before and after surgical catheterization of animals is summarized in Table 5. At rest, $\dot{V}O_2$ varied from 36.1 ± 4.9 (range 30.3 to 46.2) $ml \cdot kg^{-1} \cdot min^{-1}$ in control animals to 39.1 ± 6.6 (range 31.0 to 47.9) $ml \cdot kg^{-1} \cdot min^{-1}$ in catheterised animals (Figure 2). Initially, the treadmill velocity was 15 $m \cdot min^{-1}$, and was increased by 5 $m \cdot min^{-1}$ every 2 minutes until the animals could no longer keep pace with the treadmill. This occurred after maximum running times of 18 minutes for control and 16 minutes for catheterised animals, at maximum running velocities of 60 $m \cdot min^{-1}$ for control animals, and 55 $m \cdot min^{-1}$, respectively. At exhaustion, $\dot{V}O_2$ max was 92.8 ± 4.7 (range 88.1 to 99.7) $ml \cdot kg^{-1} \cdot min^{-1}$ in control animals and 81.9 ± 5.9 (range 74.9 to 91.5) $ml \cdot kg^{-1} \cdot min^{-1}$ in catheterised rats. At rest, the differences in $\dot{V}O_2$ between non-catheterised and catheterised animals was not significant. However, there were significant differences between $\dot{V}O_2$ max values of the different groups ($p<0.05$).

In control animals, the mean respiratory exchange ratio (RER) was 0.82 ± 0.07 and 1.02 ± 0.11 during resting conditions and maximum exercise. Corresponding values for RER in catheterised animals were 0.82 ± 0.04 and 1.13 ± 0.06 respectively.

Table 5: Oxygen uptake and respiratory exchange ratios (mean ± SEM) measured in female rats pre- (n=7) and post-catheterization (N=6) respectively, in animals running to exhaustion.

	Pre-Catheterization	Post-Catheterization
REST		
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	36.1 ± 4.9	39.1 ± 6.6
RER	0.82 ± 0.07	0.82 ± 0.04
EXHAUSTION		
$\dot{V}O_2$ max ($ml \cdot kg^{-1} \cdot min^{-1}$)	92.8 ± 4.7	* 81.9 ± 5.9
RER	1.02 ± 0.12	1.13 ± 0.06

* indicates significant difference from pre-catheterization value.

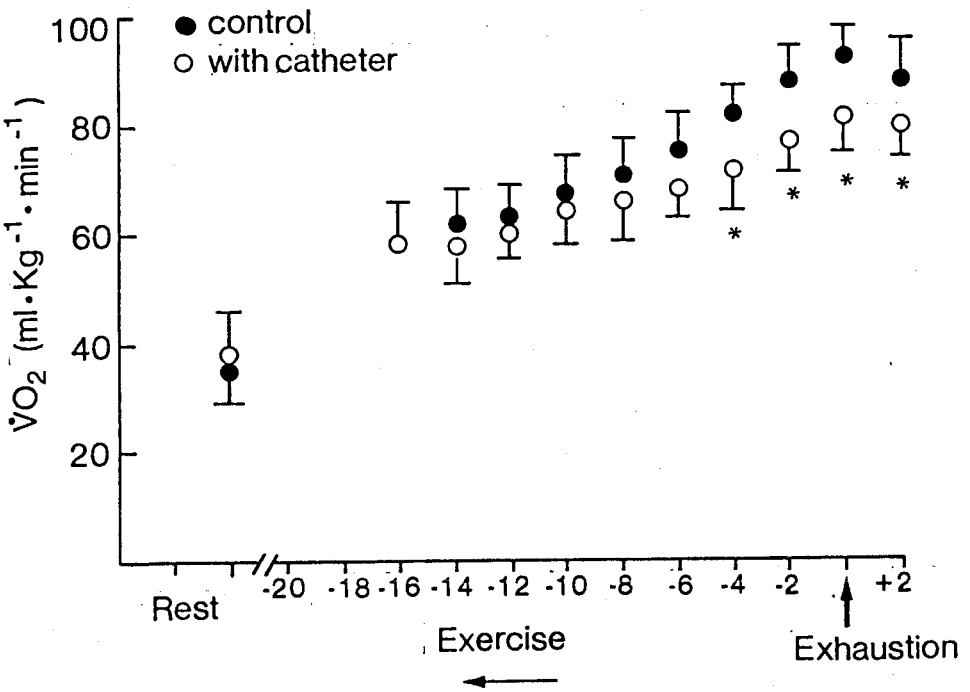


Figure 2: Oxygen uptake (mean ± SEM) measured in female rats measured during a progressive treadmill run to exhaustion pre- (n=7) and post-catheterization (N=6), respecively.
* indicates significant difference from pre-catheterization value.

4.3 Blood Ammonia and Lactate

The time course of both ammonia and lactate accumulation in rat blood during progressively increasing exercise to exhausiton is shown in Figure 3. Exercise duration ranged from 14 to 20 minutes before exhaustion. Running time was normalized to the point of exhaustion so that blood metabolite concentrations referred to a similar stage of exertion relative to this point in animals of dissimilar running ability. Catheterised animals ran an average of 2 to 4 minutes longer when blood was being sampled and reinfused than animals in the $\dot{V}O_2$ max protocol.

4.3.1 Control Animals: Non-Exercised

Blood was sampled every 2 minutes during a 20 minute period in control rats at rest, a period which corresponded to the maximum time of exercise before exhaustion in the exercised groups. During this entire period, ammonia and lactate concentrations remained at their resting values of 57.1 ± 8.5 (range 41.5 - 67.5) μM and 1.5 ± 0.2 (range 1.4 - 2.0) mM respectively. At no time point were these values significantly different from each other (Figure 3).

4.3.2 Exercised Animals

Under resting conditions immediately prior to exercise, blood ammonia was 36.4 ± 13.2 (range 18.2 - 54.5) μM and blood

lactate was 1.4 ± 0.5 (range 0.9 - 2.2) mM. These values were similar to those reported under control conditions (Figure 3).

At t = 10 minutes during exercise, the mean group ammonia concentration had increased to 67.5 ± 18.2 μM, almost double that of the resting value (36.4 ± 13.2), but was not appreciably greater than that of the non-exercised control animals (57.1 ± 8.5) at a similar time point. Corresponding blood lactate concentration at t = 10 minutes in this group was 2.9 ± 1.1 mM, double both the resting and non-exercised control concentrations.

In this group at exhaustion, the concentration of blood ammonia was 349.7 ± 61.9 (range 267.4 - 402.4) μM, and blood lactate measured 12.0 ± 1.8 (range 8.9 - 14.1) mM. These values represented a 9 fold increase in blood ammonia and an 8 fold increase in blood lactate respectively compared to resting values in the exercising group. The relative increases are slightly less compared to resting concentrations of ammonia (6 fold) and lactate (8 fold) respectively in control animals. The concentration of ammonia and lactate at the point of exhaustion in exercising animals was significantly different from the resting controls ($p<0.05$). During recovery, a rapid decrease in both ammonia and lactate was observed. After 2 minutes recovery, ammonia had decreased by 30% from its peak concentration, and lactate had decreased by 25% at the same time point. However, ammonia and lactate were not significantly different from the peak concentrations of exercise until the 6th minute of

recovery, at which time both ammonia and lactate were 50% lower than their peak exercise concentration.

Exercise resulted in an accumulation of lactate in blood which was significantly different from resting control values ($p<0.05$) between t=-4 (4 minutes prior to exhaustion) to t=0 (the point of exhaustion). Group mean differences between resting ammonia and exercise ammonia concentration were not significantly different ($p<0.05$) until t=-2 and t=0 minutes. There is, however, some difficultly in interpreting the significance of this result because of the large within-group variability in concentration. However, comparison of the time course of the rising blood concentration profiles of ammonia and lactate respectively suggests that there is very little difference between them during running in the rat (Figure 3).

Regression analysis showed that there was a significant intersubstrate correlation between concentrations of blood ammonia and lactate from exercising animals, according to the equation:

$$\text{Lactate (mM)} = 0.029(\text{Ammonia } \mu\text{M}) + 1.77 \qquad (6)$$

$(n=75,\ r=0.72,\ p<0.001)$

These data and regression line are illustrated in Figure 4.

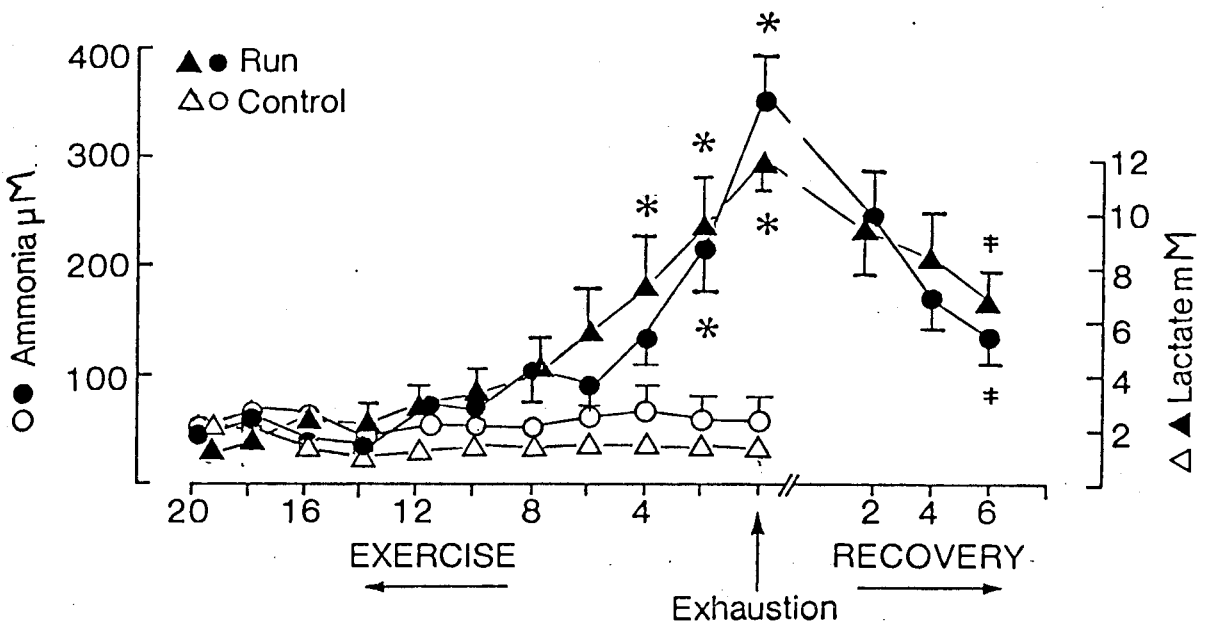


Figure 3: Time course of accumulation of blood ammonia and lactate during a progressive treadmill run to exhaustion in female rats. The values are means + SEM. Exercise Time (abscissa) was normalized to exhaustion at time zero. The other times (i.e. t = -4) indicate minutes prior to exhaustion, or minutes of recovery.
* indicates significant differences from control values.
+ indicates significant differences from peak concentrations.

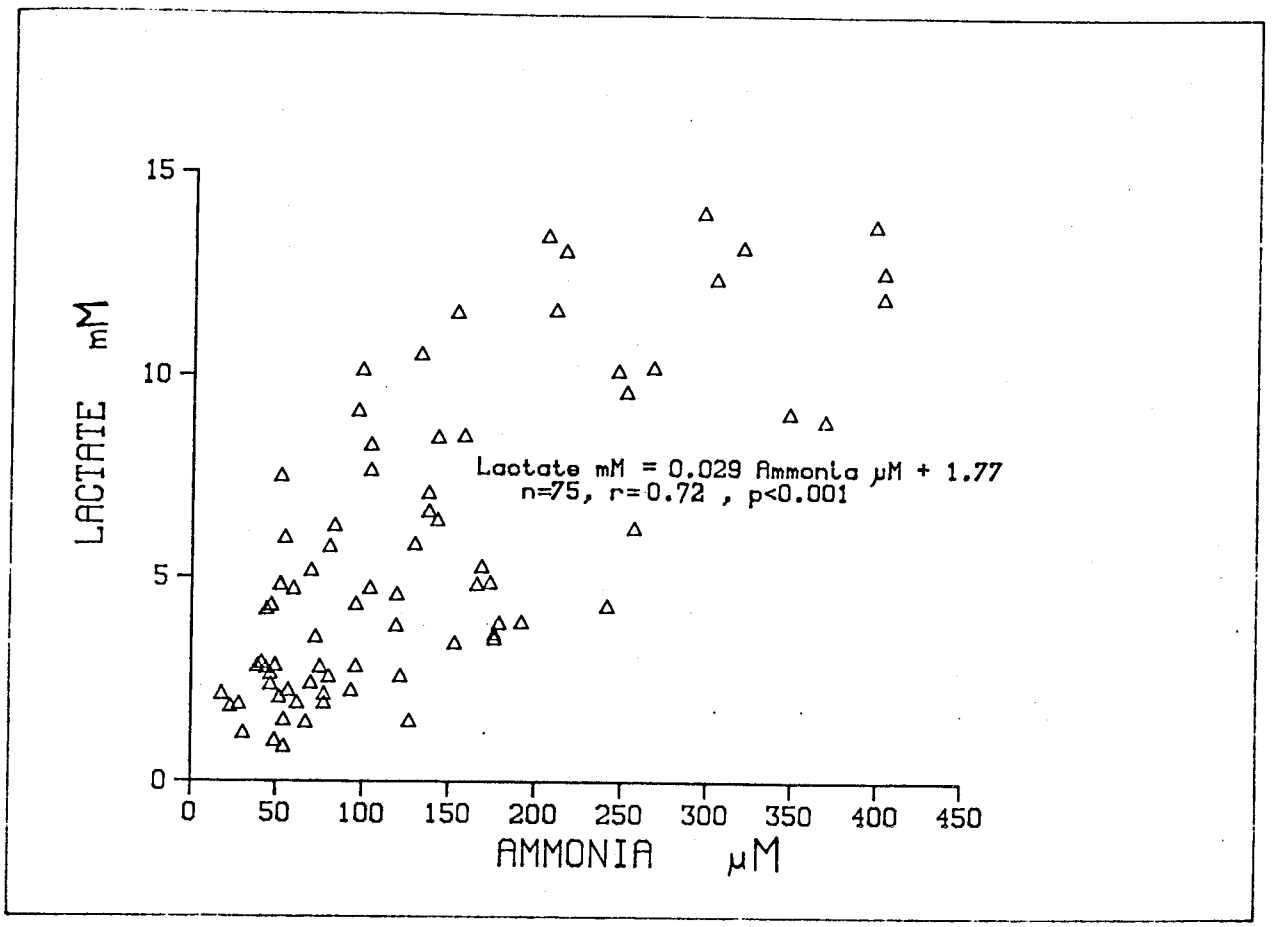


Figure 4: Comparison of the relative rate of increase of blood ammonia and lactate in female rats as they accumulated during a progressive treadmill run to exhaustion. The equation represents the line of best fit for these data.

4.4 Experiment 2: General

4.4.1 Accumulation of Ammonia and Lactate in Tissue While Working at a Constant Workrate

This experiment examined the time course of accumulation of ammonia and lactate in various tissues resulting from exercise at a constant workrate. The workrate was preset as as strenuous a condition at which animals could exercise continuously for ten minutes. Pats were sacrificed at time points corresponding to 0, 2, 4, 6, 8, and 10 minutes of exercise, and also following 5 minutes of resting recovery. Control animals were animals of the same strain and age, but with no exposure to running on the treadmill, who were sacrificed at rest. These were designated as control-sedentary.

At the time of the experiment, the running ability of the animals was evaluated subjectively as being either poor, fair, good, or excellent. Only those animals evaluated as giving either a "good" or "excellent" running performance were included in data analysis. The criterion of running performance was simply that of a willingness by the animal to keep pace with the treadmill, and to run continuously without frequent pauses to "ride" the treadmill back to the shocking device before running again. Using this criterion, nine animals were eliminated, two from the 2 minute, two from the 4 minute, one from the 6 minute, three from the 8 minute, and one from the 10 minute run group.

The study also began with n=6 in the control sedentary group. This number was reduced to 4 when one escaped during handling, and one supposedly "virgin" female gave birth to six pups eighteen days after arrival.

There were no significant differences in ammonia or lactate concentrations in any of the tissues studied between Control (sedentary) and Zero (resting - trained), and they will not be discussed in further sections.

4.4.2 Muscle Water Content

Water content of muscle was estimated in eighteen experimental animals. From the measures obtained, a comparison was made between two groups (n=6) within this sample. Group 1 consisted of animals at rest and at two minutes of exercise, and group 2 consisted of animals measured after eight and ten minutes of exercise. Muscle water content was measured in the medial gastrocnemius muscle. There was no significant change in the percentage of water in the muscle between the Group 1 (77.1 $\pm$ 0.5%) and Group 2 (77.3 $\pm$ 1.1%). Values obtained within the entire sample ranged from 75.9% (six minutes of exercise) to 78.1% (ten minutes of exercise).

4.4.3 Blood Ammonia and Lactate During Submaximal Exercise

At the onset of exercise, both ammonia and lactate increased in blood from pre-exercise values of 62.3 $\pm$ 11.6

(range 20.7 to 114.2) μM and 3.0 ± 0.3 (range 1.7 to 4.1) mM reaching peak concentrations after 8 minutes of exercise of 203.5 ± 46.6 (range 38.9 to 329.7) μM, and 10.8 ± 0.9 (range 7.3 to 12.2) mM respectively (Figure 5). Similar values were reported by Meyer, Dudley, and Terjung (1980) in untrained rats exercising at 45 $m \cdot min^{-1}$.

Due to significant differences of variance between groups, a oneway ANOVA was invalid as a statistical test of significant accumulation for blood ammonia data. The Cochran-Cox procedure also did not show significant differences between the group means. The large variance within groups was the reason for the lack of statistically significant differences in ammonia concentration between exercise groups, even though there were obvious absolute changes in blood ammonia concentrations due to the exercise protocol.

Blood lactate concentration was significantly different ($p<0.05$) from resting (zero) values at all points during exercise. After five minutes of recovery a reduction in blood lactate from its peak values at 6 and 8 minutes of exercise occurred.

In relative terms, the exercise in Experiment 2 was less intense than that reported in Experiment 1. The maximum concentrations of ammonia and lactate in blood observed in Experiment 2 (submaximal exercise) corresponded to the second to last workrate (t=-2) accomplished before exhaustion in Experiment 1 (progressive work to exhaustion). The values

reported for ammonia and lactate at t=-2 in Experiment 1 during maximum exercise tests (ie. the second to last timepoint of exercise) were 223.3 ± 26.3 μM and 9.5 + 3.2 mM respectively.

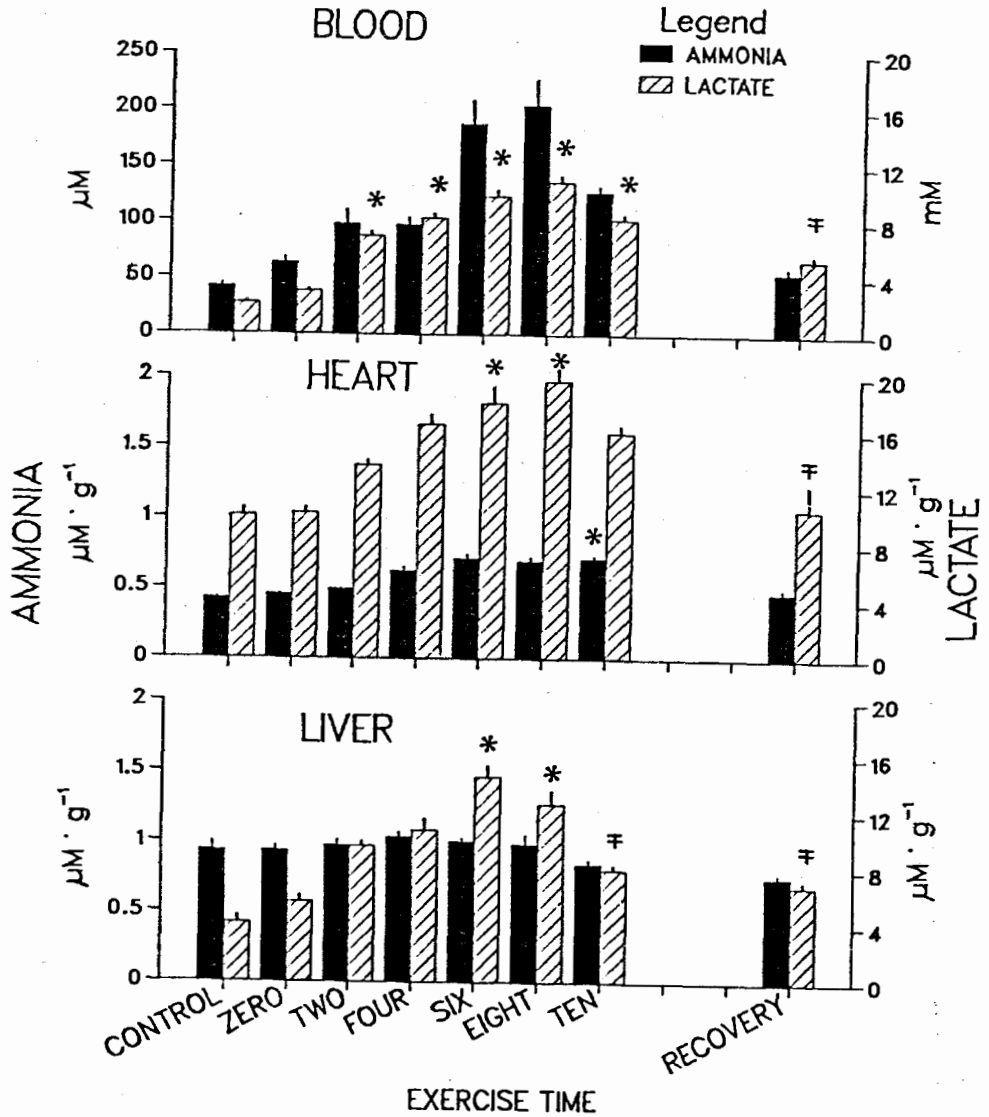


Figure 5: Showing the accumulation of ammonia and lactate in the blood, liver, and heart of rats during submaximal exercise. The wide bars represent mean values, and the narrow bars indicate SEM.

* indicates significant difference from resting concentrations.

∓ indicates significant difference from peak concentrations.

4.4.4 Liver and Heart Ammonia and Lactate

In heart, the resting concentrations of ammonia and lactate were 0.45 ± 0.02 $\mu M \cdot g^{-1}$, (range 0.39 to 0.51) and 10.3 ± 1.9 $\mu M \cdot g^{-1}$, (range 7.3 to 13.9) respectively. In liver, the resting concentration of ammonia was 0.93 ± 0.08 $\mu M \cdot g^{-1}$, (range 0.65 to 1.35), while the resting concentration of lactate was 5.7 ± 0.9 $\mu M \cdot g^{-1}$, (range 2.3 to 9.7). In both liver and heart, a rapid rise in lactate concentration was observed early in exercise. Lactate reached its maximum concentration during the 8th minute of exercise in heart tissue (19.7 ± 1.8 $\mu M \cdot g^{-1}$, range 14.3 to 25.1) , and during the 6th minute in the liver (14.6 ± 1.6 $\mu M \cdot g^{-1}$, range 10.4 to 20.8). There was a substantial decrease in lactate between 8 and 10 minutes of exercise and a further decrease during the 5 minutes of recovery. In both tissues, lactate had returned to resting concentrations following 5 minutes of recovery (Figure 5).

In liver, the changes in lactate concentration were statistically significant ($p<0.05$) between rest(zero), and 6 (peak) and 8 minutes of exercise. The peak value for liver concentration (6 minutes) was also significantly above the 10 minute exercise value, and that measured in the recovery period ($p<0.05$). In heart, similar significant changes were observed between rest (zero) and the 6 and 8 minute points of exercise, and between 8 minutes (peak value) and recovery lactate concentration.

Ammonia concentration on the other hand, did not change as markedly in heart and liver as was observed in other tissues. Of all the tissues studied, these latter tissues were the only two in which the profile of ammonia and lactate changes were dissimilar. In liver, ammonia remained relatively constant throughout the exercise period (Pest: 0.93 $\pm$ 0.08 $\mu M \cdot g^{-1}$, range 0.65 to 1.35; Maximum: 1.03 $\pm$ 0.08 $\mu M \cdot g^{-1}$, range 0.87 to 1.23), decreasing slightly during recovery to below resting values (Recovery: 0.75 $\pm$ 0.05 $\mu M \cdot g^{-1}$, range 0.62 to 0.96). None of the differences in ammonia concentration in liver were statistically significant.

In heart, there was a gradual, but steady increase in ammonia concentration throughout the exercise from 0.45 $\pm$ 0.02 $\mu M \cdot g^{-1}$ (range 0.39 to 0.51) at rest to 0.71 $\pm$ 0.04 $\mu M \cdot g^{-1}$ (range 0.55 to 0.87) at 10 minutes of exercise followed by a marked decrease in ammonia to resting concentration during recovery (0.47 $\pm$ 0.06 $\mu M \cdot g^{-1}$, range 0.33 to 0.68). Statistical differences between the group means of heart tissue ammonia were evaluated by Cochran-Cox procedure for evaluating differences between means, because data from this tissue also showed significant differences in variance between groups. From this analysis, significant differences were observed between resting and ten minutes of exercise. The decrease in ammonia in heart tissue during recovery, while distinctive, was not statistically significant.

4.4.5 Muscle Ammonia and Lactate

The three muscles studied in this experiment, soleus, plantaris, and white vastus lateralis, were chosen to represent slow-twitch oxidative, fast-twitch oxidative, and fast-twitch glycolytic muscle respectively.

In resting animals, the concentration of ammonia was 0.88 ± 0.09 $\mu M \cdot g^{-1}$ while lactate was undetectable in soleus. In plantaris, resting ammonia and lactate were 0.91 ± 0.07 $\mu M \cdot g^{-1}$ and 2.36 ± 0.56 $\mu M \cdot g^{-1}$, respectively (Figure 6). In vastus lateralis, resting ammonia and lactate were 0.62 ± 0.07 $\mu M \cdot g^{-1}$ and 6.45 ± 0.90$\mu M \cdot g^{-1}$, respectively.

Ammonia: Ammonia reached its peak concentration following 6 minutes of exercise in vastus (1.26 ± 0.24 $\mu M \cdot g^{-1}$) and after 8 minutes of exercise in both plantaris (1.26 ± 0.12 $\mu M \cdot g^{-1}$) and soleus (1.41 ± 0.13) (Figure 6). Ammonia concentrations were statistically different ($p<0.05$) from resting values at 8 minutes of exercise in soleus, and at 6 and 8 minutes of exercise in vastus tissue. In addition, ammonia concentration during recovery was significantly reduced from these peak values. No significant differences in ammonia concentration were detected between any two exercise groups in plantaris muscle.

Lactate: Peak lactate values were observed after 6 minutes of exercise in plantaris (12.3 ± 3.4 $\mu M \cdot g^{-1}$) and soleus (8.2 ± 2.3 $\mu M \cdot g^{-1}$), and after 8 minutes of exercise in vastus (18.0 ± 3.3) (Figure 6). Significant differences ($p<0.05$) in lactate values were observed between zero (resting) concentrations and 6 and 8

minutes of exercise in plantaris, and vastus muscles, and at 2, 4, 6, 8, and 10 minutes of exercise in soleus. During recovery, lactate decreased significantly ($p<0.05$) from the peak exercise concentration measured in these three muscle groups to approximatly the same values observed during rest.

Maximum changes in concentration in skeletal muscle tissue, calculated as the peak concentration minus the resting, or zero value, are shown in Table 6. Table 6 also compares these data with similar studies reported by others (Meyer, Dudley and Terjung, 1980). In the present study, the greatest change in ammonia concentration in skeletal muscle was observed in the vastus lateralis muscle. Smaller changes were observed in soleus and plantaris muscle. Lactate changes were also greatest in vastus lateralis. Smaller changes occurred in plantaris, and soleus exhibited the least change in lactate concentration as a result of this exercise protocol.

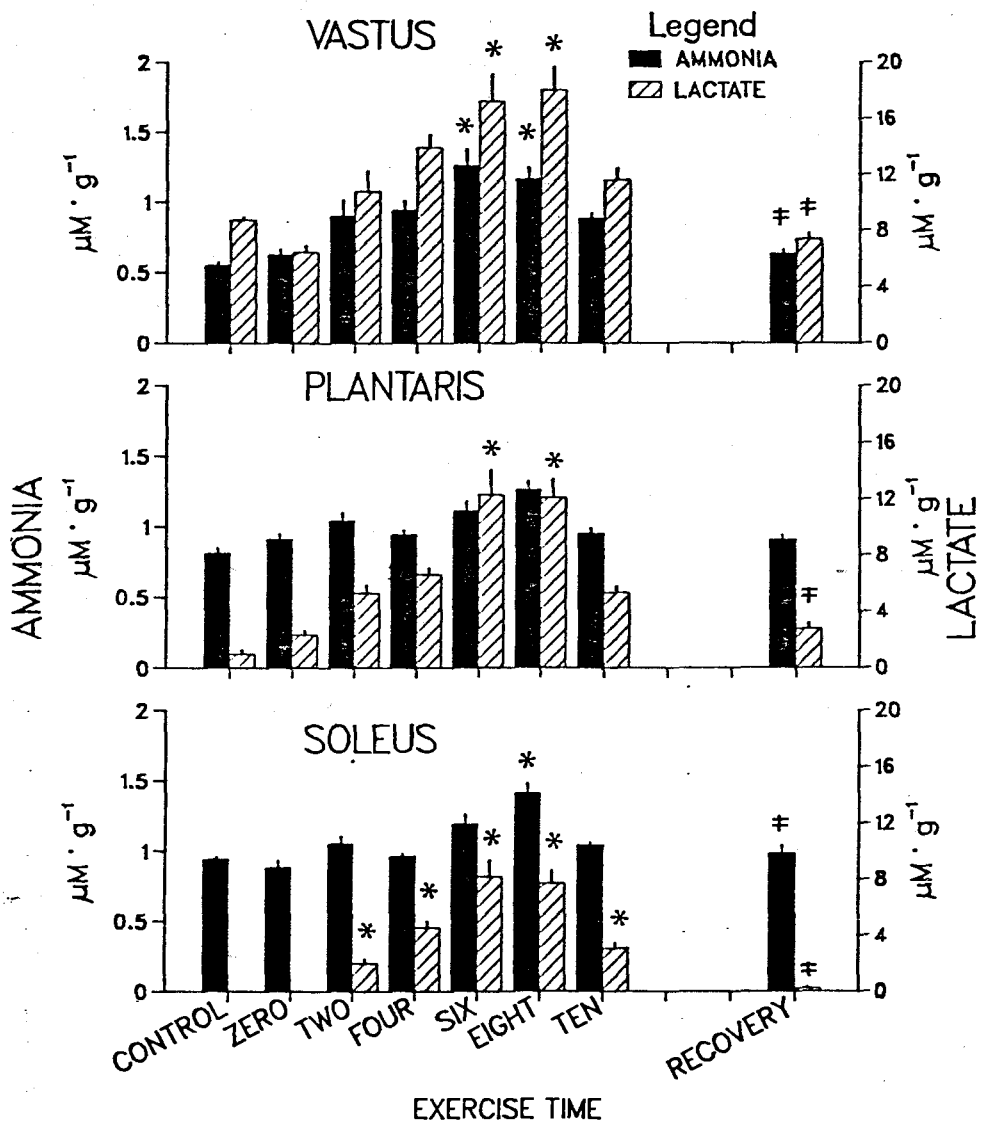


Figure 6: Showing the accumulation of ammonia and lactate in the white vastus lateralis, plantaris, and soleus rat muscle after submaximal exercise. In soleus, lactate concentration at control and zero time points was not detectable. Wide bars represent means and the narrow bars indicate SEM.

* indicates significant differences from resting concentrations.

\+ indicates significant differences from peak concentrations.

Table 6: Peak changes in ammonia and lactate contents observed in three skeletal muscle groups after exercise. Values were calculated from the peak concentration observed minus the resting concentration value.
Values in parenthesis are peak changes in ammonia and lactate concentrations observed after 4 minutes of similar exercise at 45 m.min-1 reported by Meyer, Dudley, and Turjung, 1980.

CHANGE IN AMMONIA: ($\mu M \cdot g^{-1}$ wet weight)	Vastus	>	Soleus	>	Plantaris
	0.64 (1.94)		0.53 (0.51)		0.35 (1.25)
CHANGE IN LACTATE: ($\mu M \cdot g^{-1}$ wet weight)	Vastus	>	Plantaris	>	Soleus
	11.5 (19.2)		9.9 (9.9)		8.2 (6.6)

In resting muscle, the concentration of ammonia was significantly greater ($p<0.05$) in soleus than in vastus tissue, but no significant differences were observed in resting ammonia concentration between soleus and plantaris or plantaris and vastus tissue. Resting lactate in muscle was significantly greater in vastus ($p<0.05$) than in either plantaris or soleus, however no significant differences were observed between resting lactate concentration in soleus and plantaris. At the peak concentration observed during exercise, there were no significant differences between ammonia concentration between vastus, plantaris, and soleus tissue. Peak concentrations of lactate, however, were significantly greater ($p<0.05$) in vastus

compared to soleus, but not between vastus and plantaris, or between plantaris and soleus tissue. Significant differences ($p<0.05$) were observed in the change in lactate concentration observed between resting and peak values, between vastus and soleus tissue.

4.4.6 The Relationship Between Ammonia and Lactate Concentrations in Tissue

The correlation between ammonia and lactate accumulation in tissue resulting from exercise was examined by regression analysis of ammonia with lactate in each particular tissue. Significant correlations were apparent in blood, liver, heart, vastus, plantaris, and soleus tissues as illustrated by the following linear equations:

Blood: Lactate mM = 0.033 (Ammonia μM) + 3.28 (7)
($n=44$, $r = 0.81$, $p<0.001$)

Liver: Lactate $\mu M \cdot g^{-1}$ = 8.20 (Ammonia $\mu M \cdot g^{-1}$) + 1.58 (8)
($n=46$, $r = 0.35$, $p<0.02$)

Heart: Lactate $\mu M \cdot g^{-1}$ = 19.66 (Ammonia $\mu M \cdot g^{-1}$) + 2.89 (9)
($n=46$, $r = 0.71$, $p<0.001$)

Vastus: Lactate $\mu M \cdot g^{-1}$ = 10.59 (Ammonia $\mu M \cdot g^{-1}$) + 1.77 (10)
($n=41$, $r = 0.67$, $p<0.001$)

Plantaris: Lactate $\mu M \cdot g^{-1}$ = 15.05 (Ammonia $\mu M \cdot g^{-1}$) -8.89 (11)
($n=47$, $r = 0.68$, $p<0.001$)

Soleus: Lactate $\mu M \cdot g^{-1}$ = 9.97 (Ammonia $\mu M \cdot g^{-1}$) -7.24 (12)
($n=46$, $r=0.67$, p 0.001)

These data are shown in Figure 8. The gradient of the regression line for blood slope appears to be different from the other tissues. This difference probably results from the different units of concentration used for blood and the magnitude of the difference between ammonia and lactate in in molar concentration normally found in this tissue. Heart tissue lactate concentration rises much more rapidly than ammonia, due to exercise. Changes in ammonia relative to lactate in plantaris and soleus muscle are somewhat obscured by the relatively high resting ammonia concentration found in these tissues.

Liver has the least significant relationship between between ammonia and lactate concentration ($p<0.02$), which is not surprising since no significant changes in liver ammonia were observed as a result of the exercise. In all other tissue observed, the correlation between ammonia and lactate was significant at the $p<0.001$ level.

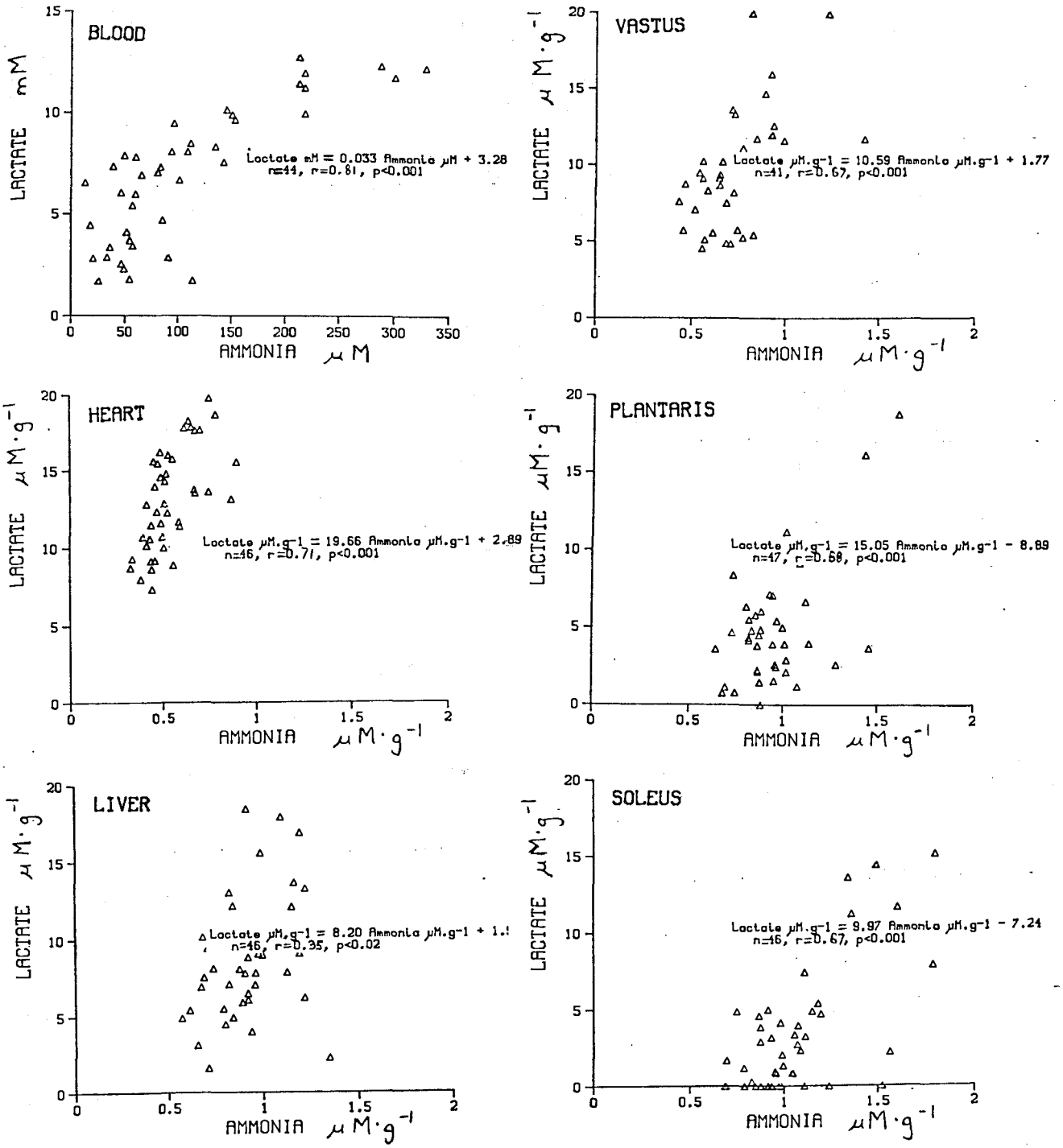


Figure 7: Scattergrams of data expressing the relation betweem lactate and ammonia in various rat tissues after submaximal running at 45 $m \cdot min^{-1}$ for 10 minutes.

4.4.7 Brain Ammonia Accumulation

Brain ammonia concentration, measured in the rat brain stem, cerebellum, and cortex, remained relatively constant throughout the duration of exercise and recovery. (Figure 7). In the cortex, there appeared to be a greater degree of variability in ammonia concentration both at the onset of exercise, from 0 to 4 minutes, and during post-exercise recovery. However, these changes were not significant, probably due to the large within-groups variance at each exercise time.

In the cerebellum, ammonia was significantly increased during recovery, in comparison with resting values, or those at six minutes of exercise. This is particularly interesting, since in most of the tissues examined, ammonia reached its peak values between six and eight minutes of exercise, and showed significant decreases following the recovery period.

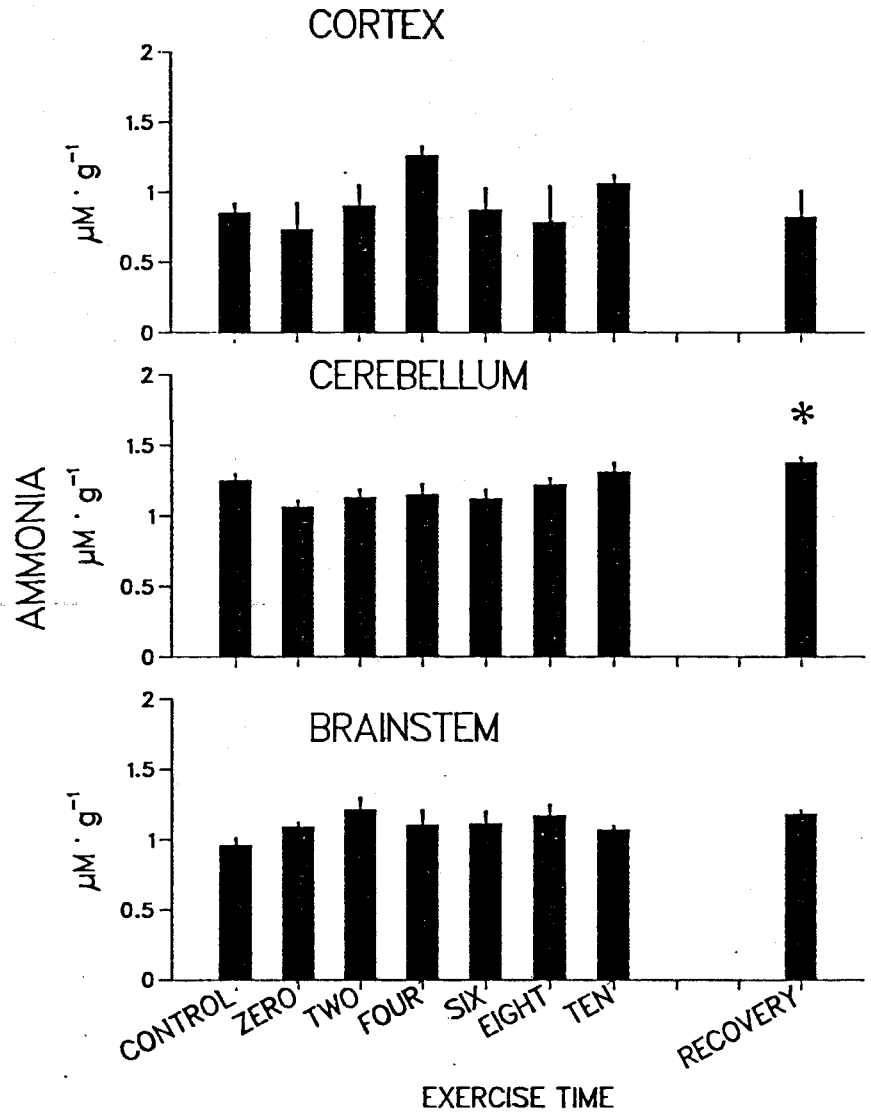


Figure 8: Accumulation of ammonia and lactate in the cortex cerebellum and brain stem of rats resulting from submaximal exercise. Wide bars represent means, and narrow bars indicate SEM.
* indicates significant differences from resting concentrations.

5.0 Discussion

5.1 General

The procedure used to obtain tissue samples from exercised animals in this study was designed to minimize the interval between cessation of exercise and sampling of tissues, to standardize the order in which samples were taken from each animal, and to reduce inter-animal variability in the time of tissue sampling. Since muscle water content is known to increase by 2 to 3 %, from about 77% w/w at rest to 79-80% w/w after intense exercise (Bergstrom, Granieri and Hultman, 1971), metabolite tissue concentrations are frequently adjusted relative to some reference compound. Muscle content of total creatine (TCr = PCr + Cr) is usually chosen as the marker substance since it is reported to remain constant during exercise (Harris, et al., 1976).

When a comparison of wet weight to dry weight of muscle tissue was made in this study, no difference in water content was observed between rest conditions and those of exercise. No adjustment in various tissue metabolite concentrations has been made, therefore, relative to a standard such as total creatine, for any potential tissue water volume changes.

5.2 Animal Body Weights

All animals in this study (Experiment 1 and 2) showed significant increases in their body weight from the time of their arrival to the termination of the experiment. This was expected, since animals were received at 8 weeks of age. Female Wistar rats reach sexual maturity between 60 to 72 days of age, and are considered to be developmentally mature at body weights above 200 g.

There were no significant differences in body weights between any experimental groups in either Experiment 1 or 2. Particularly noteworthy was the fact that in Experiment 2, control animals who had not been exposed to treadmill running, were not significantly different in weight from other animals. Male rats usually demonstrate significant reductions in body weight as a result of training (Harpur, 1980). This complication was avoided by the choice of female experimental animals in this study.

5.3 Oxygen Consumption

During progressively increasing work in the running rat, $\dot{V}O_2$ increases exponentially as a function of work intensity (running speed) to a maximum asymtotic value. Further increases in running speed have no effect in increasing $\dot{V}O_2$, and might even cause slight decreases in its value (Shepherd and Gollnick, 1976; Brooks and White, 1978; Gleeson and Baldwin, 1978; Patch and Brooks, 1980).

Gleeson and Baldwin (1981) measured $\dot{V}O_2$ max of 93.1 ± 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$ at a speed of 27 to 28 $m \cdot min^{-1}$, 0% grade, in untrained female Wistar rats (the breed of animal used in these experiments). The highest rate of oxygen consumption in rats has been reported by Shepherd and Gollnick (1976), at 95.4 ± 1.4 $ml \cdot kg^{-1} \cdot min^{-1}$ for male Sprague-Dawley rats running at 49.5 $m \cdot min^{-1}$, although these rats could continue to run up to speed of 67.0 $m \cdot min^{-1}$ without further increases in oxygen uptake. Harpur (1980) reported a range of maximum oxygen uptake values in exercising rats between 81.7 and 95.4 $ml \cdot kg^{-1} \cdot min^{-1}$ at speeds ranging from 43 to 50 $m \cdot min^{-1}$ on a level surface (Harpur, 1980).

The animals of the present study exhibited $\dot{V}O_2$ max values within the range reported in the literature (92.8 ± 4.7, control, and 81.9 ± 5.9, catheterised) (Table 5).

The 11.7% reduction in $\dot{V}O_2$ max in cannulated rats compared with controls ($p<0.05$) cannot be completely accounted for, although it was probably a result of the surgical procedure. Gleeson and Baldwin (1981), who reported a comparable reduction in $\dot{V}O_2$ max following catheterization of female Wistar rats, suggested that it could be related to appetite-suppresion following Ketamine anesthesia.

Cannulated, rats were behaviourally indistinguishable from non-cannulated animals after a 24 hour recovery period, although each had lost an average of 4.2 ± 0.9% and 3.7 ± 0.6% of their body weight on the first day post surgery (Table 3). Weight reduction accompanying a possible anesthesia-induced appetite

loss may have reduced the endurance running ability of the rats.

Resting values for $\dot{V}O_2$ reported in this study are generally 10 to 15 $ml \cdot kg^{-1} \cdot min^{-1}$ higher than those reported previously (Brooks and White, 1978; Harpur, 1980; Patch and Brooks, 1980; Gleeson and Baldwin, 1981). The difference may be, that in the present study, animals were alert in the anticipation of exercise, whereas other investigators have measured resting $\dot{V}O_2$ values when animals were resting on a treadmill for an hour, when animals were sleeping, or were one hour post-exercise.

Serial measurements of $\dot{V}O_2$ during treadmill running at progressive intensity are shown in Figure 2. In both groups of animals, the kinetics of oxygen uptake shows a familiar normal approach to an asymptotic value as exercise increases. This suggests that reliable measures of $\dot{V}O_2$ max were determined (Shepherd and Gollnick, 1976). Since the $\dot{V}O_2$ max values measured in this study were within the range of values previously reported for normal and catheterised rats (Harpur, 1980; Gleeson and Baldwin, 1981), the values were considered to be a good representation of the oxygen uptake capacity of the animals. During heavy exercise, the RER values were 1.02 ± 0.11 in control, and 1.13 ± 0.06 in catheterised rats. RER values greater than or equal to 1 also suggest maximum effort in running was achieved by the animals. Values obtained in this study support the assessment that the animals did in fact exercise to exhaustion.

5.4 Lactate and Ammonia Accumulation in Blood During Maximal Exercise

The present data indicate that during progressive exercise to exhaustion (Experiment 1), blood ammonia and lactate concentrations rise in blood exponentially with time. Using the exercise protocol described, blood ammonia was 349.7 $\pm$ 61.9 uM and blood lactate was 12.0 $\pm$ 1.8 mM at exhaustion (Figure 4). Similar results have been reported by others using the end point of exercise as the criterion point of exhaustion, both in rats (Laborit, et al., 1957 and 1958; Barnes, et al., 1964; Meyer, Dudley, and Terjung, 1980) and humans (Allen and Conn, 1960; Brodan, et al., 1974; Wilkerson, et al., 1975 and 1977). Recently, two papers have reported a similar time-course of blood lactate and ammonia accumulation during exercise and recovery in humans (Babij, Matthews and Rennie, 1983; Banister, et al., 1983), although in humans, lactate continued to increase in the initial post-exercise period. In rats, both ammonia and lactate decreased rapidly post-exercise, which suggests that rats have a better clearance mechanism for lactate than humans do.

Non-significant increases in blood ammonia and lactate were first observed at t=-12 minutes (Figure 4), which corresponded to a mean workrate of 25 $m \cdot min^{-1}$. Greater increases in ammonia and lactate then developed between t=-10 and t=-8 minutes of exercise, corresponding to a mean work rate of 30 and 35 $m \cdot min^{-1}$ at each time point. Since oxygen uptake was not measured

simultaneously with blood sampling, and because animals ran for a greater length of time during the blood sampling procedures than during the oxygen uptake measurements in this experiment, the oxygen uptake corresponding to a particular workrate (speed) in this study can only be estimated.

Figure 9 illustrates the relationship between ammonia (9A) and lactate (9B) accumulation and % $\dot{V}O_2$ max during a progressive treadmill run to exhaustion. The $\dot{V}O_2$ data were extrapolated from those observed during maximum oxygen uptake studies using a similar progressive treadmill run to exhaustion with the same increments in running speed at each time point. As previously discussed, the animals ran for a longer duration when blood was sampled and reinfused. Thus it was necessary to extrapolate $\dot{V}O_2$ data from the time of exhaustion (100% $\dot{V}O_2$ max) to the resting concentration (48% $\dot{V}O_2$ max) and apply them to the time points of exercise in this study. With these limitations, the following observations were made.

An initial increase was observed in both blood ammonia and lactate concentration at t=-12, at a workrate (speed) corresponding to 74% $\dot{V}O_2$ max. This increase developed distinctly between time -10 and -8 minutes, respectively corresponding to 78 and 80% of $\dot{V}O_2$ max. It was not possible to determine the pre-eminance of either lactate or ammonia accumulation in the blood.

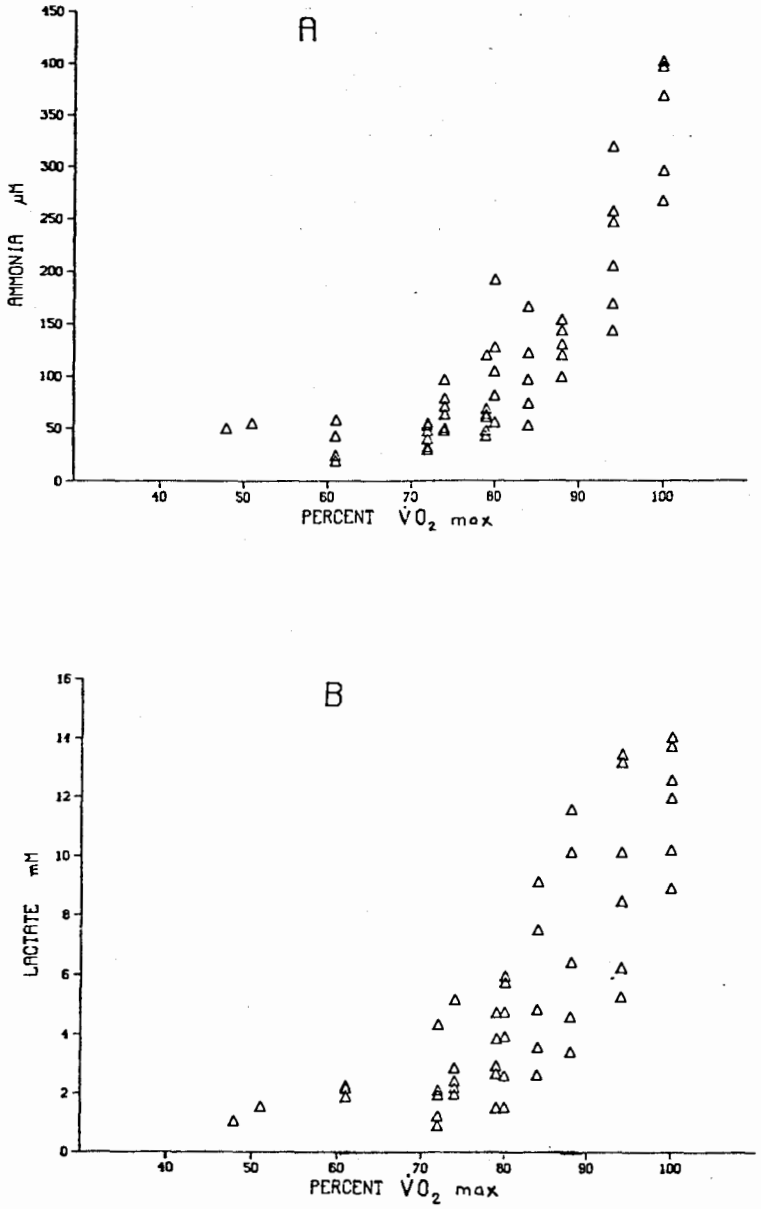


Figure 9: Showing the relation between blood ammonia (9A) and blood lactate (9B) concentrations and $\%\dot{V}O_2$ max in female rats during a progressive treadmill run to exhaustion.

In this study, catheterised animals ran for an average of 2 to 4 minuts longer during the procedure involving blood sampling and reinfusion than during the oxygen uptake protocol. This may have resulted from two factors. First, the reinfusion of "clean" blood may have diluted the fatiguing end-products of metabolism, or second, the infusion of cold blood (4°C) may have helped to maintain the body temperature at a lower value. Either of these might have aided in delaying the onset of fatigue.

The observed scatter of blood ammonia and lactate concentration relative to each other at coincident time points of the exercise regime in Experiment 1, (Figure 4, Figure 9) emphasizes the limitations of group data analysis of physiological phenomena. Blood ammonia and lactate concentration variability at the point of exhaustion reflects the fact that not all animals could exercise for the same length of time (range 14 to 20 minutes) and stopped at different relative workrates. Increased reliability would probably derive from using multiple tests on a smaller number of animals, so that both inter-, and intra-test variability might be determined.

5.5 Ammonia and Lactate Accumulation in Tissue During Submaximal Exercise

In Experiment 2, which examined the time course of accumulation of metabolites in tissue at a constant workrate, the exercise protocol caused the content of ammonia and lactate

in blood and muscle tissue to increase. Workrate quantitatively affected peak ammonia and lactate accumulation. The submaximal workrate in Experiment 2 is reflected in lower peak concentrations of ammonia and lactate in blood (Figure 5), compared with Experiment 1 (Figure 3), when animals exercised to exhaustion.

In Experiment 1, there was less variance in blood ammonia concentrations within groups than was observed in Experiment 2. The difference in the homogeneity of variance within groups may have been a result of normalizing the point of exhaustion in Experiment 1, which meant that blood was sampled from animals relative to a similar state of fatigue. Because of this, similar overall changes in blood ammonia concentration which were significantly different from resting values in Experiment 1 were not found to be significantly different in Experiment 2.

5.6 Accumulation of Ammonia and Lactate in Heart and Liver

Lactate accumulated significantly in heart and in liver as a result of the exercise. Maximum changes in tissue lactate concentration were in heart muscle from 10.3 $\pm$ 0.4 $\mu M \cdot g^{-1}$ at rest to 19.7 $\pm$ 1.8 $\mu M \cdot g^{-1}$) by the 8th minute of exercise ($p<0.05$) and in liver from 5.7 $\pm$ 0.5 $\mu M \cdot g^{-1}$ at rest to 14.6 $\pm$1.6 $\mu M \cdot g^{-1}$) by the 6th minute of exercise ($p<0.05$). There was also a small but significant increase in ammonia concentration observed in heart muscle (0.45 $\pm$0.02 $\mu M \cdot g^{-1}$ at rest - 0.71 $\pm$0.04 $\mu M \cdot g^{-1}$ at the end of the exercise, $p<0.05$). No significant changes

occurred in ammonia concentration in liver.

The observed changes in lactate and ammonia in liver and heart may partially reflect the function and metabolic capabilities of these tissues. Muscle is considered to be the major source of both lactate and ammonia production during exercise. During exercise, lactate may be taken up by liver and heart tissue for recycling as a carbon source for oxidative metabolism. Both liver and heart tissue appear to be capable of tolerating relatively high levels of lactic acid. In this study, for example, the peak lactate concentration in heart tissue was greater than in any of the 3 skeletal muscles, and in liver, peak lactate was greater than that observed in either soleus or plantaris muscles (Figure 5,6). In liver, lactate may be oxidized, or utilised for glucose production by gluconeogenesis. Gluconeogenesis appears to be the dominant fate of lactate in liver.

Carlsten, et al., (1961) studied the uptake of lactate by cardiac muscle. They observed an uptake of lactate by cardiac muscle at rest which increased during exercise. The increase was directly correlated to the arterial concentration of lactate. Utilization of lactate by tissue as an energy source depends upon the enzymatic profile of the muscle fibres. The isoenzyme pattern of lactate dehydrogenase within heart (predominantly the H-LDH isozyme) favours the oxidation of lactate (Hultman and Sahlin, 1980).

Ammonia accumulation, however, was considerably less in both liver and heart compared with skeletal muscle (Figure 5,6). AMP deaminase activity is low in both liver and heart (Table 1), so there probably was little production of ammonia in these tissues by this pathway. Glutamate dehydrogenase activity is high in both these tissues, which might give heart and liver a greater capacity to convert ammonia to glutamate (Table 1). In liver, the absence of ammonia accumulation suggests that this tissue, which is also a primary site of ammonia detoxification by urea formation, was capable of handling the metabolic load of ammonia delivered to it by blood, where an increase in ammonia was observed as a result of exercise (Figure 5). It is also possible that blood flow to the liver was not compromised by the submaximal exercise in this study.

The very slow accumulation of ammonia in heart from rest (0.45 ± 0.02 $\mu M \cdot g^{-1}$) to its maximum value (0.71 ± 0.04 $\mu M \cdot g^{-1}$) during the 10th minute of exercise, was not unexpected. Heart is primarily a slow twitch oxidative muscle, therefore it would be expected to produce very limited amounts of ammonia. As previously discussed, cardiac tissue appears to have the intrinsic enzymatic capacity to buffer ammonia to form less toxic compounds (i.e. glutamate). It may also be physiologically undesireable for ammonia to accumulate in cardiac tissue because of the toxic nature of ammonia (Watanabe, Yamazeki, and Aoyama, 1969).

5.7 Accumulation of Ammonia and Lactate in Skeletal Muscle During Submaximal Exercise

During 10 minutes of submaximal exercise at 45 $m \cdot min^{-1}$, changes in ammonia and lactate concentrations followed a similar profile in the three skeletal muscles studied (Figure 6). Soleus, plantaris and white vastus lateralis were selected in this study because they were representative of all 3 muscle fibre types, *i.e.* slow-twitch oxidative (SO), fast-twitch oxidative (FOG) and fast-twitch glycolytic (FG) muscles respectively. In the rat, soleus consists of 84% SO and 16% FOG fibres. Plantaris consists of 53% FOG, 41% FG, and 6% SO fibres. White vastus lateralis, while not reported for rat muscle, usually consists of 77% FG and 23% FOG (Guinea pig - Ariano, Armstrong, and Edgerton, 1973). The three distinct fibre types differ in their capacity to produce ammonia (Lowenstein, 1972) and lactate (Tesch, 1980) in the order FG > FOG > SO, as previously discussed.

In skeletal muscle tissue, progressive increases in ammonia and lactate were observed througout the exercise, reaching peak values at 6 or 8 minutes of exercise, after which both ammonia and lactate decreased up to the end of exercise (10th minute), and continued to decrease to near resting concentrations during recovery. The decrease observed in both metabolites may have been a result of the intrinsic buffering, (*i.e.* conversion of ammonia to glutamate or glutamine or the oxidation of lactate), within the tissues surpassing the metabolic production of

lactate and ammonia.

The data reported in Table 6 shows the maximum changes of lactate and ammonia concentrations observed in skeletal muscle as a result of submaximal exercise (running at 45 $m \cdot min^{-1}$). Differences between data from this study and those previously reported (Meyer, Dudley and Terjung, 1980) are apparent both with respect to peak changes observed, particularly in ammonia concentration, and the relative changes between fibre types. Meyer, Dudley and Terjung (1980) (Table 6) observed maximum changes in ammonia concentration of 1.94 $\mu M \cdot g^{-1}$ in vastus, 1.25 $\mu M \cdot g^{-1}$ in plantaris, and 0.51 $\mu M \cdot g^{-1}$ in soleus, compared with the maximum changes reported in this study of 0.65 $\mu M \cdot g^{-1}$ in vastus, 0.35 $\mu M \cdot g^{-1}$ in plantaris, and 0.53 $\mu M \cdot g^{-1}$ in soleus. These authors also reported maximum changes in lactate accumulation of 19.2 $\mu M \cdot g^{-1}$ in vastus, 9.9 $\mu M \cdot g^{-1}$ in plantaris, and 6.6 $\mu M \cdot g^{-1}$ in soleus tissue, compared to 11.5 $\mu M \cdot g^{-1}$ in vastus, 9.9 $\mu M \cdot g^{-1}$ in plantaris, and 8.2 $\mu M \cdot g^{-1}$ in soleus observed in this study. While these differences cannot be completely accounted for, they may be due to the fact that resting concentrations for rat muscle ammonia and lactate were higher in this study than in others (Meyer and Terjung, 1979, 1980; Meyer, Dudley and Terjung, 1980). Elevated resting concentrations of ammonia and lactate could in turn be a result of the time required to freeze the tissue after sacrificing the animals, and the possibility that the animals were in some way disturbed or excited before they were sacrificed. The latter

possibility is less likely. Since soleus tissue is more vascular than plantaris or vastus, it is also possible that more residual blood was retained in soleus tissue following the dissection, affecting the resting metabolite concentrations. Additionally, in the Meyer, Dudley, and Terjung study, animals were given only 2 or 3 preliminary exercise bouts in order to aquaint the animals with treadmill running. A training effect, resulting in less ammonia and lactate production during exercise may have occurred in the present study where, in fact, the animals underwent considerable training previous to sacrifice.

The observed maximum change in ammonia concentration was unaccountably greater in soleus than in plantaris tissue.

5.8 Ammonia Accumulation in Brain Tissue

Ammonia accumulation in brain tissue was examined in this study because of the known toxic effects of ammonia on the central nervous system (Iles and Jack, 1980).

In the present study only cerebellar tissue showed significant increase in ammonia concentration in brain during post-exercise recovery (Figure 7). The actual source of this additional ammonia could not be identified however. More accurate analysis might have been possible if the concentration of other metabolites taking part in the buffering process for ammonia had been determined (glutamate, glutamine and GABA). Although some change in brain ammonia concentration was observed it is possible that the exercise undertaken was not of

sufficient intensity to induce marked changes in brain ammonia metabolism. The hypothesis that ammonia accumulation might be involved in disruption of CNS function during exercise has been proposed in relationship to the gross ventilatory and neurotransmitter disturbances which accompany all-out exhaustive exercise (Mutch and Banister, 1983). No definitive conclusions on this topic may be made however from these limited data from a sub-maximal exercise performance. In brain tissue, high levels of ammonia have been associated with neurological disturbances and convulsions (Iles and Jack, 1980; Singh and Banister, 1983). In this tissue, ammonia may first be buffered by conversion to glutamate, followed by glutamate conversion to either glutamine or γ-amino butyric acid (GABA) by the enzyme L-glutamate α-decarboxylase. Therefore, the relative concentrations of glutamate, glutamine, and GABA concentrations should also be evaluated in brain tissue relative to changing blood and brain ammonia concentration, in order to integrate the role that exercise produced ammonia may play in controlling events of the CNS.

LIMITATIONS

This study examined the time course of changes in ammonia and lactate as a result of two intensites of exercise in rats. The validity of extrapolating conclusions from in vivo processes in animals to humans is limited in exercise studies for the following reasons:

1. Consistent performance, and evaluation of exercise intensity in animals is obviously affected by an inability to communicate. Maximum effort, in humans, depends upon motivational drive, a factor unable to be assessed in animals.
2. The biomechanical differences in running between bipeds and quadrupeds limits comparisons of metabolic changes in specific muscle groups between the species.
3. The rate of metabolic reactions, and the efficiency of buffering processes may not be directly comparable, as is suggested by the rapid clearance of lactate from animals in this study, even after maximum effort.
4. Conclusions about changes in ammonia production are limited without additional relevant data on those metabolites which serve to remove ammonia from tissue and blood. Of particular interest is a broad profile of amino acid concentration changes, among which changes in glutamate, glutamine, aspartate, and GABA (brain) levels are most relevant. The changes in urea formation accompanying exercise are also an important consideration in appreciating the complex biochemical adjustments accompanying severe exercise.

5. More intense exercise, such as repeated high intensity interval running, for example, may be necessary to determine whether ammonia accumulates in brain tissue as a result of exercise.

6. Neither the source of ammonia production, nor the pattern of ammonia movement between tissue compartments could be determined in this study. Tracer studies for ammonia metabolism use an ^{13}-N radiolabel. Unfortunately, this isotope has a half-life of only 10 minutes, making its use difficult and expensive.

7. Reinfusion of cold whole blood (Experiment 1) may have delayed the onset of fatique, either by dilution of fatiguing metabolites, or by reducing body temperature of the animals. The absolute measurements of ammonia and lactate may have been affected by this process.

8. Normalization of exercise time relative to the point of exhaustion should be considered in the interpretation of data in Experiment 1.

CONCLUSIONS AND RECOMMENDATIONS

Within the limitations of this study, the following conclusions and were made:

1. the accumulation of ammonia and lactate is affected by exercise intensity.
2. progressively increasing workrate to exhaustion results in an exponential increase in both ammonia and lactate in blood.
3. during submaximal exercise, the time course of ammonia and lactate accumulation are similar in blood and skeletal muscles.
4. during submaximal exercise, ammonia accumulation is more gradual in heart tissue than in blood or in skeletal muscle.
5. submaximal exercise does not produce marked changes in brain ammonia concentrations.

The following recommendations were made with respect to future research on ammonia metabolism:

1. Since intrinsic tissue buffering may mask changes in metabolic production, evaluation of gross ammonia production during maximal and submaximal exercise should include quantitative analysis of metabolites known to act as buffers for ammonia both in the blood and relevant tissues known to be sources of ammonia production.
2. More intensive exercise may be needed for a better assessment of ammonia accumulation in brain tissue.

APPENDIX 1: ANIMAL TREADMILL AND SHOCKER

A diagram of the treadmill is shown in Figure A-1. It consists of a continuous belt forming the running surface. The belt rides on copper rollers supported by a metal frame. A plexiglas compartment is suspended over the belt with electric prods protruding into the rear of the compartment.

The frame is made of 12 guage aluminum, the base of which measured 50cm X 35cm. The platform onto which the rollers attached is hinged to the base at the rear of the unit. This allows graded elevation of the running surface of the treadmill. The dimensions of the platform are the same as the base, 50cm X 35 cm.

Two copper rollers, 8cm in diameter and 11cm in length and made of copper tubing, constructed with a slight crown, ensures that the treadmill belt does not drift laterally. At each end of the rollers a brass flange is soldered to the copper tubing. A 1.5cm brass shaft protrudes from the centre of the flange. The rollers are fitted to the platform by a brass shaft surrounded by bearings housed in aluminium blocks. At the point of attachment of the rear roller to the platform, the housing blocks are slotted to permit adjustment of the rollers and the tension of the belt. The shaft of one roller extends to permit connection with the motor and power unit. An aluminum stage measuring 33cm in length by 16cm in width by 9cm in height attaches to the platform between the two rollers and assists in supporting the treadmill belt thus providing a stable running

surface.

The belt is constructed of one-ply polyvinyl chloride which retains its tension and can be easily cleaned. It is spliced and joined as an endless belt to provide a smooth running surface.

The plexiglas compartment measuring 31.5 X10 X 15 cm and is constructed from 0.6cm plexiglas. The compartment suspends over the belt by means of four angle-brackets secured to the stage-platform.

An electrical A.C. shocking device is attached to the rear of the suspended compartment. The shocking rods are inserted directly into the rear of the compartment through holes drilled in the plexiglas. The rods are separated by 1.5 cm and connect in series to a 250 volt transformer secondary through a current limiting resistor. Maximum output voltage is 250 V at 2.5 ma and 60 Hz. A schematic diagram of the shocker is shown in Fig. A-2.

Elevation of the treadmill is accomplished by means of an upright post at the front of the treadmill. A pin securs the platform of the treadmill at fixed increments of elevation on the upright post. Two interchangable posts are available, one which read in degrees of elevation (0, 5, 10, 15, and 20) and the second which read as percentage of grade elevation (0, 5, 10, 15, 20, and 25).

The treadmill is powered by a 6,000 rpm sewing machine motor (Nakajima All Co., Ltd.. NA-35, Input 70 W, 6,000 rpm). Connection of the motor to the rollers of the treadmill is made through the shaft using a beltdriven rpm reduction ratio of

25:1, in a two-stage reduction of 5:1:5:1. The speed is controlled with a variable autotransformer (Superior Powerstat, 10C).

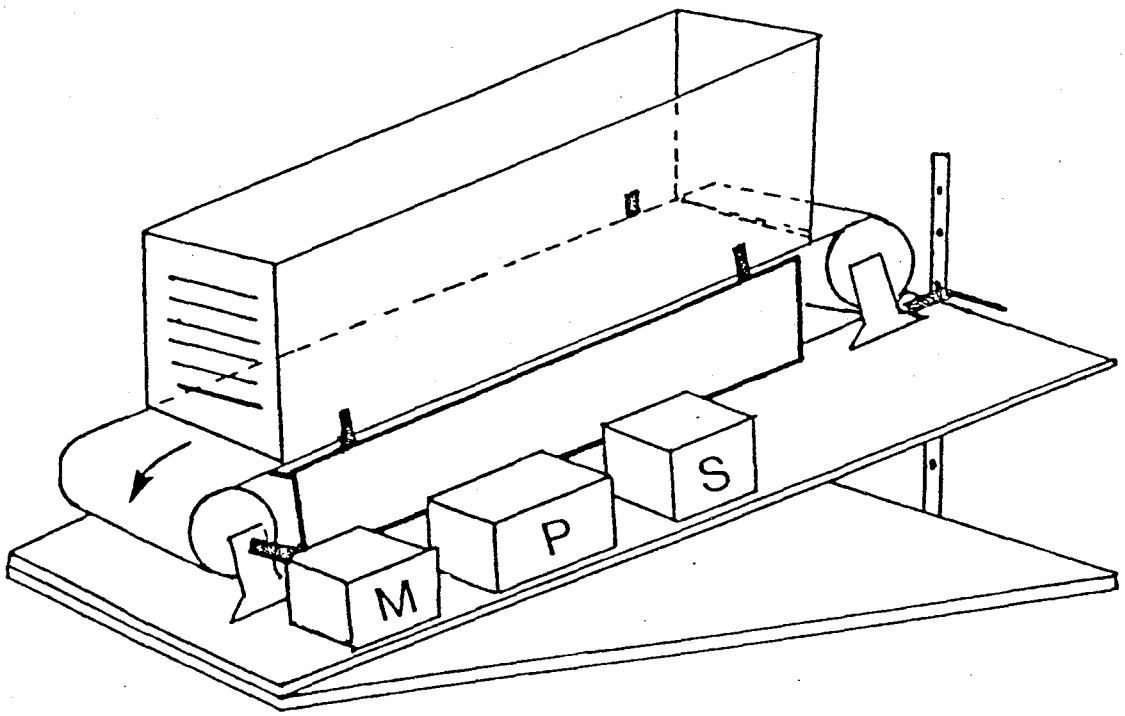


Figure A-1: Showing a schematic diagram of the animal treadmill which was designed for use in these experiments.

M - motor

P - power source

S - electrical motivation control

→ - belt direction

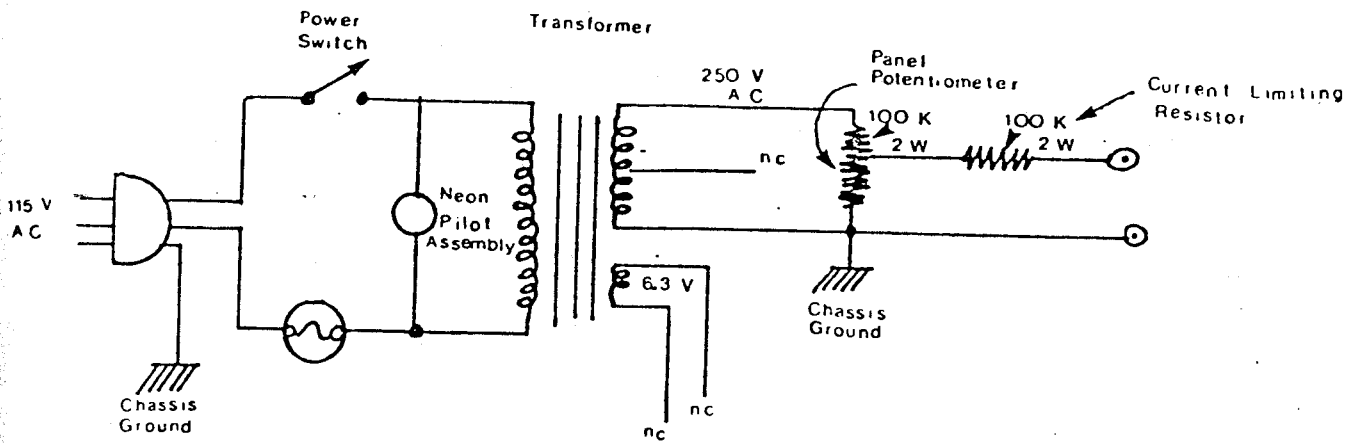


Figure A-2: Schematic Diagram For Current-Limited Animal Shocker. Showing the electronic schematic diagram used in the transformer of the current-limited shocking device attatched to the animal treadmill.

APPENDIX 2: CANNULATION TECHNIQUES

Cannula Preparation

The cannula preparation and completed cannula are illustrated schematically in Figure A-3.

One length of polyethylene tubing (PE-90, i.d. = 0.86 mm, o.d. = 1.27 mm) is threaded through a rectangular piece of silicon sheeting (Silastic Brand: 5mm X 10mm, 0.002mm thick. The silicon tab limits the distance for which the catheter could be inserted into a vein. The length of the catheter from the tab to the tip of the PE-90 tubing is able to be adjusted according to the size of the rat. A finished length from the tab to the end of the cannula of 28mm is appropriate for animals between 260 to 280 g. The silicon sheet is divided into three approximately equal parts, as illustrated in Fig. A-3. Part A is folded back upon the tubing and bonded to Part B with silicone adhesive (Silastic brand, Medical adhesive Type A). When the adhesive is cured, the sheet is trimmed to form a smooth tab. This tab is sutured to subcutaneous tissue to provide an anchor for the cannula near the point of entry of the cannula to the jugular vein.

After the free end of the cannula is exteriorized at the back of the neck, the tubing is threaded through a second silicon sheet, (Silastic brand: 5mm X 10mm, 0.002mm thick) which is trimmed in the shape of an oval. This tab provids an anchor for the cannula at the point at which the cannula is

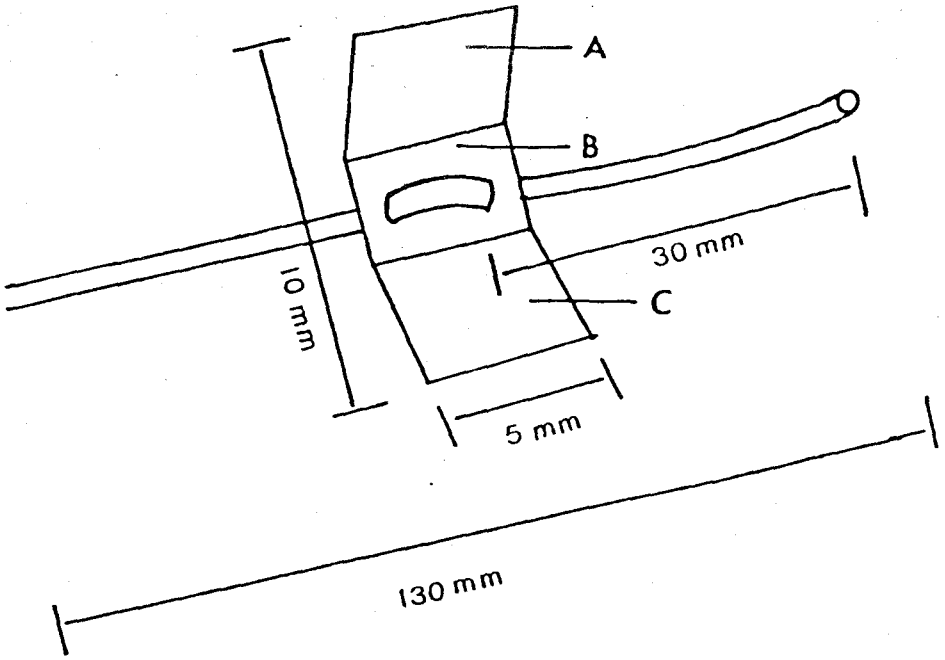
exteriorized on the animal's back.

The cannula is plugged with 24 guage stainless steel tubing, 10 mm in length, which prevents leakage of fluid from the cannula. The plug can easily removed to allow blood sampling.

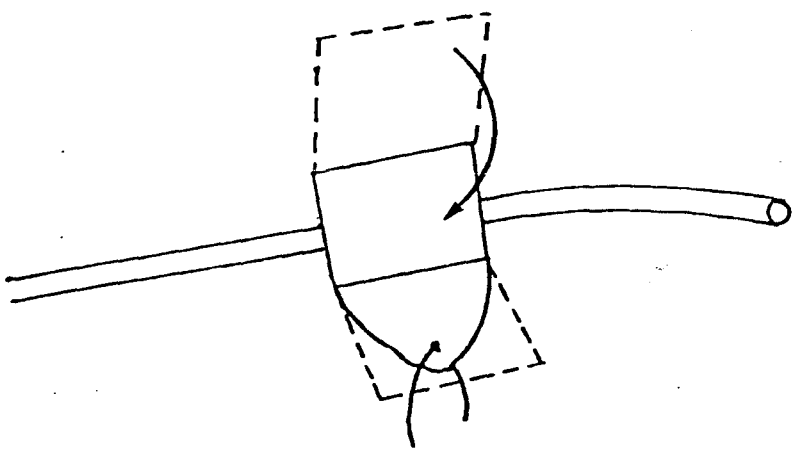
Surgical Implantation

Rats were anaesthetized with ketamine hydrochloride (Ketaset, Fogar/STB) intraperitoneally (10mg/100g body weight). Animals were shaved in two regions, at the throat and right clavical region, and at the back of the neck. A longitudinal incision was made at the area where the right external jugular vein passes dorsal to the pectoralis major muscle. The right jugular vein was freed from subcutaneous tissue, and isolated by slipping a pair of curved forceps under the vein. Two ligatures of silk suture thread were made loosely around the vein. After the vein was isolated in this manner, a small incision was made in the vein which was expanded by insertion of fine forceps. The cannula, described above, was inserted into the vein through the incision, and guided inwards until the silicon tab touched the vein. The cannula was flushed with heparinized saline (250 Units. ml^{-1}) to ensure that adequate blood flow could be established. The two ligatures were tightened to anchor the cannula and to prevent leakage of blood from the vein. The cannula was further stabilized by suturing the tab to tissue in the neck.

A longitudinal incision was then made in the dorsum of the neck. A pair of curved forceps was inserted through the incision, and guided under the skin until the tips were exteriorized at the incision in the neck. With the forceps, the free end of the cannula was then guided underneath the skin at the side of the neck until the cannula was drawn through the incision at the back of the neck. The tubing was threaded through the second silicon sheet, as described above. This tab was anchored by a single suture between the scapulae. The cannula was then flushed with heparinized saline (250 $U \cdot ml^{-1}$) and the metal plug was inserted. The incisions were sutured closed, and the rats were given a prophylactic dose of antibiotic (Derepen, Ayerst. 0.4 ml/kg.) At the termination of the experiment, cannula placement was confirmed by autopsy.



A



B

Figure A-3: Showing the procedure used in the preparation of the cannula and the tab which aids in the placement of the cannula in the animal.

APPENDIX 3: MAXIMUM OXYGEN UPTAKE PROTOCOL

The determination of respiratory gas exchange in the exercising rat was be made using a flow-through respirometry system. Measurement of $\dot{V}O_2$ and $\dot{V}CO_2$ have been made in a similar procedure using an open-flow mask for hummingbirds and lizards (Withers, 1977) and exercising rats (Pussel, Campagna and Wenger, 1980; Gleeson and Baldwin, 1981).

In the calculation of oxygen uptake and carbon dioxide production, the following terms are used:

$\dot{V}O_2$	-	oxygen uptake per minute
$\dot{V}_E$	-	rate of airflow out of mask
$\dot{V}CO_2$	-	carbon dioxide production per minute
F_IO_2	-	fraction inspired oxygen
F_ICO_2	-	fraction inspired carbon dioxide
F_IN2	-	fraction inspired nitrogen
F_EO_2	-	fraction expired oxygen
F_ECO_2	-	fraction expired carbon dioxide
F_EN2	-	fraction expired nitrogen

Throughout the text, volumes are expressed at standard temperature and pressure dry (STPD), by converting to standard temperature (0° C) and pressure (760 Torr), and adjusting for ambient relative humidity.

Calculation of $\dot{V}O_2$ and $\dot{V}CO_2$ were made using the premise of pulmonary nitrogen (N_2) constancy during gas exchange, essentially as described by Consolazio, et al., (1963), and as

used by Brooks and White (1978) to study the metabolic rates of rats at rest and with exercise using open-circuit, indirect calorimetry.

$$\dot{V}O2 = \dot{V}E(STPD)\left[\frac{1.00 - (FECO2 + FEO2)}{FIN2}\right] FIO2 - FEO2$$

$$\dot{V}CO2 = \dot{V}E(STPD) \quad FECO2-\left[\frac{1.00 - (FECO2 + FEO2)}{FIN2}\right] FICO2$$

Ventilatory gas exchange was measured by fitting the rats with a light-weight clear plastic mask made from a light acetate sheet. The mask was retained by a simple collar made from flexible wire attatched behind the ears of the animal. A mask of similar design was used by Russel, Campagna, and Wenger (1980) and by Gleeson and Baldwin, (1981). The combined weight of the mask and collar was 1.9 g. Animals were conditioned to wear the mask and were trained to run while wearing it before oxygen uptake studies were performed.

Pespiratory gases were measured by extracting air from the front of the nose cone close to the rat's nostrils at a constant rate of 5,000 ml.min-1 (STPD) and drawning it through a 0.5 litre gas mixing chamber. This flow rate was chosen following the reference of Russel, Campagna and Wenger (1980), and Gleeson and Baldwin (1981). A continuous aliquot of mixed expired gas

(500 ml.min-1) was drawn from the expiration side of the mixing chamber, through a dessicant to an Applied Electrochemistry O_2 analyser and a Beckman LB-2 CO_2 analyzer.

The arrangement of equipment for gas analysis is illustrated in Figure A-4.

Oxygen uptake and carbon dioxide production were expressed as $ml.kg^{-1}.\ min^{-1}$. Respiratory exchange ratios were calculated as the ratio $\dot{V}CO_2/\dot{V}O_2$.

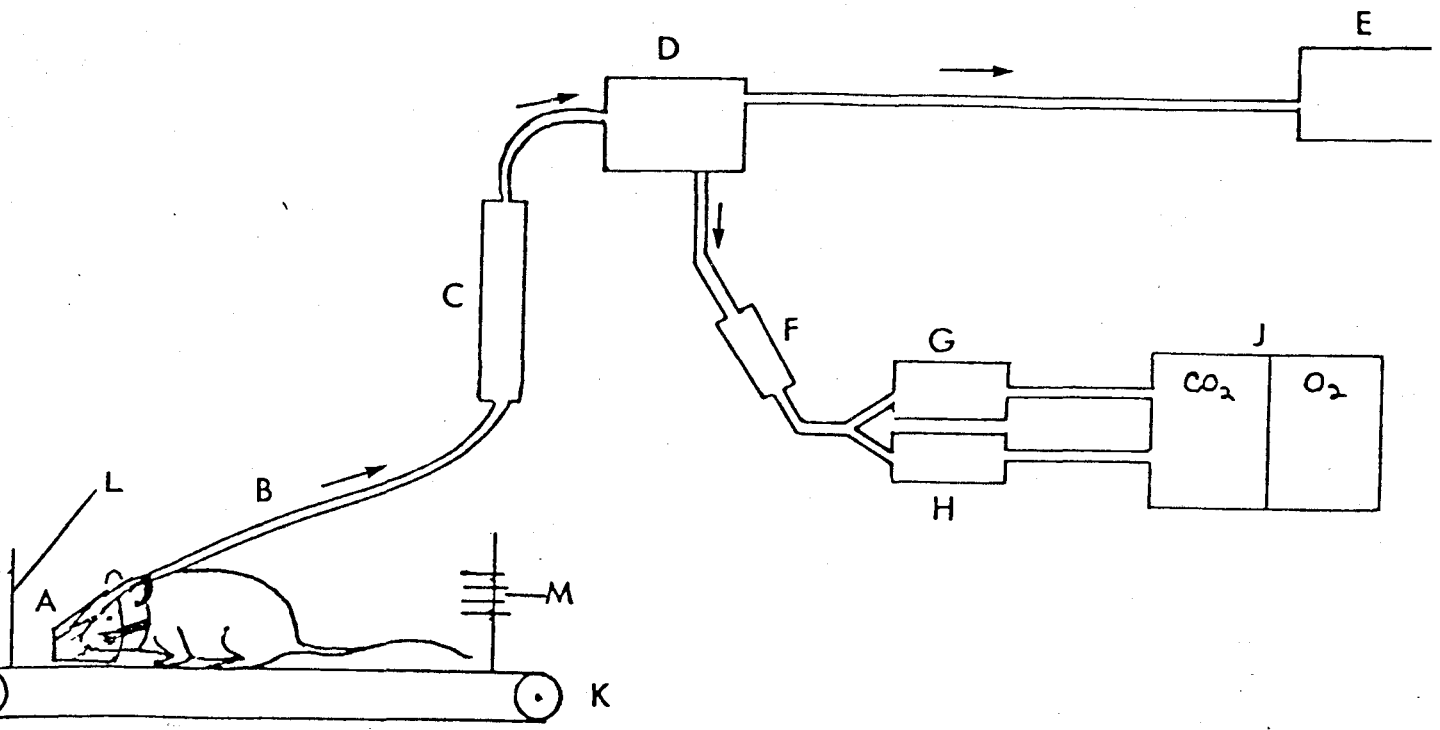


Figure A-4: Showing the arrangement of equipment as it was used in the measurement of oxygen uptake in the running rat.

A - face mask
B - outlet tubing
C - flowmeter
D - mixing chamber
E - pump
F - drying tube
G - O_2 analyzer
H - CO_2 analyzer
J - recorder
K - treadmill
L - cage
M - electrical prods
→ - direction of air flow

A Graded Treadmill Test for Rats

A test was designed to evaluate maximal work capacity in rats. A small animal treadmill, as described in Appendix 1, was used in the protocol below.

Animals were transported to the laboratory, and remained in individual cages prior to experimentation. For the measurement of $\dot{V}O_2$, animals were allowed to rest quietly for approximately 10 minutes before resting measurements were taken.

After a brief warmup period of easy running, to ensure that rats run in the correct direction and not become tangled in the gas analysis equipment, the test velocity of the treadmill was set at 15 $m \cdot min^{-1}$. This speed is comparable to easy exercise for the rat. The speed of the treadmill was then increased by 5 $m \cdot min^{-1}$ at the end of each 2 minute period. Stepwise increments in velocity were used to allow the rate of O_2 consumption to reach a plateau before the next increment in work. In the running rat, $\dot{V}O_2$ increases as a linear function of work intensity (running speed) up to a maximum value, after which further increased in running speed will cause the $\dot{V}O_2$ values to plateau, or to decrease slightly (Shepherd and Gollnick, 1976; Brooks and White, 1978; Gleeson and Baldwin, 1978; Patch and Brooks, 1980).

APPENDIX 4: COLLECTION, STORAGE AND PREPARATION OF TISSUE SAMPLES FOR ANALYSIS

Tissue Sampling Procedures

Animals were anesthetized with light ether anaesthesia, and exsanguinated. The animals were anesthetized in a 5 litre container in which a wad of cotton saturated in ether was placed. Anaesthesia was considered to be complete when the blink reflex had disappeared. The chest of the animal was then wet, and the abdomen opened with scissors. Blood was withdrawn from the abdominal aorta. Heart, and a piece of liver were removed and frozen in liquid nitrogen. The animal was then quickly decapitated, the brain removed intact, and separated into cortex, cerebellum and brain stem. Brain samples were frozen immediately in liquid nitrogen. The left hindlimb muscles were exposed by removal of the skin, and the white vastus lateralis, soleus, and plantaris muscles were excised, and frozen in liquid nitrogen. All tissues were stored at -80° C until analysis.

Two investigators performed the disection procedure to reduce the time to process the tissue. It required six to seven minutes from termination of the experimental procedure to the completion of disection and freezing of the tissue. Tissues were removed in the same order from each animal to maintain consistency in the time of sampling.

Treatment of Blood for Analysis

In Experiment 1 (Catheter - Run Protocol), blood samples (0.5 ml) were withdrawn in a heparinized 1 ml syringe from the indwelling catheter to obtain samples of mixed venous blood. In Experiment 2, (Tissue -Time Course Protocol) following ether anaesthesia blood samples were withdrawn from the abdominal aorta in a heparinized 10 ml syringe using a 21 guage needle. Each blood sample was divided into two portions for preparation for ammonia and lactate analysis.

For the determination of ammonia, blood was centrifuged at 4° C for 10 minutes in a refrigerated centrifuge. The plasma was transferred into test tubes and frozen over dry ice for storage at -80° C.

For the determination of lactate, whole blood was deproteinized in ice-cold perchloric acid (0.6 N, 1:2 ratio blood:$HClO_4$) and after mixing, centrifuged at 4° C for 10 min. in a refrigerated centrifuge. The supernatent was transferred into separate tubes, frozen over dry ice, then stored at -80° C.

Tissue Homogenization

Tissue samples were prepared for chemical analysis by homogenization in 10% trichloroacetic acid (TCA). While frozen, a piece of tissue was dissected, weighed, and homogenized in ice-cold TCA at 0° C. Temperature was maintained by immersing all tubes in an ice bath. The ratio of tissue weight to TCA was approximately 1:10. The homogenate was centrifuged, and the

supernatent was drawn off and neutralized with 2 M $KHCO_3$ (potassium bicarbonate). The neutralized extract was divided into two portions and immediately frozen over dry ice, then stored at -80° C until analysis for lactate and ammonia.

APPENDIX 5: CHEMICAL ASSAYS

Blood was assayed for ammonia by the methodology described by Sigma, (1980). Blood lactate was determined using the enzymatic method described by Gutmann and Wahlefeld (1974) and Boehringer Mannheim Diagnostica, (1979).

Muscle, brain, liver and heart tissue were assayed for ammonia by enzymatic determination (Kun and Kearney, 1974). Tissues were assayed for lactate according to the method of Gutmann and Wahlefeld (1974).

Ammonia Assay - Blood

The enzymatic technique described in Sigma Technical bulletin No. 170-UV was developed to achieve greater specificity in quantitation of circulating ammonia concentrations. By avoiding the use of alkali, the liberation of amines as well as formation of ammonia by deamidation of acid amides or by deamination reactions are minimized. The method is based on the reductive amination of 2-Oxoglutarate, using Glutamate Dehydrogenase and reduced nicotinamide adenine dinucleotide (NADH). The reaction is dependent on ammonium ion (Kun and Kearney, 1974; Sigma, 1980).

The reductive amination of 2-Oxoglutarate is catalyzed by L-Glutamate Dehydrogenase as follows:

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NADH} \longrightarrow \text{Glutamate} + NAD^+ + H2O$$

The decrease in absorbance at 340 nm due to oxidation of NADH is proportional to the ammonium ion concentration.

Reagents

1. Ammonia Assay Solution, Sigma Stock No. 170-UV

   2-oxoglutarate, 2mM

   NADH, 0.12mM

   Buffers and salt stabilizers

   (note: Upon request, Sigma would not give more detailed information)

2. L-Glutamic Dehydrogenase Solution, Sigma Stock No. 170-4

   L-glutamic dehydrogenase (bovine liver), 1200 units·ml^{-1} in 50% glycerol and phosphate buffer, pH 7.4

Instruments

Beckman DU-8 Spectrophotometer

Wavelenght 340 nm

Procedure

1. Reactions were carried out at 25°C.
2. To each cuvette, the following solutions were added:

   0.1 ml plasma. An equivalent volume of water was added to the BLANK cuvette.
3. The solution was mixed and allowed to sit for 5 minutes for equilibration.
4. Absorbance 1 (A1) of the cuvette was read and recorded at 340 nm.
5. 0.01 m. L-Glutamic Dehydrogenase was added to the cuvette.
6. The solution was mixed and allowed to stand for 5 minutes for equilibration.
7. Absorbance 2 (A2) of the cuvette was read and recorded

at 340 nm.

8. The concentration of ammonia was determined by the following calculation:

$$\text{Ammonia } (\mu g \cdot ml^{-1}) = (\Delta A \text{ plasma} - \Delta A \text{ blank}) \times 44$$

To convert the concentration of ammonia from $\mu g \cdot ml^{-1}$ to μM, the following conversion was used.

$$\text{Ammonia } (\mu M) = \text{Ammonia } (\mu g \cdot ml^{-1}) \times (1000/17)$$

$$= \text{Ammonia } (\mu g \cdot ml^{-1}) \times 58.8$$

Lactate Assay - Blood

Blood was assayed for lactate using assay kits available commercially from Boehringer Mannheim. This method was based on the oxidation of lactate to form pyruvate. The increase in absorbance at 340 nm is due to the reduction of NAD^+ to NADH and is proportional to the amount of lactate present (Boehringer Mannheim, 1979).

Reagents

1. Hydrazine - Glycine Buffer

   Glycine Buffer 0.5 M, pH 9.0

   Hydrazine 0.4 M

2. B-Nicotinamide adenine dinucleotide (NAD)

   NAD^+ 27 mM

3. Lactate Dehydrogenase

   LDH (rabbit muscle) : 650 units$\cdot ml^{-1}$,

   in ammonium sulphate, 3.2 mM pH 6.5

Instruments

Beckman DU-8 Spectrophotometer

Wavelength 340 nm.

Procedure

1. Reactions were carried out at 25°C.
2. To each cuvette, the following solutions were added:

   1.0 ml Hydrazine - Glycine Buffer

   0.1 ml supernatent (from blood) An equivalent volume of $HClO_4$ was added to blank cuvette.

   0.1 ml NAD

0.01 ml LDH

3. The solutions were mixed and incubated for 1 hour in a water bath at 25°C.

4. Absorbance of the sample was read at 340 nm against reagent BLANK (=ΔA).

5. The concentration of lactate was calculated as follows:

Lactate $mg \cdot ml^{-1}$ = ΔA X 0.49

To convert the concentration of lactate from $mg \cdot ml^{-1}$ to mM the following conversion was used:

Lactate mM = Lactate $mg \cdot ml^{-1}$ X (1000/90.1)

= Lactate $mg \cdot ml^{-1}$ X 11.1

Ammonia Assay - Tissue

This enzymatic determination of ammonia was used for the analysis of tissue extracts (Kun and Kearney, 1974). Tissue extracts were prepared for analysis as described above.

This assay is based on the same principle as described for Ammonia Assay - Blood, ie. that the reductive amination of 2-oxoglutarate is catalysed by the enzyme glutamate dehydrogenase. The decrease of NADH, as measured by the change in absorbance at 340 nm, is a measure of the reaction, which is:

$$NADH + NH4 + 2\text{-Oxoglutarate} \longrightarrow L\text{-Glutamate} + NAD^{+} + H2O$$

Reagents

1. Tris Buffer Solution

   Tris-hydroxymethyl-aminomethane, tris 0.5 M, pH 8.0

2. 2-Oxoglutarate Solution, 0.1 M, ph 7.4

3. NADH Solution

   Reduced nicotinamide-adenine denucleotide,

   ca. 8mM B-NADH

4. Glutamate Dehydrogenase, GlDH

   from liver, 90 units/mg.

Instruments

Beckman DU-8 Spectrophotometer

Wavelength 340 nm

Procedure

1. Reactons were carried out at 37°C.

2. To each cuvette, the following solutions were added:

   0.2 ml Tris Buffer Solution

0.1 ml 2-Oxoglutarate Solution

0.03 ml NADH Solution

0.15 ml Deionized Water

0.50 ml Tissue Extract - An equivalent volume of neutralized TCA was added to the BLANK cuvette.

3. The samples were mixed and Absorbance 1 (A1) was read and recorded at 340 nm.
4. To each cuvette, 0.02 ml GlDH Solution was added.
5. The samples were mixed and incubated at 37°C in a water bath for 30 minutes.
6. Absorbance 2 (A2) was read and recorded at 340 nm.
7. The concentration of ammonia was calculated as follows:

$$NH_3\ \mu M = 488.8\ (\Delta A \text{ sample} - \Delta A \text{ blank}) - 5.09$$

$$NH_3\ \mu M \cdot g^{-1} \text{ wet weight} = NH_3\ \mu M \times \frac{\text{Extract Volume}}{\text{Tissue Weight}}$$

Lactate Assay - Tissue

Tissue extracts were analyzed for lactate concentration by a method modified from that described in Lowry and Passoneau (1972). This assay is a two step enzyme reaction, in which any pyruvate which may interfere with the measurement of lactate is initially removed by a transamination reaction with glutamate, using

glutamate - pyruvate transaminase to catalyze the reaction. The second reaction is catalysed by lactate dehydrogenase (Lowrey and Passoneau, 1972).

The overall principle of this reaction is the oxidation of lactate to form pyruvate as follows:

Lactate + NAD^+ ⟶ Pyruvate + NADH + H+

As mentioned above, this method is coupled to a reaction which results in the removal of any pyruvate which could interfere with the measurement of lactate because of an unfavorable equilibrium toward pyruvate reduction. This reaction is:

Pyruvate + Glutamate ⟶ Alanine + 2-Oxoglutarate

Reagents

1. Glutamate Solution

   Glutamate (monosodium), 1 M

   NaOH, 0.9 M, to pH 10

2. Neutralized Trichloroacetic Acid (TCA), pH 7.2

   10% TCA : H20 : 2M $KHCO_3$, in the ratio

   1.3 : 34 : 1.52 (per volume)

3. Reagent Buffer Solution

2-Amino-2-Methyl Propanol 100 mM

NAD^+ 1.5 mM

Glutamate Solution (above) 10% (vol)

4. Glutamic-Pyruvic Transaminase (GPT), pig heart, 136 units·ml^{-1}

5. Lactate Dehydrogenase (LDH) 500 units·ml^{-1}

Instruments

Beckman DU-8 Spectrophotometer

Wavelength 340 nm

Procedure

1. All reactions were carried out at 37°C.
2. To each cuvette, the following solutions were added:

   0.25 ml Neutralized tissue extract. An equivalent volume of neutralized TCA was added to the BLANK cuvette.

   1.20 ml Reagent Buffer Solution

   0.05 ml GPT
3. Solutions were mixed and allowed to sit for 10 minutes for equilibration. Absorbance 1 (A1) was read and recorded at 340 nm.
4. To each cuvette, 0.05 ml LDH was added.
5. Solutions were mixed and were incubated in a water bath at 37°C for 30 minutes.
6. Absorbance 2 (A2) was read and recorded at 340 nm.
7. The concentration of lactate in the extract was determined by the following equation:

Lactate $\mu M = 1.18\ (\Delta A) - 0.16$

Lactate $\mu M \cdot g^{-1}$ wet weight =

$$\text{Lactate } \mu M \times \frac{\text{Extract Volume}}{\text{Tissue Weight}}$$

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