LACTATE TURNOVER IN FAST-MOVING VERTEBRATES: THE CONTROL OF PLASMA METABOLITE FLUXES.

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ABSTRACT

During sustained exercise, working muscles must be supplied with adequate kinds and amounts of exogenous fuels, and the delivery rates of oxygen and oxidizable substrates should be matched. The study of metabolite fluxes and their regulation is therefore critical to the understanding of exercise metabolism. Lactate has received renewed attention from physiologists and biochemists with the realization that it is not only an end product of glycolysis, but also an important fuel for aerobic work. As an oxidizable fuel, this substrate may provide some performance advantage over other fuels such as glucose and free fatty acids. The goals of this thesis were: 1) to determine whether endurance-adapted animals can support higher plasma lactate turnover rates than sedentary animals; and 2) to investigate the major factors involved in the regulation of plasma metabolite turnover at the whole-organism level - using lactate as a model. Lactate turnover rates were measured by bolus injection of [U-^{14}C]lactate in skipjack tuna, Katsuwonus pelamis, and in thoroughbred racehorses, Equus caballus. In tuna, turnover rates ranged from 112 to 431 umol min^{-1} kg^{-1}, and they were positively correlated with lactate concentration (slope = 15.1, r = 0.92). This teleost is able to support higher plasma lactate turnover rates than expected for a mammalian species of equivalent size, even though it operates at a much

lower temperature, and lactate is probably an important oxidizable fuel in this species. For comparative purposes, resting turnover rates of lactate and glucose were plotted versus body mass on a log-log scale for a wide range of mammalian species. These plots were linear, and they showed the same slope as the classic body mass vs metabolic rate relationship.

Thoroughbred horses are likely to have an aerobic scope of 40-fold or more. One of their main physiological adaptations to exercise is the ability to increase hematocrit by more than one and a half-fold in response to exercise. In the present study, this adjustment allowed them to reach an A-V difference in O_2 content of more than 23 vol% during maximal exercise, a much higher value than other mammals. Their lactate turnover rate and cardiac output were measured at rest and two levels of submaximal exercise (45 and 55 $\dot{V}O_2max$) to investigate the relationship between cardiovascular adjustments on plasma lactate turnover rate. Cardiac output ranged from 106 to 571 ml min^{-1} kg^{-1}, and mean lactate turnover rate from 9.3 at rest, to 75.9 umol min^{-1} kg^{-1} at 55% $\dot{V}O_2max$. In contrast with the situation found in tuna, the lactate turnover rates of thoroughbreds were not elevated compared with other mammals, showing that the metabolic adaptations of these outstanding athletes do not include the ability to sustain higher lactate fluxes than sedentary animals. In horses, the contribution of

plasma lactate oxidation to $\dot{V}O_2$ is minimal, and this substrate is not an important oxidative fuel; lipid oxidation may represent their major pathway for aerobic energy production during exercise. The ability to oxidize plasma lactate at high rates is therefore not necessarily required for the "elite" performance of endurance exercise. This study also shows that both, plasma lactate concentration and cardiac output are positively correlated with turnover rate. The correlation between cardiac output and lactate turnover rate is independent of the relationship between plasma lactate concentration and turnover rate. Plasma metabolite concentration and cardiac output can be regulators of plasma metabolite turnover rate. It is proposed that these two variables are, respectively, the fine and coarse controls for flux rate adjustments during exercise.

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CHAPTER 1:

GENERAL INTRODUCTION

1.1 STATEMENT OF PROBLEM.

During sustained exercise, contracting muscles must be supplied with adequate kinds and amounts of exogenous fuels to minimize the use of endogenous substrates and delay the onset of fatigue. In addition, the delivery rates of oxygen and oxidizable fuels to the active musculature should be matched. Oxygen fluxes have been studied thoroughly, but oxidative fuel kinetics have not. The understanding of exercise metabolism depends critically on a large amount of quantitative information concerning the flux rates of carbon fuels and their regulation. This information is still quite fragmentary because the measurement of metabolite fluxes is technically difficult and labor intensive. For these reasons, a decision had to be made to limit this study to the investigation of one metabolite (lactate), and two experimental species (tuna and horse). The goals of this thesis are: 1) to determine lactate turnover rates in two species adapted for fast locomotion, and to compare their ability to move this metabolite through their plasma compartment (from sites of production to sites of utilization) with the corresponding ability of more sedentary

species, and 2) to investigate the major factors involved in the regulation of whole-organism plasma metabolite turnover - using lactate as a model. Before giving some background information concerning lactate metabolism studies and the measurement of lactate fluxes in vivo, a more detailed explanation of the aims of this work will be presented in the next few paragraphs.

The first goal of this thesis (#1 cited above) deals with the ability of animals adapted for fast endurance locomotion to support high lactate fluxes in their plasma. - Do such animals support higher plasma lactate fluxes than sedentary organisms ? Animals able to support endurance-type exercise over long periods of time, and at moderately high speeds, would be expected to be able to use oxidizable fuels from their plasma at high rates because, under such exercise conditions, their working muscles operate as open systems, and these active muscles have the opportunity to oxidize blood-borne substrates in addition to endogenous (intramuscular) fuels. The depletion of endogenous fuels has been shown to be one of the limiting factors for endurance exercise, and "good" endurance performers could have the ability to utilize exogenous fuels at higher rates than sedentary animals to slow down the use of intramuscular fuels in their oxidative muscle fibers. Because plasma lactate can be a very good oxidizable fuel for many tissues (oxidative muscle fibers in particular), the lactate turnover rate of endurance performers could be higher than in sedentary species that rarely exercise long enough to deplete their

endogenous fuel stores.

Recently, the importance of lactate as an oxidizable fuel during exercise has been emphasized by a number of researchers investigating whole-body metabolism of rats, dogs, and humans (for example see Brooks, 1985; Issekutz et al, 1976; Okajima et al, 1981). Results from Georges Brooks laboratory, in particular (see Brooks, 1985 for a brief summary), show that lactate could be an ideal oxidizable fuel for exercising animals because it can be transported at high rates to working muscles. It can be mobilized faster than plasma free fatty acids, and animals can sustain higher turnover rates of lactate than glucose or free fatty acids. Because lactate could provide some advantages over other oxidizable fuels found in plasma, good endurance performers may use lactate at a higher rate than sedentary animals for a given level of work, and such "animal athletes" would therefore have to be able to transport this substrate rapidly through their plasma compartment. Tuna and thoroughbred horses were chosen as two examples of "elite animal athletes" representing two widely different groups of organisms to investigate this aspect of the problem. This thesis shows that, as expected for an endurance athlete, tuna can support high lactate turnover rates. In contrast, thoroughbred horses do not show such an ability, and the use of lactate as an oxidizable fuel in this species is not important.

The second goal of this study (#2 cited above) is to characterize the major ways used by vertebrates to alter

the flux rates of oxidizable fuels to their working muscles in response to submaximal exercise at the whole-organism level. The aim of the study is neither to determine what limits maximal lactate flux rates in plasma, nor to investigate directly the regulation of lactate production and utilization at the cellular level. Instead, the following question is asked: - How do animals support different steady-state rates of plasma substrate turnover in response to different levels of sustainable physical activity ? It is hypothesized that an increase in plasma lactate concentration should allow them to augment the rate of lactate supply to oxidative muscles, therefore, lactate concentration should be correlated with the rate of plasma lactate turnover. Maintaining different steady-state lactate levels in their plasma should allow the animal to determine different rates of lactate delivery rate to the working muscles (for a given rate of muscle perfusion) in response to different rates of exercise. The effect of changes in plasma concentration on lactate turnover rate would be most clearly demonstrated in a species showing a very wide range of lactate concentrations under natural conditions. Tuna was chosen for this purpose (Chap. 2) because this teleost has the ability to bring its plasma lactate concentrations to values exceeding 30 mM (see Chapter 2, and Guppy et al. 1979).

An increase in cardiac output should also allow animals to augment the supply rate of lactate to their working muscles by increasing perfusion in lactate-utilizing tissues during exercise. Almost all the increase in cardiac

output elicited by exercise goes to working muscles, and the oxidative fibers of these muscles should be better supplied with oxidizable substrates (including lactate) when cardiac output is augmented. Therefore, cardiac output should also be correlated with lactate turnover rate, and a change in cardiac output should modify the slope of the relationship between lactate turnover rate and plasma lactate concentration. Tuna does not represent a good experimental animal to investigate this problem because the cardiac output of this fish cannot be manipulated experimentally under controlled conditions, and it is difficult to measure blood flow accurately in this species. A mammalian model in which cardiac output can be altered over a wide range of values would be more appropriate, and the thoroughbred racehorse was chosen as an experimental animal to investigate the effect of changes in whole-body perfusion rate (i.e. changes in cardiac output) on plasma metabolite turnover rate (Chap. 4 and 5). More specific goals for each part of this study will be given in the respective introductions to the different chapters.

1.2 LACTATE METABOLISM

Lactate has occupied a prominent position in the study of muscular activity for a long time. Its production by working muscle provides a means for ATP synthesis through the glycolytic pathway. This can occur without the presence of oxygen, with the net formation of 2 ATP molecules per

glucose, and 3 ATP per glycosyl unit utilized (Molé, 1983). The primary substrate for lactate production in muscle is glucosyl units derived from intramuscular glycogen stores. The main origin of lactate is most clearly demonstrated in experiments dealing with patients afflicted with a metabolic disorder called McArdle's disease. These patients lack the enzyme glycogen phosphorylase in their skeletal muscles, and they cannot utilize muscle glycogen as a substrate for energy metabolism during work (Lewis and Haller, 1986). The muscles of these patients do not produce lactate, even under maximal exercise conditions.

Until recently, the investigation of lactate metabolism in vivo during exercise relied solely on concentration measurements, and information concerning lactate fluxes was lacking. Over the last decade, flux measurements have become more widely available via tracer techniques, and a new picture of lactate metabolism has emerged. In particular, metabolic tracers have allowed to quantify the rates of lactate production and utilization at the whole organism level, something that had not been possible with the use of lactate concentration measurements only. Metabolic tracer techniques have permitted to clearly demonstrate that lactate was not simply an end-product of glycolysis (its unique and traditional role for very long), but that this metabolite was also a very important oxidizable fuel for muscle at rest, and during submaximal exercise. Several recent studies have emphasized this second role of lactate (see for example Brooks, 1985; Eldridge, 1974;

Issekutz et al, 1976; Jorfeldt, 1970). The modern study of lactate metabolism has been reviewed by several authors (the best accounts can be found in DiPrampero, 1981; Hultman and Sahlin, 1980; Krebs et al, 1975; Minaire, 1973; Molé, 1983; Moret et al, 1980). However, no updated discussion of turnover data is presented in these papers. Before describing in more detail the findings and the significance of recent work, the history of lactate metabolism studies will be reviewed.

The study of lactate started in the 18th century when this metabolite was first isolated from sour milk. The original procedure used at that time was first described by Scheele (see Scheele, 1786 for an english translation of the original paper published in swedish in 1780). In 1807, Berzelius identified lactate as a product of muscle contraction (see Needham, 1971). However, Berzelius did not continue his study of chemical changes in muscle tissue, and the cause of lactate formation in muscles during contraction could not be determined then. Eighty years later, and independently from each other, Gaglio (1886), and Berlinerblau (1887) isolated lactic acid from living tissues as zinc lactate, and they measured its concentration in the blood of resting dogs for the first time. In the late 1800's, it took a minimum of 85 ml of blood, but preferably 100 to 200 ml, to make one lactate concentration measurement! Several days were necessary to perform one analysis. Today, thanks to the analytical use of pure enzymes, 0.1 ml of blood or less, and a few minutes, are sufficient to give accurate

values (Krebs et al, 1975). After lactate was discovered and isolated, researchers started to investigate the specific conditions under which it is produced by muscle. Araki was one of the first physiologists to study these conditions in intact animals. He showed that lactate concentration was increased in dogs, rabbits, and hens subjected to hypoxia (Araki, 1891). Increased lactate formation under impaired oxygen supply conditions was confirmed by several other authors in different species. In 1920, Meyerhof and Hill proposed their concept of the "oxygen debt" and demonstrated that stimulated frog muscle shows a decrease in glycogen while lactate is being produced (Meyerhof, 1920). They deduced that lactate formation was an indication of oxygen lack caused by exercise, and that the removal of lactate during recovery was the direct cause of post-exercise excess oxygen consumption. Hill and Meyerhof (see Meyerhof, 1930) showed a few years later that lactate was the final product of glycolysis. Until the 1930's, lactate formation was assumed to be necessary for the conversion of chemical energy to mechanical energy in contracting muscles, and the role of ATP as immediate source of chemical energy had not yet been discovered. A few years later, skeletal muscles were shown to release lactate in the resting state (Kramer et al, 1939). Some attention was focussed around the problem of lactate movements between muscle and blood. Hill, Long and Lupton (1924) hypothesized that muscle and blood lactate concentrations should be equal under steady-state exercise conditions. Several researchers tried to measure lactate

concentration in muscle and blood simultaneously during submaximal exercise, but they usually failed to find similar concentrations in both tissues (see for example Sacks and Sacks, 1937). Most studies concluded that lactate was not in equilibrium between muscle and blood. However, it was not clear whether the elevated lactate concentrations found in muscle tissue were not simply due to the different sampling procedures utilized for blood and muscle. In light of their results, Sacks and Sacks (1937) criticized the approach used by Margaria's group. Already then, they recognized that it was impossible to quantify the rate of lactate production from changes in blood lactate concentration as Margaria had done in his studies. Net release (estimated from A-V differences in lactate concentration) appeared to increase approximately exponentially with exercise intensity (Bang, 1936; Wahren, 1966; Wahren and Hagenfeldt, 1968). Bang (1936) showed that lactate concentration went through a transient increase at the beginning of sustained submaximal exercise before decreasing back to a steady value.

Already at the beginning of the century, there was some indications that oxygen lack was not a required condition for the formation of lactate. For example, the oral administration of sucrose to human subjects was shown to increase blood lactate concentration (Collazo and Lewicki, 1925). The same phenomenon was observed after glucose infusion in rats (Cori and Cori, 1929). Dill et al (1932) observed that the accumulation of lactate was not necessarily caused by low oxygen availability. Despite these early

results, the production of lactate has been associated almost automatically with oxygen lack by a large number of people including several contemporary researchers. Lactate production is now known to occur in the presence as well as the absence of oxygen (see for example Connett et al, 1984; Jobsis and Stainsby, 1968). Recent results from tracer kinetics experiments have confirmed that lactate can be produced at high rates in the presence of oxygen (Brooks and Donovan, 1983; Depocas et al, 1969; Donovan and Brooks, 1983; Eldridge et al, 1974; Freminet et al, 1972; Freminet et al, 1974; Issekutz et al, 1976; Issekutz, 1984).

As indicated previously, recent tracer studies allowed to quantify the role of lactate as an oxidizable fuel. However, this role had been recognized earlier in resting muscle by a number of researchers who observed that, during exercise, the muscles that were not involved in active work had the ability to utilize lactate (Barr and Himwich, 1923; Carlson and Pernow, 1959; Margaria et al, 1933). It was later measured that active recovery (at low exercise intensities) was more effective in removing lactate from the blood than resting recovery (Ahlborg et al, 1976; Bang, 1936; Jorfeldt, 1970; McGrail et al, 1978). Also, studies conducted during the seventies showed that all muscles (active and resting) had an increased ability to take up lactate from the blood when the recovery period was active (Ahlborg et al, 1976; also, see McGrail et al, 1978). Without using metabolic tracers, these experiments already supported the idea that lactate can be a good substrate for energy metabolism in

skeletal muscle.

Over the years, a lot of attention has been devoted to the investigation of lactate production and removal at the onset of exercise, during exercise, and during the recovery process following exercise. Many factors influence the rate and magnitude of the accumulation of lactate in blood and muscles. These factors include diet, state of physical fitness, and the type and duration of the exercise (Gollnick et al, 1986). For very long, it was thought that no lactate was produced at rest or during submaximal exercise because no lactate accumulation in muscle or blood could be measured under these conditions. Margaria (1976) was still supporting this view recently. Wasserman and co-workers proposed the concept of "anaerobic threshold" (Wasserman et al, 1973; Wasserman et al, 1981) to explain the sudden increase in blood lactate concentration observed when augmenting work beyond about 55 to 60% maximal oxygen consumption. This concept has been challenged over the last few years because the observed threshold does not indicate the sudden onset of lactate production due to anaerobiosis (see for example Brooks and Fahey, 1984). Several lines of evidence show that lactate is produced under all conditions of activity (Eldridge, 1975; Issekutz et al, 1976; Jorfeldt, 1970). Even at rest, the tissue concentrations of lactate are maintained above zero by a balance between production and removal. In man, the major tissues contributing to lactate production at rest are: skeletal muscle, erythrocytes, brain, and leucocytes (Molé, 1983). Skeletal muscle has been estimated

to produce about 35% of total lactate appearing in the blood of resting humans (Hultman and Sahlin, 1980). Thus, at least some muscle cells do produce lactate when the organism is at rest. During submaximal exercise, the production and release of lactate from these cells is greatly increased, but lactate concentration stays constant because other cells take up lactate and oxidize it faster during work (thereby maintaining a situation in which net production in the whole muscle is nil). In this way, it is possible to have lactate being produced and released in some fibers while other fibers are taking up and oxidizing lactate in an active mixed muscle, with no change in blood lactate concentration (see Molé, 1983).

The fate of lactate during and after exercise is still an open question, particularly with regard to the quantification of the different pathways utilized for lactate disposal. The removal of lactate from the blood compartment is performed mainly via oxidation, gluconeogenesis (lactate - glucose conversion in the liver with direct release of glucose into the blood), and glyconeogenesis (glycogen synthesis in liver and skeletal muscle occuring mainly during recovery from exercise to replenish the carbohydrate stores in these tissues). The amount of lactate lost via renal excretion is negligible in mammals (Dies et al, 1969). The same is true in fish for both renal and gill excretion (Cameron and Kormanik, 1982; Kobayashi and Wood, 1980). The design of systems avoiding the loss of lactate to the environment makes sense because this metabolite represents a

very valuable source of energy. The respective contributions of oxidation, gluconeogenesis, and glyconeogenesis to total lactate removal are still under investigation, but it is now clear that they depend upon several factors including the activity level of the animal, and its state of training. The fate of lactate at the whole organism level can only be understood if kinetic parameters are quantified. Most of the recent work on lactate kinetics relevant to this thesis has been performed in the laboratories of G. Brooks, B. Issekutz, and J. Katz, exclusively on mammalian species. Lactate fluxes of resting rats have been investigated by Okajima et al (1981). Donovan and Brooks (1983) have looked at the effects of exercise and endurance training on turnover rate in the same animal. More recently, flux rates have been measured in exercising humans (Stanley et al, 1985; and Mazzeo et al, 1986), and Issekutz (1984) has studied the effect of beta-adrenergic blockade on lactate turnover of exercising dogs. Interspecific comparisons of lactate kinetics are restricted to mammals, as no other group of vertebrates has been studied. To summarize in vivo flux studies: plasma lactate fluxes are high compared with the fluxes of other substrates, even in resting animals, and they increase drastically with exercise. At least 50% of total lactate produced is oxidized at rest, and this percentage increases to more than 80% at submaximal work intensities (Donovan and Brooks, 1983; Issekutz et al, 1976; Mazzeo et al, 1986; Stanley et al, 1985). These results emphasize the fact that lactate is not only an end product of glycolysis, but it is

also an important oxidizable substrate for skeletal muscle, particularly during submaximal exercise in rats, dogs, and humans.

Lactate production by glycolytic muscle fibers could represent an important means of supplying carbon substrate to oxidative muscles during work (Brooks, 1985). If organisms can derive a performance advantage from their ability to support high plasma lactate fluxes, the study of trained or natural athletes should be especially instructive; metabolic adaptations to exercise involving the production, movement, or utilization of lactate should be emphasized in elite animal performers compared with sedentary species.

Lactate was chosen as a model metabolite for this study because 1): it is a good exogenous fuel for oxidative muscles, and 2): because the turnover rate of lactate varies over a very wide range of values in response to different levels of activity. Therefore, the turnover rate of lactate can be manipulated over a wide range using exercise as an experimental tool to control this rate. Also, the regulatory mechanisms involved in the control of plasma metabolite turnover rate (at the whole-organism level) should be exaggerated in the case of lactate.

1.3 METABOLITE TURNOVER

All the body constituents of a living organism are continually produced and utilized in a process called

turnover (see Hetenyi et al, 1983). Like all other compounds, plasma metabolites are therefore in a dynamic state, undergoing constant replacement. For decades however, researchers have used changes in the concentration of substrates in plasma to draw conclusions about their rate of release in the circulation and their rate of uptake therefrom. Such conclusions are often invalid because concentration changes do not allow the quantification of rates of production and utilization by the organism (Wolfe, 1984). Metabolite flux rates can be altered drastically without noticeable changes in their plasma concentration. In addition, large changes in concentration may only reflect minor imbalances between the rates of appearance (R_a) and disappearance (R_d) into and from the plasma compartment, particularly if these rates are high. The understanding of muscle fuel metabolism at the whole-organism level is only possible if substrate fluxes are quantified.

The turnover rate of a plasma metabolite shows how rapidly this particular compound is exchanged between cells, tissues, or organs via the circulation. It is a measure of its rate of movement through the blood compartment from site(s) of production to site(s) of utilization or excretion if this compound is indeed excreted (which is not the case for a metabolite such as lactate). The turnover of an oxidizable substrate represents the maximal rate at which this exogenous fuel is being supplied to oxidative working muscles from various storage areas such as liver, adipose tissue, or other muscles. In the case of lactate, a large

fraction of total turnover rate is oxidized [50% to more than 80%: see Donovan and Brooks (1983), Issekutz et al (1976), and Mazzeo et al (1986)] and the importance of lactate as an oxidizable fuel can be estimated from turnover rate measurements (Mazzeo et al, 1986; Stanley et al, 1985; Wolfe, 1984). A good understanding of exogenous substrate supply is particularly relevant to the study of sustainable exercise which can last longer if endogenous fuels are used sparingly.

1.4 THESIS OUTLINE

This thesis investigates plasma lactate turnover in two outstanding animal "athletes": the skipjack tuna and the thoroughbred horse. The purpose of this work is not to compare the two animals but to use each one of them to solve different problems concerning the regulation of lactate fluxes as explained in the previous sections. In both cases, lactate is used as a model to study flux regulation in general. In addition, lactate turnover rate is quantified in two species whose plasma metabolite kinetics have never been investigated.

Chapter 2 examines lactate turnover rate in the skipjack tuna. The primary goal of this chapter is to study the relationship between plasma lactate concentration and the rate of lactate turnover in this teleost whose plasma lactate concentration can reach values exceeding 30 mM. A close examination of the results from this first study shows that

cardiovascular adjustments may also influence plasma metabolite turnover rate. The effects of changes in cardiac output on metabolite turnover rate have never been investigated at the whole organism level. These changes may have an important impact on the transfer of oxidizable fuels from their storage sites to their utilization sites: the working muscles. Also, they may alter the relationship between lactate concentration and plasma lactate turnover rate. Unfortunately, the study of cardiovascular changes, and their effect on lactate turnover rate could not be performed on tuna, and a different experimental animal was needed to study this problem in more detail (mainly because tuna could not be exercised under controlled conditions). With a mammalian model, exercise could be used as a tool to elicit cardiovascular adjustments, and to experimentally alter metabolite flux rates. The thoroughbred racehorse was chosen for the second part of this investigation (Chapters 3 to 5) because 1): this animal is known to undergo large cardiovascular changes in response to exercise, 2): lactate turnover rate and cardiac output have never been measured in this species, and 3): the thoroughbred horse is a very good example of an "elite" mammalian athlete. Chapter 2 also deals with the comparative aspects of metabolite kinetics studies, and it discusses problems associated with the comparison of kinetic data between species (in particular between homeothermic and ectothermic organisms). It is shown that tuna have the ability to move lactate across their plasma compartment at rates equal or exceeding the rates measured in

rats, dogs, and humans.

In the second part of the thesis (Chapters 3 to 5), lactate turnover is measured in the thoroughbred horse, an animal both genetically geared for high performance, and trained for racing. Chapter 3 characterizes the response of thoroughbred horses to submaximal exercise on a treadmill in order to determine the necessary conditions for the measurement of lactate turnover rate, and of the cardiovascular parameters relevant to this work. In later chapters, lactate turnover rate is measured by use of the bolus injection technique. Because this technique requires plasma lactate steady-state conditions, Chapter 3 examines when, and how such conditions are established. It focusses on the measurement of the cardiovascular and metabolic events associated with the onset of submaximal exercise, particularly with respect to the impact these events have on plasma lactate concentration. In this part of the thesis, the specific response of the animals used for the lactate turnover measurements is assessed. The data from chapter 3 allowed to determine the timing of injections and sampling for all the measurements reported in chapter 4 and 5. To complete this study of the general response of thoroughbred horses to treadmill exercise, muscle biopsies were taken to assess the metabolic status of one of the main locomotory muscles. These biopsies were sampled to see whether the lactate steady state observed in plasma after a few minutes of submaximal exercise was also present in working muscle. This preliminary work was necessary because, at the time

these experiments were conducted, no thoroughbred horse had been exercised on a treadmill, and the metabolic response of these animals to treadmill work was unknown.

In Chapter 4, the cardiac output of resting and exercising thoroughbred horses is determined by use of the thermodilution technique. The cardiac output measurements made here are used in the following chapter (5) to determine the impact of cardiovascular changes on plasma lactate turnover rate. Chapter 4 presents the first cardiac output measurements performed in thoroughbred racehorses. In addition, arterio-venous differences in oxygen content are measured at the different levels of activity chosen for the lactate turnover study. These measurements, together with cardiac output values, allow to calculate the metabolic rate of the animals for the different exercise protocols used in this study. The quantitative assessment of the oxygen consumption of these animals was necessary to be able to compare the exercise intensities selected for the horses with exercise regimes used in other studies done on different species including man. A valid comparison of lactate turnover rates could only be made if metabolic rates were known.

In Chapter 5, plasma lactate turnover is quantified in resting and exercising thoroughbreds by use of the bolus injection technique. An integrated analysis of the turnover rate and cardiac output data is presented in this chapter. The major factors involved in the regulation of plasma metabolite turnover rate are identified in the final analysis. The impact of changes in plasma lactate

concentration and in cardiac output on the turnover rate of oxidizable substrates is discussed. Unlike what is found in Chapter 2 for tuna, this last chapter shows that lactate is not an important metabolic fuel for muscle in exercising thoroughbred horses.

CHAPTER 2:

LACTATE AND GLUCOSE TURNOVER IN SKIPJACK TUNA.

2.1 INTRODUCTION

Over the past few years, exercise physiologists and biochemists have shown renewed interest in the study of lactate with the realization that this compound plays a double role in muscle metabolism. It is not simply an end product of glycolysis but also an important fuel for aerobic work (Hochachka et al, 1985). In mammals, muscle lactate production starts long before O_2 becomes limiting (Connett et al, 1984), and lactate turnover rate increases with blood lactate concentration (Brooks et al, 1984; Donovan and Brooks, 1983; Eldridge et al, 1974; Issekutz et al, 1976; Okajima et al, 1981).

As elite swimmers, tuna are characterized by a combination of endurance and sprint capabilities. Not only can they sustain moderate swimming speeds over very long distances, but they can reach top velocities of over 20 body lengths/s (Waters and Fierstine, 1964). The metabolic machinery supporting tuna locomotion must meet the challenges imposed by this lifestyle, and it should include: 1) fast fluxes of fuels to the working muscles during aerobic endurance swimming and 2) fast fluxes of anaerobic end

products to their sites of catabolism during recovery from burst exercise.

While swimming at high speed, skipjack tuna can generate lactate very rapidly, up to record concentrations of 90 umol/g wet weight in white muscle (Guppy et al, 1979). During recovery, blood lactate clearance occurs much faster in tuna than in other teleosts. Minimum blood lactate concentrations are reached after less than 2 h of recovery in exhausted skipjacks (Barrett and Connor, 1964). Even with a much smaller lactate load, clearance takes 12 h in rainbow trout (Black et al, 1962).

Present views of exercise and recovery in fish rely mainly on the interpretation of metabolite concentration measurements. Estimates of metabolic fluxes would provide a new dimension to the understanding of fuel metabolism as a dynamic process. However, glucose turnover rate has only been measured in kelp bass and coho salmon (Bever et al, 1977; Lin et al, 1978), and lactate fluxes have not been investigated in fish.

The goal of this study, therefore, was to measure lactate and glucose turnover rates in skipjack tuna to: 1) extend the observed correlation between lactate turnover rate and lactate concentration found in mammals to a much wider range of lactate concentrations and to a nonmammalian species, 2) find out whether the high rate of lactate clearance reported in tuna during recovery could be accounted for by high blood lactate fluxes, 3) assess the importance of lactate and glucose as metabolic fuels in a fast pelagic fish

and compare their flux rates with values measured in mammals, and 4) obtain some insight into the possible operation of the Cori cycle in tuna.

2.2 MATERIALS AND METHODS

2.2.1 EXPERIMENTAL ANIMALS. Skipjack tuna, Katsuwonus pelamis, were caught with barbless hooks off the coast of Oahu, Hawaii, from May to August 1984. They were brought back to the Kewalo Research Facility (National Marine Fisheries Service, Honolulu laboratory) in the bait wells of commercial fishing vessels and transferred to 75,000-liter circular holding tanks supplied with well-aerated 25^{0}C seawater. The fish were not fed in captivity and were used in the first 4 days after capture. The status of their carbohydrate stores was determined in a control group of five fish to assess whether capture and captivity caused glycogen or glucose depletion. The control animals were kept for 4 days in captivity under the same conditions as the fish used in turnover experiments, therefore, their carbohydrate stores were similar to the stores of the fish used for the measurement of lactate turnover. The control fish were caught with a net and killed with a sharp blow on the head. A blood sample was immediately taken by cardiac puncture. Approximately 1 g of white muscle, red muscle, and liver were freeze clamped in liquid N_2 and extracted in perchloric acid by use of previously described procedures (Guppy et al,

1979). Glucose, glucose 6-phosphate, and glycogen concentrations were determined in these tissues.

Fish to be used in turnover experiments swam vigorously around their holding tanks (from 1 to 10 min) before being directed to a large funnel leading to a 10-liter plastic bag containing O_2-supersaturated water with MS-222 (tricaine methanesulfonate, 1:2,000, wt/vol): an effective fish anesthetic.

2.2.2 CATHETERIZATION. As soon as the fish stopped moving, it was brought into the laboratory and placed ventral side up on an operating table. The gills were irrigated with aerated 24^{0}C recirculating seawater containing MS-222 (1:15,000). A catheter (Surflo I.V. Catheter 20 g X 2 in., ID 0.80 X 51 mm, Terumo, Japan) was inserted at a 45^{0} angle approximately 2 cm in front of the pelvic fins, directed towards the head for the percutaneous cannulation of the ventral aorta just anterior to the heart. The catheter was connected to a pressure transducer for pressure verification of catheter position. A 40-cm piece of PE-160 tubing was attached to the catheter and sutured to the underside of the animal. Double cannulation (ventral and dorsal aorta) was attempted on a few individuals. The dorsal aorta cannulation was done through the mouth by use of a technique described in Jones et al (1986). This procedure was difficult to perform, and success rate was low (under 30%).

Catheters, PE tubing, and syringes were always rinsed with heparinized saline (10 U/ml) before use. Total

cannulation time never exceeded 20 and 35 min for single and double cannulations, respectively. Once the catheter(s) was in place, heart rate and blood pressure were monitored via the transducer. The tuna was turned upright, strapped to a Plexiglass holder, and submerged. The anesthetic concentration was then reduced to approximately 1:30,000 and was adjusted individually for each fish throughout the turnover experiments. The animals were allowed to recover until they slowly moved their tail without thrashing. They were maintained in this state by continuously adjusting the anaesthetic concentration.

Preinjection measurements of heart rate and blood pressure together with blood glucose concentration were valuable indicators of the state of the animal after surgery. Animals showing a blood glucose concentration of less than 1 mM were not used in turnover experiments. The limit was set at this value because no fish with blood glucose levels of 1 mM or more showed signs of stress at any occasion. In some cases, blood glucose concentration started to decrease from its original steady-state value after several hours of sampling in a turnover experiment. Whenever this occurred the tail ends of decay curves were not included in the analysis. After this operation, sampling times for all turnover experiments ranged from 2 to 6 h (i.e. a minimum of 2 hours of sampling was always available to derive a decay curve), and the assumptions of steady state kinetics required by the bolus injection technique were met.

2.2.3 INJECTION OF LABELED METABOLITES AND BLOOD SAMPLING. A bolus of 25-35 uCi [U-^{14}C]lactate (specific activity > 100 mCi/mmol), 10 uCi [U-^{14}C]glucose (4 mCi/mmol) or 25-30 uCi [6-^{3}H]glucose (300 mCi/mmol) was injected via the catheter at time 0. In two specimens [U-^{14}C]glucose and [6-^{3}H]glucose were injected simultaneously to estimate glucose carbon recycling. The line was flushed with 3 ml heparinized saline immediately after injection. Blood samples (0.5 ml) were drawn starting 1 min after injection to allow the bolus to equilibrate in the rapidly mixing pool. This time was sufficient to allow total blood volume to be circulated across the entire body, and the bolus was assumed to be mixed after this period. Samples were drawn about every 40 s during the first 5 min and then at appropriate intervals. This choice of sampling times was made in order to obtain a good measurement of lactate specific activity decay without drawing excessive volumes of blood from the animal. The catheter was flushed with 0.5 ml saline between samples. The amount of blood drawn throughout an experiment never exceeded 10% of total blood volume. Radiochemicals were purchased from New England Nuclear (Boston, Mass.) and Amersham (Oakville, Ontario).

2.2.4 METABOLITE ASSAYS AND COUNTING. Blood samples were deproteinized immediately in 1-2 parts cold perchloric acid (8%) and spun down. The supernatant was stored at -4^{0}C. Lactate and glucose concentrations were determined enzymatically at 340 nm by use of the procedure of

Bergmeyer (1974). Tissue glycogen of the control fish was determined with the amyloglucosidase hydrolysis technique (Bergmeyer, 1974). All metabolite assays were performed in duplicate, at the latest 7 days after sampling and the mean of these measurements was used in the calculations. For each determination of metabolite concentration, a new set of two measurements was made if the first two values were differing by more than 10%. Glucose determinations never showed such variability, but it was necessary to repeat lactate determinations (two assays) in about 5% of the cases.

The specific activity of lactate was estimated by subtracting the activity found in glucose from total blood activity. This method is adequate when measuring lactate turnover with ^{14}C-lactate by use of the bolus injection technique because other carbon pools than lactate and glucose never reach significant specific activities (in a bolus injection experiment, the animal is in contact with high isotopic activities for a very short period only, compared with a continuous infusion experiment in which a long equilibration period is necessary before a measurement of turnover can be made). When using the continuous infusion method the specific activity of several other carbon pools than lactate or glucose have time to equilibrate (because the animal is in contact with high isotopic activities for several hours). However, even under such conditions, more than 90% of total blood activity is still found in lactate, and about 6-8% is found in glucose (Davis, 1983; Donovan and Brooks, 1983; Okajima et al, 1981). The remaining activity

can be measured in other compounds such as alanine, pyruvate, and others, but, when taken together, these secondary compounds only represent a very small fraction of total activity (i.e. a maximum of 5% in extreme cases). In the bolus injection technique, the contribution of these other compounds will be even smaller than 5% for reasons explained previously, and their effect can be considered as negligible. In bolus injection experiments, the specific activity of lactate can therefore be estimated by subtracting the activity found in glucose from total activity. The maximal possible error caused by ignoring these secondary metabolites would be a 2 to 3 % overestimation of lactate specific activity in the tail ends of the decay curves. This overestimation of specific activity would cause a minor underestimation of lactate turnover (when dividing the injected dose by the surface area under the decay curve), and this effect has been considered as negligible in the present study. In three randomly chosen decay curves, all the measured specific activities of lactate were decreased by 5%, and turnover rate was recalculated with these new curves; the new "corrected" values for turnover rate were not significantly different from the original "uncorrected" turnover rates. As explained before, it is very unlikely however, that the specific activity of alanine, pyruvate, and other secondary compounds would represent 5% of total activity in a bolus injection experiment.

Blood glucose was separated to determine its activity. Blood perchloric acid extract (200 ul) was

incubated with 4 ml 1 M glucose and Ø.6 g Amberlite MB-3 resin (Sigma Chemicals). The mixture was shaken for 2 h at 25^{Ø}C, allowing all the charged compounds to bind to the resin before it was spun down and 1 ml of the supernatant was counted. After separation 9Ø.1±3.2% of the total radioactive glucose was recovered as determined with [6-^{3}H]glucose standards.

Lactate activity was measured by counting 4Ø ul perchloric acid extract and correcting for the activity found in glucose. Scintillation counting was performed on a Beckman LS-9ØØØ liquid scintillation counter using external standard quench correction. All samples were mixed with 1Ø ml aqueous counting scintillant (ACS II, Amersham) and left in the dark for at least 12 h before counting. Single-label counting showed an efficiency of 94% for ^{14}C and 42% for ^{3}H (dual-label: 73 and 35%, respectively). No attempt was made to measure the appearance of ^{14}C in carbon dioxide because no reliable technique is available for the quantitative trapping of CO_2 in salt water. However, like in mammals, the activity found in blood and in other tissues decays very rapidly after a bolus injection of ^{14}C-lactate, showing that the major fate of plasma lactate in tuna is also oxidation (this compound is not excreted in fish: see Cameron and Kormanik, 1982; Kobayashi and Wood, 198Ø). In some preliminary experiments, 6 skipjack tuna were injected with ^{14}C-lactate and they were allowed to quietly swim in a large tank (1-2 body length per second) for 6 hours. Glycogen was isolated from their muscles and liver to determine the

activity in this compound. Almost no lactate had been incorporated, and the specific activity of glycogen was extremely low in these tissues. Furthermore, the activity measured was too variable to reliably quantify glycogen synthesis from lactate and this experimental approach had to be abandonned. These results clearly showed, however, that ^{14}C was not accumulating in an unknown carbon pool in this species.

2.2.5 TERMINOLOGY, CALCULATIONS AND STATISTICS. The concept of metabolite turnover can only be applied to a system in steady state (i.e. when the plasma concentration of this compound does not change over time). Under these conditions, the rate of appearance (R_a) is equal to the rate of disappearance (R_d), and can be called turnover rate or replacement rate. If the animal comes out of steady state, R_a and R_d must be referred to and measured independently because they are no longer equal. Hetenyi *et al* (1983) give a good summary of the theory and terminology associated with flux measurements in the study of metabolism *in vivo*.

Two major techniques are available for the measurement of turnover rate: bolus injection and continuous infusion (Katz *et al*, 1981; Okajima *et al*, 1981; Wolfe, 1984). They lead to the same results but differ in their assumptions and in their usefulness for various experimental situations. Unlike continuous infusion, bolus injection requires steady-state metabolite concentration, and it does not allow the independent mesurement of rates of appearance

and disappearance. Here, bolus injection was chosen over continuous infusion because of surgical difficulties; implanting the second catheter required by the infusion technique was too difficult in the species investigated.

An excellent analysis of current methods used for the in vivo measurement of fluxes with radio- and stable isotopes can be found in R.R. Wolfe's book: Tracers in Metabolic Research (1984). These modern methods allow the investigation of metabolism in vivo, and, over the last 10 years, they have been used by a large number of investigators (reviews by Hetenyi et al, 1983; Katz, 1979; Wolfe, 1984). Both stable and radioisotope tracer techniques have been applied to the measurement of metabolite turnover in plasma and they have been validated for a number of common blood metabolites (Hetenyi et al, 1983; Katz, 1979; Wolfe, 1984). Because the continuous infusion technique had been developed purely theoretically, it had to be validated experimentally. Dogs were anaesthetized, and all sources of endogenous glucose (liver and kidney) were removed surgically (Allsop et al, 1978). Glucose was then infused at a known rate, and the continuous infusion technique was used to calculate the rate of glucose appearance. The maximum difference between the true rate and the calculated rate was 5% and the coefficient of variation of the tracer calculated rate about the actual rate was 3.4% (see Wolfe, 1984). Several laboratories have quantified the rate of plasma lactate turnover in different species, and under various experimental conditions (Davis, 1983; Donovan and Brooks, 1983; Eldridge, 1974; Eldridge et

al, 1974; Freminet and Leclerc, 1980; Issekutz, 1984; Issekutz et al, 1976; Katz et al, 1981; Mazzeo et al, 1982; Mazzeo et al, 1986; Okajima et al, 1981; Reilly and Chandrasena, 1977; Stanley et al, 1985). The specific use of tracer techniques for the investigation of lactate kinetics has been reviewed in Brooks and Fahey (1984), Freminet and Minaire (1984), Katz (1986), and Wolfe (1984). The use of bolus injection for lactate turnover measurements may seem questionable because the labeled compound may not have time to reach the intracellular fluid which contains a fraction of the total lactate pool (i.e. isotopic equilibrium may not have time to occur). However, the bolus injection technique has been shown to lead to the same results as the continuous infusion technique for which this problem is absent (in continuous infusion, the label equilibrates between extra- and intracellular fluids over several hours). The turnover rates measured with the two techniques were found to be identical for several metabolites including glucose (Katz, Rostami, and Dunn, 1974; Katz, 1979), and lactate (Katz et al, 1981; Katz, 1986; Okajima et al, 1981). These two techniques give identical results because labeled lactate equilibrates quickly between blood and muscle compartments (Wolfe, pers. comm.; also see Koch et al, 1981), as indicated by the K_m values reported for lactate transporters in various cell membranes (Dubinsky and Racker, 1978; Koch et al, 1981; Roos, 1975; Spencer and Lehninger, 1976).

In the present study, turnover rate was calculated as the dose injected (in dpm) divided by the surface area

under the specific activity decay curve (in dpm $umol^{-1}$ min, see Katz et al, 1981). To calculate this surface area the decay curve was first fitted with a multiexponential function by use of the nonlinear regression program P3R from BioMedical Data Processing (BMDP; see Wolfe, 1984), which determines the least-squares estimates of the function parameters by a modified Gauss-Newton algorithm. The fitted function was then integrated between time Ø and the time when 5% of the maximum possible specific activity was reached. The maximum possible activity was calculated as the dose injected divided by the volume of the rapidly mixing pool estimated to be 8% of the body volume. Metabolic clearance rate (MCR) was calculated as turnover rate divided by the steady state metabolite concentration (Katz et al, 1981).

2.3 RESULTS

Glucose, glucose 6-phosphate, and glycogen concentrations in tissues of animals from the control group are given in Table 1. Skipjack tuna blood glucose concentration appeared to be regulated because it was relatively constant throughout the turnover experiments (Tables 2 and 3, Fig. 3B). However, the set point was different between individuals, and the mean blood glucose concentration ranged from 1.2 to 6.8 mM. In each turnover experiment a blood sample was drawn before injection of the radioactive bolus. Lactate and glucose concentrations of the preinjection sample were always within 2% of the means given in Tables 2 and 3.

2.3.1 LACTATE TURNOVER. After injection of [U-^{14}C]lactate the activity recovered in glucose never exceeded 5% of total blood activity, even after 6 h. The lactate specific activity decay curves were best fitted with the sum of three exponential functions (Fig. 1A). In all cases the calculated functions fitted the observed values extremely well. Fish No. 4 had a relatively low blood lactate concentration and the lowest turnover rate. Specific activity decreased sharply over the first 10 min after injection and then more gradually (Fig. 1A). Blood lactate concentration was relatively stable throughout the experiment (Fig. 1B).

Turnover rates and MCR are given for seven individuals in Table 2. These animals were selected because

TABLE 1. TISSUE METABOLITE CONCENTRATIONS IN THE CONTROL GROUP OF SKIPJACK TUNA.

Values are means ± SEM for 5 animals. Concentrations given in umol/g wet weight for muscle and liver, and umol/ml for blood. Tuna were handled and kept in captivity under the same conditions as animals used in the turnover experiments.

TISSUE	GLUCOSE	GLUCOSE 6-PHOSPHATE	GLYCOGEN (GLUCOSYL UNITS)
White muscle	Ø.68 ± Ø.12	4.16 ± Ø.97	92.54 ± 9.17
Red muscle	Ø.84 ± Ø.17	1.3Ø ± Ø.34	24.22 ± 5.2Ø
Liver	3.54 ± Ø.34	-----	4.Ø6 ± Ø.87
Blood	3.Ø4 ± Ø.39	-----	-----

TABLE 2. BLOOD METABOLITE CONCENTRATIONS AND LACTATE TURNOVER RATES IN SKIPJACK TUNA.

Values are means ± SEM, number of blood samples (N) in parentheses. [1] Sampling site also given: VA = Ventral Aorta, DA = Dorsal Aorta. Fish No. 6 and 7 were sampled simultaneously from VA and DA. Turnover rate was determined by bolus injection of [U-^{14}C]lactate.

FISH No.[1]	WEIGHT (g)	GLUCOSE mM	LACTATE mM	(N)	LACTATE TURNOVER RATE (umol min^{-1} kg^{-1})	METABOLIC CLEARANCE RATE (ml min^{-1} kg^{-1})
1 VA	1065	4.35±0.08	27.5±1.0	(11)	380.9	13.9
2 VA	1298	4.26±0.12	25.0±0.4	(13)	296.0	11.8
3 VA	1440	2.30±0.07	19.1±0.5	(12)	299.8	15.7
4 VA	1436	6.31±0.14	14.6±0.3	(15)	112.6	7.7
5 VA	1880	1.85±0.03	12.0±0.5	(13)	199.9	16.7
6 VA	1405	4.10±0.11	11.6±0.3	(11)	180.8	15.6
6 DA		4.12±0.09	10.2±0.4	(11)	131.8	12.9
7 VA	1412	1.23±0.02	26.5±0.3	(10)	415.2	15.7
7 DA		1.22±0.02	27.6±0.5	(11)	431.4	15.6

FIG. 1 Lactate turnover rate measurement in skipjack tuna (fish No. 4). Radioactive bolus injected and blood sampled via the ventral aorta catheter. A: blood lactate specific activity decay curve after injection of 25 uCi [U-^{14}C]lactate at time Ø. Curve fitted with the sum of 3 exponential functions. B: blood lactate concentration during the sampling period.

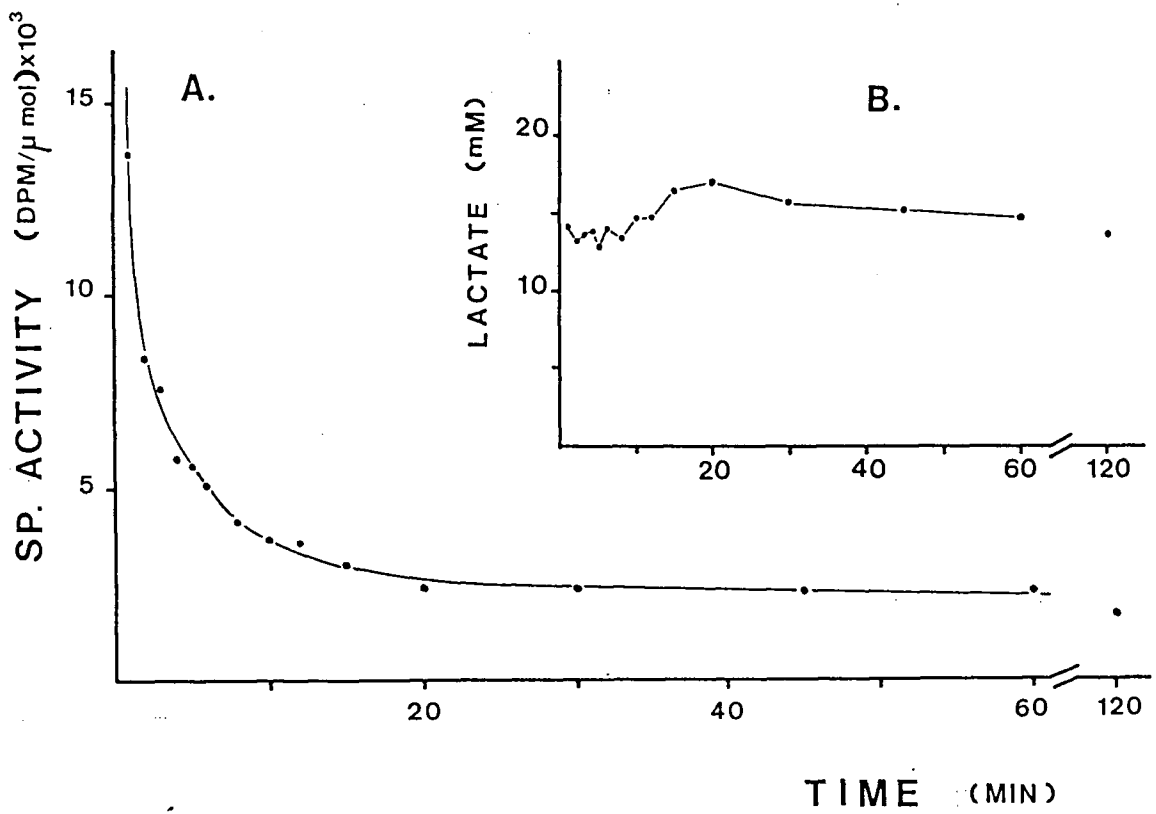
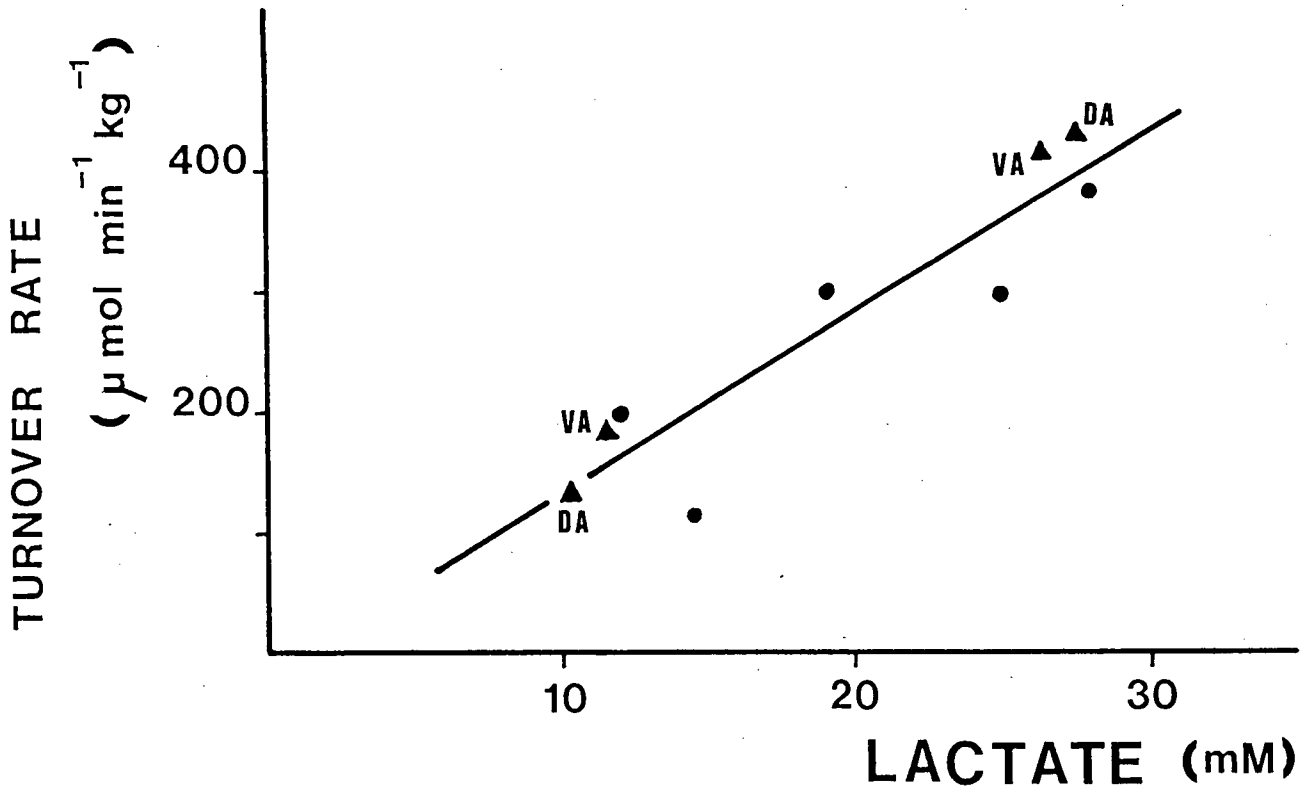

A.
B.
SP. ACTIVITY (DPM/μ mol)x10³
LACTATE (mM)
15
10
5
20
10
20
40
60
120
20
40
60
120
TIME (MIN)

FIG. 2 Relationship between lactate turnover rate and blood lactate concentration in skipjack tuna. Circles: animals with single ventral aorta catheter; triangles: 2 animals (fish No. 6 and 7) sampled simultaneously from ventral aorta (VA) and dorsal aorta (DA). The line was fitted by linear regression (slope = 15.1, r^2 = Ø.85).


TURNOVER RATE
(μ mol min⁻¹ kg⁻¹)
400
200
VA
DA
VA
DA
10
20
30
LACTATE (mM)

the coefficient of variation of their mean blood lactate concentration was less than 15%. These seven individuals had stable enough lactate concentrations to justify the use of steady state kinetics in our calculations (3 expts. were rejected). Mean blood lactate concentrations in individual fish ranged from 10.2 to 27.6 mM. Turnover rate was positively correlated with lactate concentration (Fig. 2), and the slope of the relationship was highly significantly different from zero ($F = 40$, $P < 0.001$, 1 df/7 df, ANOVA).

MCR was not affected by blood lactate concentration. A linear regression for blood lactate vs. MCR (graph not shown) had a slope of 0.044 ($r^2 = 0.0134$), which is not significantly different from zero ($F = 0$, $P > 0.05$, 1 df/7 df, ANOVA). Mean MCR was 14.0 $\pm$ 0.9 (SEM) ml min^{-1} kg^{-1} (N = 9).

Simultaneous sampling from the dorsal aorta and the ventral aorta was performed on fish No. 6 and 7. Turnover rates determined from both sampling sites were similar (Table 2, Fig. 2), indicating that these two sites could be used interchangeably. Differences between the dorsal aorta and ventral aorta lactate specific activity decay curves were not sufficient to quantify lactate utilization by the gills.

2.3.2 GLUCOSE TURNOVER. As in mammalian species, the turnover rate for glucose was much lower than for lactate. A typical specific activity decay curve is given for fish No. 9 (Fig. 3A), which exhibited an average turnover rate. The decay curves for glucose were best fitted with the

sum of two exponential functions. Turnover rate and MCR for glucose are given in Table 3. Turnover rate was independent of glucose concentration ($F = 1$, $P > 0.25$, 1 df/5 df, ANOVA) and averaged 15.3 $\pm$ 1.2 (SEM) umol min^{-1} kg^{-1} as determined with [6-^{3}H]glucose ($N = 5$). MCR was 4.7 $\pm$ 1.0 (SEM) ml min^{-1} kg^{-1} ($N = 5$). Fish No. 10 and 12 showed a high blood glucose concentration, but their turnover rate was not elevated. Consequently, glucose MCR was lower than average for these two fish.

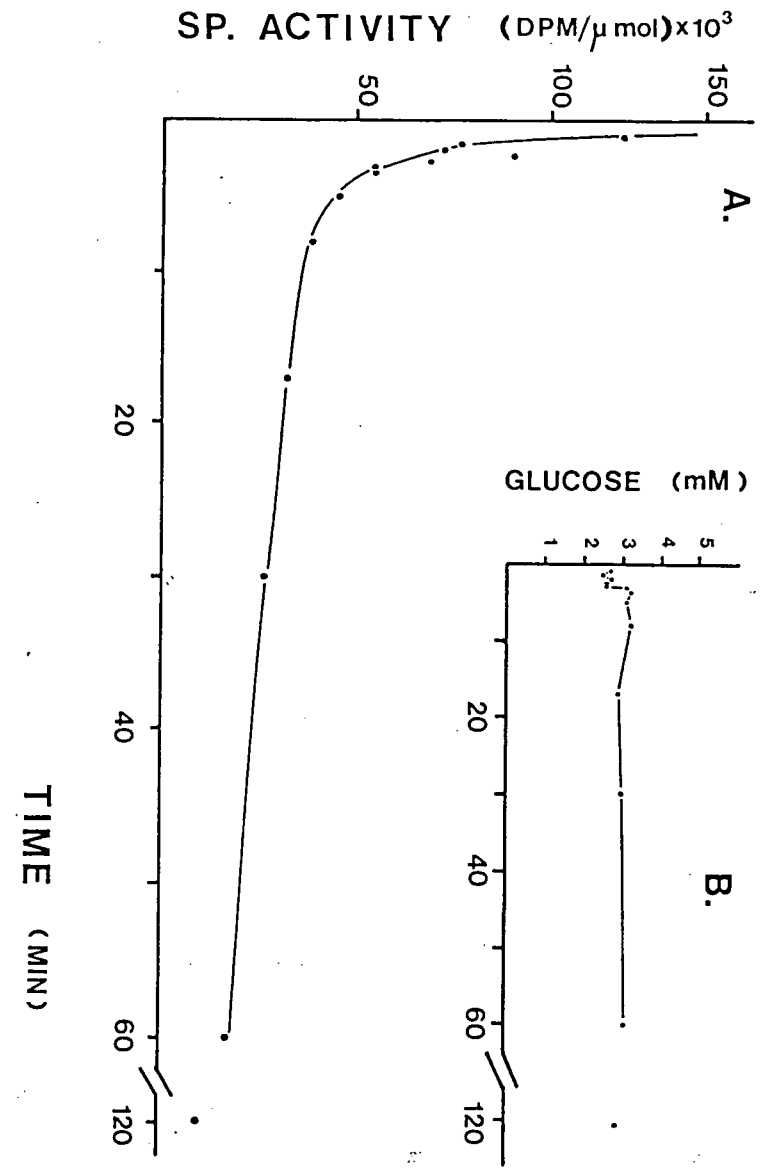
[6-^{3}H]glucose is considered an irreversible tracer because its predominant end product is tritiated H_2O. The reincorporation of labeled water into glucose is insignificant because rapid equilibration occurs within the large amount of body H_2O. [U-^{14}C]glucose is a reversible tracer because ^{14}C incorporated into other metabolic intermediates can be recycled back to glucose. This recycling slows down the decay of the specific activity curve, causing an underestimation of the true rate of glucose turnover. In this study, turnover rate determined with [6-^{3}H]glucose was much higher than with [U-^{14}C]glucose (Table 3), indicating that glucose carbon recycling is relatively high in tuna (28 and 16% for fish No. 11 and 12, respectively).

TABLE 3. BLOOD GLUCOSE CONCENTRATION AND GLUCOSE TURNOVER RATE IN SKIPJACK TUNA.

Values are means ± SEM. Number of blood samples (N) in parentheses. Turnover rates were determined by bolus injection of [6-^{3}H]glucose or [U-^{14}C]glucose. Fish No. 11 and 12 were injected with both isotopes. Injection of the radioactive bolus and blood sampling were done via ventral aorta catheters.

FISH No.	WEIGHT (g)	LABEL	GLUCOSE mM	GLUCOSE TURNOVER RATE (umol min^{-1} kg^{-1})	METABOLIC CLEARANCE RATE (ml min^{-1} kg^{-1})
8	1680	^{3}H	2.52 ± 0.08 (11)	14.8	5.9
9	1497	^{3}H	2.83 ± 0.09 (13)	15.2	5.4
10	1320	^{3}H	6.77 ± 0.17 (13)	11.0	1.6
11	1671	^{3}H	2.46 ± 0.03 (11)	17.3	7.0
11		^{14}C		12.5	5.1
12	1597	^{3}H	5.19 ± 0.10 (13)	18.1	3.5
12		^{14}C		15.2	2.9

FIG. 3 Glucose turnover rate measurement in skipjack tuna (fish No. 9). Injection and blood sampling same as Fig. 1. A: blood glucose specific activity decay curve after injection of 28.2 uCi [6-^{3}H]glucose at time Ø. The curve was fitted with the sum of two exponential functions. B: blood glucose concentration during the sampling period.


SP. ACTIVITY (DPM/μ mol)x10³
50
100
150
A.
20
40
60
120
TIME (MIN)
GLUCOSE (mM)
1
2
3
4
5
20
40
60
120
B.

2.4 DISCUSSION

The lactate turnover rates reported here for skipjack tuna range from 112 to 431 umol min^{-1} kg^{-1}. These values are higher than the rates reported by Okajima *et al* (1981) for resting rats (70 umol min^{-1} kg^{-1}) and are comparable to values obtained at rest (200 umol min^{-1} kg^{-1}) and at different levels of exercise (up to 500 umol min^{-1} kg^{-1}) by Donovan and Brooks (1983), also in rats. Because lactate is not excreted in fish (Cameron and Kormanik, 1982; Kobayashi and Wood, 1980), and because it is transported rapidly across cell membranes (Dubinsky and Racker, 1978; Koch *et al*, 1981; Roos, 1975; Spencer and Lehninger, 1976), lactate turnover rate can be used as an index of plasma lactate metabolism *in vivo*.

The animals used in this study were not overly stressed by capture, transportation, and short-term captivity without food. They were not exhausted because their carbohydrate stores were far from being depleted. Red muscle glycogen concentration was only 30% lower than in the group of "resting" skipjack tuna reported by Guppy *et al* (1979); white muscle concentration was 90% higher. Glucose and glucose 6-phosphate levels in the two muscle types were similar to the "resting" fish of Guppy *et al*. Surprisingly, liver glycogen concentrations were low compared with other teleosts, but no other values are available for tuna liver. There is no doubt that the experimental situation used for the present measurements was very artificial because the

animals were restrained and lightly anesthetized. However, fish treated with MS-222 do not show any changes in blood glucose and lactate concentrations compared with untreated individuals (Wells et al, 1984). The same anesthetic causes a reduction in the levels of circulating catecholamines (Mazeaud et al, 1977), suggesting a possible depression of carbohydrate metabolism. Until now, all attempts to carry out this type of experiment on unanesthetized free-swimming tuna have been unsuccessful. Even under the present experimental conditions (i.e. semi-anaesthetized fish artificially ventilated), it is technically difficult to investigate metabolism in this species. Therefore, the number of tuna used in this study was limited to 12 without counting the fish used for preliminary experiments and unsuccessful experiments (for example when plasma metabolite concentration was out of steady state). Also, access to live tuna for experimental purposes is extremely restricted and costly. In fact, Kewalo Research Facility in Honolulu is the only laboratory in the world where live tuna are kept in captivity on a regular basis.

Because fish possess a secondary circulation (Vogel, 1985) the bolus injection technique may be inadequate for the measurement of plasma metabolite turnover rate in this group of animals. However, the presence of the secondary circulation does not seem to invalidate the use of bolus injection in tuna because recent measurements indicate that the flow rate of blood through the secondary vascular system may be extremely low. The half-time for mixing between the

primary and secondary circulations was estimated between 1.2 and 1.7 hours in rainbow trout (D. Randall pers. comm.). In the case of rainbow trout, the secondary system can be treated as a single input (or output) of lactate into the primary system and it plays the same role as any organ using or producing lactate. The bolus injection technique is therefore a valid experimental approach to measure lactate turnover rate in rainbow trout. In the present study, it was assumed that the mixing of blood between the primary and secondary circulation of skipjack tuna was also very slow, but this assumption has not been verified experimentally.

It is important to realize that metabolite turnover rates generally depend on several critical factors that should be taken into account if meaningful comparisons are to be made, particularly with mammalian values. To begin with, the effects of exercise, body mass, and temperature will be considered in some detail. Then the possible mechanisms allowing tuna to support such high turnover rates will be discussed.

Lactate turnover rate has been shown to be positively correlated with blood lactate concentration in resting rats (Okajima *et al*, 1981) and in resting dogs infused with different lactate concentrations (Eldridge *et al*, 1974). This is not an autocorrelation because lactate concentration *per se* is not used in the calculation of turnover rate. Instead, specific activity is used, and this value is a ratio which is independent of lactate concentration (both, the numerator and denominator of this

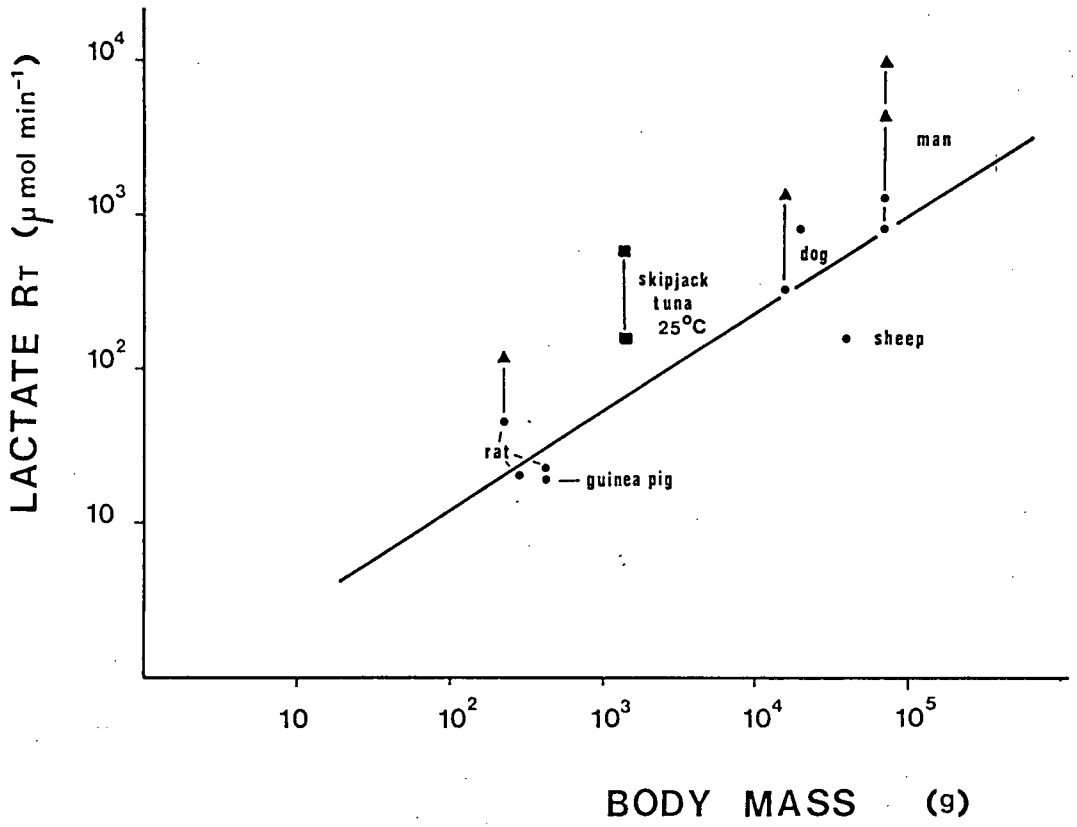
ratio depend on lactate concentration). This explains why it is possible to find cases in which metabolite concentration and turnover rate are not correlated, or cases in which a negative correlation between these two variables can be demonstrated (see for example Jenkins et al, 1986, for an example of a negative correlation between plasma glucose concentration and glucose turnover rate).

Lactate turnover rate also increases with exercise intensity (Brooks et al, 1984; Donovan and Brooks, 1983; Issekutz et al, 1976), but the effect of exercise is twofold because both perfusion and blood lactate concentration increase as work rate goes up. The present data show that lactate turnover rate is also correlated with blood lactate concentration in tuna (Fig. 2), and this is the first time such a demonstration is made for a nonmammalian species. Also, the range of plasma lactate concentrations covered by the present correlation is extended to values larger than 30 mM. Measurements of heart rate and blood pressure (results not shown) made before the bolus injections indicate that perfusion was probably similar between individuals showing different steady state blood lactate concentrations. Unfortunately, these measurements were not made throughout the turnover experiments, and cardiac output may have differed between individuals. However, MCR was not correlated with lactate concentration. When studying exercising animals a positive correlation between MCR and work rate would indicate different perfusion rates between different levels of exercise. Therefore, the constancy of MCR over the

observed range of lactate concentrations also suggests that the relationship shown in Fig. 2 probably represents the true effect of lactate concentration independent of perfusion rate. The respective contributions of lactate concentration and perfusion rate on turnover increase should be investigated separately.

The relationship between body mass and whole-body lactate turnover (R_t, in umol min^{-1}) is presented for a few mammalian species in Fig. 4. A linear regression across the turnover rates of resting mammals (circles) has been drawn (slope = Ø.64, r^2 = Ø.85). The correlation is not better because it is difficult to standardize "resting conditions" for a wide range of body shapes and sizes, knowing that lactate production and removal rates are influenced by activity. Furthermore, lactate R_t measurements are not available for many species. The effect of exercise on turnover rate is also illustrated for rat, dog, and man (triangles in Fig. 4). The range of values obtained for skipjack tuna in $25^{Ø}C$ water is also plotted. These values fall well within mammalian rates even without considering the effect of temperature.

FIG. 4 Relationship between body mass and whole-body lactate turnover (R_t) on log-log plot for mammals and skipjack tuna. Mammals at rest (circles) and exercising (triangles). The line was fitted by linear regression using resting values for mammals only. Turnover rates were measured with [^{14}C]lactate in all cases. References: rat (Donovan and Brooks, 1983; Freminet and Leclerc, 1980; Okajima et al, 1981)/ guinea pig (Freminet and Leclerc, 1980)/ dog (Eldridge et al, 1974; Issekutz et al, 1976)/ sheep (Reilly and Chandrasena, 1977)/ man (Brooks and Donovan, 1983; Stanley et al, 1984).


LACTATE R_T (μ mol min^{-1})
10^4
10^3
10^2
10
man
dog
skipjack
tuna
25°C
sheep
rat
guinea pig
10
10^2
10^3
10^4
10^5
BODY MASS (g)

Ocean-caught skipjack tuna can have a core temperature $10^{\emptyset}C$ higher than ambient, but such a high difference is only observed after feeding frenzies at sea. Captive animals subjected to violent exercise do not show more than a $5^{\emptyset}C$ excess core tremperature (Stevens and Neill, 1978). It is very unlikely that the body temperature of the fish used in this study exceeded water temperature by more than 2 or $3^{\emptyset}C$. If a Q_{10} of 2 is assumed, the lactate turnover rates measured at $25^{\emptyset}C$ should approximately double at $37^{\emptyset}C$.

It is interesting to notice that, even with a high lactate turnover rate of 400 umol min^{-1} kg^{-1}, an exhausted tuna with 1 kg of white muscle at a lactate concentration of 90 umol/g (Guppy et al, 1979) would take more than 3 h to metabolize its lactate load of 90 mmoles. However, complete recovery appears to be achieved in less than 2 h (Barrett and Connor, 1964). Also, one of the cannulated animals in this study showed a drop in blood lactate concentration from 32 to 12 mM in less than 2 h. These observations suggest that not all lactate passes via the blood compartment during recovery but that a significant portion is metabolized directly in white muscle.

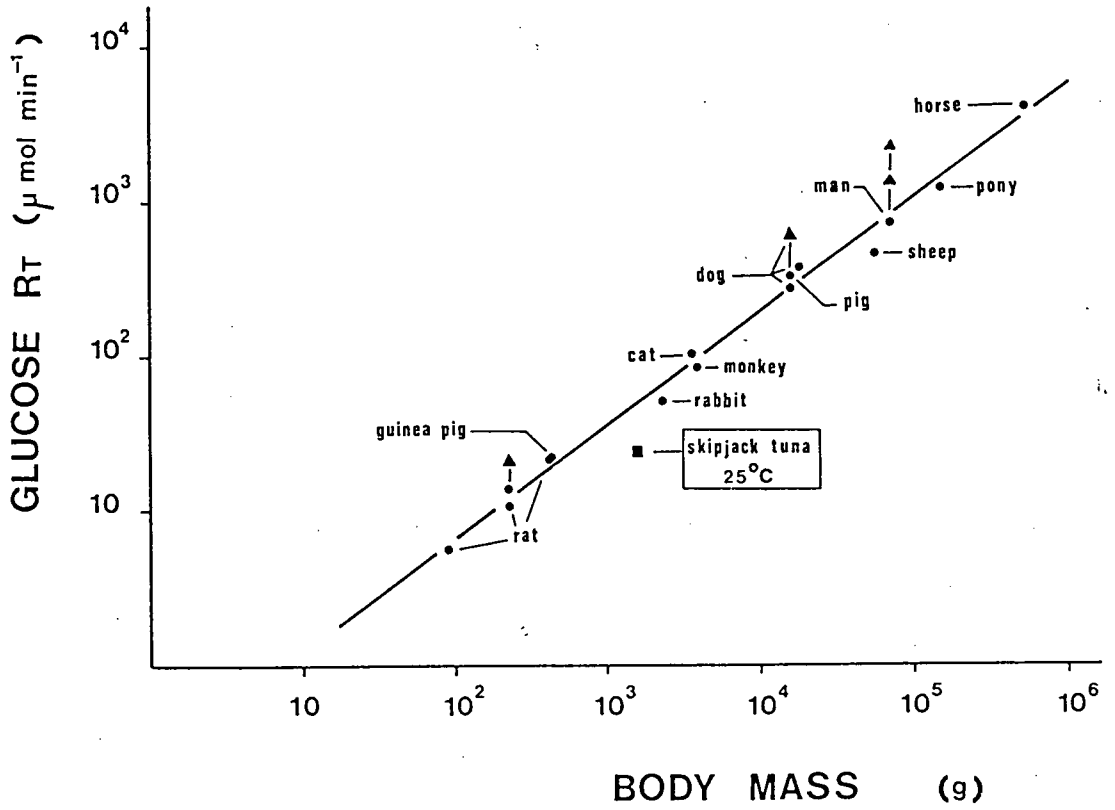
How can a tuna turn over lactate at the same rate or even faster than a $37^{\emptyset}C$ mammal? Perfusion is lower in a 2 kg tuna [cardiac output of approximately 90 ml/min (Brill et al, 1978)] than in a mammal of similar size. Blood lactate concentration can be higher in tuna, allowing faster diffusion between the blood and lactate-utilizing tissues.

However, this diffusional advantage cannot fully compensate for the effect of a $10^{\circ}C$ difference in body temperature. Therefore, tuna must have other adaptations allowing them to cycle lactate as fast as they do. The very high aerobic potential of lactate-utilizing tissues, such as red muscle (Guppy et al, 1979), could allow fast lactate diffusion by maintaining a steep concentration gradient between the blood and this "lactate sink". A second possibility involves use of the central vascular countercurrent heat exchanger to accelerate movements between lactate-producing white muscle and lactate-oxidizing red muscle (P.W. Hochachka, R.W. Brill, J.-M. Weber, B. Emmett, C. Daxboeck, S. Perry, and T.W. Moon, unpublished observations).

Tuna appear to regulate their blood glucose concentration much more tightly than other teleosts (Tables 2 and 3, Fig. 3). Cannulated kelp bass (Bever et al, 1977) and rainbow trout (Weber, unpublished data) show wide fluctuations in blood glucose levels. Tuna and mammalian insulins demonstrate similar properties (King and Kahn, 1981), which may allow these fish to achieve glucose homeostasis. Even though each tuna maintained a steady blood glucose concentration, the set points ranged from 1.2 to 6.8 mM in different individuals. The significance of these observed differences is not clear but may represent various nutritional states.

Glucose turnover rate has only been measured in two other fish species. Bever et al (1977) showed that kelp bass, Paralabrax sp., has a rate of about 2 umol min^{-1} kg^{-1}. A

FIG. 5 Relationship between body mass and whole-body glucose turnover rate (R_t) on log-log plot for mammals and skipjack tuna. Same symbols as in Fig. 4. A linear regression was fitted for resting mammals only. The turnover rates were determined with [^{3}H]glucose in all cases except for the horse (^{14}C). References: rat (Brooks and Donovan, 1983; Freminet and Leclerc, 1980; Katz et al, 1974; Kettlehut et al, 1980)/ guinea pig (Freminet and Leclerc, 1980)/ rabbit (Katz et al, 1974)/ cat (Kettlehut et al, 1980)/ monkey (Armstrong et al, 1979)/ dog (Issekutz, 1977; Issekutz et al, 1976)/ pig (Trayhurn et al, 1981)/ sheep (Brockman et al, 1975), man (Hall et al, 1979)/ pony (Anwer et al, 1976)/ horse (Evans, 1971).


GLUCOSE RT (μ mol min⁻¹)
10⁴
10³
10²
10
horse
man
pony
sheep
dog
pig
cat
monkey
rabbit
guinea pig
skipjack tuna
25°C
rat
10
10²
10³
10⁴
10⁵
10⁶
BODY MASS (g)

similar value was found for coho salmon, Oncorhynchus kisutch (Lin et al, 1978). The rates measured in fish species other than tuna are approximately 1/30 those found in resting rats (Katz et al, 1974).

Although the data base is very restricted for fish, mammalian values are available for a wide range of body sizes. The relationship between body mass and whole-body glucose turnover at rest (R_t, in umol min^{-1}) is presented in Fig. 5 on a double logarithmic scale (slope = 0.72, r^2 = 0.99, linear regression). Interestingly, this slope is not significantly different from the slope found for the classic body mass vs. metabolic rate relationship. The same analogy can be drawn for the body mass vs mass-specific glucose turnover rate (in umol min^{-1} kg^{-1}; graph not shown; slope = -0.27, r^2 = 0.94). The close correlations obtained suggest that it is of minimal importance to directly measure resting glucose turnover rate in mammals unless there are good reasons to predict that the species under study will depart from this line (extremely sluggish organism or elite animal athlete). When corrected for temperature the mean glucose R_t found for tuna falls exactly on the mammalian line. The effect of exercise on glucose R_t in mammals (triangles in Fig. 5) is much less pronounced than on lactate R_t (Fig. 4).

Under the present experimental conditions very little labeled lactate was converted to blood glucose, indicating that the role played by the Cori cycle in tuna lactate metabolism is not important. It is not clear why

glucose recycling was so high nor with what other compounds glucose may have been exchanging.

In conclusion, this study shows that skipjack tuna can support lactate and glucose turnover rates at least as high as those reported for mammalian species. Glucose is turned over much faster in tuna than in other teleosts, probably as a consequence of their high metabolic rate. Tuna lactate turnover rates are higher than values measured in mammals, and they are correlated with lactate concentration. These high turnover rates indicate that these animals can metabolize lactate at high rates. Furthermore, the major fate of plasma lactate in tuna appears to be oxidation like in mammalian species (even though $^{14}CO_2$ was not collected in this study, the main fate of plasma lactate is probably oxidation because total activity of blood decreased rapidly, and no important accumulation of ^{14}C could be measured in tissues after bolus injection of ^{14}C-lactate). Therefore, tuna may have the ability to oxidize plasma lactate at high rates (comparable to the rates reported for rats, dogs, and humans, when the appropriate temperature and body size correction are applied). These fish may be able to achieve such high turnover rates because they evolved particular anatomic and enzymatic adaptations for high-performance swimming. These high values, however, cannot fully account for the reported rates of lactate clearance during recovery, suggesting that part of the lactate produced in white muscle is metabolized in situ. The Cori cycle is probably not an important pathway for lactate clearance in tuna.

CHAPTER 3:

ONSET OF SUBMAXIMAL EXERCISE IN THOROUGHBRED HORSES.

3.1 INTRODUCTION

This chapter is the first of three dealing with exercise metabolism and physiology of thoroughbred horses. It characterizes their basic cardiovascular and metabolic responses to treadmill exercise, focussing on the effect of exercise on plasma lactate concentration. It was necessary to determine when plasma lactate concentration reaches a steady state because the bolus injection technique used in Chapter 5 for the measurement of lactate turnover rate requires steady concentration. Therefore, the behavior of lactate concentration had to be known during the exercise protocols selected for this study to be able to determine the injection and sampling times of the kinetics experiments performed in later chapters. The selection of low intensity (submaximal) exercise was made because turnover rate can only be measured under steady-state conditions when using the bolus injection technique (plasma lactate concentration cannot be in steady state at higher exercise intensities). As explained in the general introduction (Chapter 1), the next two chapters will investigate changes in cardiac output (Chapter 4) and their effect on lactate turnover rate in the same animals (Chapter 5).

A transient increase in plasma lactate concentration has been commonly observed in humans after the initiation of submaximal exercise (Bang, 1936; Hermansen and Stensvold, 1972; Rowell et al, 1966; Saiki et al, 1967). A maximum concentration is reached after a few minutes of work, followed by a decrease back to a new steady state. Remarkably little attention has been devoted to the mechanisms responsible for these changes. They are usually interpreted as the consequence of a delay in the activation of oxidative metabolism (Saiki et al, 1967) which, in turn, is presumably due to the relatively slow onset of the oxygen transport system.

In addition to the ordinary physiological adjustments to exercise shown by other mammals, horses have the ability to greatly increase their hematocrit by actively releasing red blood cells from their spleen (Persson, 1983). At the onset of submaximal exercise, their oxygen transport system is activated much more rapidly than in humans (Fregin and Thomas, 1983; Pan et al, 1984; Forster et al, 1984) and they show a heart rate overshoot which does not occur in man (Pan et al, 1984). For these reasons, the lactate overshoot observed in humans may be reduced or absent in horses.

The aim of this study was to characterize the lactate response to submaximal exercise in an elite performer, the thoroughbred racehorse, to establish if and when a lactate steady state was reached. As major indices of the oxygen transport system, heart rate and hematocrit were measured simultaneously with plasma lactate concentration to

determine whether the metabolic and cardiovascular responses coincide and to get some insights into the possible causes for the observed pattern of changes in lactate concentration. The effect of exercise intensity was also investigated because it has been shown to influence the steady state lactate concentration obtained during work (Hermansen and Stensvold, 1972). A model is presented to explain the measured concentration changes in terms of lactate fluxes to and from the plasma. Finally, the metabolic intermediates of the glycogenolytic pathway were measured in one of the main locomotory muscles to assess whether a steady state was also established in this tissue. Muscle biopsies were taken during the lactate steady state in plasma to determine whether plasma measurements reflect muscle lactate behavior.

3.2 MATERIALS AND METHODS

3.2.1 EXPERIMENTAL ANIMALS AND CATHETERIZATION.

All experiments were carried out in the summer of 1985 at the exercise unit of the Animal Health Trust, Newmarket, UK. Three thoroughbred horses (KJ, SB, and JW) were exercised 6 days a week for three months. Their regular training in the field consisted of walking and trotting with an occasional canter or gallop, and this was supplemented with treadmill work approximately twice a week to familiarize them with the experimental set-up. A fourth thoroughbred (IN) was only used for resting values and for pre- and post-canter muscle biopsies, one month after having been introduced to treadmill exercise. All horses were geldings and they were maintained on a diet of dry food and hay with unlimited access to water. Their weights and ages are given in Table 4.

Before catheterization, a small area of skin was shaved and disinfected at the site of catheter introduction just above the jugular vein. Local anaesthetic was injected subcutaneously (3 X 0.5 ml 2% lignocaine; 1:200,000 epinephrine). A stab incision was made and an I.V. catheter (Becton-Dickinson 14 g with 17 g X 8 in. inner needle) was introduced into the vein. The catheter was sutured to the skin and connected to a 100-cm extension line (Lectroflex Vygon PVC, 2 ml capacity) which was filled with saline and tightly taped around the neck of the animal. No heparin was added, but the extension line and the catheter were flushed every 10-15 min to keep them patent.

3.2.2 EXERCISE PROTOCOLS. After catheterization, the horse was fitted with a safety harness, heart rate monitor electrodes (EQB-HR7 heart rate meter), and ECG leads. He was walked to the treadmill (Sato, BIAB Industrial, Ludvika, Sweden) where the harness was secured above him to an emergency switch-off mechanism (which was never triggered in the present experiments). A fan was placed in front of the animal to simulate normal air cooling experienced during outdoor locomotion.

All experiments were carried out between 10 am and 3 pm, at least 3 h after the morning feed. Temperature ranged from 16 to 20^{0}C and relative humidity from 53 to 73%. Different combinations of speed and incline were tried for each horse before selecting two submaximal exercise regimes eliciting comfortable performances at both trotting and cantering gaits. (i) A 3-4 m/s trot at 6% incline sustainable for 40 min, and (ii) a 6.5 m/s horizontal canter for 10-15 min, which represents less than 50% of their maximal speed on the treadmill for this incline. All exercise bouts were initiated with a short warm-up period consisting of a 4-min walk at 1.6 m/s for the trot protocol and a 4-min walk (1.6 m/s) followed by a 4-min trot (4 m/s) for the canter protocol.

3.2.3 BLOOD AND TISSUE SAMPLING. Blood samples were collected in ice-cold 7-ml vacutainers containing 0.07 ml ethylenediaminetetraacetic acid (EDTA) as anticoagulant and placed on ice. They were carefully mixed before filling

hematocrit tubes to avoid problems caused by the fast sedimentation of horse red cells. After measuring hematocrit on a microcentrifuge, the rest of the sample was spun and the plasma was separated. It was placed at -20^{0}C before further analysis, and the entire procedure was completed within 10 min of sampling.

A resting blood sample (7 ml) was taken before the horse was brought on the treadmill. During the trot protocol, blood was drawn every min for the first 10 min of exercise and every 5 min thereafter (each sample was drawn in about 5 s). During the canter protocol, samples were taken every min throughout the exercise bout. The line was always flushed with saline between successive samples.

Biopsies from the middle gluteal muscle were taken from the 4 horses before and after the canter protocol by the use of previously described procedures (Snow, 1983). Before taking the resting biopsy (pre-canter), the animal was allowed to stand quietly in a stall for 15 min. Suction was applied while sampling and two muscle specimens were pooled each time to obtain the 200-300 mg of tissue necessary to perform all the metabolite assays. The biopsies were immediately frozen in liquid N_2. The post-canter muscle samples were frozen less than 1.5 min after the cessation of exercise.

The tissue samples were later ground in liquid N_2 with mortar and pestle. The fine powder obtained was immediately mixed with 6-7 parts of ice-cold 8% perchloric acid and homogenized for 2 X 15 sec on an Ultra-Turrax

homogenizer (Ika-Werk, West Germany). Two 100-ul aliquots of the suspension were frozen in liquid N_2 for glycogen determinations. The rest was spun at 11,000 g for 3 min at 4^0C in Eppendorf tubes and the supernatants were kept in liquid N_2 until the metabolite assays were performed.

3.2.4 METABOLITE ASSAYS. All the metabolic intermediates were assayed in duplicate on a Pye-Unicam spectrophotometer (SP6-500) by measuring the reduction of NAD (or NADP) or the oxidation of NADH (or NADPH) at 340 nm, using the procedures described in Bergmeyer (1974) as modified for micro-assays (total cuvette volume 0.5-0.7 ml). Lactate concentration was measured in plasma within three days of sampling. Muscle glycogen concentration was determined with the amyloglucosidase technique (Bergmeyer, 1974) and corrected for incomplete hydrolysis as measured with rabbit muscle glycogen standards (efficiency = 80.0%). Glucose, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, glyceraldehyde-3-phosphate, dihydroxyacetone-phosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, pyruvate, and lactate were also measured in the muscle samples.

3.3 RESULTS.

Resting values for plasma lactate concentration, heart rate and hematocrit of the thoroughbreds used in this study are presented in Table 4 with weights and ages.

3.3.1 TROT PROTOCOL. Results from the 40-min trot experiments are summarized in Fig. 6. KJ and SB were running at 4 m/s, but a lower speed of 3 m/s was chosen for JW, the older horse, who could not sustain 4 m/s over 40 min without experiencing articular pain caused by pounding on the relatively hard surface of the treadmill belt. The choice of this lower velocity was not a consequence of his state of training.

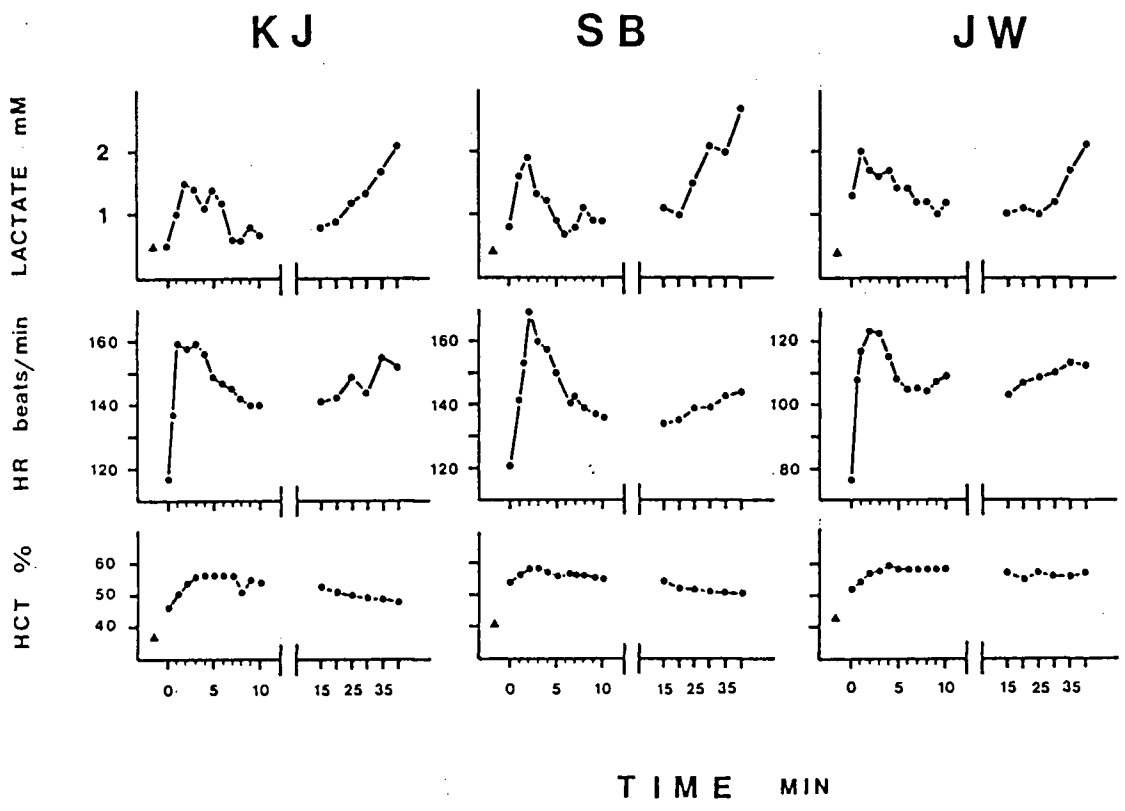
Mean resting lactate concentration was 0.45 mM. At the end of the 4 min warm-up walk (= time 0 on Fig. 6), this concentration was unchanged (KJ) or slightly elevated (KJ and SB). When the trot was started, lactate concentration increased to a maximum of 1.5 to 2 mM after 2 min of running, declined to levels above those at rest after 6 to 7 min, and stayed constant and low for about 15 min thereafter. Lactate started to accumulate slowly in plasma during the last 15-20 min of the exercise bout and reached 2-3 mM at the end of the 40-min trot.

Resting heart rate averaged 35.5 beats/min. The preliminary walk was sufficient to double (JW) or even triple that rate (KJ and SB). An additional 40 beats/min increase was seen after the first 2 min of trot before heart rate

TABLE 4. WEIGHT, AGE, AND RESTING VALUES FOR PLASMA LACTATE CONCENTRATION, HEART RATE AND HEMATOCRIT IN FOUR TRAINED THOROUGHBRED HORSES.

HORSE	WEIGHT kg	AGE y	LACTATE mM	HEART RATE beats/min	HEMATOCRIT %
KJ	453	4	Ø.5	41	37
SB	426	5	Ø.4	44	41
JW	466	13	Ø.4	32	43
IN	428	3	Ø.5	25	33

FIG. 6 Plasma lactate concentration, heart rate, and hematocrit of 3 trained thoroughbred horses during a 40-min trot at 6% incline on a treadmill (KJ and SB: 4 m/s; JW: 3 m/s). Time 0 when treadmill speed started to be increased from a 4 min warm-up walk at 1.6 m/s. Transition from 1.6 to 4 m/s (or 3 m/s for JW) was completed in 20 s. Note that on all graphs, the time scale changes after 10 min. Triangles represent resting values.


KJ
SB
JW
LACTATE mM
HR beats/min
HCT %
2
1
160
140
120
120
100
80
60
50
40
0 5 10 15 25 35
TIME MIN

decreased to a stable value of 120 beats/min (JW) and 140 beats/min (KJ and SB) which was sustained for approximately 15 min. During the last 15-20 min of the trot, heart rate slowly increased without reaching the maximum obtained after 2 min.

Mean hematocrit was 38.5% in resting animals. The warm-up walk increased this value to 52.0% and a maximum of 57.7% was reached after 3 min of trotting. Hematocrit steadily decreased thereafter at a very slow rate until the end of the 40-min exercise bout (bottom graphs on Fig. 6). The slopes of these decrements and their statistical significance are presented in Table 5.

3.3.2 CANTER PROTOCOL. All horses were running at 6.5 m/s but the older horse (JW) could not comfortably sustain that speed for more than 10 min. Plasma lactate concentration, heart rate and hematocrit during the canter experiments are shown in Fig. 7. Lactate concentration was slightly elevated (to about 1 mM) compared with resting values after the preliminary walk and trot. The pattern seen in the first few minutes of canter was similar to what was observed for the trot. Maximum concentrations were also reached at 3-4 min but they were higher than in the trot (2-3 mM). The time necessary to reach a new steady lactate concentration was greater in the canter protocol (8-10 min vs 6-7 min in the trot protocol).

Heart rate was approximately tripled during the warm-up period and reached 170 beats/min in KJ and SB after

2-3 min of cantering. Even though JW was running at the same speed as the other horses, the highest heart rate he reached was only 135 beats/min. For all animals, heart rates stabilized at some lower values between 120 and 140 beats/min after 5-10 min at 6.5 m/s.

The highest hematocrits seen in this study were measured after 3 min of cantering. KJ reached an absolute value of 61%, which represents a more than 1.5 fold increase over his resting level of 37%. JW and SB reached 56 and 59% respectively. After peaking at 3 min, hematocrit significantly decreased in all horses until the end of exercise (linear regressions and ANOVAS are given in Table 5).

3.3.3 MUSCLE METABOLITE CONCENTRATIONS. The levels of glycogen and of the glycogenolytic intermediates in the middle gluteal muscle before and after the canter protocol are given in Table 6. The values obtained for JW, who ran only for 10 min, were not significantly different from the mean concentrations obtained for the 3 other horses. Therefore, results from all 4 animals were pooled. Exercise did not cause significant changes in metabolite concentrations (paired t-test, $p > 0.05$) except for glycogen which showed a 30% decrease compared with pre-exercise values ($p < 0.05$). Even lactate concentration was not increased after 15 min at 6.5 m/s ($p = 0.85$).

TABLE 5. STATISTICAL ANALYSIS OF THE DECLINE IN HEMATOCRIT AFTER 3-4 MIN OF TREADMILL EXERCISE IN THOROUGHBRED HORSES. Linear regressions were performed on the hematocrit vs time relationships of Fig. 6 and Fig. 7 between the time when maximum hematocrit was reached and the end of the exercise bout. ANOVA was used to determine the probability that the slopes are equal to Ø. Refer to methods for exercise conditions.

	T R O T		C A N T E R	
HORSE	SLOPE	p	SLOPE	p
KJ	- Ø.22	< Ø.ØØ1	- Ø.61	< Ø.ØØ1
SB	- Ø.18	< Ø.ØØ1	- Ø.31	< Ø.Ø1
JW	- Ø.Ø6	Ø.Ø6	- Ø.18	< Ø.Ø1

FIG. 7 Same as Fig. 6, but during a horizontal canter at 6.5 m/s for all horses. Time Ø when treadmill speed started to be increased from a 4 min warm-up trot at 4 m/s. Transition from 4 to 6.5 m/s was completed in 2Ø s.

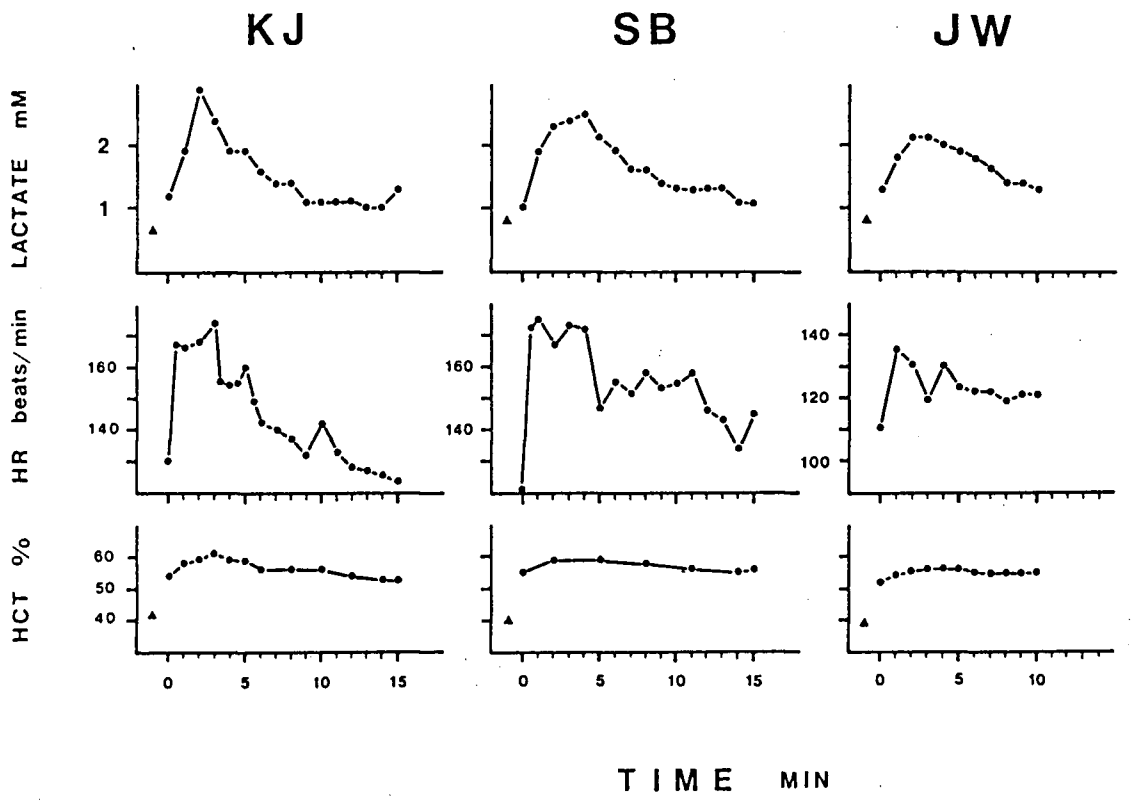

KJ
SB
JW
LACTATE mM
2
1
HR beats/min
160
140
160
140
140
120
100
HCT %
60
50
40
0
5
10
15
0
5
10
15
0
5
10
TIME MIN

TABLE 6. METABOLITE CONCENTRATIONS BEFORE AND AFTER A 15 MIN CANTER (AT 6.5 m/s) IN THE MIDDLE GLUTEAL MUSCLE OF THOROUGHBRED HORSES RUNNING ON A TREADMILL.

Values are means ± SEM (n = 4). Concentrations are given in umol/g wet weight. Glycogen given in glucosyl units. Pre- and post-exercise values were compared with a paired t-test (* indicates $p < 0.05$)

METABOLITE	PRE-EXERCISE	POST-EXERCISE
Glycogen *	131.7 ± 8.7	96.3 ± 17.0
Glucose	0.31 ± 0.082	0.30 ± 0.106
Glucose 6-phosphate	0.56 ± 0.093	0.56 ± 0.089
Fructose 6-phosphate	0.08 ± 0.014	0.06 ± 0.017
Fructose 1,6-bisphosphate	0.25 ± 0.050	0.25 ± 0.023
Glyceraldehyde 3-phosphate	0.03 ± 0.005	0.04 ± 0.005
Dihydroxyacetone phosphate	0.06 ± 0.005	0.07 ± 0.006
3-Phosphoglycerate	0.17 ± 0.016	0.20 ± 0.010
2-Phosphoglycerate	0.03 ± 0.008	0.03 ± 0.007
Phosphoenolpyruvate	0.11 ± 0.009	0.11 ± 0.007
Pyruvate	0.10 ± 0.012	0.11 ± 0.007
Lactate	2.73 ± 0.13	2.76 ± 0.04

3.4 DISCUSSION

This study allowed the simultaneous measurement of short-term changes in plasma lactate concentration, hematocrit and heart rate of thoroughbred racehorses under well controlled exercise conditions. Rapid drawing of multiple blood samples was made possible by the use of catheterized animals running on a large treadmill. The time course of the hematocrit response in thoroughbreds is presented here for the first time. In spite of their ability to mobilize the cardiovascular system very rapidly, they showed a marked plasma lactate overshoot which appeared to follow a pattern similar to what had been reported previously in humans (Bang, 1936; Hermansen and Stensvold, 1972; Rowell *et al*, 1966; Saiki *et al*, 1967) and in dogs (Issekutz *et al*, 1976). However, the low sampling frequencies of these earlier studies do not provide the necessary time resolution to make careful comparisons.

The intensity of exercise had a marked effect on the patterns of changes for all parameters investigated. When work intensity was increased: 1) the maxima reached during the onset of exercise were higher for plasma lactate concentration, heart rate and hematocrit; 2) lactate concentration and heart rate took longer to reach a steady state; and 3) the steady state lactate concentration and steady state heart rate established while exercising were both elevated.

3.4.1 CHANGES IN HEMATOCRIT DURING EXERCISE.

The ability of horses to drastically increase hematocrit when oxygen consumption goes up is one of their most striking adaptations for high-speed locomotion. More than one third of the total number of erythrocytes can be liberated from the spleen via adrenergic stimulation (Persson, 1983). The size of the splenic reservoir and consequently the magnitude of the hematocrit response to exercise are increased by training (Lykkeboe et al, 1977). The thoroughbreds used here were able to bring their hematocrit from a mean resting value of 38.5% to 52.0% after walking for 4 min. Three additional minutes of trot caused an increase to 57.7% and values above 60% were reached after 3 min of subsequent cantering. Standardbred horses show similar increases to those measured here (Persson et al, 1973; Pan et al, 1984) and hematocrits as high as 66% have been reported for thoroughbred horses after racing (Snow et al, 1983). Hematocrit adjustments are likely to occur even more rapidly during maximal exercise than what was observed here for submaximal work.

A true steady state was not established for hematocrit. After reaching maximum values, a slow but steady decrease was measured at both work intensities. This slow drift could be due to fluid shift into the plasma compartment or to a re-sequestering of the red cells by the spleen. Preliminary measurements show that total plasma protein concentration and hematocrit seem to decrease in parallel. This observation suggests that a slow influx of water into plasma is probably responsible for the decline in hematocrit.

At the end of the trot protocol, heart rate started to increase while hematocrit decreased, showing that both variables could not be regulated by circulating catecholamines at that stage of the exercise bout.

Humans can also slightly increase their hematocrit in response to exercise (Hollman and Kastner, 1969) but no response was found in rats (Brooks and Donovan, 1983). The large hematocrit adjustment seen here in thoroughbred horses should allow them to augment $\dot{V}O_2max$ by 50% if the consequent change in blood viscosity does not lower their maximum cardiac output (see Crowell and Smith, 1967). Horses may maintain low hematocrits at rest to reduce the work of the heart, only allowing higher viscosity when high oxygen carrying capacity is needed for activity.

3.4.2 PLASMA LACTATE CONCENTRATION AT THE START OF EXERCISE. During the onset of work, the overall patterns of changes observed for plasma lactate concentration and heart rate were the same. The three variables examined peaked simultaneously after 2 to 3 minutes of submaximal exercise. The rapid activation of the cardiovascular system found here in thoroughbreds (strong hematocrit response and heart rate overshoot) did not prevent these animals from relying on anaerobic metabolism in the early stages of exercise. They showed a lactate overshoot equivalent to what had been reported in humans. At the onset of work, oxygen transport by

the blood was probably not the limiting factor for oxidative metabolism of muscle cells because the same pattern of changes in lactate concentration can be elicited by very different cardiovascular responses (i.e. human vs horse). For instance, oxygen supply could be limited at the cellular level during the transfer of O_2 from capillaries to muscle mitochondria.

At the start of submaximal exercise, oxidative ATP generation does not fully support total ATP utilization by the working muscles. The accumulation of lactate in plasma could be caused by the preferential recruitment of fast-twitch glycolytic fibers or to the slow perfusion of lactate-utilizing oxidative fibers. As exercise is sustained, the type of fibers recruited or the pattern of blood flow to the different fiber types may be altered, causing plasma lactate to reach a new steady state.

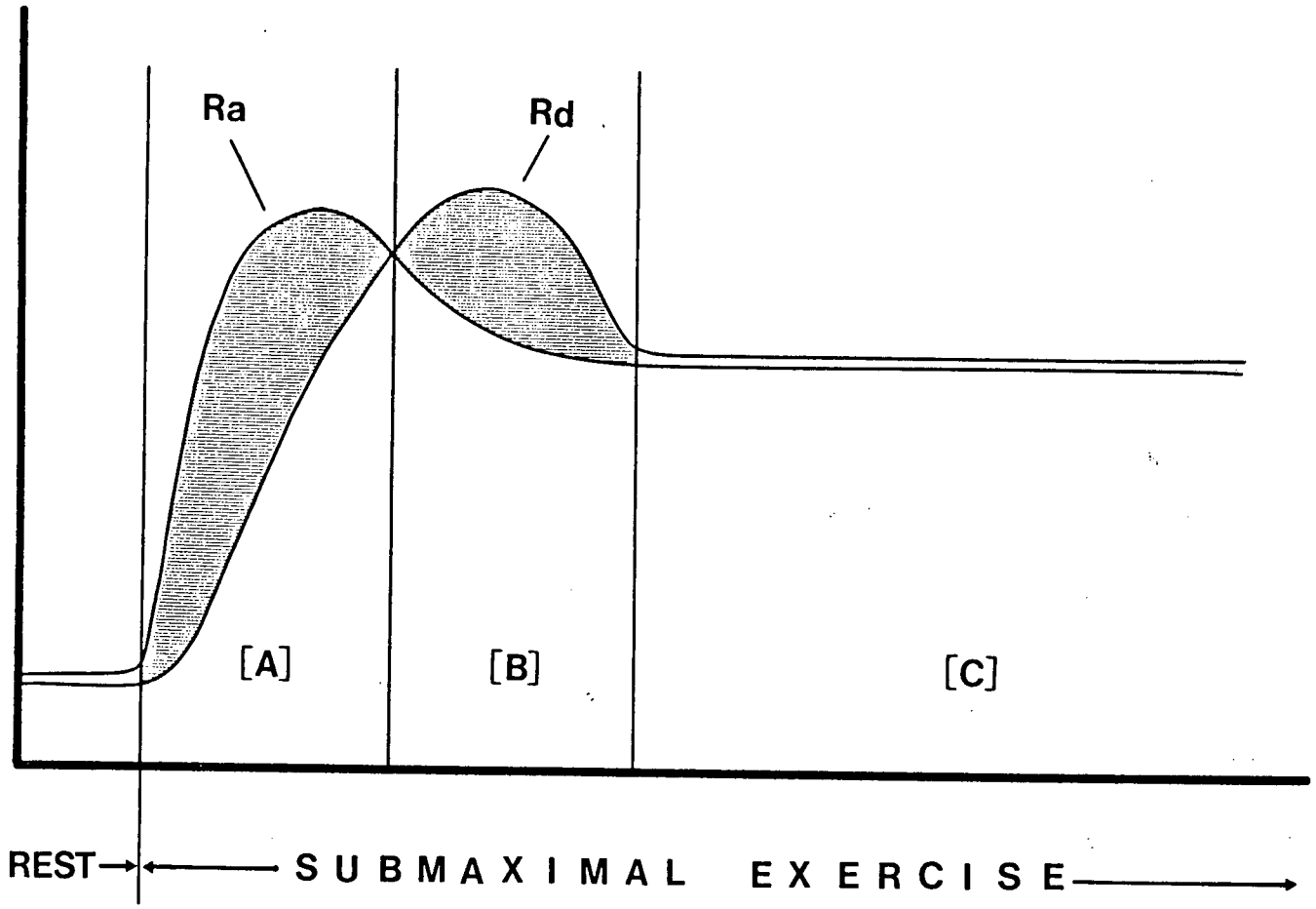
3.4.3 LACTATE FLUXES TO AND FROM THE PLASMA. Because animals continuously produce and utilize lactate at high rates (see Chapter 2; Brooks, 1985), the plasma concentration of this metabolite is determined both by its rate of appearance into the circulation (R_a) and by its rate of removal from the circulation (R_d). A number of instructive inferences about lactate fluxes can be drawn from the pattern of concentration changes reported in this study. A model is presented in Fig. 8 explaining the concentration changes elicited by constant intensity submaximal exercise in terms of flux rates. This model predicts the fluctuations of

R_a and R_d during the transition from rest to steady state exercise.

In a resting organism, lactate R_a and R_d are equal, and plasma concentration therefore stays constant (Hetenyii et al 1983). At the onset of exercise, this equilibrium is disturbed and lactate starts accumulating in the plasma compartment. For the first few minutes of work (phase [A]), R_a is greater than R_d because concentration increases (as seen in the first 2-3 min of exercise in Figs. 6 and 7). Between phase [A] and phase [B], the R_a and R_d curves cross-over and R_d is now greater than R_a throughout phase [B] during which concentration decreases (Fig.6 and 7). In the first half of phase [B], there is an overshoot in R_d causing the disappearance of part of the lactate accumulated in phase [A]. At the end of phase [B], a new steady state is obtained where R_a is again, like at rest, equivalent to R_d. The exercise flux rates are however much higher than at rest, as reported for all vertebrates studied to date (see Chapter 2).

The steady state lactate concentrations measured at rest and after the onset of exercise were slightly different (Figs. 6 and 7). The net amount of lactate added to the plasma compartment at the beginning of exercise (phases [A] and [B]) can be calculated by subtracting the surface area delimited by the R_a and R_d curves in phase [B] from the surface area delimited in phase [A] [this quantity will be in umoles if R_a and R_d are given in umol min^{-1} and if the time scale is in minutes].

FIG. 8 Model proposed for changes in the rates of lactate appearance (Ra) and removal (Rd) during the transition from rest to constant intensity submaximal work. Exercise is divided in three phases: (A) where Ra > Rd; (B) where Rd > Ra; and (C) where Ra = Rd. The surface area indicated in (A) represents the net amount of lactate added to the rapidly mixing pool during phase (A). Surface area in (B) is equal to the net amount of lactate removed from the same pool during phase (B). The difference between these 2 surface areas is directly proportional to the difference in plasma lactate concentrations between rest and steady state exercise [phase (C)].


Ra
Rd
[A]
[B]
[C]
REST
SUBMAXIMAL EXERCISE
TIME

During the last 15 to 20 min of the trot protocol, plasma lactate concentration started to increase, indicating that a slight imbalance between R_a and R_d was again present. This could have been caused by fatigue of the oxidative fibers which are now replaced by fast-twitch glycolytic fibers.

3.4.4 MUSCLE METABOLITES. The most interesting aspect of the metabolic analysis in the middle gluteal muscle was the 30% reduction in glycogen caused by the 15 min canter (Table 6). As a fuel, glycogen has two potential fates in working skeletal muscle. It can either be converted anaerobically to lactate with the net production of 3 ATP per glucosyl unit, or it can be completely oxidized in the mitochondria (Hochachka et al, 1983). The second pathway has the advantage of yielding 12.3 times more ATP than the first for the same amount of glycogen utilized: an important fact to consider during sustained exercise. The present results could be interpreted in 3 ways: i) anaerobic glycogenolysis with rapid release of lactate into the circulation for its subsequent metabolism elsewhere; ii) anaerobic glycogenolysis in a small number of fast-twitch glycolytic fibers and transfer of lactate to adjacent high oxidative fibers for complete oxidation; or iii) aerobic glycogenolysis exclusively. If the first mechanism was important, higher muscle lactate levels would have been observed after exercise. Aerobic glycogenolysis was probably the main pathway for energy metabolism in the middle gluteus,

particularly in view of its high proportion of oxidative fibers. In thoroughbred horses, it is composed of 57% fast-twitch high oxidative fibers and 11% slow oxidative fibers (Snow, 1983). The second mechanism differs from the third only in the fact that it requires the cooperative metabolism of two fiber types. Lactate exchange between glycolytic and oxidative fibers has been proposed long ago in fish (Wittenberger and Diaciuc, 1965) and it has recently received more support in exercising mammals (Brooks, 1985). The net effect of the second and third mechanisms is the aerobic breakdown of glycogen and both mechanisms could have been operating in the middle gluteus. Assuming that aerobic glycogenolysis was the only pathway utilized during the 15 min canter, this muscle was turning over ATP at a rate of 85 umol/g per min, a value somewhat higher than what has been observed in other species operating at their maximum sustained level.

No changes in the concentrations of a selected number of glycolytic intermediates were apparent after the canter relative to the pre-exercise state (Table 6). Large changes were measured in rat muscle after submaximal exercise using the same time course for freezing (Dobson, 1986). Here, the lactate/pyruvate ratio was the same before and after exercise, indicating that the cytosolic redox potential was maintained after 15 min of work. During the canter protocol, a steady state was established for plasma lactate and exercise was interrupted while plasma lactate concentration was constant. This observation, together with the

measurements of glycolytic intermediates, confirms that a true metabolic steady state was reached and maintained until the end of the exercise bout.

CHAPTER 4:

CARDIAC OUTPUT AND OXYGEN CONSUMPTION OF EXERCISING THOROUGHBRED HORSES.

4.1 INTRODUCTION

The cardiovascular adjustments elicited by exercise are usually simply interpreted as the organism's way to increase oxygen supply to the active musculature and to facilitate carbon dioxide release into the lungs. The potentially important role played by these adjustments in augmenting the provision of oxidative fuels to contracting muscles, and in accelerating the transport of other metabolic end products than CO_2 and heat has not been investigated quantitatively at the whole-organism level. In particular, increasing cardiac output should have an effect on the turnover rate of plasma metabolites as suggested in Chapter 2.

Lactate turnover has been measured in several species, and the flux rates of this metabolite in and out of the plasma compartment have been shown to vary considerably as the activity state of the animal changes (see Chapter 2). Exercise causes an increase in both the production of lactate and its utilization as an oxidative fuel (Brooks, 1985). Because the thoroughbred racehorse has been carefully selected for its aerobic and anaerobic capacity over three

centuries, it could provide a good model for the investigation of plasma lactate fluxes during exercise.

In addition, horses have a very high aerobic scope with the impressive ability to increase their oxygen consumption by 36-fold between rest and high-intensity exercise (Thomas and Fregin, 1981; Manohar, 1986). Functional adaptations of their cardiovascular system allow them to alter metabolic rate over such a wide range, but the effect of work rate on cardiac output has only been measured in standardbred horses (Thomas and Fregin, 1981; Thomas et al, 1983) and in ponies (Pan et al, 1984; Forster et al, 1984; Manohar, 1986).

The overall goal of this study (Chapters 4 and 5) was therefore to investigate the effect of changes in cardiac output on the rate of plasma lactate turnover in the thoroughbred racehorse. The present chapter characterizes the effects of exercise on cardiac output and on the rate of oxygen delivery. The following chapter deals with the measurement of plasma lactate turnover in the same animals under identical exercise conditions.

To complete the present investigation, the measurement of maximal cardiac output and $\dot{V}O_2max$ was attempted during high-intensity exercise. Data for maximal cardiac output were not obtained for technical reasons, but a lowest possible value for $\dot{V}O_2max$ and for the aerobic scope of thoroughbred horses could be estimated from heart rates and A-V differences in oxygen content.

4.2 MATERIALS AND METHODS

4.2.1 ANIMALS. The thoroughbred horses used in the previous study (JW, KJ, and SB) were also used in the present experiments. Their body weights, ages, diet, and state of training are given in Chapter 3.

4.2.2 CATHETERIZATIONS. (A) RIGHT ATRIUM. The skin was shaved on a small area above the left jugular vein and disinfected. Before catheterization, local anaesthesia and stab incision were performed as in Chapter 3. The vein was then punctured with a Monoject 200 needle (16 g X 1.5 in.). A metal spring guide-wire was introduced and a catheter introducer (USCI, Hemaquet AV percutaneous catheter introducer) was inserted in the vessel. After removing the guide-wire, the catheter (USCI, woven Dacron, closed end, NIH cardiovascular catheter, size 8F X 100 cm) was passed through the introducer into the right ventricle and connected to a pressure transducer for verification of its location. It was then slowly withdrawn into the right atrium and sutured to the neck of the animal to secure its position (the pressure wave observed on the transducer would clearly indicate whether the catheter tip was placed in the pulmonary artery, right ventricle, right atrium, or vena cava).

(B) PULMONARY ARTERY. A second catheter introducer was placed in the left jugular vein and a cardiac output catheter (Oximetrix, opticath model P7110-E size 7.5 F, flow directed thermal dilution pulmonary artery catheter

series D) was placed several inches into the pulmonary artery and sutured in position using the technique described previously.

(C) CAROTID ARTERY. Surgical transposition of the right common carotid artery to a subcutaneous location had been performed on all horses several months before the experiments. Prior to catheterization, the skin was prepared as described above. A stab incision was made before introducing a guide-wire, sliding the catheter in position (Vygon XRO intravascular catheter 18 g X 46 cm) and suturing it to the neck. The sampling port was located about 10 cm cranial to the aortic arch.

The three catheters were connected to extension lines (Lectroflex Vygon PVC) which were securely taped to the neck and filled with saline. No heparin was used but lines and catheters were flushed every 10-15 min to keep them patent.

4.2.3 EXERCISE PROTOCOLS. All experiments were carried out at least 3 h after the morning feed. Temperature ranged from 18 to 20^{o}C and relative humidity from 61 to 71%. The resting measurements were performed while the animal was standing in a stall beside the treadmill at least 30 min after catheterization. The horse was then fitted with a harness and brought on the treadmill (see Chapter 3). Four exercise protocols were used: (i) a 1.6 m/s walk at 6% incline, (ii) a 3-4 m/s trot at 6% incline sustainable for 40 min, (iii) a 6.5 m/s horizontal canter sustainable for 10 to

15 min, and (iv) a 12.5 m/s gallop at 5% incline sustainable for 2 min. Trot, canter and gallop protocols were initiated with a warm-up period consisting of a 4 min walk at 1.6 m/s for the trot, a 4 min walk (1.6 m/s) followed by a 4 min trot (4 m/s) for the canter, and a 4 min walk (1.6 m/s) followed by a 2 min trot (4 m/s) for the gallop. The increase from 4 m/s to the galloping speed of 12.5 m/s was achieved in 45 s.

During the trot protocol, KJ and SB were running at 4 m/s, but a lower speed of 3 m/s was chosen for the older horse (JW) because he could not sustain 4 m/s over 40 min without experiencing articular pain caused by pounding on the treadmill belt. When this occurred, the animal would stop having a regular gait and he would attempt to break into a very slow canter on repeated occasions (presumably to change the pattern of stress forces applied to the articulations). This alteration in the locomotory pattern was never observed if the animal was maintained at 3 m/s over 40 min. The chosen submaximal exercise regimes were designed to elicit comfortable, steady state performances sustainable for long enough to allow the measurement of lactate turnover. The investigation of plasma lactate fluxes in the same animals is presented in Chapter 5. In the present study, no significant differences could be found between values measured in JW and in the other horses. Therefore, data for all animals were pooled.

4.2.4 CARDIAC OUTPUT AND BLOOD OXYGEN CONTENT.

Cardiac output was measured by the thermodilution technique

using an American Edwards Laboratories Cardiac Output Computer (9520A) and Oximetrix Shaw Catheter Oximeter System (OS-1270A). The computer and the catheter used in this study were calibrated with a pump and thermostated bath at 37°C. The measured flow rates using the thermodilution technique were accurate within 8% of the true values. In human studies, the thermodilution technique gives the most accurate measurements of cardiac output when compared with dye dilution or non-invasive techniques such as thoracic impedance measurements (D. Wheeldon pers. comm.).

In the present study, a bolus of cold dextrose (5%) was injected into the right atrium and blood temperature was recorded from a thermistor located at the tip of the pulmonary artery catheter. Before injection, the temperature of the dextrose solution was monitored with a reference thermistor connected to the computer. Volumes and temperatures of the dextrose boluses ranged from 24 ml to 28 ml and from 4.2°C to 13.0°C respectively. Bolus injection was performed at least 3 times for each animal and for each activity level.

Arterial and mixed venous blood samples were drawn simultaneously from the right carotid and from the pulmonary artery to determine A-V differences in oxygen content. Samples were taken before (A) and after each measurement of cardiac output (B). Oxygen content corresponding to a given cardiac output was calculated as the mean between samples (A) and (B). Blood was collected in 5 ml heparinized syringes which were tightly sealed and stored on ice. Oxygen content

was determined at 37°C on a Micro Autocal pH and Blood Gas Analyzer (Instrument Laboratories # 613) and a CO-Oximeter (Instrument Laboratories # 282) used in combination. Measurements were performed no later than 3 h after sampling. Hemoglobin concentration, PO_2, PCO_2, and pH were measured directly. Hemoglobin % saturation was automatically back calculated from a human hemoglobin O_2 saturation curve adjusted for pH and PCO_2. Human and horse hemoglobins have very similar P_{50} and display similar Bohr shifts (Prosser, 1975); the error introduced by using a human dissociation curve should therefore be minimal. In addition, this error would be eliminated or at least decreased for relative values such as A-V differences or changes between rest and exercise, which were more important to this study than absolute values. Oxygen content was calculated as [1.34 X hemoglobin concentration (in g/100 ml) X hemoglobin % saturation] + [0.003 X pO_2 (in mm Hg)]. Hematocrit was determined by spinning samples in hematocrit tubes for 10 min on a microcentrifuge. Oxygen consumption was calculated from cardiac output and A-V difference in O_2 content.

Measurements were performed at rest (R), 5 min after starting the walk protocol (W), 1 min (ST) and 7 min (ET) after the animal had started the trot protocol, 7 min after starting the canter protocol (C), and 1 min into the gallop (G). The determination of cardiac output at the highest speed was not successful because the neck movements of galloping animals caused too much mechanical interference with our temperature measurements and the exact position of the catheters could not be maintained with certainty.

Therefore, only A-V difference in oxygen content, hematocrit and heart rate could be measured in the gallop.

4.2.5 CALCULATIONS AND STATISTICS. Cardiac output (CO) was calculated as follows:

$$CO = \frac{(T_b - T_i) * V}{\int_0^{\infty} T \, dt}$$

Where T_b=blood temperature, T_i=temperature of the bolus injected, and V=volume of the bolus. The surface area under the temperature vs time curve (integral T dt from time Ø to infinity) was determined with a digitizer (GTCO Corp. digitizing pad) and an IBM computer.

Means were compared with a oneway analysis of variance. The Student-Newman-Keuls test (T-method for unplanned comparisons, see Sokal and Rohlf, 1981) was used as a post hoc test when the overall F value indicated that further analysis was necessary.

4.3 RESULTS

4.3.1 CARDIAC PARAMETERS. Mean values for cardiac output, heart rate and stroke volume are presented in Fig. 9. The cardiac outputs measured at the beginning of the trot protocol (ST) and later in the same protocol (ET) were identical, therefore only one value (T) is given in Fig. 9.

Exercise had a significant effect on cardiac output (3 df/8 df, F=24.6, P<Ø.ØØ1), heart rate (F=33.Ø, P<Ø.ØØ1), and stroke volume (F=4.2, P<Ø.Ø5). Cardiac output went from a mean resting value of 66.5 l/min to 212.3 l/min during the canter protocol, which corresponds to a change from 15Ø to 475 ml min^{-1} kg^{-1}. It was significantly lower at rest compared with values measured during exercise (P<Ø.Ø1) but mean values for the walk, trot, and canter protocols were not significantly different from each other.

Heart rate went from a mean resting value of 49 beats/min to 144 beats/min in the canter protocol (Fig. 9). During the gallop protocol, heart rate reached 197±1Ø beats/min (mean ± SEM) and values of 22Ø to 23Ø beats/min have been measured in the same horses during treadmill exercise (Joyce Harman, unpublished data), clearly indicating that the canter protocol represented easy exercise for these animals. Mean heart rate was lower at rest than during any exercise protocol (P<Ø.Ø1), and it was significantly lower during the walk than during the trot and canter protocols (P<Ø.Ø5). No difference was found between trot and canter. Heart rate was higher for the gallop than for any other activity level (P<Ø.Ø1).

Stroke volume was 1.34 l at rest, and it increased to a maximum of 1.58 l during the walk protocol. Even though the overall ANOVA indicated a significant effect of exercise on stroke volume, no particular mean was found to be different from any other mean using Student-Newman-Keuls as a post hoc test.

FIG. 9 Cardiac output (CO), heart rate (HR) and stroke volume (SV) in trained thoroughbred horses at rest and during exercise on a treadmill. R = rest; W = walk (1.6 m/s, 6% incline); T = trot (3-4 m/s, 6% incline); C = canter (6.5 m/s, 0% incline). During the canter protocol heart rate reached 197 ± 10 beats/min (not shown on Fig. 9). See methods for details of the exercise protocols. Values given are means + SEM (N=3).

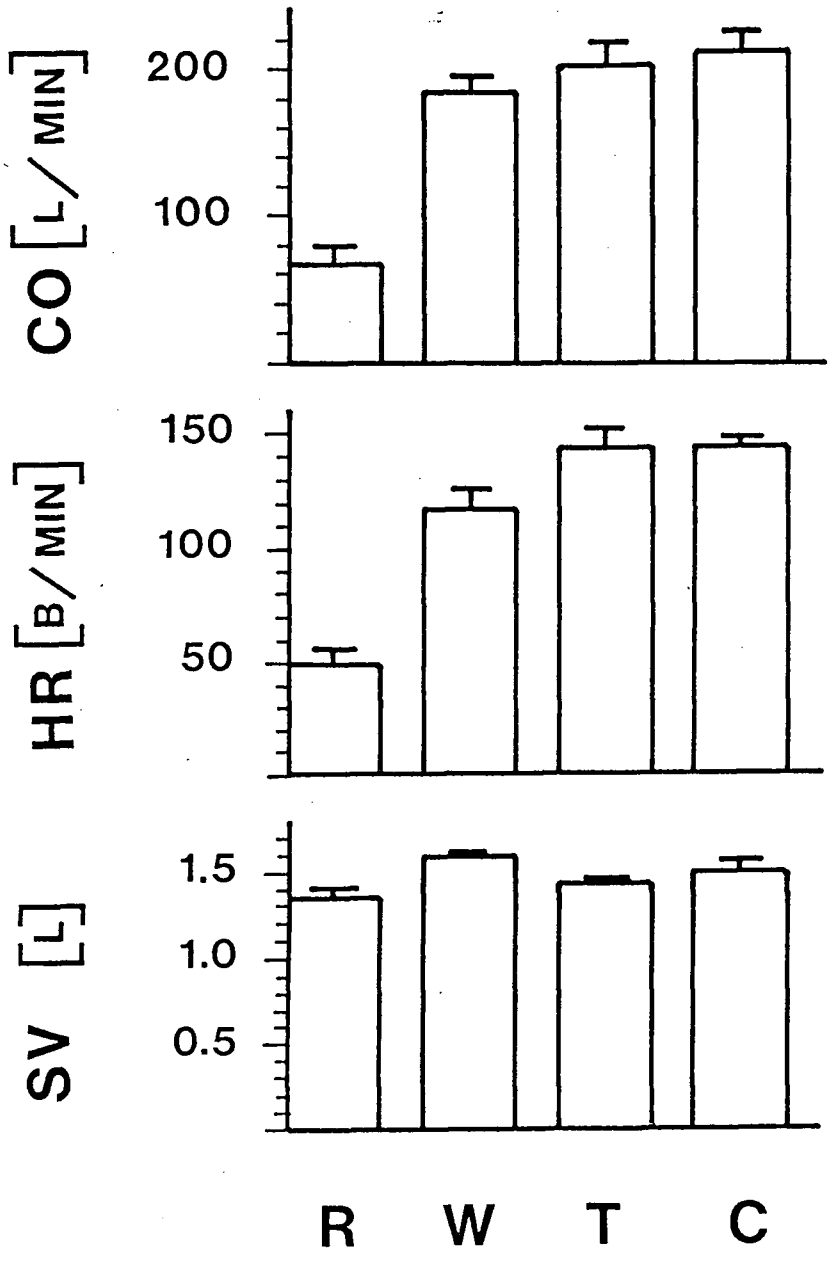
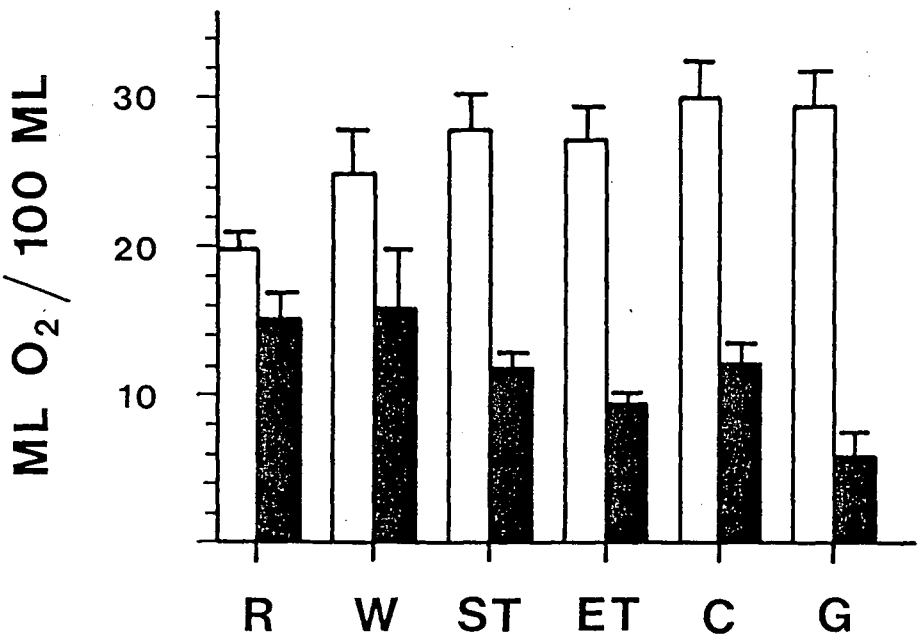

CO [L/MIN]
200
100
HR [B/MIN]
150
100
50
SV [L]
1.5
1.0
0.5
R
W
T
C

FIG. 10 Blood oxygen content in trained thoroughbred horses at rest and during treadmill exercise. Open bars: arterial blood; black bars: mixed venous blood. R = rest; W = walk; ST = start of trot protocol; ET = end of trot protocol; C = canter; G = gallop (12.5 m/s, 5% incline). Values given are means + SEM (N=3).


ML O_2 / 100 ML
30
20
10
R
W
ST
ET
C
G

4.3.2 A-V DIFFERENCES IN OXYGEN CONTENT. The oxygen contents of arterial and mixed venous blood are given in Fig. 10 for the different levels of activity. Mean arterial oxygen content went from a minimum of 19.8 vol% at rest to a maximum of 30.0 vol% during the canter protocol. At 15.8 vol%, venous oxygen content was highest during the walk protocol, and it decreased to a low value of 5.9 vol% in galloping thoroughbreds. The mean A-V difference in oxygen content increased drastically from 4.7 vol% at rest to 23.4 vol% during the gallop protocol. The statistical analysis was performed on the mean A-V differences. The overall effect of exercise was highly significant (5 df/12 df, $F=27.52$, $P<0.001$). Post hoc tests show that: (i) the mean A-V difference was higher during the walk protocol than at rest ($P<0.05$), (ii) it was higher at the beginning of the trot protocol (ST) than during the walk ($P<0.01$), and (iii) it was higher during the gallop than during the canter protocol ($P<0.01$). No other differences between adjacent means were significant at $P<0.05$.

Arterial hematocrit was often higher than venous hematocrit, but the difference was not significant. Only arterial levels are reported here. Mean values at the different work intensities are given in Fig. 11. Hematocrit greatly increased from a low resting value of 38.1% to 56.2% during the exercise protocol of lowest intensity (walk). A maximum level of 63.4% was reached in the horses galloping at 12.5 m/s. The overall effect of exercise on hematocrit was highly significant (5 df/12 df, $F=7.45$, $P<0.01$). The mean

value was lower at rest than at any work intensity ($P<0.01$), but no significant differences could be found between the various levels of exercise.

4.3.3 OXYGEN CONSUMPTION. Mass specific rates of oxygen consumption are given in Fig. 12 (top graph). The mean resting rate was 7.2 ml min^{-1} kg^{-1}, and it increased to 85.1 ml min^{-1} kg^{-1} during the canter protocol. Whole-body oxygen consumption rates are presented in the bottom graph of Fig. 12, and the statistical analysis was performed on these values. Whole-body rate of oxygen consumption went from a resting level of 3.2 l/min to 38.1 l/min in the canter protocol. The overall effect of exercise was highly significant (4 df/10 df, $F=12.83$, $P<0.001$). Post hoc tests show that oxygen consumption was significantly higher during the walk protocol than at rest ($P<0.05$), and that it was also higher at the beginning of the trot protocol (ST) compared with the walk ($P<0.05$). Mean consumption rates during the trot (ST and ET) and canter (C) were not different.

FIG. 11 Hematocrit (HCT) of trained thoroughbred horses at rest and during treadmill exercise. Same legend as in Fig. 10. Values given are means + SEM (N=3).

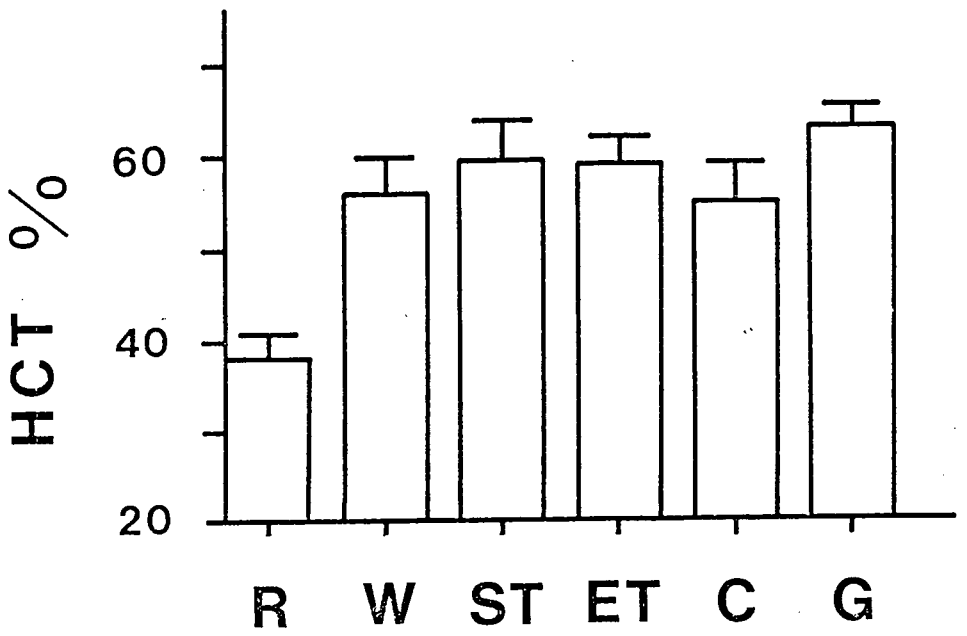
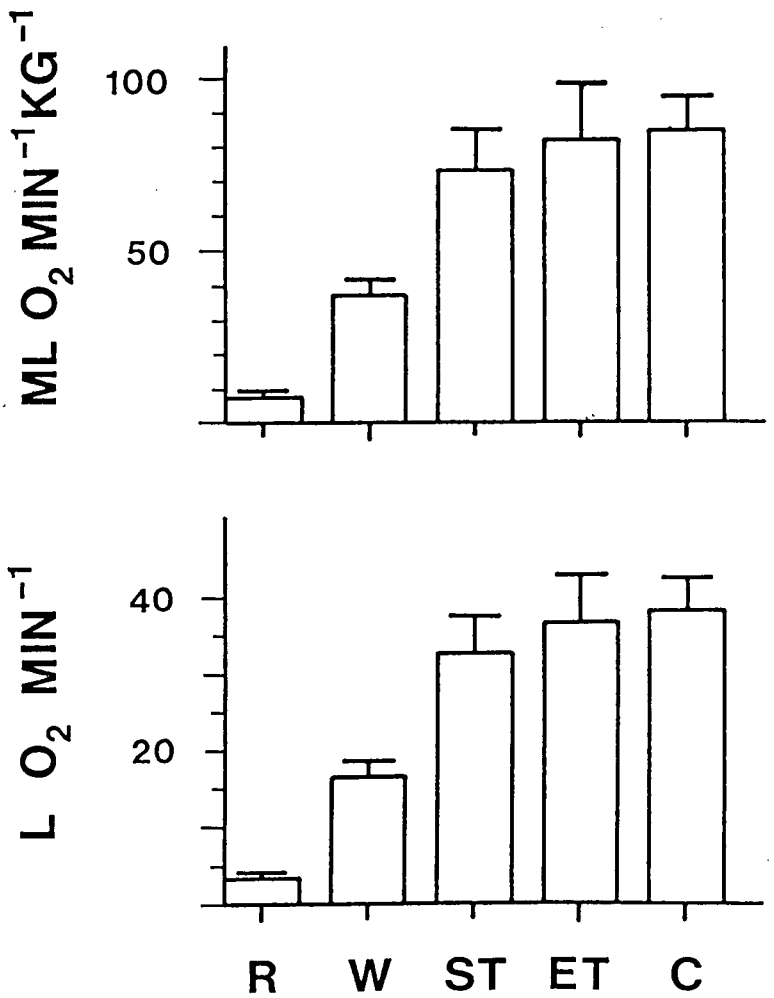

HCT %
60
40
20
R
W
ST
ET
C
G

FIG. 12 Oxygen consumption of trained thoroughbred horses at rest and during treadmill exercise. Top graph: mass specific oxygen consumption. Bottom graph: whole-body oxygen consumption. Same legend as in Fig. 10. Values given are means + SEM (N=3).


ML O_2 $MIN^{-1}KG^{-1}$
100
50
L O_2 MIN^{-1}
40
20
R
W
ST
ET
C

4.4 DISCUSSION

The cardiac output of thoroughbred racehorses was measured at rest and at different levels of submaximal exercise to complement our investigation of plasma lactate fluxes presented in the following chapter. This study shows that thoroughbred horses have the impressive ability to increase their A-V difference in oxygen content by at least 5-fold between rest and maximal exercise. They have a higher maximal stroke volume than standardbred horses, and their maximal A-V difference in O_2 content is much higher than in ponies (Manohar, 1986). Thoroughbreds can bring their arterial content up to 30 vol% via a more than 50% increase in circulating hemoglobin concentration. This response of their oxygen transport system represents one of the most striking adaptations shown by horses for aerobic performance. Thoroughbred horses may be able to augment their oxygen consumption by more than 40-fold between rest and maximal exercise: the highest aerobic scope in mammals.

4.4.1 CARDIAC OUTPUT. Thoroughbreds showed a 3-fold increase in cardiac output between rest and the canter protocol. The observed change was directly caused by an equivalent increment in heart rate because stroke volume did not vary significantly between the two activity states (Fig. 9). The stroke volumes measured here were higher than previously reported in standardbred horses for which maximum values only reach 1.27 l in untrained animals (Thomas and

Fregin, 1981) and 1.36 l after a short period of training (Thomas et al, 1983). Stroke volumes of thoroughbreds ranged from 1.34 l at rest to 1.58 l during the walk protocol. In humans, maximum stroke volume can increase by up to 20% as a result of intensive endurance training, but genetic differences in heart size seem to play an important role in determining the maximal possible cardiac output of a given individual (Brooks and Fahey, 1984). The higher maximal stroke volume found in thoroughbreds compared with the standardbreds of Thomas et al (1983) can therefore be attributed to a combination of training and genetic differences.

The values measured here at rest do not represent true resting levels because mean heart rate (49 beats/min) was 10 beats/min higher than minimal rates measured in the same animals under different experimental conditions (see Chapter 3). The true resting heart rate of these horses may even be under 30 beats/min, but such highly excitable animals could not be kept perfectly quiet in the presence of several people manipulating the different catheters. There is no doubt that the cardiac output of thoroughbred horses can vary over a much wider range of values than were reported in this study. Unfortunately, it could not be measured in galloping animals, and maximal values were far from being attained during the canter protocol which represents less than half the top speed these horses can achieve on a treadmill. At this submaximal work intensity, mean heart rate only reached 144 beats/min. The same animals were able to increase this

rate to 197 beats/min during the gallop protocol, and they have been observed to gallop at maximum heart rates of 220-230 beats/min (Joyce Harman, unpublished data).

Thomas et al (1983) reported a 6.6-fold increase in cardiac output for standardbred horses between rest and exercise, but the highest work intensity used in their study did not elicit $\dot{V}O_2max$, and the scope of these animals is probably higher. Endurance-trained human athletes can support an eight-fold increase in cardiac output between rest and $\dot{V}O_2max$ (Brooks and Fahey, 1984). Recently, Manohar demonstrated that untrained ponies have the ability to augment their cardiac output by more than eight fold between rest and maximal exercise (Manohar, 1986). It is possible to estimate maximal cardiac output of the thoroughbred horses used in the present study if we make the conservative assumption that, when galloping at $\dot{V}O_2max$, their stroke volume is equal to the lowest value reported here (i.e. 1.34 l in resting animals). With this stroke volume and a heart rate of 225 beats/min, their cardiac output would reach 300 l/min. In view of the above calculation, and when considering the scope found in ponies (Manohar, 1986) and trained humans (Brooks and Fahey, 1984), it is very likely that thoroughbred horses can also undergo an 8-fold increase in cardiac output between rest and $\dot{V}O_2max$.

4.4.2 A-V DIFFERENCE IN OXYGEN CONTENT. Results show that thoroughbred horses have the capability to increase their A-V difference in oxygen content by at least 5-fold

between rest and maximal exercise. Ponies closely approach this scope with a 4.5-fold increase (Manohar, 1986), but endurance-trained human athletes can only alter their A-V difference by 3-fold (Brooks and Fahey, 1984; Astrand and Rodahl, 1977). The scope of thoroughbred horses is probably even greater than 5-fold because true resting A-V differences should be lower than measured here for reasons stated previously [also, lower resting values of 3.7% were found in ponies (Manohar, 1986) compared with 4.7 vol% in this study]. In addition, maximum A-V differences could be higher than measured in the gallop protocol if heavy exercise was sustained for a longer period of time or if the animals were operating at their maximal heart rate of 220-230 beats/min.

This study shows that thoroughbred horses can bring their A-V difference in oxygen content to an absolute value exceeding 23 vol% (see Fig. 10). In comparison, top human athletes and ponies are only able to reach 17 vol% when running at $\dot{V}O_2max$ (Brooks and Fahey, 1984; Manohar, 1986). Thoroughbreds have the capability to boost their A-V difference to such a high level because their arterial oxygen content can attain much higher values than humans and ponies (Brooks and Fahey, 1984; Manohar, 1986). The thoroughbreds of this study were able to bring arterial content to 30.0 vol% in the canter protocol, but ponies can only reach maximal values of 22.4 vol% (Manohar, 1986). No direct measurement of arterial oxygen content is available for standardbred horses, but Lykkeboe *et al* (1977) saturated venous blood of exercising animals with oxygen and showed that it had an O_2

capacity of 30 vol%. Standardbred horses may therefore show the same range of A-V differences in oxygen content as reported here for thoroughbred horses.

It is clear that the large increase in arterial oxygen content elicited by exercise was a direct consequence of a concomitant change in hematocrit. Indeed, as hematocrit went from about 40% to 60% (Fig. 11) arterial oxygen content increased from 20 to 30 vol% (Fig. 10). During the rest to work transition, about one third of the total number of erythrocytes was released from the spleen (see Persson, 1983), causing a more than 50% increase in circulating hemoglobin concentration. Hematocrit changes throughout the trot and canter protocols in the same horses have been presented in Chapter 3.

4.4.3 METABOLIC RATE. Endurance-trained humans can show a $\dot{V}O_2$ increase of more than 12-fold in response to exercise (Brooks and Fahey, 1984). Thoroughbred horses already showed a 12-fold increase in $\dot{V}O_2$ between rest and the canter protocol, which only represents low intensity exercise for such animals. This change in the rate of oxygen supply to the working musculature was achieved via a 3.2-fold increase in cardiac output accompanied by a 3.8-fold increase in A-V O_2 difference. Oxygen consumption went up slightly between the beginning of the trot protocol (ST) and later in the same protocol (ET) because the A-V difference in O_2 content increased somewhat (see Figs. 10 and 12). No change in cardiac output could be measured between the two stages of

the trot protocol (ST and ET).

Maximal oxygen consumption of thoroughbreds would reach approximately 156 ml min^{-1} kg^{-1} (or 70 l/min) with an A-V difference of 23.4 vol% and a maximal cardiac output of 300 l/min. $\dot{V}O_2max$ of trained humans (with a body mass of 70 kg) can exceed 60 ml min^{-1} kg^{-1} in top athletes (more than 4l/min whole body consumption for 70 kg body mass; see Rowell, 1974; Brooks and Fahey, 1984). With a 6-fold difference in body mass horses would be expected to have a much lower mass specific metabolic rate than humans (see Schmidt-Nielsen 1979). Clearly, thoroughbred horses support an outstanding aerobic capacity with a mass specific $\dot{V}O_2max$ two to three times higher than for the best human athletes.

With a maximal O_2 consumption of 70 l/min, the trot protocol would represent 45 to 50% $\dot{V}O_2max$ and the canter would be 55% VO_2max. Only a small difference in $\dot{V}O_2$ was measured between the trot and the canter protocols. This difference was low because the animals switched gait, recruiting different muscle groups. They have been shown to change gait at specific velocities allowing them to minimize energy expenditure at any given speed (see Hoyt and Taylor, 1981).

With an 8-fold increase in cardiac output and a 5-fold increase of their A-V difference in oxygen content, thoroughbred horses can augment their oxygen consumption by 40-fold between rest and maximal exercise. This would represent the highest aerobic scope found in mammals, including standardbred horses and ponies. An equivalent scope

in the delivery rate of oxidizable fuels to the working muscles may be supported by thoroughbred horses. The problem of how changes in cardiac output influence plasma metabolite turnover rate during exercise is addressed in the last chapter of this thesis.

CHAPTER 5:

LACTATE TURNOVER IN EXERCISING THOROUGHBRED HORSES: EFFECT OF CHANGES IN CARDIAC OUTPUT.

5.1 INTRODUCTION

Sustained muscle work requires the adequate supply of exogenous fuels to minimize the use of endogenous substrates and postpone their depletion. Organisms must be able to regulate the flux rates of their plasma metabolites if the right mixture of oxidizable fuels is to be provided via the blood at the appropriate time and speed. To meet the energetic demands of exercise, substrate fluxes can be augmented via two mechanisms: 1) by increasing their concentration in plasma, and 2) by increasing blood flow to the working muscles. At the whole-organism level, the first mechanism is used (as seen in Chapter 2 in the case of lactate), but the second one has never been investigated. Indeed, the turnover rate of several substrates is positively correlated with their plasma concentration (Chapter 2; Verdonk et al, 1981; Bortz et al, 1972; Hagenfeldt, 1975), however, it has never been shown whether changes in cardiac output have an effect on the turnover rate of plasma metabolites when the entire organism is taken into account. If these changes have an effect, the relationship between plasma lactate concentration and lactate turnover rate would

also depend on cardiac ouput. This effect has never been shown.

Lactate fluxes provide an ideal model to study the control of metabolite turnover because they vary over a much wider range of values than the fluxes of other substrates (see Chapter 2), and they can be manipulated easily through exercise. The supply of lactate to working muscles is not diffusion limited because lactate transport across cell membranes is rapid as indicated by the high K_m values found for all the lactate carrier of all cells studied to date (Dubinsky and Racker, 1978; Koch et al, 1981; Roos, 1975; Spencer and Lehninger, 1976). Therefore, lactate supply should be perfusion limited in all situations where cellular lactate oxidation can keep up with lactate supply (as it is the case during submaximal exercise), and changes in cardiac output should have an effect on the rate of lactate turnover in plasma (note that, under steady-state conditions, turnover rate is equal to the rate of disappearance and to the rate of appearance from and into the rapidly mixing pool of lactate; see Wolfe, 1984).

Plasma lactate can be an important oxidizable substrate in some animals (Donovan and Brooks, 1983; Mazzeo et al, 1986), and its role during sustained exercise has been emphasized by Brooks (1985). It is not clear whether exercising organisms gain some performance advantage by transporting lactate at high rates through their plasma compartment. If they do, species genetically geared for high performance, like the thoroughbred horse, would be expected

to have the ability to sustain high lactate turnover rates, and training should enhance this ability. In exercising rats, albeit a species by no means noted for its athletic prowess, training has no effect on turnover rate (Donovan and Brooks, 1983). Lactate turnover has never been measured in horses.

The goal of this study was therefore to measure plasma lactate flux rates in resting and exercising thoroughbred horses: 1) to determine the effect of changes in cardiac output on lactate turnover rate using blood flow data from the previous study (Chapter 4), and 2) to assess the importance of lactate as a metabolic fuel in a trained animal athlete and to compare its flux rates with values measured in other, more sedentary species. In addition, I attempted to investigate whether lactate turnover rate decreases throughout a bout of submaximal exercise, as would be predicted if the contribution of fat oxidation to total energy metabolism increased.

5.2 MATERIALS AND METHODS

5.2.1 ANIMALS AND CATHETERIZATIONS. Measurements were performed on the four thoroughbred horses used in the previous studies (Chapter 3 and Chapter 4). Their weights and ages are given in Table 4 (see Chapter 3). During the present experiments, the horses were kept on the same diet and their regular training was identical to what has been described previously. The four animals had undergone a surgical

transposition of the right common carotid artery to a subcutaneous position several months before the experiments to facilitate the surgical procedures performed for the measurement of lactate turnover rate. The transposition of this vessel allowed a relatively easy access to the arterial system whenever arterial blood had to be collected.

Before a turnover measurement, a Vygon XRO intravascular catheter (18 g X 46 cm) was introduced in the right carotid and a cardiac catheter (USCI, size 8F X 125 cm) was placed in the right atrium using a Kontron Medical pressure transducer (Model 108). Both catheters were connected to extension lines (100 cm, 2-ml capacity) which were securely taped to the neck and filled with saline. Extension lines and catheters were flushed every 10-15 min to keep them patent. Detailed catheterization procedures are described in Chapter 4.

5.2.2 EXERCISE PROTOCOLS. Experiments were carried out at least 3 h after the morning feed. Temperature ranged from 17 to 20^{0}C and relative humidity from 53 to 71%. No measurement of lactate turnover rate was started earlier than 30 min after catheterization. The animals were fitted with a harness and a heart rate monitor. Measurement of resting turnover rate was performed while the animal was quietly standing in a stall. For the determination of lactate fluxes during work, the horse was brought on the treadmill and the harness was tied to an emergency switch-off mechanism above him. A fan simulating air cooling during normal

locomotion was placed in front of the animal.

Two exercise protocols were selected: 1) a 4 m/s trot at 6% incline sustainable for 40 min, and 2) a 6.5 m/s horizontal canter for 15 min. They were identical to the trot and canter protocols of the previous study. In two cases, these protocols had to be adjusted for individual horses. A lower trotting speed of 3 m/s had to be used for the older horse (JW) because he could not sustain 4 m/s over 40 min (see previous study for details), and a higher cantering speed of 8.3 m/s was chosen for IN because he was able to trot at 6.5 m/s and he had problems switching to a canter at the latter velocity. In these 2 cases, the values measured for JW and IN were not significantly different from the measurements performed on the other horses at the respective gaits. Therefore, data for all animals were pooled in the calculations. All exercise bouts were initiated with a short warm-up period consisting of a 4 min walk (1.6 m/s) before the trot protocol and a 4 min walk followed by a 4 min trot (4 m/s) before the canter protocol. Heart rate was recorded every 30 s during the first 10 min of each experiment and at longer intervals thereafter. The basic metabolic and cardiovascular responses of the same horses subjected to identical exercise protocols have been reported in Chapter 3.

5.2.3 MEASUREMENT OF LACTATE TURNOVER. The bolus injection technique was used to determine the rate of plasma lactate turnover (see Chapter 1, and Wolfe, 1984). Bolus injection was selected over continuous infusion because the

infusion technique requires much larger amounts of isotope and it would have made the experiments prohibitively expensive in such a large animal. Even when using bolus injection (as was done here), the high cost of a single measurement placed some important constraints on the number of turnover rates that could be measured in each horse. In addition, because continuous infusion was too expensive to perform, the quantification of lactate oxidation could not be done in the present experiments. Indeed, it is not possible to quantify the contribution of lactate oxidation to total turnover rate (by following the production rate of $^{14}CO_2$) when the bolus injection technique is used.

Separate experiments were carried out for each horse at each activity level. Measurements were performed at rest (R), at the beginning of the trot protocol (ST), later during the trot protocol (ET), and during the canter protocol (C).

For exercising animals, the radioactive bolus was injected 1 min after the beginning of the trot protocol (ST), 7 min into the same protocol (ET), and 7 min after starting the canter protocol (C). The same timing was used for the measurement of cardiac output in the previous study (Chapter 4). [U-^{14}C]lactate (specific activity = 150 mCi/mmol) was purchased from Amersham (UK), and doses of 120-150 uCi (rest), 140-220 uCi (trot) and 230-260 uCi (canter) were injected into the right atrium. The labeled metabolite was administered in a 13-20 ml saline solution, and the catheter was flushed with 50 ml saline. The total procedure (bolus

injection and flushing) was completed in 6 s. The exact size of the bolus was determined by weighing the syringe before and after injection. At the end of three randomly chosen experiments, the cardiac catheter was withdrawn and counted to determine whether part of the bolus remained in the line after flushing. The activity measured in the catheters only represented 0.1-0.6% of the total doses injected, and this loss of activity was not taken into account in the calculations.

Blood samples (5 ml) were drawn from the carotid artery starting 10 s after injection. They were taken approximately every 10 s for the first 3 min and at longer intervals thereafter. Total sampling time was 6 to 20 min during exercise and 2 h in resting animals. Samples were collected in ice-cold 7-ml vacutainers containing 0.07 ml ethylenediaminetetraacetic acid (EDTA) as anticoagulant and placed on ice. Hematocrit was measured immediately in micro-hematocrit tubes (10 min spin). Plasma was separated from the rest of the sample and stored at -20^{0}C before counting and metabolite assays.

Lactate and glucose concentrations were measured on a Pye-Unicam spectrophotometer (SP6-500) by following the reduction of NAD^{+} or $NADP^{+}$ at 340 nm (Bergmeyer 1974). All the assays were performed in duplicate (see Chapter 2 for details) within 3 days of sampling with a total cuvette volume of 1 ml. Scintillation counting was performed on an Isocap 300 scintillation counter by use of internal standard quench correction. The activity of lactate was calculated as

total plasma activity minus the activity measured in plasma glucose. Glucose activity was determined by use of previously described procedures (see Methods in Chapter 2). Plasma samples (Ø.5 ml) mixed with 1Ø ml aqueous counting scintillant (ACS II, Amersham) were left in the dark at 4ØC for at least 12 h before being counted for 2Ø min. Efficiency ranged from 65 to 82%. No activity could be measured in glucose, even 2 h after injection of the radioactive bolus in resting animals. Successive experiments on the same horse were always separated by a minimum of 4 days even though no residual activity could be measured in plasma or urine 24 h after injection.

5.2.4 CALCULATIONS AND STATISTICS. Turnover rate was calculated as the dose injected divided by the surface area under the specific activity decay curve. To calculate this surface area the decay curve was fitted with the sum of two exponential function by use of the procedure described in Chapter 2 (using the sum of three exponential functions did not improve the fit of the calculated functions, therefore, the sum of only two exponentials was used here), and this function was integrated between time Ø and the time when 5% of the maximum possible specific activity was reached. The maximum activity was calculated as the dose injected divided by the total blood volume [using the mean value of 142.1 ml of blood per kg body weight measured by Persson (1968) in thoroughbred horses]. Metabolic clearance rate (MCR) was calculated as turnover rate divided by plasma lactate

concentration (Wolfe, 1984). For each experiment, cardiac output was determined from mean heart rate by linear interpolation of the heart rate vs cardiac output relationship. This relationship was calculated for each horse (linear regressions; r=Ø.98-Ø.99) using the results of the previous study (Chapter 4). Data from JW, KJ, and SB were pooled to calculate a regression equation for IN (r=Ø.98) whose cardiac output was not measured.

Means were compared with a oneway ANOVA, and the Student-Newman-Keuls post hoc test (T-Method for unplanned comparisons, see Sokal and Rohlf 1981) was used when the overall F value indicated that further analysis was necessary. A multiple regression analysis was performed to estimate the respective contributions of changes in lactate concentration and cardiac output to the total variability in lactate turnover rate (Table 7). ANOVA was used to determine the significance of the slopes calculated by linear regression and to analyze the multiple regression model (see Sokal and Rohlf, 1981).

5.3 RESULTS

The lactate specific activity decay curves and the concentration of lactate in plasma after bolus injection of [U-^{14}C]lactate are given in Fig. 13 to 15 for one of the thoroughbreds (KJ), at rest and during exercise. In all experiments, the calculated multiexponential functions fitted

the measured specific activities extremely well. Specific activity decreased much more sharply during the canter than at rest (Fig. 14), particularly when considering that the dose of [U-^{14}C]lactate injected in the resting animal was less than half the amount administered during the canter. The lactate turnover rates measured after integrating the fitted functions were 11.2 umol min^{-1} kg^{-1} at rest (Fig. 13 and top graph Fig. 14) and 42.3 umol min^{-1} kg^{-1} during the canter (bottom graph in Fig. 14). Values of 51.3 and 45.2 umol min^{-1} kg^{-1} were measured at the beginning of the trot protocol and later during the same protocol (Fig. 15). Plasma lactate concentration was steady at the different levels of activity (insets: Fig. 13 to 15) and, for this animal, mean concentration was 25% higher during exercise than at rest.

Lactate turnover rates for each animal and each activity state are given in Table 7, which summarizes the results of this study. For each experiment, Table 7 also shows the mean values for heart rate, hematocrit and plasma lactate concentration, as well as cardiac output and metabolic clearance rate for lactate. The average heart rate of the four horses was 37.9 $\pm$ 2.1 at rest, 132.7 $\pm$ 9.0 at the beginning of the trot protocol (ST), 130.0 $\pm$ 8.0 later in the same protocol (ET), and 147.5 $\pm$ 9.6 beats/min in the canter protocol (means $\pm$ SEM, N=4). For a given level of exercise, JW had a lower heart rate than the other horses. Mean hematocrit (for the 4 animals) increased from a resting value of 36.2 $\pm$ 2.7% to a maximum of 54.7 $\pm$ 0.8% during the canter

FIG. 13 Lactate specific activity decay curve and lactate concentration (inset) in plasma of a resting thoroughbred horse (KJ) after injection of 122.14 uCi [U-^{14}C]lactate at time Ø (experiment No. 11). See Fig.2 for a detailed view of the first 5 min after injection. Radioactive bolus was injected into the right atrium at time Ø and blood was sampled from the carotid artery. The decay curve was fitted with a double exponential function.

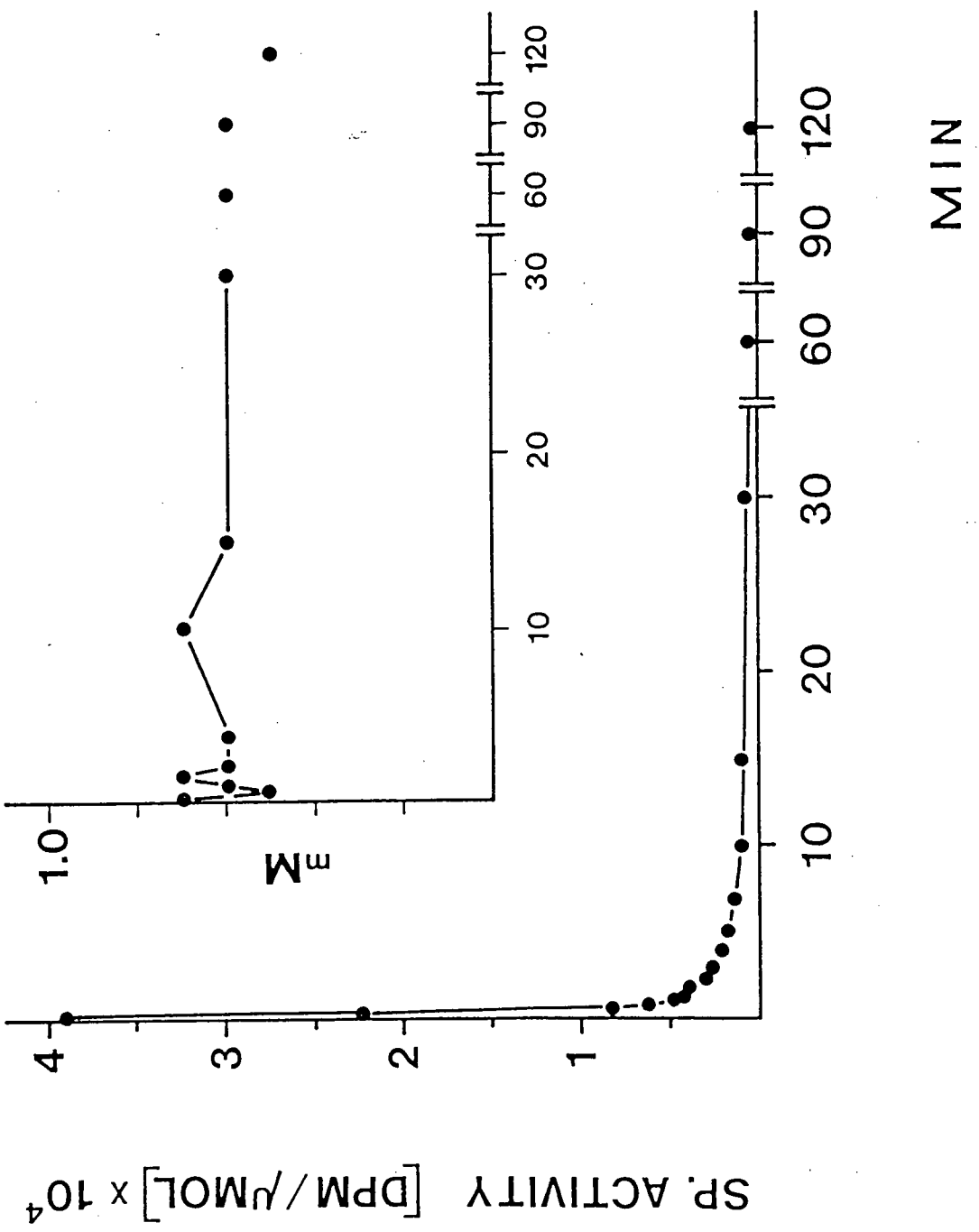
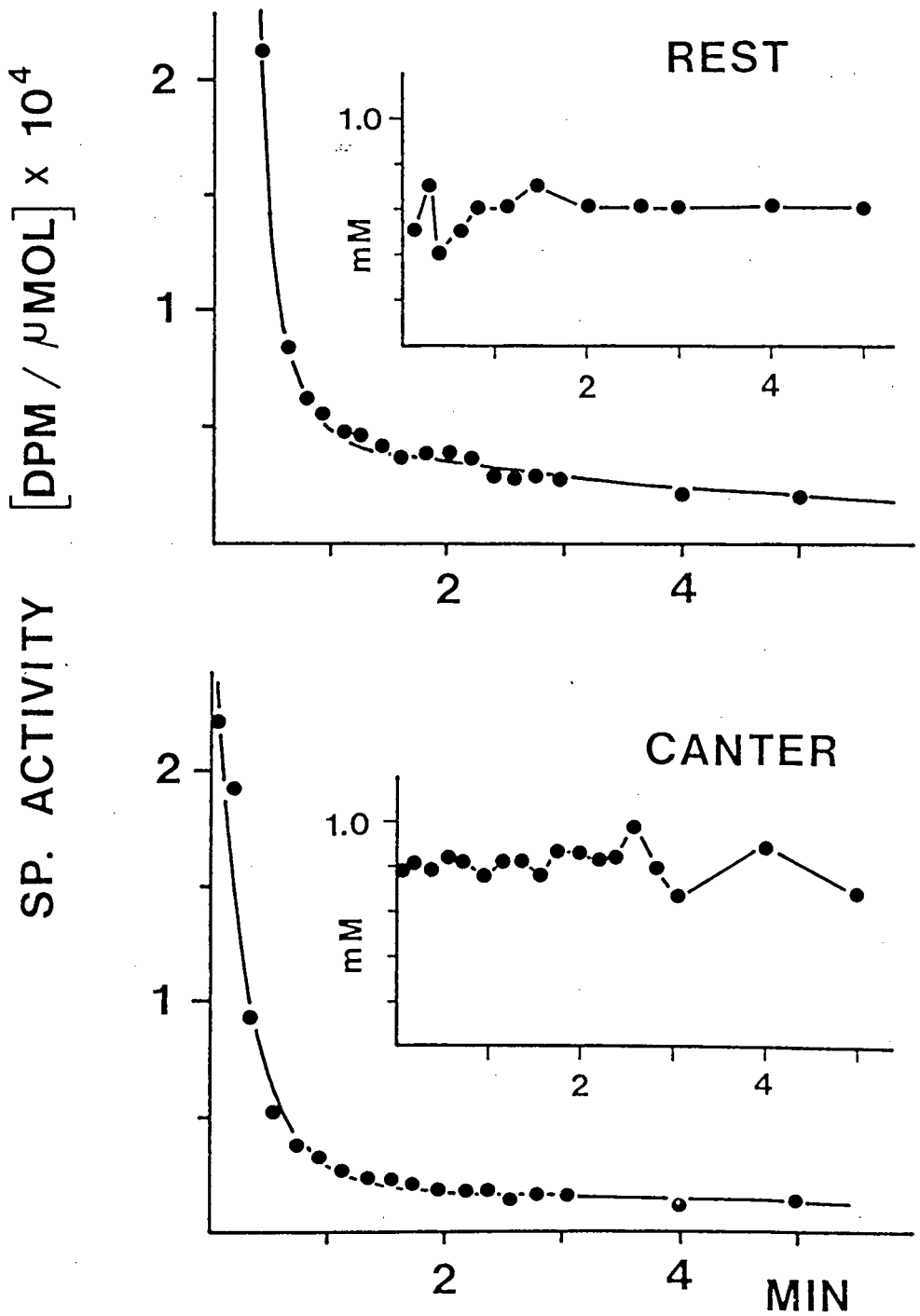

SP. ACTIVITY [DPM/μMOL] x 10⁴
4
3
2
1
10
20
30
60
90
120
MIN
mM
1.0
10
20
30
60
90
120

FIG. 14 AND 15 Lactate specific activity decay curves and lactate concentration (insets) in plasma of a thoroughbred horse (KJ) at rest and exercising on a treadmill during the first 5 min after bolus injection of [U-^{14}C]lactate. Doses of 122.14 uCi (rest: R), 169.14 uCi (start trot: ST), 144.27 uCi (end trot: ET) and 251.Ø2 uCi (canter: C) were injected at time Ø (experiments No. 11 to 14 respectively). Same injection and sampling sites as in Fig. 13. All decay curves were fitted with double exponential functions. See methods for exercise protocols and the timing of injections.


REST
CANTER
SP. ACTIVITY [DPM / μMOL] x 10⁴
mM
1.0
2
4
MIN

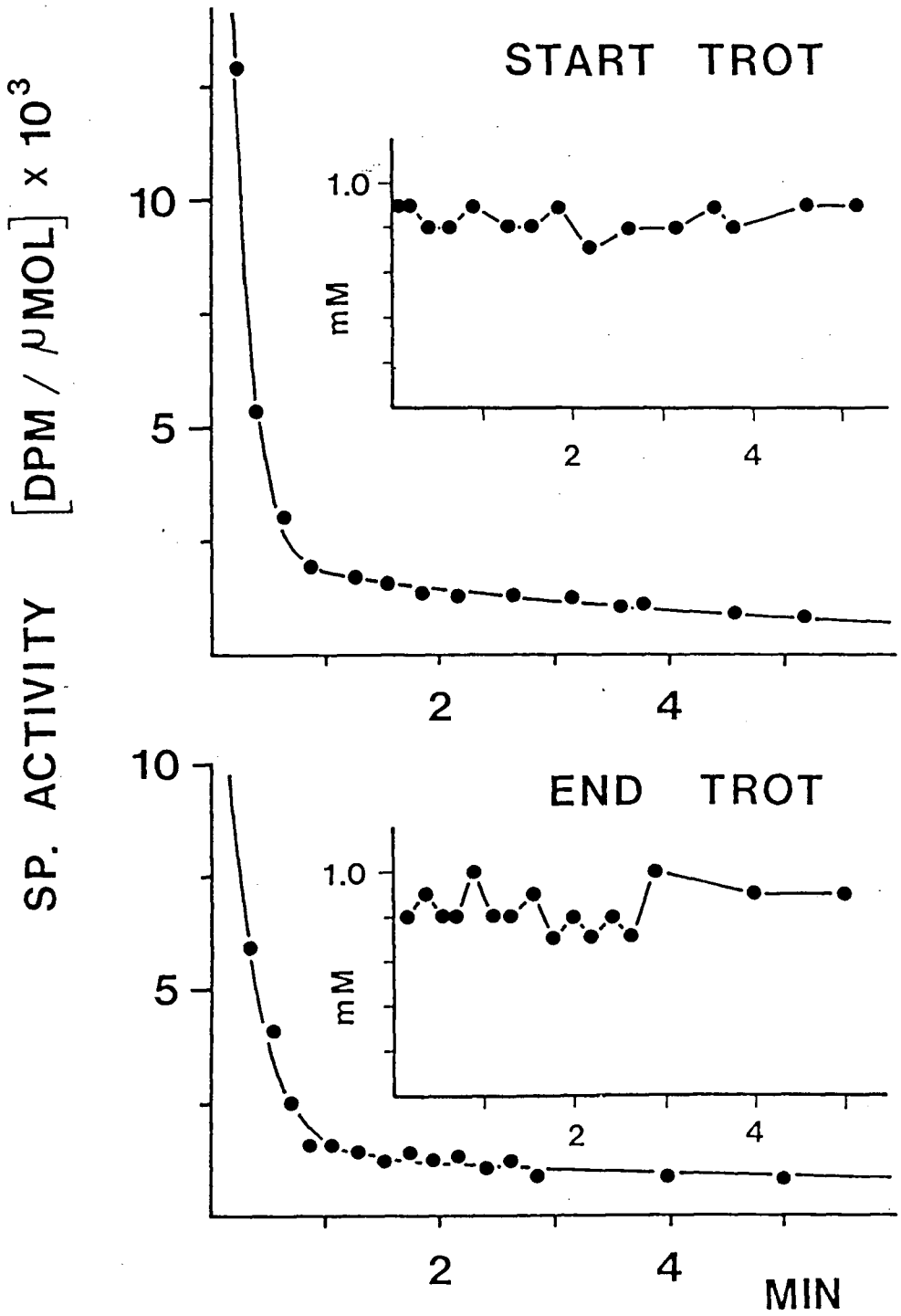

START TROT
END TROT
SP. ACTIVITY [DPM / μMOL] x 10³
10
5
2
4
1.0
mM
MIN

TABLE 7. LACTATE TURNOVER RATE, LACTATE CLEARANCE RATE, AND CARDIAC OUTPUT OF THOROUGHBRED HORSES AT REST AND DURING EXERCISE (CONT'D ON NEXT PAGE).

EXPT No.	HORSE	ACTIVITY STATE	SPEED m/s	INC-LINE %	HEART RATE b/min	HCT %	PLASMA [LACTATE] mM
1	JW	REST	-	-	38.8	44.Ø	Ø.8Ø±Ø.Ø2
2	JW	TROT (ST)	4.Ø	6	118.8	59.Ø	2.Ø7±Ø.Ø3
3	JW	TROT (ST)	3.Ø	6	118.Ø	55.3	1.47±Ø.Ø3
4	JW	TROT (ET)	3.Ø	6	1Ø8.1	57.6	Ø.74±Ø.Ø2
5	JW	CANTER	6.5	Ø	122.3	53.5	1.89±Ø.Ø8
6	SB	REST	-	-	43.3	35.1	Ø.83±Ø.Ø2
7	SB	TROT (ST)	4.Ø	6	157.5	58.Ø	1.99±Ø.Ø9
8	SB	TROT (ET)	4.Ø	6	138.4	57.9	Ø.98±Ø.Ø2
9	SB	CANTER	6.5	Ø	146.3	56.6	1.25±Ø.Ø2
1Ø	SB	CANTER	4.Ø	6	175.3	58.2	3.54±Ø.Ø7
11	KJ	REST	-	-	36.2	32.9	Ø.62±Ø.Ø2
12	KJ	TROT (ST)	4.Ø	6	134.2	54.1	Ø.84±Ø.Ø2
13	KJ	TROT (ET)	4.Ø	6	144.4	54.Ø	Ø.8Ø±Ø.Ø3
14	KJ	CANTER	6.5	Ø	168.2	55.4	Ø.81±Ø.Ø2
15	IN	REST	-	-	33.3	32.8	Ø.56±Ø.Ø2
16	IN	TROT (ST)	4.Ø	6	121.2	49.Ø	Ø.72±Ø.Ø2
17	IN	TROT (ET)	4.Ø	6	129.Ø	48.8	Ø.72±Ø.Ø2
18	IN	CANTER	8.3	Ø	153.1	53.3	Ø.98±Ø.Ø2

TABLE 7. (CONT'D).

N	CARDIAC OUTPUT ml min-1 kg-1	TURNOVER RATE (R_t) umol min-1 kg-1	METABOLIC CLEARANCE RATE (MCR) ml min-1 kg-1
20	116.5	12.2	15.3
15	367.2	127.8	61.7
13	364.8	115.4	78.5
19	333.7	34.9	47.2
17	378.1	71.7	37.9
23	153.5	7.3	8.8
11	542.3	85.5	42.9
18	477.2	70.4	71.9
17	504.2	95.2	76.2
14	--	276.2	78.0
25	106.0	11.2	18.1
14	451.2	51.3	61.1
19	487.2	45.2	56.5
19	571.1	42.3	52.2
23	107.0	6.5	11.6
19	417.3	51.3	71.2
17	444.9	50.7	70.4
19	529.7	55.1	56.3

Turnover rate was measured by bolus injection of [U-^{14}C]lactate. ST: start of trot protocol; ET: later in trot protocol (see Methods). HCT = mean hematocrit. Lactate concentration given as mean $\pm$ SEM; N = number of blood samples. Note that in canter protocol, speed was higher for IN than for the other horses (expt. No. 18). In experiment No. 10, SB was cantering at abnormally low speed and struggling. Cardiac output was calculated by linear interpolation using data from previous study (Chapter 4; see Methods for details).

protocol. Plasma lactate concentration was steady throughout all the experiments as shown by the low standard errors reported in Table 7 and by the insets in Fig. 13 to 15. The assumption of steady state required by the bolus injection technique was therefore met in each experiment. Mean lactate concentration ranged from Ø.56 to Ø.83 mM in resting animals. At the beginning of the trot protocol, lactate levels were only slightly elevated for two animals (KJ and IN) and reached about 2 mM for the other two (JW and SB). Lactate concentration was lower during the second part of the trotprotocol (ET) than at the beginning of that same protocol (ST), except for IN where no difference was measured. During the canter, mean lactate concentrations ranged from Ø.81 to 1.89 mM, with the same individuals (JW and SB) showing the highest values as in the trot protocol. Average cardiac output for all animals was 12Ø.8 $\pm$ 11.2 ml min^{-1} kg^{-1} at rest, 443.9 $\pm$ 37.3 at the beginning of the trot protocol (ST), 435.8 $\pm$ 35.2 later in the same protocol (ET), and 495.8 $\pm$ 41.6 during the canter (means $\pm$ SEM, N=4). Mean MCR was 13.5 $\pm$ 2.Ø ml min^{-1} kg^{-1} at rest, 63.4 $\pm$ 7.7 (ST) and 61.5 $\pm$ 5.9 (ET) during the trot protocol, and 55.7 $\pm$ 7.9 in the canter protocol.

Mean lactate turnover rates for the different activity states are given in Fig. 16. They ranged from a low resting value of 9.3 umol min^{-1} kg^{-1} to a maximum of 75.9 at the beginning of the trot protocol. The overall effect of exercise on turnover rate was highly significant (3 df/12 df; F=8.1; P<Ø.ØØ5). Post hoc tests show that lactate turnover

FIG. 16 Lactate turnover rate in resting and exercising thoroughbred horses. Values given are means + SEM (N = 4). R: rest; ST: start of trot protocol; ET: later during the trot protocol; C: during the canter protocol. See methods for details.

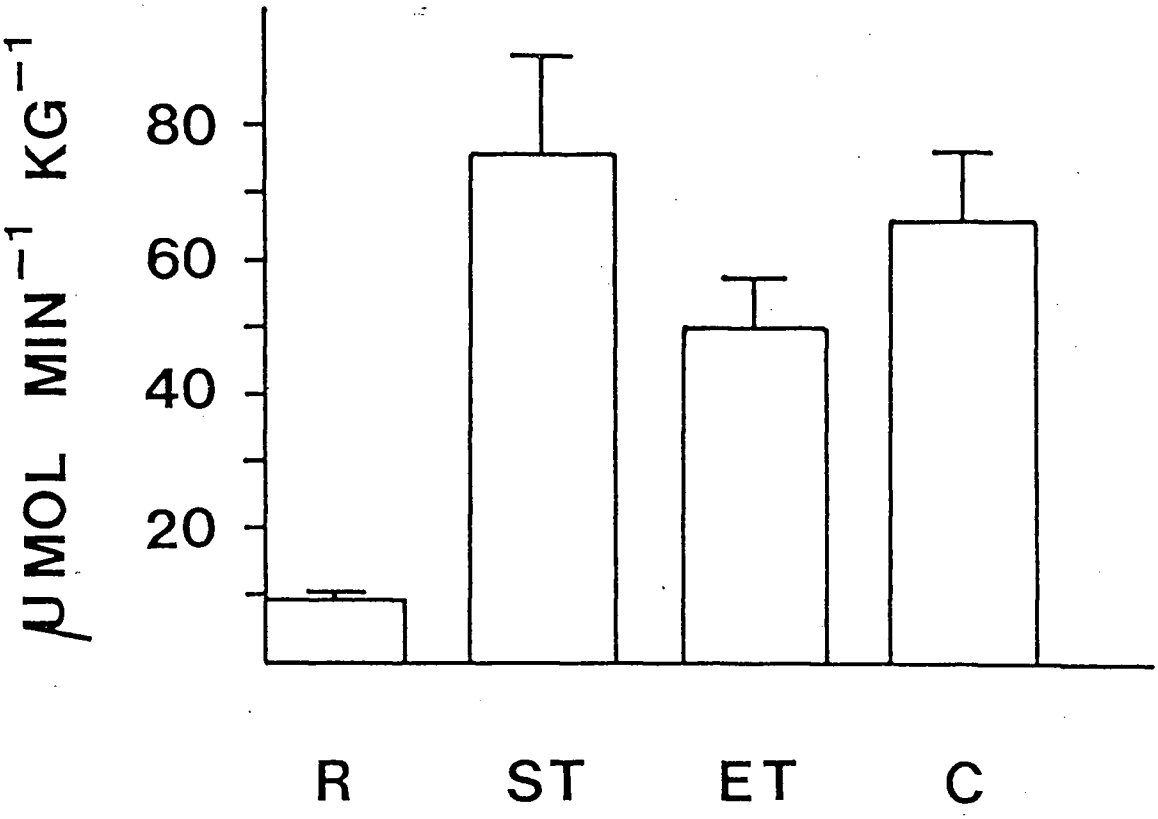

μMOL MIN^{-1} KG^{-1}
80
60
40
20
R
ST
ET
C

FIG. 17 Relationship between lactate turnover rate and mean plasma lactate concentration in resting and exercising thoroughbred horses. Line fitted by linear regression (slope = 59.02, r = 0.80). Different symbols represent individual horses: squares (JW); solid circles (SB); open circles (KJ); and triangles (IN).

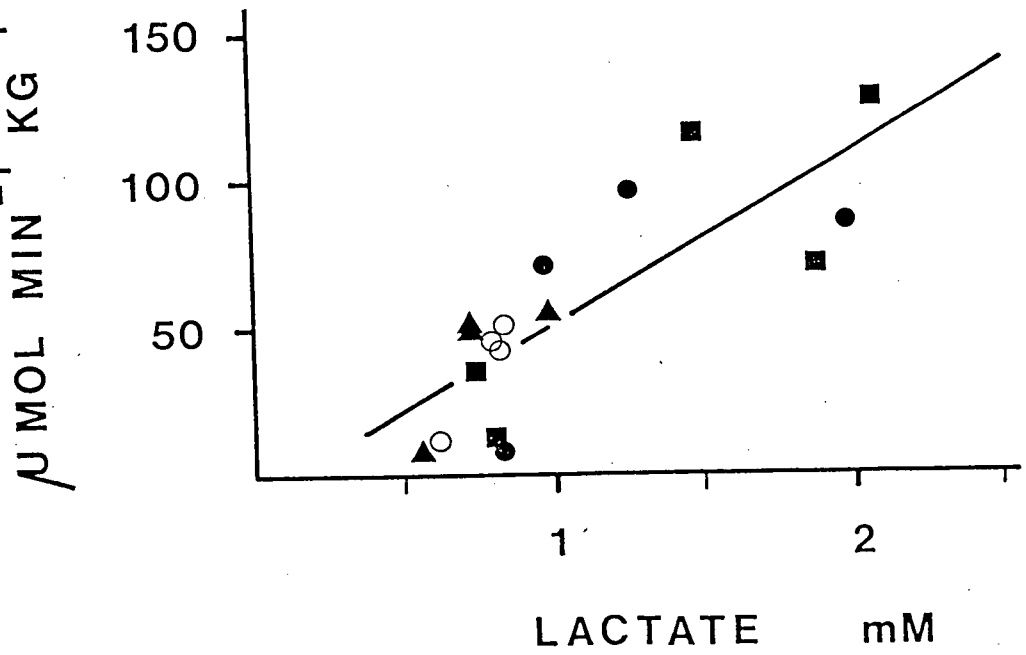

150
100
50
1
2
LACTATE mM
μ MOL MIN^{-1} KG^{-1}

TABLE 8. EFFECTS OF PLASMA LACTATE CONCENTRATION AND CARDIAC OUTPUT ON THE RATE OF LACTATE TURNOVER IN THOROUGHBRED HORSES

REGRESSION MODEL	r	% OF THE VARIABILITY IN R_t EXPLAINED BY MODEL	F	P
R_t = f([LAC])	Ø.8Ø	64	26.5	< Ø.ØØ1
R_t = f(CO)	Ø.58	34	7.6	< Ø.Ø5
R_t = f([LAC], CO)	Ø.87	76	21.7	< Ø.ØØØ1

Linear regressions were performed on the data from Table 7 (N = 17; experiment No. 1Ø was not included). R_t = lactate turnover rate; [LAC] = plasma lactate concentration; CO = cardiac output. ANOVA was used to determine whether the slopes of the regression equations were significantly different from Ø [F values and probabilities (P) are given above].

rate was significantly lower at rest than for the different exercise protocols ($P<0.05$). Mean turnover rate was not different between the canter (C) and the trot protocols (ST and ET). Mean lactate turnover was higher at the beginning of the trot protocol (ST) than later during the same protocol (ET), but the difference was not significant at $P=0.05$. In experiment 10, the animal was cantering at an unnaturally low velocity for this gait (4 m/s). In addition, he was trying to go faster than the treadmill by constantly pushing against a metal rail placed in front of him. His mean heart rate was much higher than for horses cantering normally at 6.5 m/s, and he maintained a high but steady plasma lactate concentration of 3.5 mM throughout the experiment. Under these conditions, his rate of lactate turnover reached 276.2 umol min^{-1} kg^{-1}.

The relationship between plasma lactate concentration and lactate turnover rate is given in Fig. 17. The line was fitted by least squares (linear regression: slope=59.0, r=0.80). A complete regression analysis of the effects of changes in plasma lactate concentration and cardiac output on lactate turnover rate is summarized in Table 8. The effect of changes in plasma lactate concentration was highly significant and could explain 64% of the variability in turnover rate. The effect of changes in cardiac output was also significant but could only account for 34% of the variability in turnover rate. When both, lactate concentration and cardiac output, were used in a multiple regression, their combined effect was highly

significant and could explain 76% of the variability in lactate turnover rate (Table 8). The coefficient of multiple determination (R^2) was significantly increased by the addition of cardiac output as a second independent variable to the simpler regression model only using lactate concentration as a single independent variable (1 df/14 df; F=6.8; P<Ø.Ø25; see Sokal and Rohlf 1981, p. 634). This analysis shows that the inclusion of cardiac output in the regression model caused a significant increase in the determination of the variability in lactate turnover rate (see Table 8).

5.4 DISCUSSION.

This study shows that cardiac output is correlated with the rate of lactate turnover. It also provides the first measurements of plasma lactate fluxes in thoroughbred racehorses and confirms the positive correlation between lactate concentration and turnover rate of plasma lactate reported for tuna in Chapter 2.

Because horses are very expensive to keep, and particularly because thoroughbred racehorses are not commonly used for invasive experiments, the present study had to be carried out on four animals only. In addition, the high cost of the isotope required for the measurement of lactate turnover rate limited the total number of experiments; turnover rate could only be measured four times in each

animal under different exercise conditions. Finally, very few large animal treadmills are available for the type of studies carried out in this thesis, and such treadmills are expensive to maintain and to operate. Therefore, the total amount of time available for the present work was also limiting.

5.4.1 LACTATE TURNOVER RATE AND ROLE OF PLASMA LACTATE AS AN OXIDATIVE FUEL. In thoroughbred horses, submaximal exercise of up to 55% $\dot{V}O_2max$ caused a 5 to 7-fold increase in lactate turnover rate over the mean resting flux rate of 9.3 umol min^{-1} kg^{-1}. This value is lower than the rate reported by Stanley et al (1985) for resting humans (15 umol min^{-1} kg^{-1}), but such a discrepancy is expected when considering the 6-fold difference in body mass (see Chapter 2). The turnover rates of 50 umol min^{-1} kg^{-1} (trot) and 66 umol min^{-1} kg^{-1} (canter) measured here in running horses are similar to values found in exercising humans for the same work intensity (45-50 umol min^{-1} kg^{-1}; see Stanley et al, 1985; and Mazzeo et al, 1986). When the range of turnover rates found in thoroughbreds is presented against body mass on a log-log plot (see Chapter 2) and compared with other species, it closely matches the standard mammalian pattern expected for their body size.

At rest, about fifty percent of the lactate turned over is oxidized in rats (Donovan and Brooks, 1983), in dogs (Issekutz et al, 1976) and in humans (Mazzeo et al, 1986). Lactate oxidation is responsible for 13 to 20% of the oxygen

consumption of a resting human [calculated from Stanley et al (1985) and Mazzeo et al (1986)] and for 39% of the resting $\dot{V}O_2$ in rats (calculated from Donovan and Brooks, 1983). If a similar fraction of lactate turnover rate is oxidized in thoroughbred horses, only 7% of their resting $\dot{V}O_2$ can be accounted for by lactate oxidation (using a mean resting turnover rate of 9.3 umol min^{-1} kg^{-1} and a resting $\dot{V}O_2$ of 5 ml min^{-1} kg^{-1}). Note that even if 100% of the rate of lactate turnover was oxidized, the contribution of lactate oxidation to total oxygen consumption in thoroughbreds would be 14% (the lowest estimate found for humans, but a much lower value than in rats). Interspecific differences in the relative use of lactate as an oxidizable fuel are even more striking during exercise. At 50% $\dot{V}O_2max$, 75 to 80% of lactate turnover rate is oxidized in rats (Donovan and Brooks, 1983) and in humans (Mazzeo et al, 1986). If the same percentage of the lactate flux is oxidized in thoroughbreds, the contribution of lactate oxidation to $\dot{V}O_2$ decreases to only 4.5% in a cantering horse. For the same exercise intensity (i.e. 50-60% $\dot{V}O_2max$), humans use 12% (calculated from Stanley et al, 1985, and Mazzeo et al, 1986) and rats use an astounding 65% of their $\dot{V}O_2$ to oxidize lactate alone (from Donovan and Brooks, 1983). Unfortunately, it was impossible to quantify lactate oxidation directly in the present study because the bolus injection technique was used (continuous infusion is necessary when oxidation has to be quantified by collection of $^{14}CO_2$, but, as explained previously, infusion would have been far too costly in such

large animals). However, even if 100% of the lactate turnover measured here was going to oxidation, this pathway of lactate metabolism would only account for less than 6% of $\dot{V}O_2$ in a cantering thoroughbred horse. Even though the contribution of plasma lactate as an oxidizable fuel is significant in horses, the above calculations clearly show that this substrate only plays a minor role during submaximal exercise compared with other mammalian species, and it is not necessary to have direct oxidation measurements to draw this conclusion.

Resting glucose turnover rates of 7 umol min^{-1} kg^{-1} have been measured in untrained ponies and horses (Anwer et al, 1976; Evans, 1971). Trained horses can reach 12 umol min^{-1} kg^{-1} at rest (Evans, 1971), but no data are available during work. Exercise causes glucose turnover rate to maximally increase by 2 to 3-fold in other mammals and 50 to 80% of the total flux of glucose is oxidized depending on work intensity (Brooks, 1985). If the glucose kinetics of horses follow the standard mammalian pattern (i.e. 50% and 80% of glucose turnover rate is oxidized at rest and at 50% VO_2max, respectively), the combined contributions of exogenous lactate and exogenous glucose supplies can only account for 25% of $\dot{V}O_2$ at rest and for about 10% of $\dot{V}O_2$ during the canter [assuming a glucose turnover of 36 umol min^{-1} kg^{-1}: 3 times the resting value reported by Evans (1971) in trained horses]. At rest and during submaximal exercise, it is clear that exogenous fuels other than carbohydrates or endogenous substrates must play a major role

in oxidative ATP production. Because the contribution of amino acids is likely to be minor, thoroughbred horses probably use fat as their major oxidizable substrate for muscle work. The R_Q of untrained standardbred horses increases from a resting value of 0.83 to 0.98 during high-intensity exercise (Thomas and Fregin, 1981) suggesting that, in the untrained state, carbohydrate oxidation becomes more important when work rate is elevated. However, the same investigators have shown that this increase in R_Q was suppressed by training (Thomas et al, 1983), indicating that fat could remain an important oxidizable fuel during high-intensity exercise in trained standardbred horses. A different kind of evidence also suggests that fat oxidation may be an important source of energy in exercising horses. A very large increase in plasma glycerol concentration has been observed during and after exercise in thoroughbred horses (Snow et al, 1985), indicating that triglyceride utilization could be very high in these animals. Horses may increase their reliance on lactate oxidation at higher exercise intensities than used in the present study. The high lactate turnover rate of 276.2 umol min^{-1} kg^{-1} measured under unusual conditions in experiment 10 (see Results section) indicates that they have the ability to boost lactate flux rates much higher than observed during the trot and canter protocols. The highest rates of lactate disappearance measured in humans by Stanley et al (1985) only reached 230 umol min^{-1} kg^{-1} in trained subjects. Here, SB was already able to reach 276 umol min^{-1} kg^{-1} at a relatively low

plasma lactate concentration of 3.5 mM (Expt. 1Ø) while the highest human values were measured at concentrations of 9 to 11 mM.

The present study suggests that lactate turnover rate decreases throughout a bout of submaximal work. As exercise is sustained, rats, dogs, and humans have been shown to progressively increase their reliance upon fat as a substrate for energy metabolism (Holloszy et al, 1986). Plasma free fatty acid concentration increases and R_Q goes down throughout exercise. A concomitant decrease in plasma carbohydrate turnover would therefore be expected during prolonged exercise.

5.4.2 LACTATE CLEARANCE RATE. Metabolic clearance rates (MCR) showed a 4.5-fold increase between rest and the trot protocol (13.5 to 63.4 ml min^{-1} kg^{-1}). At a higher exercise intensity (canter), this rate decreased to 55.7 ml min^{-1} kg^{-1}. The same pattern of changes in MCR as a function of work intensity has been observed in humans (Mazzeo et al, 1986; Stanley et al, 1985). The resting MCR of horses was about half the value found in humans, but the rate measured during the trot protocol was 70% higher than the largest MCR reported in man (Stanley et al, 1985). Exercising thoroughbred horses therefore have the ability to support a given rate of lactate turnover with a lower plasma lactate concentration than humans. Endurance training has been shown to lower the plasma concentration for a given turnover rate in rats, thereby increasing MCR (Donovan and Brooks, 1983).

The higher MCR shown by thoroughbreds may therefore simply reflect the highly trained condition of these animals.

5.4.3 EFFECT OF PLASMA METABOLITE CONCENTRATION ON TURNOVER RATE. The turnover rate and the plasma concentration of lactate are positively correlated in thoroughbred horses. The same relationship has been observed in a number of animals including rats (Okajima et al, 1981; Donovan and Brooks 1983), dogs (Eldridge et al, 1974; Issekutz et al, 1976), seals (Davis, 1983), humans (Mazzeo et al, 1986), and even tuna (Chapter 2). The flux rates of glucose (Verdonk et al, 1981), glycerol (Bortz et al, 1972) and free fatty acids (Paul and Issekutz, 1967; Hagenfeldt, 1975) have also been shown to increase as their plasma concentration goes up. A simple mass action effect of these metabolites could explain the observed relationships. The rate of cellular uptake by working muscles should be proportional to substrate concentration in plasma for any given rate of oxidative metabolism in these muscles. Indeed, Stanley et al (1985) observed that the rate of lactate disappearance seems to be directly controlled by plasma lactate concentration in humans. Some contradictory evidence is available for glucose however, where an inverse relationship between concentration and turnover rate has also been reported recently (Jenkins et al, 1986). These authors showed that insulin has an effect on the slope of the relationship, and their results suggest that hormonal influences can override the mass action effect of glucose concentration.

Most of the studies investigating hormonal effects on substrate fluxes deal with glucose metabolism (see Hetenyi et al, 1983 for a review) but limited information is available for lactate. Challiss et al (1986) showed that the rate of lactate production by the rat hindlimb depends on the concentration of insulin in plasma, and Issekutz (1984) reported that the increase in lactate turnover rate elicited by exercise was abolished by beta-adrenergic blockade. Issekutz's data suggest that epinephrine affects lactate flux rates indirectly via changes in plasma lactate concentration. Indeed, the complex pattern of concentration changes caused by his experimental protocol is exactly matched by concomitant fluctuations in flux rates (Fig. 2 in Issekutz, 1984). Hormonal effects must be studied more thoroughly if the control of metabolite fluxes is to be fully understood.

5.4.4 EFFECT OF CHANGES IN CARDIAC OUTPUT. This study represents the first investigation of the role played by total perfusion rate on plasma substrate turnover at the whole organism level. It shows that cardiac output is correlated with plasma lactate fluxes, and that this correlation is independent of the lactate concentration versus turnover rate relationship (see Table 8). These results agree with the kinetics data of other studies (see next paragraph) in which the effect of changes in cardiac ouput was not specifically investigated (in particular Issekutz et al, 1976 in the case of lactate turnover). It is well known that the large increase in cardiac output elicited

by exercise goes to the working muscles (Brooks and Fahey, 1984). Therefore, changes in cardiac output must have an effect on the delivery rate of lactate to these muscles. In fact, the turnover rates of all plasma metabolites should also be influenced by changes in cardiac output, and recent evidence shows that this is probably true for glucose. Challiss et al (1986) showed that the rate of glucose utilization by the rat hindlimb depends on blood flow at rest and during isometric contractions. Furthermore, glucose turnover rate increases with exercise in the absence of a significant change in plasma glucose concentration (Brooks and Donovan, 1983; Jenkins et al, 1986), and this adjustment in flux rate could be mediated by perfusion changes. An increase in cardiac output not only provides more oxygen to the contracting muscles, but it also accelerates the supply of oxidizable substrates to meet the energetic demands of exercise.

The slopes reported for the relationship between lactate concentration and turnover rate vary over a very wide range depending on the animal and on the experimental conditions. This variability can be partly accounted for by interspecific differences but also, and more importantly, by the effect of cardiac output on lactate fluxes which has been ignored in metabolite kinetics studies. For instance, Issekutz et al (1976) showed that the slope was much higher when the different lactate concentrations were elicited by exercise than when varying amounts of Na-lactate were infused in resting dogs (Fig. 4 in Issekutz et al, 1976). Their

results can be explained easily when the effect of perfusion rate on turnover is considered.

Why would changes in cardiac output influence lactate turnover? Lactate transport across cell membranes is rapid, and it occurs by diffusion (mainly carrier-mediated, but to a lesser extent by simple diffusion: see Dubinsky and Racker, 1978; Koch et al, 1981; Roos, 1975; and Spencer and Lehninger, 1976). The transfer rate of lactate therefore depends on concentration gradients between extra- and intracellular fluid. During exercise, local dissipation of these gradients may occur if lactate is oxidized faster in the muscle cells than it is supplied by surrounding capillaries. A high blood flow in these vessels would ensure that gradients are maintained, thereby maximizing the rate of lactate translocation.

5.4.5 CONTROL OF EXOGENOUS FUEL SUPPLY. Both cardiac output and plasma lactate concentration influence the rate of lactate turnover. Because the fluxes of all plasma substrates are probably equally dependent on perfusion changes, cardiac output adjustments can be viewed as the coarse control for plasma metabolite flux rates. These adjustments do not allow the turnover of an individual metabolite to be altered without affecting the fluxes of all the others. Augmenting cardiac output therefore causes an overall increase in the supply of oxidative fuels as well as oxygen. Changes in plasma metabolite concentration, on the other hand, allow a finer, specific adjustment for the flux

rate of each individual exogenous fuel. The adjustment of concentration therefore represents the fine control for metabolite flux rates because it allows the supply rate of each oxidative fuel to be altered separately. The respective contribution of individual fuels to overall oxidative energy production can be finely regulated via this mechanism to supply the right combination of fuels at the right time.

5.4.6 CONCLUSIONS. If lactate provided some performance advantage over other oxidizable fuels such as glucose or free fatty acids, animals adapted for endurance exercise would be expected to support high lactate turnover rates during steady-state work. This thesis shows that "good" endurance performers do not necessarily support high lactate turnover rates when operating at sustainable exercise intensities: skipjack tuna have higher turnover rates than sedentary mammals, but thoroughbred horses do not show an increased ability to transfer this compound across their plasma compartment from sites of production to sites of utilization compared with other mammals. In Chapter 2, skipjack tuna is shown to have a range of plasma lactate flux rates that is higher than would be expected for a mammalian species of equivalent size, even though this teleost operates at a much lower temperature (approximately 10^{o}C). Because lactate turnover rates of tuna are high, and because a large proportion of this turnover is known to be accounted for by lactate oxidation in several other species under different experimental conditions, the skipjack tuna is very likely to

be able to support high rates of plasma lactate oxidation, and lactate is probably an important oxidizable fuel in this species. Therefore, results from the tuna study do not provide evidence against the idea that "elite animal athletes" could prefer lactate over other oxidizable fuels. In contrast, Chapter 5 shows that the lactate turnover rates of thoroughbred horses fall well within the expected values for sedentary mammalian species. Therefore, the metabolic adaptations of thoroughbred horses for exercise do not include the ability to sustain higher lactate turnover rates than sedentary mammals. In addition, it is shown in the last chapter of the present work that plasma lactate only plays a minor role as an oxidizable fuel for muscle energy metabolism in thoroughbred horses. The oxidation of this metabolite represents less than 5% of total oxygen consumption during submaximal work, and fat could be the major oxidative substrate in exercising horses. This low utilization of lactate as a fuel in horses is in sharp contrast with the higher reliance on this substrate shown by other species, but particularly by exercising rats. These results emphasize the fact that, even among mammals, large interspecific differences in oxidizable fuel preference are present. The lactate kinetics measurements of Chapter 5 show that the ability to oxidize plasma lactate at high rates is not necessarily required for the "elite" performance of endurance exercise.

A positive correlation between plasma lactate concentration and lactate turnover rate is demonstrated in both species studied in this thesis. In Chapter 2, this correlation is shown for the first time in a non-mammalian system, and the relationship given for tuna covers a much wider range of concentrations than previously reported for mammals (a range of more than 20 mM). Results from Chapters 4 and 5 show that cardiac output is also correlated with lactate turnover rate, and that this correlation is independent of the relationship between plasma lactate concentration and turnover rate. Changes in cardiac output have an effect on the slope of this relationship, but this effect had not been recognized previously. Differences in the slopes obtained for the concentration versus turnover relationship in earlier studies of lactate kinetics from the literature can be explained easily if the effect of cardiac output changes is taken into account. Because both cardiac output and metabolite concentration influence the rate of metabolite turnover in plasma, it is proposed that adjustments in cardiac output and in metabolite concentration represent the coarse and fine controls for the regulation of plasma metabolite turnover.

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