THE PHYSIOLOGICAL AND BIOCHEMICAL CONSTRAINTS ON ACTIVITY IN SPIDERS

BY

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Dedication: To Jumper, Alix, Cuca, and Frisca, all of whom like to smash spiders.

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Abstract of Dissertation Presented to the Graduate Council
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Most spiders can be characterized as animals that fatigue rapidly, especially when compared to insects. The maximum duration and rates of a spider's burst activities (*e.g.*, behaviors involved in attacks or escape) are limited by biochemical factors. Rapid depletion of stores of high-energy phosphate compounds (principally arginine phosphate and adenosine triphosphate) early in maximal activities quickly results in a marked slowing of the spider's movements. As the spider continues to struggle, the build-up of anaerobic by-products (principally D-lactic acid) and associated effects result in complete fatigue within *ca.* two minutes. Defects in the hydraulic leg extension mechanism of spiders resulting in insufficient hemolymph to extend the legs (due to loss of hemolymph from the prosoma and legs to the opisthosoma) do not appear to cause fatigue directly. Instead, the high prosomal hemolymph pressures needed for vigorous activity prevent the heart from pumping freshly oxygenated hemolymph to the prosoma and its active muscles. The

result is that over a two minute bout of struggling probably less than 10% of a spider's energy is derived from aerobic sources, with the remainder coming from anaerobic glycolysis and high-energy phosphate stores. This contrasts with a *ca.* 99% aerobic dependence in non-burst activity such as web-building. The exact relative dependence of a given species on each of these three energy sources during peak activity appears to be correlated with respiratory surface area: the smaller this area, the more dependency on anaerobic metabolism. Correlations probably also exist with the circulatory system.

During recovery, lactate diffuses from the prosoma to the opisthosoma where it is metabolized, probably to complex carbohydrates. The reverse process occurs during activity. Recovery takes 30 minutes or longer in completely exhausted spiders, the exact time being correlated inversely with the respiratory surface area. Transport of lactate from the prosoma is analogous to the process of blood transport of lactate from muscles to the liver in vertebrates and is an adaptation to allow quick recovery of a spider's running ability.

Temperature has less effect on spider locomotion than would be expected based on the temperature dependence of a spider's aerobic process. However, recovery is slowed in spiders that are at temperatures away from that to which they are acclimated.

The metabolic abilities of spiders, both aerobic and anaerobic, are low to moderate when compared to other groups of predatory animals (vertebrates, insects). However, a spider's use of silk and poisons probably have been major factors in lowering the need for highly developed metabolic capacities.

CHAPTER I
INTRODUCTION

The Problem

Most spiders are incapable of prolonged periods of vigorous activity. For example, two minutes of vigorous running or struggling in many species results in complete exhaustion (Anderson and Prestwich 1982). This study examines both the proximal physiological-biochemical constraints and speculates on the ultimate ecological and evolutionary reasons for this inability to sustain prolonged or high aerobic work loads.

There are two suggestions as to the cause of the constraints on a spider's activity. They are the fluid insufficiency hypothesis (Wilson 1970; Wilson and Bullock 1973) and the anaerobic products limitation hypothesis (Linzen and Gallowitz 1975).

The fluid insufficiency hypothesis argues that spiders are constrained by problems related to their locomotory system. Spiders lack direct extensor muscles in several of their leg joints (Petrunkevitch 1909) and must rely upon hydrostatic pressure to extend legs (Ellis 1944; Parry and Brown 1959a, b). High pressures (450-500 mm Hg) are generated by the contraction of the prosomal (cephalothoracic) muscles, principally the *musculi laterales* (Wilson 1970; Anderson and Prestwich 1975). Contraction depresses the prosomal carapace towards the sternum resulting in an increased pressure of the fluid of the prosoma. The legs will extend

if flexor muscles are relaxed (Wilson 1970; Anderson and Prestwich 1975). Unlike the prosoma, pressure in the opisthosoma (abdomen) seldom exceeds 100 mm Hg. Since maximum heart pressure during systole is only slightly greater than 100 mm Hg (Wilson 1962; Stewart and Martin 1974; Anderson and Prestwich 1975), Wilson (1970) argued that during maximum activity there would be a loss of hemolymph from the prosoma due to the pressure gradient. Wilson and Bullock (1973) presented indirect evidence for a gain of volume by the opisthosoma with a concurrent loss by the prosoma during maximal activity by the spider *Amaurobius ferox*. They argued that this spider was largely limited in its maximal activity by the loss of so much hemolymph to the opisthosoma that insufficient fluid for leg extension remained.

Linzen and Gallowitz (1975) emphasized a different cause for fatigue. They observed a small number of poorly developed mitochondria in the leg muscles of a wolf spider, *Cupiennius salei*. They also found a well developed glycolytic pathway along with a high activity of lactate dehydrogenase (LDH), a result confirmed by Prestwich and Ing (in press) in eleven species of spiders representing diverse taxa.

High activities of LDH are associated with the ability to oxidize cytosol-produced NADH under anaerobic conditions with the concomittant reduction of pyruvate to lactate. Linzen and Gallowitz (1975) hypothesized that since spiders possess relatively low aerobic abilities (also see Anderson 1970; Anderson and Prestwich 1982) and apparently large anaerobic capacities, they are limited in activity by anaerobic accumulations of lactate and associated effects. This idea is consistent with observations

of Cloudsley-Thompson (1957). He found that spiders exposed to pure oxygen could struggle longer and recover more quickly.

Wilson (1970) and Wilson and Bullock's (1973) fluid insufficiency hypothesis does not exclude the possibility of constraints related to anaerobic metabolism. They point out that one of the problems inherent in the design of a spider's circulatory system is the inability of the heart to pump freshly oxygenated hemolymph into the prosoma of a vigorously active spider. Thus, they implied that two processes might be involved in fatigue: fluid (hydrostatic) insufficiency occurs quickly (after less than 10 sec of struggle), slows the spider, and the second constraint, a biochemical fatigue related to anaerobiosis is involved later in activity.

I suggest a third possible constraint, namely, depletion of stores of high-energy phosphate compounds and substrates. Phosphagens (arginine phosphate in spiders, Di Jeso *et al.* 1967) are depleted during the initial phases of activity in both vertebrates and insects (Flock *et al.* 1939; Sacktor and Hurlbut 1966). Also, carbohydrates are the only suitable substrate for anaerobic glycolysis, but are not found in high concentrations in spiders (Collatz and Speck 1970; Stewart and Martin 1970; Collatz and Mommsen 1975; Rakotovao 1975). Shifts to usage of alternative substances such as fats and amino acids involve mainly aerobic processes. It is unlikely that high rates of ATP production could be maintained solely by aerobic processes, given the small number of mitochondria in spider muscles (Linzen and Gallowitz 1975). It is possible that phosphagen and/or carbohydrate depletion could result in fatigue of maximally active spiders.

Approach

It is the purpose of this study to partition out the degree each of these factors operates to constrain activity. To accomplish this and to make my results and conclusions as general as possible, I have used seven species of spiders representing diverse taxa and very different habits. A listing of spiders used and a brief description of their size, respiratory exchange system, resting rate of oxygen consumption ($\dot{V}O_2$) and prey capture mode are given in Table I-1. These features are included due to their association with the aerobic and anaerobic capabilities and activity patterns of the spider. For comparison sake, some data are included from a non-spider arachnid, the scorpion *Centruroides hentzi*.

The most detailed analyses in the subsequent chapters will revolve around three species: *Filistata hibernalis*, *Lycosa lenta*, and *Phidippus audax*. These species were chosen because of their differing habits, respiratory anatomy, availability, and because they have been extensively studied (Anderson 1970, 1974; Anderson and Prestwich 1975, 1980, 1982; Harper 1970).

I have chosen to do the biochemical analyses on either the entire animal or the functional subunits of prosoma, legs, and opisthosoma. There is a disadvantage to this procedure in that each of these compartments contains varying proportions of muscle and other tissues (Table I-2). Nevertheless, the method has the advantages of being (1) more rapid than isolation of separate muscles for analyses and (2) it fits in line with the general thrust of other studies on spider locomotion, *i.e.*, other work has focused on the relative roles of the legs, prosoma, and opisthosoma

Table 1-1. Species used and a brief summary of the relevant features of their life histories and respiration.

Prey Capture Method Family Species	Mass (g)	Relative[a] $\dot{V}O_2$	Respiratory System[c,e]	Description of Normal Activity
Orb Weavers				
Family Araneidae				
Argiope arantia	0.7-1.5	60%[b]	2 book lungs + trachea, limited to abdomen	Wait in center of viscid web, catch prey with brief rapid motions using silk and poison. Web renewal every 1-3 days involves *ca.* 1 hr moderate activity.[h],
Neoscona domiciliorium	0.3-1.0	90%[b]		
Nephila clavipes	0.5-1.5	95%[b]		
Funnel Webs				
Trapping web (cribellate) Family Filistatidae				
Filistata hibernalis	0.4-1.0	32%[b]	2 book lungs + vestigal trachea	Flat silk sheets with one or more funnel retreats, enlarged and repaired continuously. Web ensnarls prey, cautious bites.
Hunters				
Family Lycosidae				
Lycosa lenta	0.6-1.2	60%[b]	2 book lungs + trachea in abdomen only	Wait, ambush prey; large items are seized with all 8 legs.[f]
Family Oxyopidae				
Peucetia abboti	0.3-0.6	70%[d]	as above; unknown tracheal development	Ambush prey, venom apparently very fast-acting.
Family Salticidae				
Phidippus audax	0.1-0.6	55%[c]	2 book lungs + trachea extend throughout body	Waits or actively pursues prey, the most active species in this study.[g]

Table 1-1 continued

[a]Relative to $\dot{V}O_2$ predicted by Hemmingsen (1960) as cited in Anderson (1970).
[b]Anderson and Prestwich (1982).
[c]Anderson (1970).
[d]Prestwich (unpublished).
[e]Petrunkevitch (1933).
[f]Rovner (1980).
[g]Hill (1979).
[h]Peakall and Witt (1976).

Table 1-2. A description of the tissue contents of the legs, prosoma, and opisthosoma of spiders. Estimation of leg and prosomal musculature should be regarded as representative of the lower limit of their proportion of the total compartment mass.

Compartment	Tissues[a]	Functions[a]	Venous Oxygen During Activity[b]
Legs	Muscles (30% total)[c]	Support and movement	Very low
	Vascular system		
	Exoskeleton		
Prosoma	Muscles (45% total)[c]	Pressure generation for movement and feeding, also direct movement of the legs	Low
	Digestive system (pumping stomach and digestive diverticulum)	Nutrition	
	Central nervous system	Responsiveness and coordination	
	Hemolymph channels	Food, O_2, waste transport; movement	
Opisthosoma	Digestive diverticula	Digestion and biosynthesis	Probably variable, with the heart well-supplied. Other regions, unknown.
	Silk glands	Silk synthesis and storage.	
	Gonads	Reproduction	
	Book lungs and/or trachea	Gas exchange	
	Heart and Hemolymph sinuses	Hemolymph movement and exchange	
	Subcuticular and other muscles	Abdominal movement and assistance in pressure generation	
	Malpigian tubules	Excretion	

Table 1-2 continued

[a]Millot (1949)
[b]Angersbach (1978)
[c]From preserved *Lycosa lenta*

in terms of their separate contributions to movement in spiders instead of the exact roles of different muscles (Parry and Brown 1959 a, b; Wilson 1962, 1965, 1970; Wilson and Bullock 1973; Stewart and Martin 1974; Anderson and Prestwich 1975; Prestwich and Ing in press).

The organization of this dissertation is in seven chapters: Chapter II deals with the identification of anaerobic pathways in spiders and with the validation of a non-enzymatic technique for lactate determination; Chapter III presents measurements of oxygen consumption in struggling spiders and estimates the relative importance of aerobic and anaerobic metabolism in two minute struggles and in web building; Chapter IV details the actual pattern of fatigue as it occurs in spiders and a scorpion and presents measurements of heart rates and lactate production during exercise and recovery; Chapter V tests the fluid insufficiency hypothesis; Chapter VI presents measurements of exercise and recovery levels of phosphagens, glycolytic substrates, and intermediates during exercise and recovery; and Chapter VII seeks to integrate this work with previous studies and presents a model of the physiological-biochemical events that occur in spiders when they are maximally active and during recovery. Additionaly, I propose some correlations between behavior, physiology, and the ecology of spiders. Finally there are two Appendices: Appendix I deals with estimation of cardiac output in tarantulas and Appendix II with the regulation of glycolytic metabolism in araneomorph spiders.

CHAPTER II
ANAEROBIC PRODUCTS IN SPIDERS

Summary

1. The major anaerobic by-product of spiders is D-lactic acid. Minor accumulations of L-glycerol-3-phosphate may account for *ca.* five percent of the anaerobic ATP production. The possibility remains for the presence of other pathways, particularly one that results in the accumulation of alanine and succinate.
2. Lactate accumulations after maximal activity are greatest in the legs and prosoma and can be as large as 15 µmols/g; accumulations of lactate in the opisthosomas seldom exceed 4 µmols/g and average near 2.5 µmols/g.
3. At a common intensity of exercise, an inverse relationship exists between lactate accumulation and book lung surface area (Fig. II-2). This relationship is accounted for by a co-adapted respiratory-circulatory system that is adapted to supply some oxygenated hemolymph to the prosoma.

Introduction

The evidence for anaerobic metabolism in spiders is based on enzymology. Long and Kaplan (1968) identified the presence of a D-optical isomer-specific lactate dehydrogenase (D-LDH) in spiders and scorpions. This observation was confirmed for spiders by Gleason *et al.* (1971) and

Prestwich and Ing (in press). They showed, along with Linzen and Gallowitz (1975), that LDH was present in high activity and could therefore be regarded as important in anaerobic metabolism (Fig. II-1).

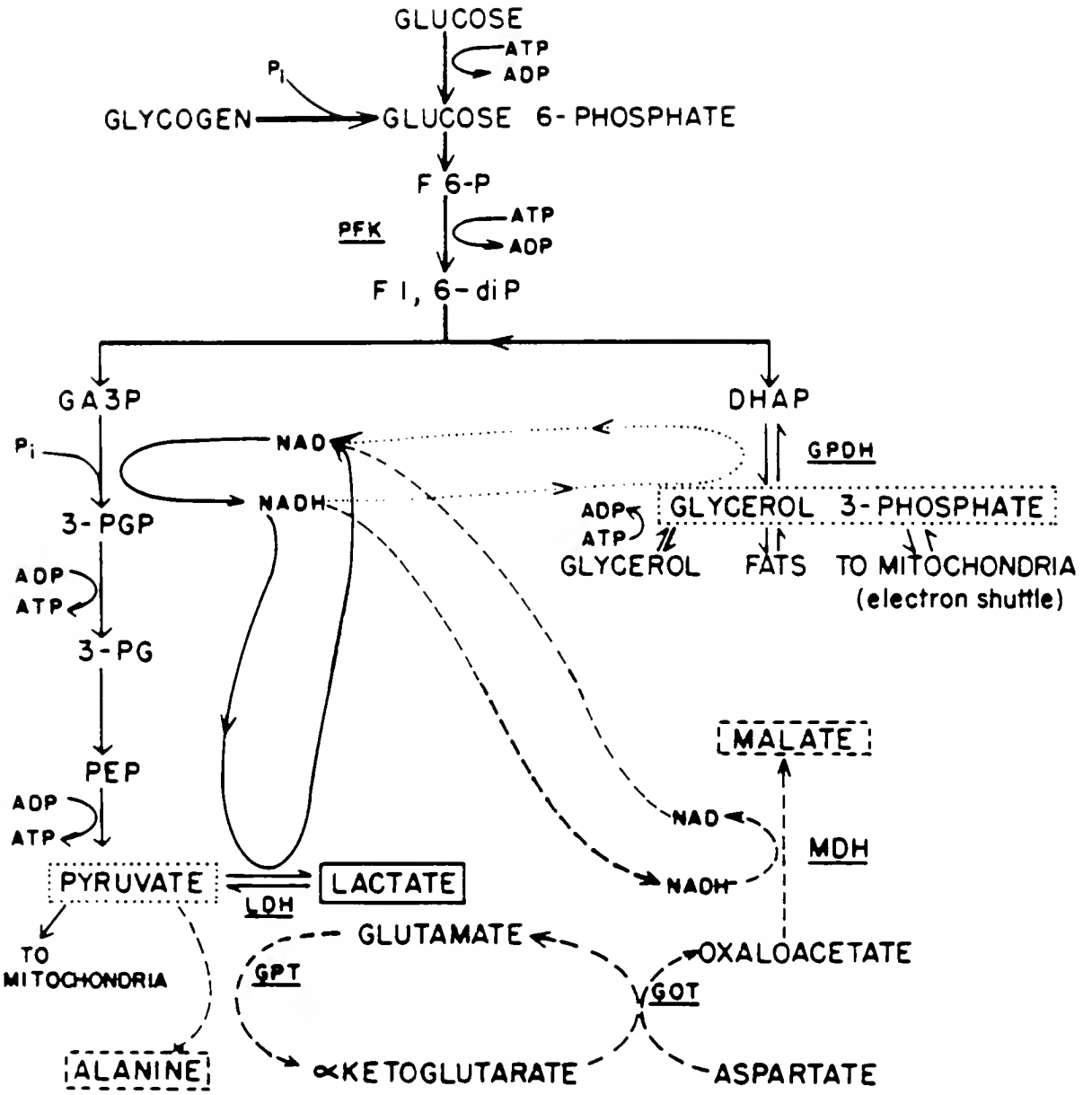
Two other anaerobic pathways have also been proposed to operate in spiders. Based on the activities of the cytosol form of malate dehydrogenase (MDH) and the transaminases glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase (GPT and GOT), Linzen and Gallowitz (1975) proposed a pathway based on the work of Sacktor (1965). This scheme results in the anaerobic accumulation of alanine and malate at the expense of aspartate and pyruvate (Fig. II-1). After examining 11 species of spiders, Prestwich and Ing (in press) found similar activities for these enzymes and agreed that spiders in general might use this mechanism to help maintain redox under anaerobic conditions. Both studies suggested that the MDH-transaminase pathway was about 10 to 20% as active as the LDH pathway.

A third pathway was suggested by Prestwich and Ing (in press). They noted sufficiently high activities of cytosolic glycerol-3-phosphate dehydrogenase (GPDH) to suspect that it might play a role in oxidation of NADH during anaerobiosis. The products of this pathway would be glycerol-3-phosphate and pyruvate (Fig. II-1). Based on the relative activities of GPDH and LDH, the GPDH scheme appeared to be only about 10 to 20% as active as the LDH-catalyzed scheme.

Enzymological results such as these can only suggest the identity of anaerobic products. Regulatory schemes can result in potential anaerobic pathways being shut down under *in vivo* conditions. For example, in tuna, both LDH and GPDH are found in high activities; yet, only lactate

Figure II-1. A simplified schematic of glycolysis including three possible anaerobic schemes. The first pathway (lower left) is the reduction of pyruvate to lactate (solid box) utilizing lactate dehydrogenase (LDH). In the second scheme (shown at the bottom of the figure), NADH is oxidized in the production of malate from oxaloacetate, mediated by malate dehydrogenase (MDH). Oxaloacetate is generated through two transaminations mediated by the enzymes glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT). Malate and alanine accumulate in equimolar amounts at the expense of pyruvate produced during glycolysis and asparate from reserves in the cell. This pathway and the LDH mediated scheme result in a net gain of 3 ATP per molecule of glucose-6-phosphate (G6P) entering glycolysis. A third scheme involves the reduction of dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate (G3P, dotted box, middle right of figure) with an equal accumulation of pyruvate (lower left). This scheme depends upon glycerol-3-phosphate dehydrogenase (GPDH) and results in the net gain of 1 ATP per G6P metabolized.

A possible variation exists for the MDH-transaminase pathway (not tested in the experiments described in this chapter). In this scheme, malate is converted to fumarate and then reduced to succinate. This scheme would consume another pair of electrons and thus help maintain redox. It could also explain the decrease of malate under anaerobic conditions (Table II-2).


GLUCOSE
ATP
ADP
Pi
GLYCOGEN
GLUCOSE 6-PHOSPHATE
F 6-P
PFK
ATP
ADP
F 1, 6-diP
GA3P
DHAP
Pi
NAD
NADH
GPDH
ADP
ATP
GLYCEROL 3-PHOSPHATE
GLYCEROL
FATS
TO MITOCHONDRIA
(electron shuttle)
3-PGP
ADP
ATP
3-PG
PEP
ADP
ATP
MALATE
NAD
NADH
MDH
PYRUVATE
LACTATE
LDH
TO
MITOCHONDRIA
GLUTAMATE
OXALOACETATE
GPT
GOT
ALANINE
∝KETOGLUTARATE
ASPARTATE

accumulated under anaerobic conditions (Guppy and Hochachka 1978). Therefore, the purpose of the experiments outlined in this chapter was to determine the relative development of these three pathways by measuring the quantities of product produced as a result of vigorous activity. Additionally, I used the results to postulate a relationship between the anaerobic capabilities of different species of spiders and their respiratory systems and behaviors.

Materials and Methods

Animals

Spiders were collected in Alachua County, Florida, and were brought into the lab at least two weeks prior to experimentation. They were kept at *ca.* 25°C; this should be regarded as the acclimation temperature (Anderson 1970). Spiders were maintained on a diet of crickets and were last fed 7 to 10 days prior to their use to assure they were all in comparable nutritional and metabolic states (Anderson 1974). Three species were selected for extensive biochemical analysis; *Filistata hibernalis*, *Lycosa lenta*, and *Phidippus audax*. They were selected due to their different activity patterns and respiratory anatomies (Table 1-1).

Enzymatic Determination of Anaerobic Products

Spiders were placed in large beakers. After they remained motionless for at least one half hour (T_a = 25°C), they were instantly frozen using liquid nitrogen. Other spiders were stimulated to maximal activity by prodding with a metal rod and air puffs for two minutes and then

frozen. This procedure generally produces maximal activity (Anderson and Prestwich 1982). The frozen spiders were broken into sections, weighed rapidly to reduce condensation, and homogenized in 4°C 0.6M $HClO_4$ (1:4 or 1:8 w/v).

Cold homogenates were neutralized using 4°C 6M KOH, the solution was thoroughly mixed, allowed to stand several hours at 4°C and then filtered using a 934 AH glass fiber Whatman Filter. The filtrate was analyzed for the following substances using the method cited after each substance: L-alanine, Williamson (1974); L-glycerol 3-phosphate (Michal and Lang 1974); D-lactate (Gawehn and Bergmeyer 1974); L-malate (Gutmann and Wahlefeld 1974); and pyruvate (Czok and Lamprecht 1974). Readings were at 340 nm using a Beckman DU spectrophotometer.

Colorimetric Analysis of Anaerobic Products

Spiders were treated as described for enzymatic analysis except some individuals were run for periods that did not result in exhaustion. After freezing, spiders were homogenized in 10% TCA and filtered as described above. Analysis was according to the technique of Harrower and Brown (1972) with the exception that each sample was determined in duplicate along with an individual blank that did not receive the p-phenylphenol indicator. Samples were read on a Model 6/20 Coleman Jr. II Spectrophotometer at 585 nm.

The technique was validated by comparison of the results obtained against those obtained enzymatically. I was unable to compare homogenates directly by analyzing them both enzymatically and colorimetrically because of residual ClO_4^- which strongly interferes with the colorimetric test. Even after precipitation of this ion with KOH, some ClO_4^- remains in

solution (K_{sp} for $KClO_4$ at pH 7.0 at 0°C = 0.0029) and I found that even minute quantities of ClO_4^- affected the amount of color developed from lactate standards. Conversely, samples in TCA could not be used for enzymatic analysis since TCA inhibits LDH. Comparison of samples in both acids, when taken from spiders under identical conditions, revealed an overestimate of *ca.* 20%. Corrections were made for substances known to affect the intensity of color (Barker and Summerson 1941) if they were known to either accumulate anaerobically (Tables II-2, Ch. VI) or be present in spiders (Collatz and Speck 1970; Stewart and Martin 1970; Collatz and Mommsen 1975). The resultant values were consistently 10% greater than those obtained enzymatically. Nevertheless, given the advantages of low cost and repeatability, I felt justified in using the colorimetric method.

Results

Since lactate (presumably the L isomer since a Boehringer test kit had been used) was reported for an agelenid spider (Collatz and Mommsen 1975), first analyses were for L(+)lactate. I found none in any of my samples. This is in agreement with Long and Kaplan (1968), Gleason *et al.* (1971) and Prestwich and Ing (in press), all of whom found only D-LDH in spiders.

Subsequent enzymatic analysis for D-lactate confirmed its presence. Table II-1 shows the results for *F. hibernalis* and *L. lenta*. Values for *P. audax* are not included since I was only able to analyze the prosomas plus legs of five animals. Of these, resting (N = 2) levels of lactate

Table II-1. Changes in concentration of D-(-)-lactate as a consequence of activity in *F. hibernalis* and *L. lenta* (P = prosoma, L = legs, t = time of activity in seconds).

Species (N) Mass at t = 0; t = 120 sec	D-(-)-Lactate Concentration µmols/g (± SE) 0 sec	120 sec	Increase Arithmetic[a] (Factorial)
F. hibernalis (6) 0.270 g; 0.251 g (P + L)	1.7 (± 0.4)	16.2 (± 1.5)	14.5 (9.4X)
L. lenta (6) 0.523 g; 0.445 g (P + L)	0.9 (± 0.0)	10.9 (± 1.16)	10.0 (12.2X)
0.293 g; 0.246 g (P)	0.7 (± 0.0)	9.3 (± 0.7)	8.6 (13.1)
0.230 g; 0.200 g (L)	1.1 (± 0.1)	12.4 (± 1.3)	11.3 (11.2X)
	Opisthosomal D-Lactate Concentrations		
F. hibernalis (6) 0.318 g; 0.186 g	0.7 (± 0.3)	2.7 (± 1.1)	2.0 (3.9X)
L. lenta (6) 0.302 g; 0.186 g	0.6 (± 0.4)	3.5 (± 0.7)	2.9 (5.8X)
	Whole Spider D-Lactate Concentrations		
F. hibernalis (6) 0.588 g; 0.437 g	1.5 (± 0.4)	10.4 (± 1.3)	8.9 (7.1X)
L. lenta (6) 0.825 g; 0.631 g	0.7 (± 0.0)	9.6 (± 1.0)	8.9 (13.6X)

[a]Arithmetic scope = anaerobic capacity (Bennett 1978)
factorial scope = (t = 120 sec)/(t = 0 sec)

Table II-2. The concentrations of three possible anaerobic products as a function of activity. Data are for the prosoma and legs only.

Species (N) Mass at t = 0; t = 120 sec	Prosoma and Leg Concentrations µmols/g (± SE) Glycerol-3-Phosphate		Malate		Alanine	
	0 sec	120 sec	0 sec	120 sec	0 sec	120 sec
F. hibernalis (11) 0.290 g; 0.290 g	0.52 (± 0.09)	1.07 (± 0.17)	0.41 (± 0.05)	0.16 (± 0.06)	2.37 (± 1.27)	4.00 (± 1.56)
L. lenta (11) 0.578 g; 0.503 g	0.31 (± 0.07)	0.71 (± 0.14)	0.29 (± 0.09)	0.09 (± 0.01)	1.20 (± 0.22)	1.41 (± 0.19)
P. audax (4) 0.093 g; 0.091 g	0.37 (± 0.13)	0.68 (± 0.13)	0.63 (± 0.09)	0.43 (± 0.18)	--- ---	--- ---

were similar to those of the two other species while active (N = 3) levels were 20% less than those of *L. lenta*.

I also analyzed the same homogenates for the products of the other proposed anaerobic pathways (Fig. II-1). The results are reported in Table II-2. Malate showed a significant decrease during activity in both species, dropping 0.25 µmols/g in *Filistata* and 0.2 in *Lycosa*. Small, non-significant increases in alanine occurred in both species. The largest changes were in G3P where significant increases of 0.55 and 0.4 µmols/g occurred in *Filistata* and *Lycosa*, respectively. Pyruvate concentrations were below the limit of detection (0.05 µmols/g). There were no significant changes in any of these substances in the opisthosoma.

Colorimetrically Determined Lactate

Table II-3 shows the colorimetrically determined lactate concentrations of seven species at rest and after exercise. Lactate concentrations in resting spiders ranged as follows: 0.3 to 1.5 µmols/g (whole spider); 1.0 to 2.2 (prosoma plus legs); and 0.5 to 2.6 (opisthosomas). The resting concentrations were usually higher (within any species) in the prosoma plus legs. The range of lactate concentrations at the completion of exercise was 1.6 to 10.4 µmols/g (whole spiders); 5.2 to 16.2 (prosoma plus legs); and 1.2 to 5.2 (opisthosomas). The increases in lactate concentration after activity were significant in all species for both whole animals and for the prosomas plus legs. Also, concentrations after exercise were always significantly greater in the prosoma plus legs than in the opisthosoma.

In the opisthosoma, lactate increased but this was usually not statistically significant. Lactate concentrations in this compartment

Table II-3. The concentration of lactate (non-enzymatic determinations) as a function of activity in spiders.

Family Species	Total Mass (g) (% Opisthosoma)	N	Activity Time (sec)	Lactate Concentration (μmols/g) (± SE) Prosoma + Legs	Opisthosoma	Whole Spider
Araneidae						
Argiope aruntia	0.730	4	0	---	---	0.31 (± 0.08)
	0.720	5	120	---	---	1.63 (± 0.20)
Neoscona domiciliorium	0.450 (70%)	6	0	2.22 (± 0.52)	0.89 (± 0.15)	1.36 (± 0.19)
	0.360 (62%)	6	120	9.28 (± 0.23)	1.23 (± 0.19)	4.43 (± 0.23)
Nephila clavipes	0.517 (60%)	3	0	1.84 (± 1.15)	0.71 (± 0.27)	1.11 (± 0.29)
	0.560 (61%)	3	120	5.22 (± 1.78)	1.32 (± 0.12)	2.94 (± 0.32)
	0.639 (61%)	2	385	7.70 (± 0.53)	1.40 (± 1.12)	4.40[a] (± 0.49)
Filistatidae						
Filistata hibernalis	0.588 (54%)	5	0	1.72 (± 0.36)	0.70 (± 0.29)	1.46 (± 0.45)
	0.437 (43%)	5	120	16.20 (± 0.15)	2.79 (± 1.11)	10.40 (± 1.30)

Table II-3 continued

Family Species	Total Mass (g) (% Opisthosoma)	N	Activity Time (sec)	Lactate Concentration (μmols/g) Prosoma + Legs	Opisthosoma	Whole Spider
Lycosidae						
Lycosa lenta	0.516 (38%)	9	0	1.18 (± 0.20)	1.10 (± 0.46)	1.13 (± 0.18)
	0.598 (45%)	9	120	14.10 (± 0.66)	3.32 (± 0.61)	9.31 (± 0.54)
Oxyopidae						
Peucetia abboti	0.407 (74%)	6	0	1.38 (± 0.33)	0.54 (± 0.10)	0.75 (± 0.10)
	0.470 (75%)	6	120	10.80 (± 1.07)	1.63 (± 0.25)	3.94 (± 0.34)
Salticidae						
Phidippus audax	0.123 (30%)	6	0	1.20 (± 0.17)	2.63 (± 0.29)	1.40 (± 0.12)
	0.129 (29%)	6	120	12.36 (± 1.21)	5.19 (± 1.02)	10.31 (± 0.64)
	0.186	10	200	---	---	7.12 (± 0.81)

[a]N = 4

were negatively correlated with its relative size (relative size range was 29 to 75% of total mass). These differences in relative opisthosomal size are a function of interspecific differences (orb weavers tend to possess larger opisthosomas) and differences in nutritional history (Anderson 1974).

Increases in lactate concentration varied as a function of exercise level. The greatest increases resulted from activity culminating in exhaustion. These increases are referred to as anaerobic capacities (Bennett 1978) and range from 3.0 to 8.9 µmols/g for whole spiders. Accumulations resulting from sub-maximal exercise are given for *A. arantia* and *N. clavipes* at 120 seconds and *P. audax* at 200 seconds.

Discussion

Development of Anaerobic Pathways in Spiders

Barring the presence of other anaerobic pathways not investigated in this study, D-lactate appears to be the main by-product of anaerobic metabolism in spiders. Resting and exhaustion levels of this substance are within the range reported for L-lactate in reptiles and amphibians (Bennett 1978).

Lactate accumulations in each body compartment (Table II-3) are correlated with LDH activity (see Prestwich and Ing in press). Pierson product-moment correlation coefficients were 0.68 (leg LDH) and 0.71 (cephalothorax LDH) with the leg plus prosomal lactate accumulations and 0.89 between opisthosomal LDH and lactate accumulation. Only the opisthosomal correlation is significant ($P < 0.05$); however, the other results are suggestive.

Two other anaerobic pathways have been proposed to operate in spiders (Linzen and Gallowitz 1975; Prestwich and Ing in press). One of these schemes, catalyzed by MDH and the transaminases GOT and GPT should result in the equal production of alanine and malate (Fig. II-1). The data on Table II-2 for prosomas show that malate concentrations decrease during activity while alanine remains constant. These results argue against the use of this pathway in spiders. They are suggestive of the possibility that malate itself becomes an electron acceptor and is thereby converted to succinate. Glycerol phosphate dehydrogenase (GPDH) appears to help maintain cytosol redox. Use of this enzyme to oxidize NADH results in equal production of G3P and pyruvate. Since pyruvate is a substrate for LDH, its accumulation is not an accurate measure of the activity of this pathway. However, the only possible sinks for G3P during anaerobiosis are fat or glycerol synthesis (Fig. II-1). Since neither seems likely, the G3P accumulation should be a good measure of the development of this pathway. Prosomal accumulations of G3P are 0.55 (*F. hibernalis*) and 0.4 (*L. lenta*) µmols/g compared to simultaneous lactate accumulations of 12.8 and 9.1 µmols/g respectively (Table II-1). Thus, G3P accumulations are only about 5% of those of lactate in both species. The ratios of the activities of GPDH to LDH are, on the other hand, slightly larger (Prestwich and Ing in press) being about 10% in *Filistata* and 16% in *Lycosa*. This discrepancy could be due either to experimental error or inhibition of GPDH during anaerobiosis in order to prevent competition between the two enzymes for NADH (Guppy and Hochachka 1978).

A weighted calculation should be used to evaluate the relative contributions of the LDH- and GPDH-catalyzed pathways to the total

anaerobic production of high-energy phosphate bonds (~P) (assuming, of course, that no unknown pathways are involved). This is necessary since 1.5 ~P are gained per lactate that accumulates and 1 ~P per G3P (both calculations assume glycogen as the starting point for glycolysis: Stewart and Martin 1970; Collatz and Speck 1970). The proportion of the total anaerobic production of ~P due to the GPDH pathway is then only three percent of the total in both species and can be ignored.

The question arises as to why the lactate scheme is favored. Its superiority over the G3P-pyruvate scheme is obvious: formation of lactate yields three times as much ATP as the alternative scheme (Fig. II-1) and it produces smaller osmotic and pH changes in the cell on a per ATP basis. The differences do not exist between the LDH and the MDH-transaminase schemes as ATP gains and osmotic effects are comparable. The lack of an important MDH-transaminase pathway may relate to both its complexity and its requirement for a substrate (asparate) that is not part of glycolysis (Fig. II-1).

Determinants of Lactate Accumulations

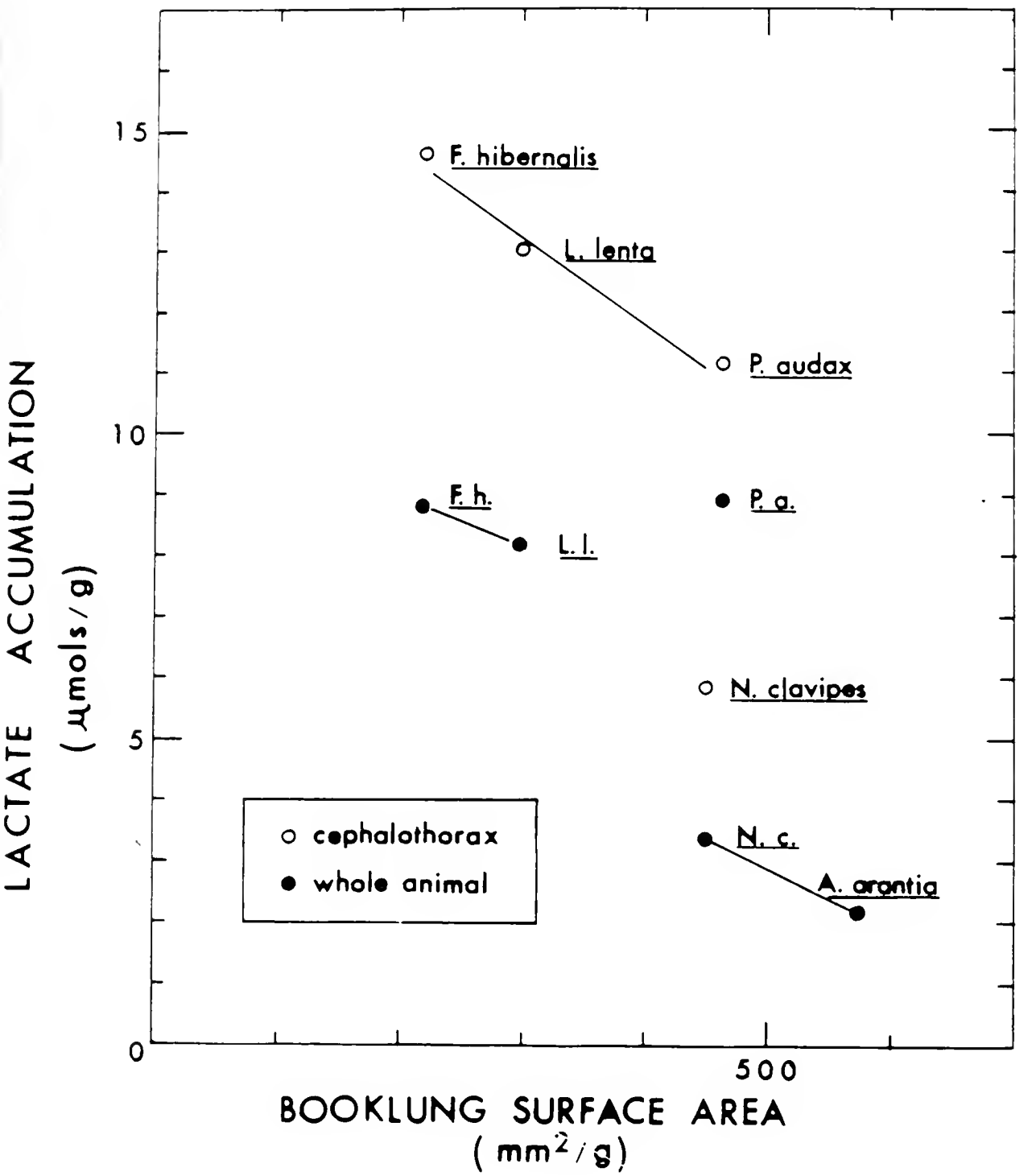
The increases in lactate in the prosoma and legs are associated with muscular activity. The smaller increases in opisthosomal lactate concentrations could be due to two factors: anaerobic activity of opisthosomal muscles indirectly associated with locomotion (Wilson 1970; Anderson and Prestwich 1975) or the transport of lactate through the hemolymph to the heart and digestive diverticulum for reoxidation (Long and Kaplan 1968) and perhaps gluconeogenesis. The inverse relationship between lactate concentration and relative opisthosomal mass is consistent with both possibilities.

Lactate accumulation is related to both the intensity of activity and the ability of a spider to deliver oxygen to its muscles, *i.e.* its aerobic capacity. The interplay between activity level and the aerobic and anaerobic capacities of animals is complex (Taigen *et al.* in press) and are the subject of the remainder of this chapter.

Aerobic capacity in spiders is proximally determined by the same factors as in vertebrates; respiratory surface area, ventilation, and circulatory capacities. Respiratory surface areas are known to vary interspecifically in spiders (Anderson 1970; Anderson and Prestwich 1982; also see Table I-1). At a common intensity of activity, anaerobic accumulation should be negatively correlated with respiratory surface area. I tested this hypothesis by constructing Figure II-2 for the five species (Table I-1) where these data are available.

Figure II-2 is consistent with the expected relationship for both whole animals and prosomas plus legs. The only exception is that the whole animal anaerobic capacity of *P. audax* is higher than predicted from the other species, probably due to a proportionally smaller abdomen (Table II-3). Figure II-2 indicates a distinct difference between orb weavers versus non-orb weavers. *Phidippus audax*, a non-orb weaver, has a book lung surface area similar to *N. clavipes*, an orb weaver; yet it accumulates more than twice the lactate in one third the time (Table II-2). The orb weavers and non-orb weavers represent two distinct, homogeneous behavioral groups. While the relative speeds (prosoma lengths/sec) *within* either group were the same, the non-orb weavers were excellent runners. The orb weavers were simply not morphologically adapted to move as rapidly. As they ran or climbed they spent a great

Figure II-2. The relationship between anaerobic capacity and book lung surface area. Data are plotted on a mass-specific basis due to differences in size (Table II-3) and on both on a whole animal (solid circles) and a prosoma plus legs (open circles) basis. The latter approach is favored due to a "dilution effect" from the largely inactive abdomens of widely variable size (Table II-3). The figure indicates two important trends: first, larger mass-specific book lung surface areas are associated with smaller lactate accumulations since proportionately more O_2 should be delivered to the tissues in these species; and secondly, no obvious influence of tracheal development is apparent since *P. audax* has a much larger tracheal system than any of the other spiders but it falls on a line predicted by other non-orb weavers possessing greatly reduced tracheal systems. Finally, the lower lactate accumulations in orb weavers are interpreted as due to the slower movements of these spiders.


LACTATE ACCUMULATION
(μmols/g)
15
10
5
0
F. hibernalis
L. lenta
P. audax
F. h.
L. l.
P. a.
N. clavipes
N. c.
A. arantia
cephalothorax
whole animal
500
BOOKLUNG SURFACE AREA
(mm²/g)

deal of time flailing about with their legs. This behavior is probably not very costly since the spider does not need to pull its entire mass. Its function is probably related to searching out paths through the foliage into which these spiders may retreat when continually threatened.

One surprise from Figure II-3 was that *P. audax* prosoma plus leg lactate accumulations fell on a line predicted by the accumulations and book lung surface areas of the other two non-orb weavers. Since *P. audax* alone possesses an extensive cephalothoracic tracheal development (Table II-2), I expected O_2 delivery in this species to be more constant than in species without significant trachea (Anderson 1970). Given greater O_2 availability and a common level of activity, I expected much smaller lactate accumulations, but they were predictable based on book lung surface area. The role of trachea in spiders needs further study. One possibility relates to aerobic scope. Since the aerobic scope of jumping spiders is higher than that of *F. hibernalis* and *L. lenta* (see Ch. III), the trachea may allow a higher threshold for the transition between aerobic and anaerobic conditions. Also, they could help decrease the time needed to repay an oxygen debt.

The presence of a relationship between book lung surface area and lactate accumulation implies a co-adapted respiratory-circulatory system. Increased respiratory surface area will not by itself provide more oxygen to the tissues. More circulatory transport of O_2 is required. This can be achieved via enhancing both the cardiac output and hemolymph O_2 capacity.

To minimize anaerobic accumulations, the proposed co-adapted respiratory-circulatory system must be capable of delivering well-oxygenated hemolymph at least intermittently to the active prosoma. In

spiders, this implies a correlation between the book lung surface area, the strength of the myocardium, and the opisthosomal sub-cuticular muscle sheet. The muscle sheet and heart are vital in this scheme because they operate together to produce the hemolymph pressures great enough to pump blood to the prosoma of active individuals (Stewart and Martin 1974).

There are no comparative data on heart strength in spiders. However, Wilson (1970) showed marked differences in the development of the opisthosomal sub-cuticular muscle sheet that correlated directly with the activeness of a given species of spider. He interpreted this as an adaptation to reduce fluid loss from the prosoma to the opisthosoma during maximal exercise (see Chs. I and V). I propose that an equally (if not more likely) function is to develop high opisthosomal pressures to assure hemolymph flow for a greater proportion of activity and thereby reduce the need for anaerobic metabolism. Studies on the relative development of opisthosomal and heart musculature, prosomal pressures, the book lung surface area, and anaerobic accumulations are needed to test this hypothesis.

CHAPTER III
THE RATES OF ANAEROBIC AND AEROBIC METABOLISM DURING ACTIVITY

Summary

1. The relative importance of aerobic and anaerobic metabolism in active spiders was investigated in four species, *Filistata hibernalis*, *Lycosa lenta*, *Phidippus audax* and *Neoscona domiciliorium*.
2. Peak $\dot{V}O_2$ varied from 2.3 to 5.8 times the resting $\dot{V}O_2$. Within any one species this ratio decreased with T_A. Aerobic capacities were directly related to book lung surface area.
3. Estimates of the relative importance of anaerobic metabolism to total power generation during short, maximal struggles (less than two minutes) varied from between 55 to 94% of the total power generation. Anaerobic dependence was inversely associated with respiratory surface area.
4. The anaerobic contribution to construction of orb webs in *Neoscona domiciliorium* was estimated to be *ca.* 1% of the total cost of the web.
5. Calculations based on lactate accumulations, recovery oxygen, and the known stoichiometry of gluconeogenesis and complete oxidation of lactate suggest most of the lactate accumulated during struggle is reconverted to hexose during the recovery period.

Introduction

Many spiders rely on a mix of aerobic and anaerobic metabolism to fuel their activities. The relative importance of these two sources of high energy phosphates (~P) have not been studied in spiders.

Spiders are characterized by relatively limited aerobic capabilities. Their resting rates of oxygen consumption ($\dot{V}O_2$) are low, ranging from 24 to 122% of the predicted $\dot{V}O_2$ for ectotherms of their size (Dresco-Derouet 1960; Anderson 1970; Greenstone and Bennett 1980; Anderson and Prestwich 1982). Their maximum $\dot{V}O_2$ values are likewise low: factorial aerobic scopes (the ratio of maximal to resting $\dot{V}O_2$) are below 10 and most are below 6 (Seymour and Vinegar 1972; Peakall and Witt 1976; Ford 1977a, b; Prestwich 1977). These compare with factorial aerobic scopes of 20X in mammals and several hundred in insects (McArdle 1981; Weis-Fogh 1964).

To compensate for their low aerobic abilities, spiders have reasonably well-developed anaerobic capacities (Linzen and Gallowitz 1975; Prestwich and Ing in press; Ch. II). Anaerobic accumulations in spiders may be negatively correlated with both respiratory surface area and resting $\dot{V}O_2$ (Fig. II-2; and see Anderson and Prestwich 1982), given a common level of activity. Thus, spiders with the poorest O_2 exchange ability, being less able to generate ~P compounds aerobically, rely to a greater degree on anaerobic metabolism.

This study seeks to partition the contributions of aerobic and anaerobic metabolism to total ~P production during both maximal and sub-maximal activities. Three species (*Filistata hibernalis*, *Lycosa lenta*, and *Phidippus audax*) that possess different respiratory surface

areas and anatomies were used to study near-maximal activity. Evaluation of relative anaerobic dependency in these species allowed for testing of the hypothesis that anaerobic dependence is inversely correlated with respiratory surface area. Because the relative anaerobic dependence of poikilotherms is frequently temperature dependent (Bennett 1978), these measurements were made at three different temperatures. Estimation of the anaerobic contributions to long-term activities in spiders were obtained by measuring the anaerobic accumulations during orb-weaving in *Neoscona domiciliorium* and comparing these with the estimated aerobic costs. Finally, the data on oxygen consumption and lactate removal during recovery allowed speculation as to the metabolic fate of lactate in spiders.

Methods

Animals

Individuals of the species *Filistata hibernalis*, *Lycosa lenta*, and *Phidippus audax* were used for the experiments involving maximal struggles. These spiders were brought into the lab and treated as described in Chapter II. Lactate accumulations incurred during orb weaving were made by freezing individuals of the araneid species *Neoscona domiciliorium* as they finished construction of their webs in the field. A summary of the characteristics of these four species is given in Table I-1.

Oxygen Consumption ($\dot{V}O_2$)

Oxygen consumption was measured manometrically using a Gilson Differential Respirometer. Spiders were placed in either 15, 50, or

120 ml respirometer flasks depending on their size. This permitted roughly equal areas for spiders to move about during their struggles. Each respirometer flask contained a measured amount of soda lime as a CO_2 absorbant and also a number of glass beads or ball bearings.

After several hours of temperature equilibration, resting $\dot{V}O_2$ was measured using the shortest feasible time intervals (five or ten minutes). Exercise was initiated by manually shaking each flask for two minutes. This caused the balls to bounce about the flask and induced the spiders to struggle.

The shaking was done with the flasks out of the water bath in order to observe the spider's activities. At 15 and 33°C it was necessary to periodically reimerse the flasks for *ca.* 10 sec (with swirling) in order to prevent manometer fluid overflow. Blanks were treated identically to the animal flasks.

Most spiders struggled for the majority of the two minutes. However, some spiders wedged themselves into the neck of the flask to avoid the balls. If a spider avoided moving for more than 20 sec, it was dropped from the experiment.

At the end of the two minute activity period, recovery $\dot{V}O_2$ was recorded for the next 30 to 55 min. Measurements of $\dot{V}O_2$ were made every five or ten minutes depending on my ability to discern a measurable change in the manometric level. All data were converted into $\mu l\ O_2/(g \cdot h)$ at STPD and mean $\dot{V}O_2$ and standard errors (SE) for each interval were calculated. The results were plotted as a function of rest, exercise, and recovery.

An interesting phenomenon occurred during many measurements. Near the completion of exercise or more often, early in the recovery period,

many individuals of all species expelled large (> 100 µl) volumes of gas. The gas release caused a manometer deflection opposite to that produced by consumption of O_2. Some of the deflections persisted for five to ten minutes and thereby obfuscated any changes in $\dot{V}O_2$. If the deflections lasted more than five minutes, the experiment was terminated and the data discarded. These deflections were never observed in the blanks.

To determine if these gas pulses were due to a burst release of CO_2 [perhaps related to lactate-caused changes in hemolymph pH (Angersbach 1978)], I loaded the flasks identically to the oxygen consumption experiments, except that in place of the spider there were two small vials containing solutions of $NaHCO_3$ and HCl. The quantity of $NaHCO_3$ was adjusted to result in the evolution of *ca.* 150 µl of CO_2 when the solutions were mixed. I then recorded the time course for reabsorption of the gas. The removal of CO_2 generally took less than 3 minutes and was faster than for a similar volume of gas produced by the spiders. There are two likely explanations for this: (a) the spiders released CO_2 over a long time span and/or (b) other gases were also released. Option (a) requires that volumes of CO_2 in excess of 100 µl be frequently released by the exhausted spiders. A calculation based on the estimated blood volume (Stewart and Martin 1970), CO_2 carrying capacities (Loewe and deEggert 1979), and estimated tissue $\dot{V}CO_2$ (based on $\dot{V}O_2$ measurements) shows this to be impossible. There is not enough CO_2 stored in the hemolymph or being produced via respiration to account for such a large release of CO_2. Thus, the most plausible explanation is that spiders release a mixture of gases during these pulses. However, their exact compositions remain unknown.

The practical implication of these observations was that it introduced both a degree of uncertainty and also made the measurement of $\dot{V}O_2$ more difficult. Given the different time courses for the removal of these evolved gases, it is likely that the exact gas composition was variable. If CO_2 composed a relatively small proportion of a gas pulse, the result would be an artificially low $\dot{V}O_2$ (because this gas would not be absorbed). Thus, I decided to discard the data from any run where the positive pressure deflection persisted for over five minutes.

Due to the common occurrence of pulsed gas release, I was usually unable to obtain a measurement of $\dot{V}O_2$ immediately at the end of the activity period. Therefore, I used a five or ten minute interval for measurement of the $\dot{V}O_2$ for activity: five minute intervals were possible in *Lycosa* and *Phidippus* at all temperatures because they had high $\dot{V}O_2$, but were feasible for *F. hibernalis* only at 33°C. Thus, the exercise interval also includes the first three to eight minutes of recovery. However, this was not a problem in *Lycosa* and *Filistata*. In cases where no gas release was apparent, noticeable increases in $\dot{V}O_2$ in both *Lycosa* and *Filistata* did not occur until one or two minutes after the completion of exercise (see Results and Discussion). However, increases in $\dot{V}O_2$ in *P. audax* did occur during activity periods and their full magnitude may have been partially obscured by gas releases.

Recovery oxygen (recovery VO_2) was difficult to measure precisely in *Phidippus* and *Filistata* due to the common occurrence of activity during recovery (see Results). I estimated recovery VO_2 as the total oxygen used above resting levels between the end of the exercise-early recovery $\dot{V}O_2$ measurement (at +3 minutes of recovery) until either

resting $\dot{V}O_2$ was reached or until a time when activity caused $\dot{V}O_2$ to increase (see Results and Discussion). Other experiments will show that in *Filistata* and *Lycosa* lactate concentrations remain constant during the first 3 to 5 minutes of recovery (Ch. IV). The principal uses of the first few minutes' recovery O_2 may be to fully saturate the spiders hemocyanin (Angersbach 1978) and resynthesize depleted stores of ~P compounds. Thus, the recovery VO_2 I report are probably good measures of the O_2 needed to remove the lactate.

Anaerobic Accumulations

Spiders were placed in respirometer flasks and treated identically as in the $\dot{V}O_2$ measurements. After either two minutes of stimulated activity or after two minutes of activity followed by 15 minutes of recovery, the respirometer flasks were opened and the spiders instantly frozen in liquid N_2. This operation required less than 15 sec and the spiders did not move significantly during the time required to open the flask and freeze them. The frozen spiders were homogenized and analyzed using the colorimetric method of Harrower and Brown (1972) as modified and described in Chapter II. Resting levels of lactate were not measured given the constancy of these values for individuals within any species. To obtain anaerobic accumulations, resting lactate concentrations given in Table II-3 were subtracted from the values I obtained for this chapter. Factorial increases in lactate were calculated by dividing active or recovery lactate concentrations by resting concentrations.

Heart Rates

Heart rates of resting and recovering *F. hibernalis* and *L. lenta* were measured at 25°C. After measuring resting heart rates, these spiders were exercised identically to those in the $\dot{V}O_2$ experiments. At the end of the activity period, they were quickly placed in glass cages and their heart rates monitored by the use of a laser as described in Anderson and Prestwich (1982). These rates were compared with those given for free-running or restrained spiders in Chapter IV.

Calculations of Energy Equivalents of Aerobic and Anaerobic Metabolism and the Energetics of Lactate Oxidation and Gluconeogenesis

Assuming that the substrate for energy metabolism during exercise is glycogen (Stewart and Martin 1970; Collatz and Speck 1970), the total number of high energy phosphate bonds (~P) synthesized per hexose residue cleaved from a glycogen polymer can be estimated as follows:

Aerobic metabolism: results in (maximally) a synthesis of 38 ~P per hexose residue. This requires six O_2; therefore, 0.282 µmols ~P are produced per µl O_2 used.

Anaerobic metabolism: results in a net synthesis of 3 ~P per hexose residue removed from glycogen. Thus, 1.5 µmols ~P are formed per µmol of lactate produced.

To calculate the amount of O_2 needed to drive gluconeogenesis from lactate to glucose-6-phosphate (this ignores the final costs involved with glycogen synthesis) or the amount of O_2 needed to fully oxidize lactate, the following relationships can be used:

Gluconeogenesis: Three ~P per lactate are needed to make glucose-6-phosphate. These could be formed aerobically

by the use of 1/2 of a mol of O_2. Therefore, gluconeogenesis requires 11.2 µl O_2 per mol of lactate.

Oxidation: This process requires 3.0 mols of O_2 per mol of lactate or 67.2 µl O_2 per µmol lactate.

Anaerobic Contribution to Orb-Web Building

Lactate contribution to orb-web construction was measured by collecting adult *Neoscona domiciliorium* in the field as they finished building their webs. They were dropped into a large volume of swirling liquid N_2 and kept frozen until returned to the lab. There, they were thawed while being homogenized in cold TCA. Lactate analysis was by the colorimetric method (Ch. II).

Results

Oxygen Consumption

The spiders struggled vigorously during most of the two minute stimulation period but they reduced their movements in the last minute. Plots of $\dot{V}O_2$ as a function of exercise and recovery are shown in Figs. III-1, 2, and 3 for temperatures of 15, 25, and 33°C respectively. Generally, resting and active $\dot{V}O_2$ were highest in *P. audax* and lowest in *F. hibernalis*.

Increases in $\dot{V}O_2$ during the two minutes of activity were evident mainly in *P. audax*. In the other two species increases in metabolic rate were usually not seen until one to five minutes after the end of activity. Delayed increases in $\dot{V}O_2$ were especially obvious in

Figure III-1. Oxygen consumption, before, during, and after a two minute struggle in respirometer flasks at 15°C. Spiders were acclimated at 25°C. The black bar is the activity period, vertical bars through means are standard errors.

Key: ■ *F. hibernalis*

○ *L. lenta*

△ *P. audax*

For each point, N $\geq$ 20.

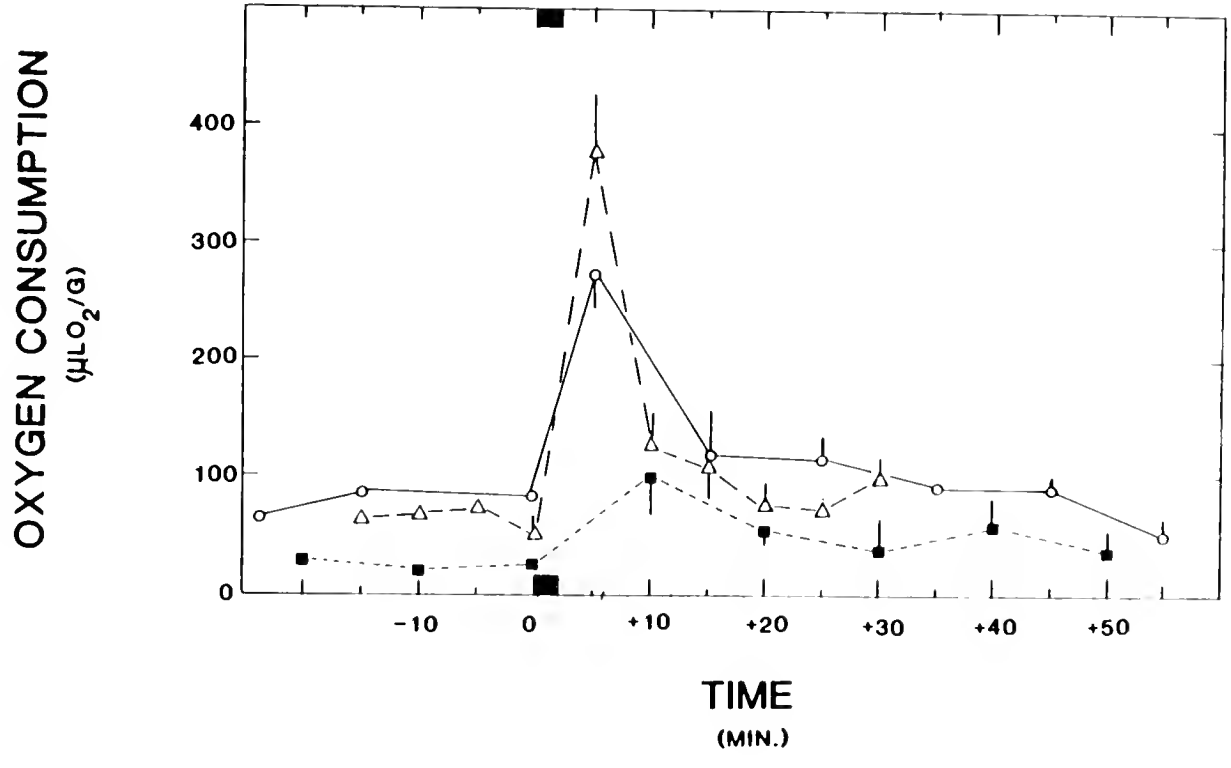

OXYGEN CONSUMPTION
(μL O2/G)
400
300
200
100
0
-10
0
+10
+20
+30
+40
+50
TIME
(MIN.)

Figure III-2. Oxygen consumption before, during, and after a two minute struggle in respirometer flasks at 25°C. Spiders were acclimated at 25°C. The black bar is the activity period, verticle bars through means are standard errors.

Key: ■ *F. hibernalis*

○ *L. lenta*

△ *P. audax*

For each point, N $\geq$ 20.

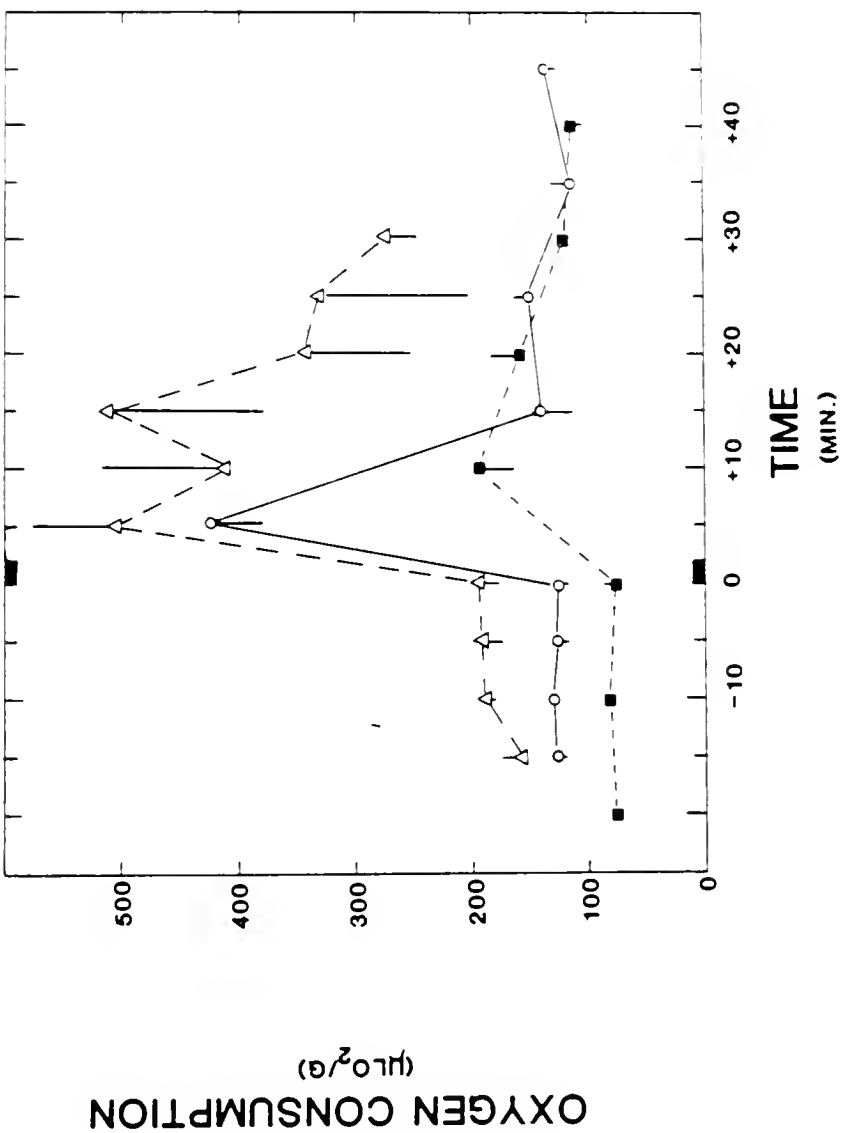

OXYGEN CONSUMPTION
(μLO2/g)
500
400
300
200
100
0
-10
0
+10
+20
+30
+40
TIME
(MIN.)

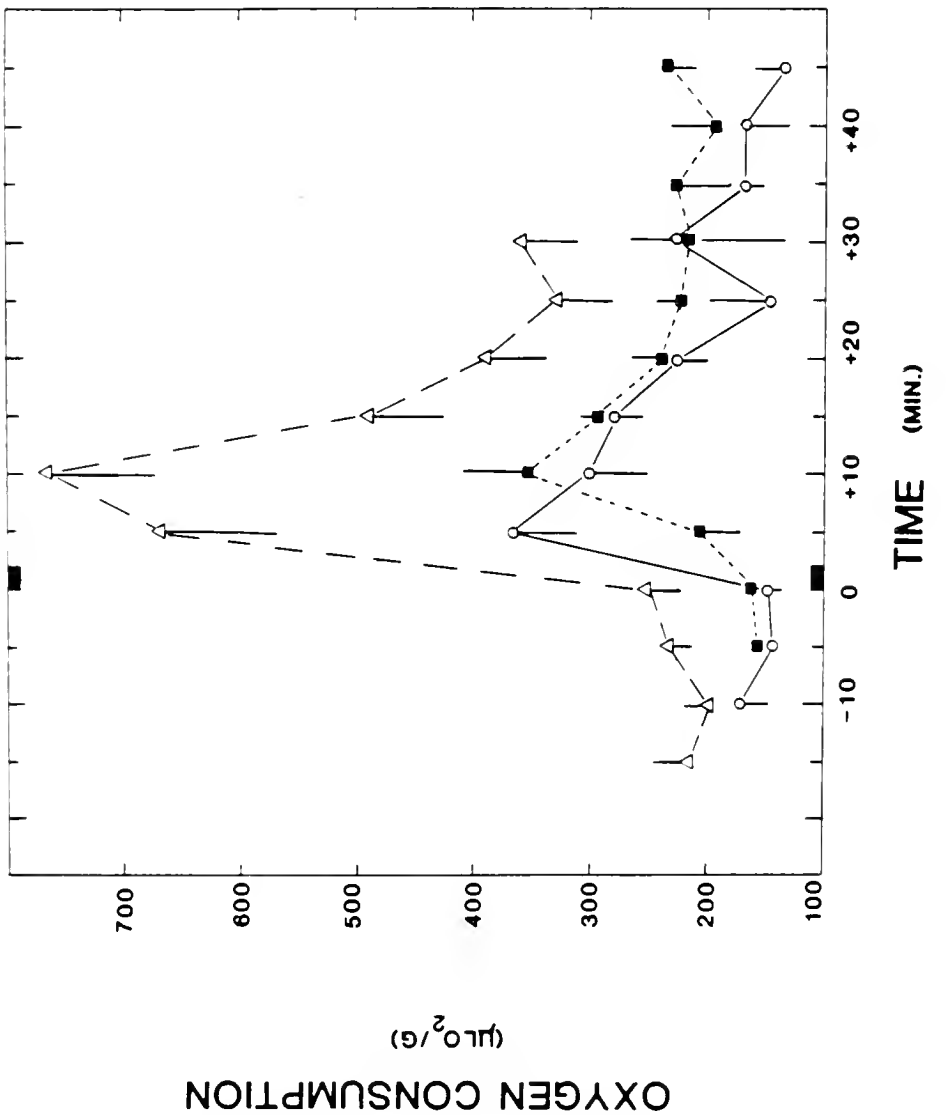
Figure III-3. Oxygen consumption before, during, and after a two minute struggle in respirometer flasks at 33°C. Spiders were acclimated at 25°C. The black bar is the activity period, verticle bars through means are standard errors.

Key: ■ *F. hibernalis*

○ *L. lenta*

△ *P. audax*

For each point, $N \geq 20$.


OXYGEN CONSUMPTION
(μLO2/G)
700
600
500
400
300
200
100
-10
0
+10
+20
+30
+40
TIME (MIN.)

F. hibernalis. This was most evident at 33°C (Fig. III-3) where it was possible to use five minute measurement intervals. Peak $\dot{V}O_2$ was not reached until after three minutes of recovery.

The length of the recovery period appeared to be shortest in *P. audax*, intermediate in *Lycosa*, and longest in *Filistata* especially where *P. audax* recovery data are not complicated by activity (Fig. III-1). Recovering individuals of *Filistata* and *Phidippus* tended to become active before their $\dot{V}O_2$ had returned to pre-struggle levels. In *P. audax* this occurred at 25° and 33°C as individuals continued activity after the end of stimulation and never calmed down. This accounts for the continued high $\dot{V}O_2$ shown for this species in Figures III-2 and 3. In *Filistata* exploratory and grooming behavior commonly appeared after 40 minutes of recovery. This prevented me from fully measuring the recovery O_2 in this species. Therefore, the data for recovery O_2 in *Filistata* represent a minimum estimate. Recovery and activity VO_2 for all three species are given in Table III-2. Finally, the resting $\dot{V}O_2$ in *Neoscona domiciliorium* measured at 25°C was 245 $\pm$ 22 µl O_2(g·h) at STPD (N = 20, mass = 0.492 g $\pm$ 0.003 g). These rates were taken from spiders hanging in "webs" (silk attached to wooden rods in the respirometer flasks).

Anaerobic Metabolism

Lactate concentrations immediately at the end of exercise and 15 minutes later are shown for *F. hibernalis*, *L. lenta*, and *P. audax* in Table III-1. In *Neoscona*, lactate concentrations at the completion of web-building (T_A = 23°C, field collected samples) were 4.7 $\pm$ 0.7, prosoma: 1.5 $\pm$ 0.1 opisthosoma; and 2.1 $\pm$ 0.25 µmols/g whole animal,

Table III-1. Anaerobic metabolism in spiders that struggled for two minutes in respirometer flasks.

Species	N	Mass ± SE (g)	T_A (°C)	Lactate Concentrations (μmols/g) 2 min	(percent)[a]	15 min	(percent)[a]
F. hibernalis	4	0.252 ± 0.03	15	2.52	(44%)	---	
	4	0.294 ± 0.04	25	6.64	(63%)	4.9	(61%)
	4	0.345 ± 0.04	33	6.78	(69%)	---	
L. lenta	4	0.483 ± 0.05	15	3.27	(51%)	---	
	4	0.498 ± 0.02	25	5.18	(47%)	3.9	(58%)
	4	0.465 ± 0.03	33	5.79	(55%)	---	
P. audax	4	0.164 ± 0.02	25	4.41	(34%)	---	

[a]Percentages express the size of the anaerobic accumulation for spiders in respirometer flasks compared to free-running spiders (Ch. IV).

Table III-2. Oxygen and lactate metabolism during exercise and recovery in spiders. Exercise VO_2 is calculated for a five minute interval including the two minutes of exercise and first three minutes of recovery (see Methods). Data are derived from Figures III-1, 2, and 3 and represents the amount of oxygen used above the resting VO_2 (*i.e.* the net recovery O_2). Lactate concentrations are all changes from the previous reading. Positive readings are increases in lactate.

		Δ VO_2 (µl O_2/g)		Δ Lactate (µmols/g)	
Species	T_A (°C)	Active (0-5 min)	Recovery (5-40 min)	0-2 min	2-15
F. hibernalis	15	1.7	26.0	2.2	--
	25	2.5	40.8	5.2	-1.7
	33	1.9	60.6	5.1	--
L. lenta	15	9.0	28.8	3.0	--
	25	12.1	29.2	4.1	-1.3
	33	9.4	43.3	3.9	--
P. audax	15	15.6	21.2	--	--
	25	14.8	59.2[a]	3.4	--
	33	18.5	90.7[a]	--	--

[a]Estimated by straight line extrapolation. This was necessary due to large amounts of activity starting 10 to 15 min after the end of forced activity.

N = 4 for all measurements. Anaerobic accumulations and factorial increases (values in parentheses) of lactate during web-building were as follows: prosoma, 2.5 µmols/g (2.1X); opisthosoma 0.6 (1.7X); and whole spiders 0.7 (1.5X).

Heart Rates

Heart rates of alert *Filistata* and *Lycosa* at 25°C were 40 and 48 beats per minute (bpm) respectively. At the completion of exercise the respective rates were 114 and 122 bpm. After 25 minutes of recovery the rates had dropped to about 45 bpm in both species.

Discussion

Aerobic Metabolism

The pre-exercise (or routine) $\dot{V}O_2$ shown in Figures III-1, 2, and 3 agree with resting rates calculated for these species using the appropriate $\dot{V}O_2$ and Q_{10} values from Anderson (1970) and Anderson and Prestwich (1982).

The factorial aerobic scopes vary between 2.3 to 5.8X resting and are within the range reported for spiders of 2 to 6X resting $\dot{V}O_2$ (Seymour and Vinegar 1973; Peakall and Witt 1976; Ford 1977a, b; Prestwich 1977). Aerobic scopes and capacities (maximum $\dot{V}O_2$) are consistently largest in *P. audax* and smallest in *F. hibernalis*.

In all three species aerobic capacity increases with T_A. However, aerobic scope declines with temperature because resting $\dot{V}O_2$ increases at a faster rate than active $\dot{V}O_2$. This is consistent with the idea that maximum O_2 exchange is somewhat temperature dependent due to changes

in ventilation and circulation, but is finally limited by the respiratory exchange surface area (Ultsch 1973).

It is important to know how close $\dot{V}O_2$ was to the maximum for each species. It was partially for this reason that heart rates and lactate accumulations were measured. Accumulation of lactate implies insufficient delivery of O_2 to the muscles and, therefore, near maximal $\dot{V}O_2$. Heart rates in spiders are elevated after activity in order to (a) redistribute hemolymph between prosoma and opisthosoma (Wilson and Bullock 1973), (b) fully saturate the hemocyanin with O_2 (Angersbach 1978), (c) to provide O_2 necessary for reactions that replenish depleted pools of ~P, and (d) to circulate lactate from the muscles and to provide O_2 to metabolize this lactate (Ch. IV). Factors a through c are probably resolved during the first few minutes of recovery (Wilson and Bullock 1973; Stewart and Martin 1974; Angersbach 1978; Chs. IV and VI) while the oxidation of lactate may take longer than one half hour (Ch. IV).

Together, the heart rate and lactate data indicate that exercise was near maximal. Heart rates at the completion of activity were *ca.* 95% those found in free-running spiders and lactate concentrations ranged between 35 and 70% those found in exhausted spiders. Any large accumulations of lactate such as these indicate a relative insufficiency of O_2 delivery to active muscles. This implies O_2 consumption is at peak level and further increases in work could only be fueled by greater production of lactate.

In spiders not possessing extensive trachea, high work loads are not compatible with the highest rates of oxygen consumption. This is because maximal activity is accompanied by high prosomal hemolymph

pressures that prevent delivery of oxygenated hemolymph to the active prosomal muscles (Wilson 1970; Stewart and Martin 1974; Anderson and Prestwich 1975; Ch. V). Thus, the physiological state of a spider's prosoma during vigorous activity resembles the situation in diving animals: muscles reach peak activity while relying only on O_2 already present in the animal. In spiders this O_2 is bound to the hemocyanin already present in the prosoma. This is probably a small amount of O_2 because (a) in resting spiders the arterial hemocyanin may be only half saturated (Angersbach 1978) and (b) the O_2 capacity of spider hemolymph is low. Thus, peak $\dot{V}O_2$ for maximal activity will be measured late in exercise as prosomal pressure drops (Stewart and Martin 1974; Anderson and Prestwich 1975; Ch. V) and normal gas exchange and circulation is resumed (Angersbach 1978). This unusual arrangement in part explains why peak $\dot{V}O_2$ is observed late in the exercise period or early in recovery (Figs. III-1, 2, and 3). The highest rates of oxygen consumption in spiders may occur when they are engaged in less than maximal activities that require low prosomal hemolymph pressures and thus permit constant circulation and exchange of O_2 (Anderson pers. comm.; see Appendix I).

Estimated total mass-specific VO_2 ($\mu l\ O_2/g$) for activity and recovery are shown in Table III-2 (see Methods for a description of the measurement interval). The differences in the exchange abilities between the three species are evident with *F. hibernalis* using the least and *P. audax* the most O_2 in what appeared to be comparable activity. The opposite pattern occurred in recovery. *Filistata hibernalis* generally used the greatest amount of O_2, consistent with its usually largest lactate accumulations (this ignores the 25° and 33°C data for *P. audax*

because continued activity of these spiders prevented measurement of recovery $\dot{V}O_2$). The actual difference in recovery VO_2 between *F. hibernalis* and the other two species is even greater than indicated in Table III-2. At the end of 40 minutes *Filistata* still had a slightly elevated $\dot{V}O_2$ while *Lycosa* and *Phidippus* (15°C only) had returned to resting values.

Lactate Removal

In *Lycosa* (and *Phidippus* at 15°C), it is probable that all lactate was removed after 35 minutes of recovery because $\dot{V}O_2$ was back to resting levels by this time and because this is more than sufficient time to remove greater amounts of lactate from free-ranging spiders (Ch. IV). Total recovery VO_2 is probably a reasonable estimate of the amount of O_2 required to remove the accumulated lactate.

In *Filistata*, the $\dot{V}O_2$ was still elevated at the end of 45 minutes (Fig. III-1-3) when spontaneous activity increased the $\dot{V}O_2$ of most individuals. However, some of the lactate accumulation was probably still present at this time given that lactate removal is slower in free-ranging individuals of this species than it is in *Lycosa* and *Phidippus*. Therefore, recovery VO_2 on Table III-2 is probably an underestimate of the actual requirements for recovery in this species.

The pathway(s) used to remove lactate during the recovery period cannot be actually demonstrated without the use of labelled compound. However, a reasonable guess as to the fate of lactate can be made by calculating the amount of O_2 required to remove lactate through either gluconeogenesis or oxidation and then comparing these numbers to the measured recovery oxygen. This is done in Table III-3 for each lactate

Table III-3. Estimated and actual recovery oxygen volumes (VO_2) derived from Table III-2.

Species	T_A (°C)	Actual VO_2 0-2	Actual VO_2 2-15	Estimated VO_2 For Lactate Accumulated 0-2 min	Estimated VO_2 For Lactate Removed between 2-15 min
F. hibernalis	15	26	--	25-148	---
	25	41	16	58-349	19-114
	33	61	--	57-342	---
L. lenta	15	29	--	34-202	---
	25	29	26	46-276	15-87
	33	43	--	44-262	---
P. audax	15	21	--	---	---
	25	59	--	38-228	---
	33	91	--	---	---

[a]These values are all from Figures III-1, 2, and 3.

[b]The range of estimated VO_2 is based on the exercise lactate (0-2 min) accumulation and on the removal of lactate between minutes 2 and 15 given in Tables III-1 and 2. The range of estimated VO_2 values starts with all lactate being reconverted to glucose (low value) to the estimated VO_2 for complete oxidation of the lactate (large value). The calculations assume 0.5 µmol O_2/µmol lactate (11.2 µl O_2/µmol lactate) for gluconeogenesis and 3.0 µmol O_2/µmol lactate (67.2 µl O_2/µmol lactate) for oxidation to CO_2 and H_2O.

accumulation. The measured VO_2 are close to those required to use most of the lactate in gluconeogenesis and completely oxidize only a small portion. If this is indeed the case, then spiders resemble vertebrates where about 80% of the lactate is reconverted to hexose and 20% is completely oxidized (Bennett 1978).

Relative Contributions of Aerobic and Anaerobic Metabolism During Peak Activity

High-energy phosphate compounds for burst activity come from three sources: aerobic metabolism, anaerobic "lactacid" metabolism, and depletion of phosphagen stores (principally arginine phosphate and ATP). Calculations of the relative importance of aerobic and lactacid power generation for the conditions used in this study are given on Table III-4. At 25°C estimates of total high-energy phosphate production are similar in all three species. This is consistent with the observation that the spiders all underwent similar intensities of struggles during the two minute activity period.

Consistent differences in anaerobic dependencies exist between the species: *Filistata* is definitely the least aerobic spider deriving between 87 to 94% of its power from lactate production. The other two species have lower anaerobic dependencies, utilizing anaerobiosis for *ca.* 55% (*Phidippus*) and 65% (*Lycosa*) of the total. These figures compare to a range of 58 to 96% anaerobic dependence for burst activities in lower vertebrates (Bennett 1978). The calculations indicate that the relative importance of anaerobic power generation does not change as a function of temperature.

For three reasons these estimates should be treated with caution: (a) aerobic inputs during activity are to a degree uncertain (see

Table III-4. Estimated anaerobic dependences during a two minute struggle in a respirometer flask. High-energy phosphate figures are derived from the VO_2 and lactate accumulations on Table II-2.

		~P Bonds Formed			
Species	T_A	Via Aerobic	Via Anaerobic	Total	% Anaerobic
F. hibernalis	15	0.5	3.3	3.8	87%
	25	0.7	7.8	8.5	92%
	33	0.5	7.6	8.1	94%
L. lenta	15	2.5	4.5	7.0	64%
	25	3.4	6.2	9.6	65%
	33	2.7	5.8	8.5	68%
P. audax	15	4.2	5.1	9.3	55%

Methods and Discussion), (b) free-ranging spiders have lactate accumulations that are about twice as great as those used in the calculations in Table III-4, and (c) inputs from phosphagens are ignored. Biases due to factors a and b tend to cancel each other. Factor c could be significant. Using values for whole spider concentrations of arginine phosphate (AP) reported by Di Jeso *et al.* (1967) and assuming all AP is hydrolyzed during a struggle, the proportional dependence on lactate drops to between 43 and 70% with AP providing between 21 and 39% of the power. Thus, the contribution of phosphagens may be important.

Respiratory Surface Area and Anaerobic Dependence

All results are consistent with the hypothesis that an inverse relationship exists between anaerobic dependence and respiratory surface area (Fig. II-2). Relative dependencies of *Lycosa* and *Filistata* are also consistent with the observations of Anderson and Prestwich (1982) on the abilities of the two spider's respiratory-circulatory systems to deliver O_2 to the tissues. They found that at 20°C it took resting *Filistata* an average of 28 heart beats to deliver 1 µl of O_2 compared to 16 beats for *Lycosa*. These two species have similar maximum heart rates; therefore, the rate of O_2 delivery to the tissues should be less in *Filistata* in agreement with the much slower recovery and lower peak $\dot{V}O_2$ in this species (Table II-2; Figs. III-1, 2, and 3).

Anaerobic Dependence During Orb-Web Building

Long-term activities such as orb-web construction contrast sharply with the burst activities discussed above. Table III-5 shows the estimated anaerobic dependence of orb-web building to be low in *Neoscona*

Table III-5. Estimated anaerobic dependence of *Neoscona domiciliorium* during a two minute struggle and 1 hour of web-building.

Activity	Activity VO_2 ($\frac{\mu l\ O_2}{g}$)	Lactate Accumulation (μmols/g)	Estimated High-Energy Phosphate Production[d]			
			Aerobic	Anaerobic	Total	Anaerobic
Free ranging struggle (2 min)	8.2[a]	4.2[c]	9.2	6.3	15.5	41%
Web-building (~ 1 h)	245[b]	0.7	69.1	1.0	70.1	1%

[a] Assumes peak $\dot{V}O_2$ = 5X resting $\dot{V}O_2$, increment = 4X resting $\dot{V}O_2$

[b] Assumes active $\dot{V}O_2$ = 2X resting (Peakall and Witt 1976; Prestwich 1977); therefore, the increment = 1X resting VO_2

[c] Data from Table II-3

[d] Calculations as in Table III-4.

domiciliorium. In this species, web-building is essentially entirely aerobic if costs are estimated at the moment the web is completed. In contrast, burst activity is approximately 40% anaerobic (using data for *Neoscona* from Table II-3). By reliance on aerobic metabolism, an orb-weaver uses a more energy- and substrate-efficient process and maintains a reserve (anaerobic) power generating capacity for use if it is threatened during web-building and needs to escape.

CHAPTER IV
ACTIVITY AND RECOVERY IN SPIDERS

Summary

1. The relationship between lactate accumulation, heart rate, and fatigue in maximally active spiders was investigated to learn the cause of fatigue in spiders.
2. Spiders that were forced to run for two minutes show two stages of fatigue. In the phase I, they lose nearly two thirds of their maximal speed in 20 to 30 seconds (*Lycosa* and *Filistata*, respectively). This striking decrease in speed is followed by a slower phase of fatigue that takes the rest of the activity period and is characterized by a much slower rate of decrease in speed. Scorpions have a similar pattern of maximal activity (Fig. IV-1).
3. Maximum heart rates are not reached until late in the two minute activity bout or, more commonly, early in recovery.
4. Lactate accumulations in the prosoma similar to levels known to cause fatigue in other animals are reached after about one minute of maximal activity (Figs. IV-5, 6, and 7). However, lactate accumulations in *Lycosa* during the first 20 sec of activity (Fig. IV-8) are not high enough to explain the rapid fatigue that occurs during this time (phase I; see above).
5. During recovery, lactate is apparently circulated to the opisthosoma from the prosoma. In the opisthosoma it is probably either completely

oxidized or resynthesized into glucose. This arrangement allows rapid removal of lactate from the muscles where it is probably responsible for fatigue to an area where it can be metabolized without affecting the ability of the spider to locomote. This decreases the time necessary for recovery.

Introduction

In the preceding chapters I have demonstrated that the major anaerobic by-product in spiders is D-lactate (Ch. II) and that anaerobic metabolism is the dominant energy supply during maximal activity (Ch. III). The purpose of the experiments described in this chapter are to detail the changes in running speed, lactate concentration, and heart rate that occur during two minute struggles and during the recovery period following these struggles. Specifically, the data will be used to test the hypothesis that lactate accumulations are the main limitation on maximal exercise in spiders.

Methods

Animals

Three species of spiders (*F. hibernalis*, *L. lenta*, and *P. audax*) and one scorpion (*C. hentzi*) were used. All individuals were maintained in plastic boxes or Petri dishes in the lab at a temperature of *ca.* 25°C. They were last fed 7 to 10 days before use in an experiment (Anderson 1974). Water was freely available to all individuals except *F. hibernalis* (this species dies when exposed to moist conditions).

Running Speeds

Animals were exercised in an area measuring 1.0 x 0.3 x 0.2 meters having an interior marked in 0.1 m grids. Spiders were placed in the runway and then prodded with a blunt rod to force them to run. Speeds were determined by measuring the distance the spider traveled over a five second period and were expressed as either cm/s or prosoma length/s. In spiders the latter measure is preferable to body lengths/s since the size of the opisthosoma varies greatly in different species and according to the nutritional status of an individual (Anderson 1974). Prosomal lengths were determined on CO_2-anesthesized spiders after the completion of speed measurements by using a Gaertner measuring microscope. All measurements were made to 0.01 mm.

Not all spiders ran continuously during the two minute exercise period. Some individuals spent a great deal of time attacking the rod. However, the attacks were usually brief. If any attack lasted more than 10 sec the data were discarded. At the end of the two minute exercise period most spiders moved only very slowly and only when sharply prodded. I considered this to be exhaustion and it occurred in most spiders after between 90 to 150 sec of activity.

At the completion of 120 sec activity, spiders were placed in a vial for either five or ten minutes to recover. They were then put back in the runway and their speed was measured over the initial five second period of activity to provide an index of recovery.

Heart Rates

Heart rates were measured on both free and restrained spiders using the methods of Carrell and Heathcote (1976) and Anderson and Prestwich

(1982). Restrained spiders were glued to glass rods as described in Chapter V Methods except that no other attachments were made to the animals. Heart rates in alert spiders were about 1.5 to 2 times those obtained for resting spiders of the same species (Anderson and Prestwich 1982).

Changes in heart rate in active and recovering spiders were determined by mechanically stimulating the restrained spiders for two minutes and then allowing them to remain undisturbed for up to one hour while they recovered. As a control, several *L. lenta* were exercised at 25°C in the runway described above, quickly placed in a glass box, and their heart rate during recovery was measured. No differences were found between these and the restrained spiders heart rates during recovery.

Lactate Determinations

Lactate concentrations were measured on spiders at rest and after various intervals of running and recovery. Samples were obtained by freezing the spiders in liquid N_2 and then dividing them into prosomal and opisthosomal sections. These pieces were quickly weighed to prevent condensation, ground to a fine powder under liquid N_2, and then homogenized in cold 10% TCA. This solution was filtered and later analyzed according to the procedures for the colorimetric determinations outlined in the Chapter II Methods.

Statistics

Standard errors were calculated for all means. Tests of significance were based on Student's T-test and the level of significance was defined at 0.05.

Results

Running

Prodding results in activity patterns that, while maximal, are nevertheless somewhat abnormal, especially in *P. audax*. In the field, *Phidippus* actively hunts in shrubbery using vision to locate prey and avoid enemies. A dragline of silk is constantly laid down by the spider as it runs and jumps about. When placed in a running arena and touched, they often sidestepped and dodged the prod, frequently making 180° turns instead of running. While turning, they often became entangled in their own draglines. The result of these maneuvers was to slow them down. However, these jumping spiders were maximally engaged in constant activity and most individuals refused to move more than minimally at the end of the two minute stimulation period.

Lycosa lenta apparently also has good vision but individuals of this species attacked the prod instead of sidestepping it. These attacks were frequent and violent: the rod was tightly seized and repeatedly bitten, reminiscent of prey capture (Rovner 1980). When these spiders were shaken loose, they would run rapidly. This pattern was similar to what I observed in the field. When I disturbed a *L. lenta*, it fled to its burrow or some nearby cover. If continuously pursued, *Lycosa* attacked. Thus, the forced activity in the arena approximated field behavior in this species.

Finally, *F. hibernalis* individuals ran continuously as long as prodded. After repeated prods some assumed a tight, ball-like posture and never attempted to attack. In field conditions, a threat is met by

retreat into the tubular sanctuary of the web. If a spider cannot locate the retreat, it will run a considerable distance.

The results of forced running at 25°C are shown in Figure IV-1. Speeds are expressed as prosomal lengths per sec. Integration of each curve gives the total relative distance (prosomal lengths) traveled in two minutes. By making the total distance traveled by *Lycosa* equal to 100%, the relative distances covered by *Filistata* and *Phidippus* were 96% and 104% respectively with no statistically significant differences amongst these species.

The effect of T_A on running speed is illustrated for *Filistata* in Figure IV-2 and summarized for all species on Table IV-1. For the table, the running speeds have been fitted to an equation for exponential decay:

$$\log_{10} S = i + d(t) \qquad (1)$$

where S is the absolute speed in mm/sec, i is the $\log_{10}$ of the initial (maximum) speed, d is the rate of change in speed and t is the total time since the start of running in seconds. Coefficients of determination (r^2) range from 0.75 to 0.96 with most above 0.86 indicating that the data fit the model.

The values of i can be used to compare the initial running speeds. In each species, the initial speed increases with T_a and *L. lenta* always has the fastest initial speed. The rate of change of speed (d) is generally similar in all species at any common temperature. In both *Lycosa* and *Filistata*, the smallest decreases in speed with respect to running time occur at 25°C and the greatest rates of decrease are at 33°C.

Another measure of the decrease in speed as a function of running time is the time required for running speed to decrease to a value that is about a third of the initial speed (see Table IV-1 for a more rigorous definition of this measure of exhaustion). By this measure, *L. lenta*

Figure IV-1. Running speed at 25°C during two minutes of forced activity and after five and ten minutes of recovery (+ 5 and +10). Speeds are relative speeds given as prosoma lengths/sec. Standard errors (SE) are shown as bars; when not shown the SE is smaller than the symbol.

Key:

◑ *C. hentzi*

■ *F. hibernalis*

○ *L. lenta*

△ *P. audax*

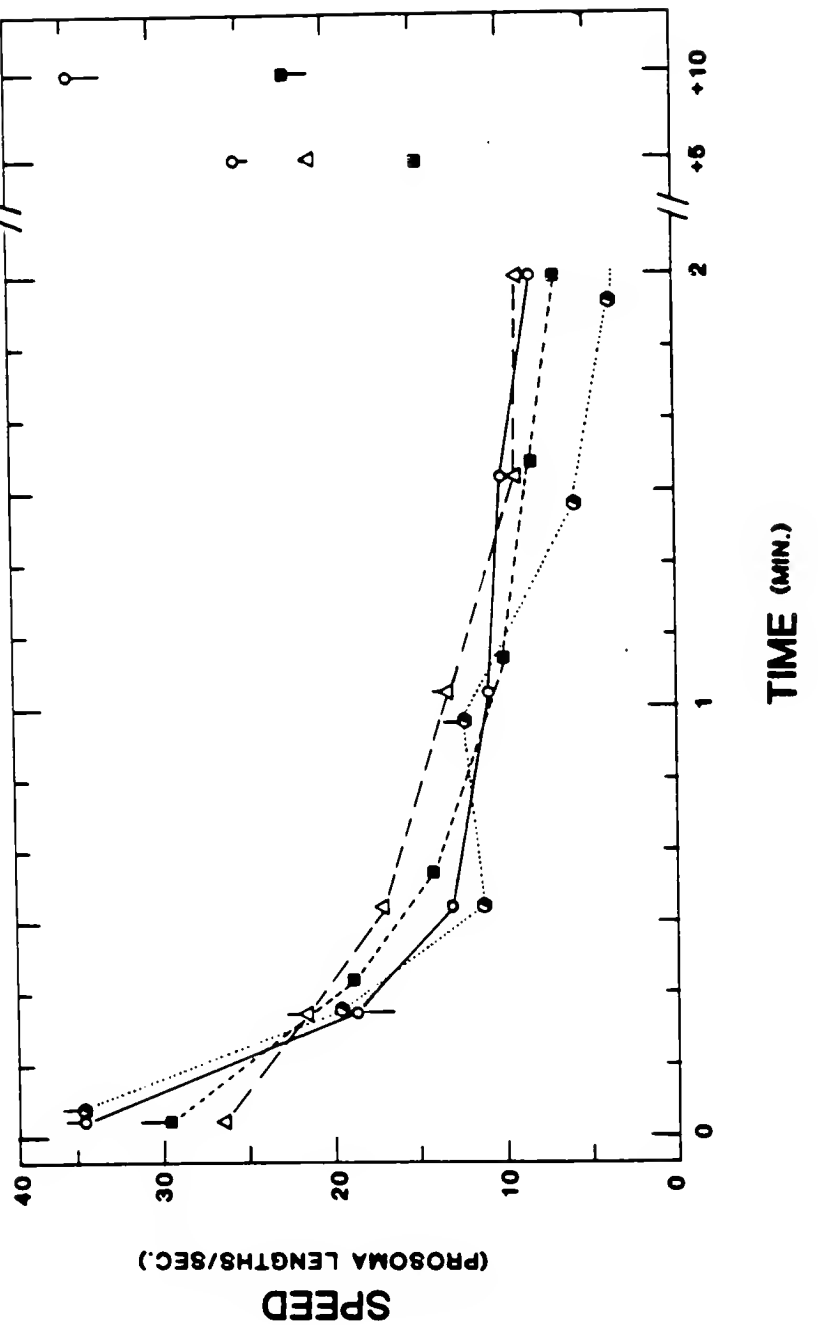

SPEED
(PROSOMA LENGTHS/SEC.)
40
30
20
10
0
0
1
2
+5
+10
TIME (MIN.)

Figure IV-2. Running speeds at different temperatures in 25°C acclimated *F. hibernalis*. The top curve is for 33°C, middle 25°C, and lowest curve 15°C.

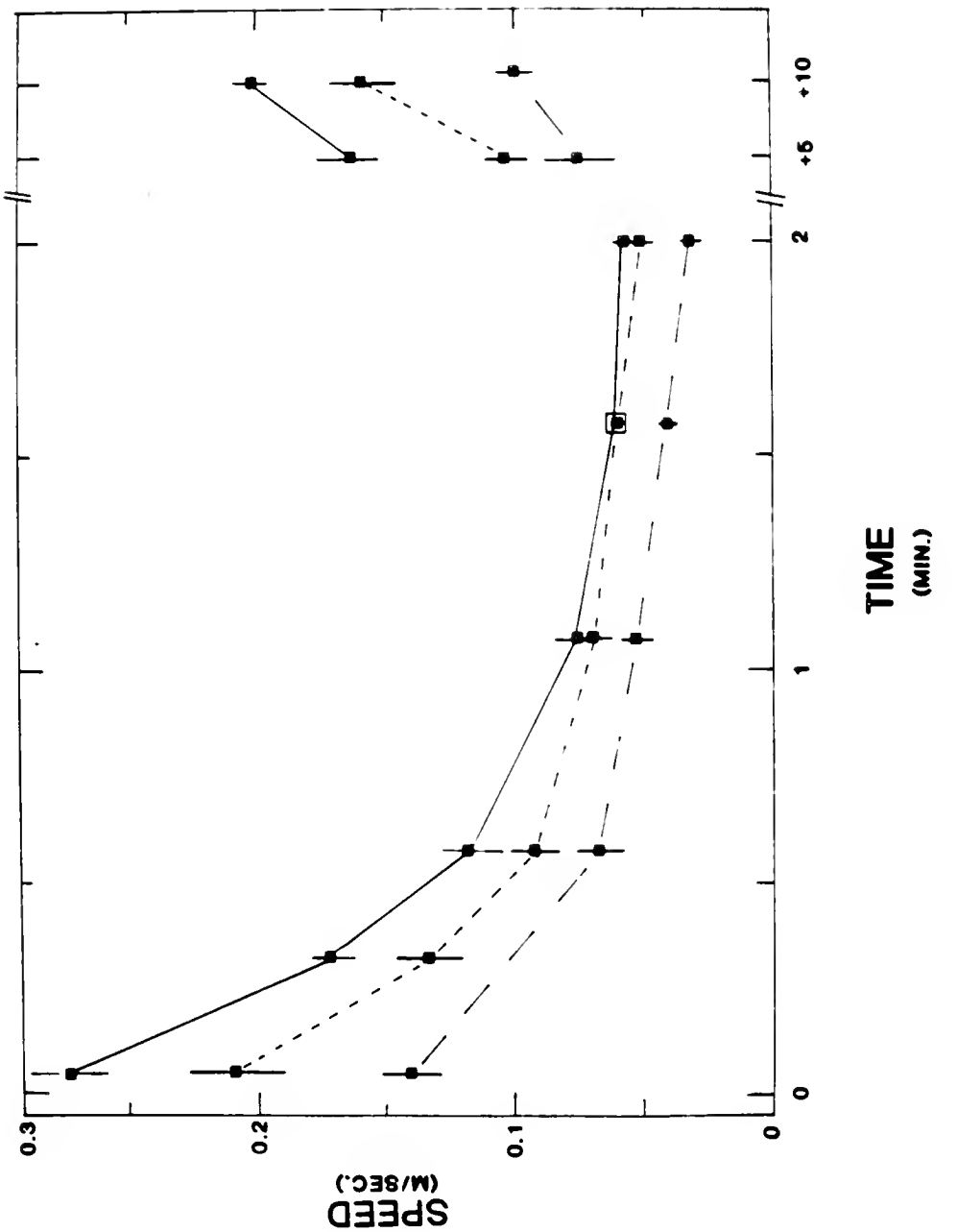

SPEED
(M/SEC.)
0.3
0.2
0.1
0
0
1
2
+5
+10
TIME
(MIN.)

Table IV-1. Running speeds and recovery in spiders and a scorpion. The data are fitted to a mathematical model of the form: $\log_{10} S = i + d(t)$ where S is running speed in cm/sec, i is the initial speed (average over 0 to 5 sec), d is the rate of change of speed, and t is the running time in seconds. Coefficients of determination (r^2) are given for each regression. The last columns are measures of fatigue and recovery and are explained below and in the text.

Species	N	T_A	Regression Equation i	d	r^2	Length of Phase I[a] (seconds)	Percent of Maximum Speed[b] +5	+10
C. hentzi	10	25°C	2.1151	0.0077	0.93	28	---	---
F. hibernalis	10	15°C	2.1653	0.0051	0.94	37	54%	71%
	12	25°C	2.2951	0.0048	0.87	30	49%	76%
	9	33°C	2.4222	0.0059	0.92	31	60%	74%
L. lenta	12	15°C	2.2067	0.0051	0.89	38	61%	78%
	12	25°C	2.4957	0.0044	0.97	20	73%	100%
	12	33°C	2.5998	0.0056	0.86	18	63%	84%
P. audax	7	25°C	2.1889	0.0042	0.96	45	84%	---

[a]Phase I is defined as the time required for a spider to exhaust such that it is only running at 133% of its final speed. Thus, it has decreased in speed 2/3s of the way to a minimum (phase II fatigue) speed.

[b]Percent of maximum speed is the maximum running speed after five or ten minutes of recovery divided by the initial (maximum speed, see Figs. IV-1 and 2) times 100.

slows the most rapidly, especially at 25 and 33°C, having reached this speed in 20 sec or less. This is largely a reflection of the high initial running speed in this species. By contrast, it takes *P. audax* 45 sec to have slowed by an equal proportion (also see Fig. IV-1).

By the time the speed of *Lycosa* and *Filistata* had slowed by two thirds, *ca.* 10% of the individuals were plainly dragging their IVth pair of legs. When this occurred, it had always happened before the completion of 20 sec of running. There was no correlation between initial running speed and the development of leg dragging behavior. In the remaining 90% of the individuals of both these species, this behavior was not observed.

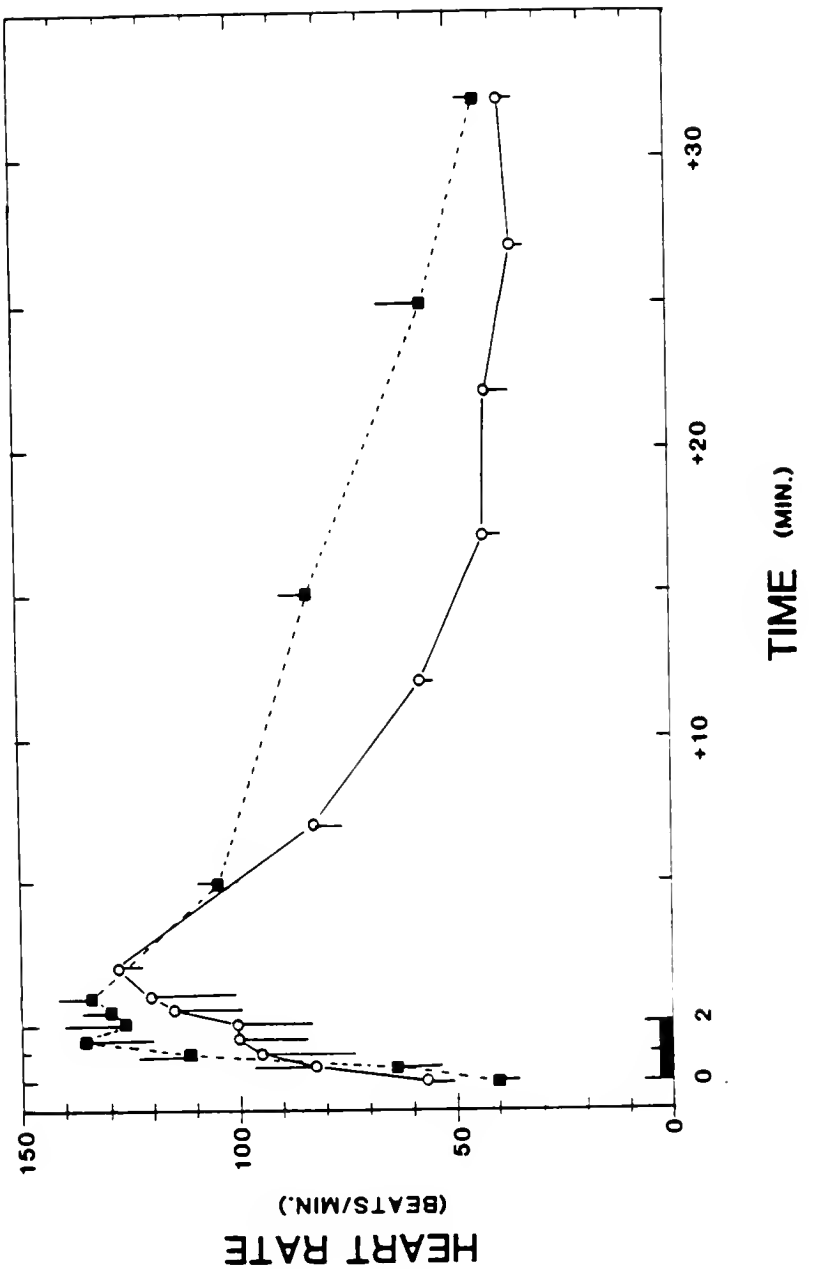
Comparison of running speeds after five or ten minutes of recovery with the initial (0-5 sec) running speeds gives a measure of recovery (Figs. IV-1, 2; Table IV-1). Although all spiders did comparable amounts of exercise over two minutes (except at 33°C--see Table IV-6), *P. audax* and *L. lenta* tend to recover the most rapidly. Recovery rates (speed after resting/initial running speed) are similar at all three temperatures in *F. hibernalis*; however, in *Lycosa* recovery is fastest at 25°C.

Finally, the running and recovery performances of *C. hentzi*, a scorpion, is similar to the three species of spiders in terms of both relative speed (Fig. IV-1) and total distance covered (91% of *Lycosa*). However, the rate of decrease in speed is greater in this species (Table IV-1).

Heart Rates

Figure IV-3 shows the effect of exercise and recovery on heart rates of restrained *L. lenta* and *F. hibernalis* at 25°C. Maximum rates are

Figure IV-3. Heart rates at 25°C in active and recovering *F. hibernalis* (■) and *L. lenta* (O). Note that peak heart rates are not reached until late in exercise when the spider is already fatigued (Fig. IV-1) or during recovery.


HEART RATE
(BEATS/MIN.)
150
100
50
0
0
2
+10
+20
+30
TIME (MIN.)

similar in both species and occur either near the end of exercise or early in the recovery period. However, the magnitude and rate of the increase in heart rate is greater and the recovery is slower in *F. hibernalis*. During recovery, interspecific heart rates are significantly higher in *Filistata* after five minutes of recovery and remain so until +33 min into the recovery period. At this time, *Lycosa* has a rate below its routine heart rate while *Filistata*'s rate is still slightly (but not significantly) elevated.

The effect of temperature on heart rate is shown for *F. hibernalis* in Fig. IV-4 and the data for both species are summarized in Table IV-2. Maximum rate and pattern of recovery is similar in both species (*Lycosa* and *Filistata*) at all three temperatures. Both species recover most rapidly at their acclimation temperature (25°C), although *Lycosa* recovers sooner than *Filistata*. Finally, the Q_{10} for both alert and maximal heart rates are between 1.3 and 1.8.

Lactate Production

Figure IV-5, bottom panel, shows change in lactate concentrations for whole spiders at 25°C. At any given time there is usually no significant difference in lactate concentrations between species. It is not until 15 to 25 min into the recovery period that lactate concentrations are significantly lower than those found at the end of exercise. Complete removal of lactate (recovery) probably takes over 30 minutes in all three species.

Because the relative opisthosomal sizes differ in these three species, whole animal lactate concentrations are misleading as they can obscure

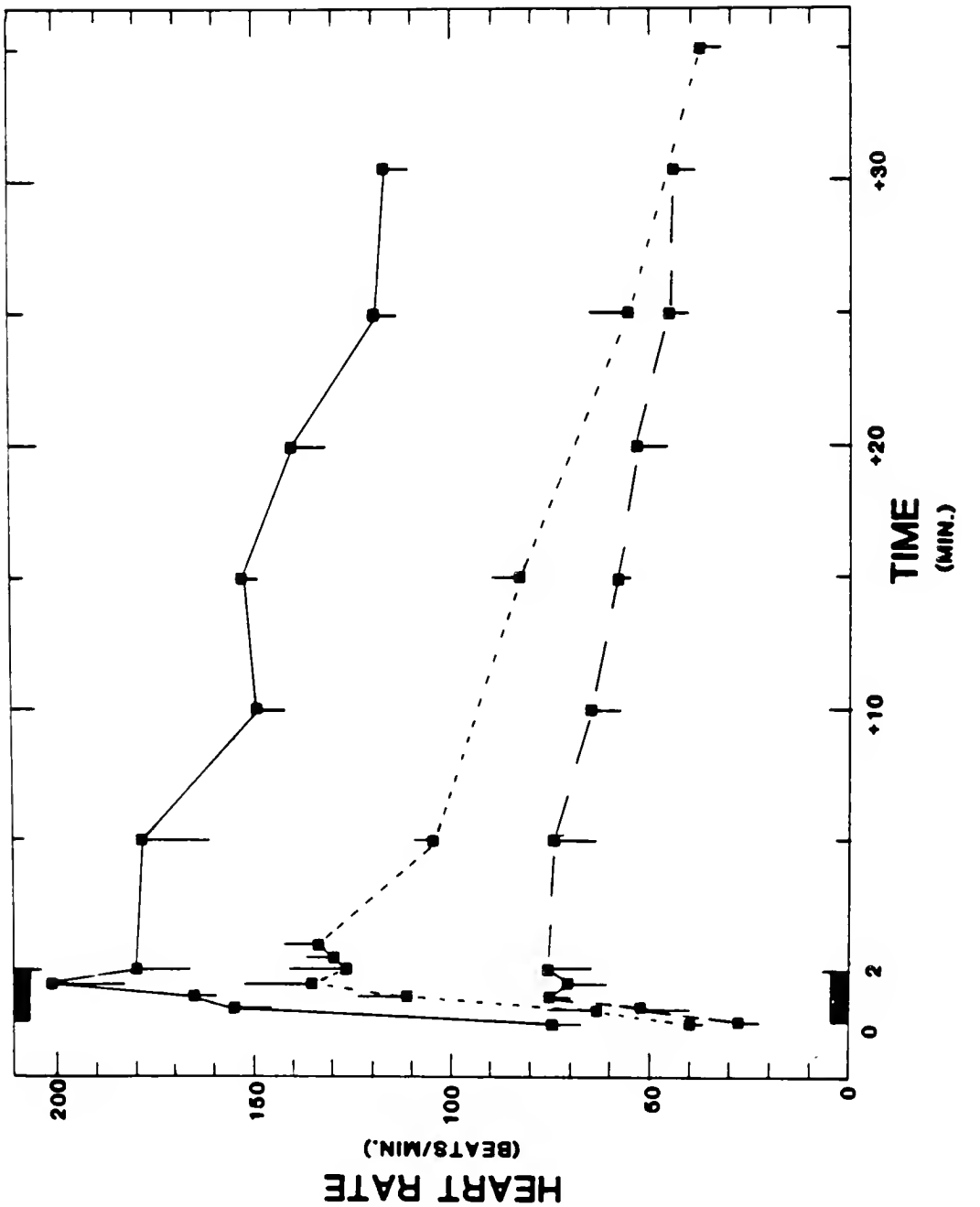
Table IV-2. Heart rates in two species of spiders as a function of activity and temperature. All spiders were acclimated at 25°C.

Species	N	T_a	Heart Rates (beats/min) Alert	Maximum	Percent of Maximum Rate[a] + 5	+ 10	66% Decreased[b] Time (min)
F. hibernalis	10	15°C	27	75	99%	87%	> 30
	14	25°C	40	135	77%	69%	19
	8	33°C	74	180	88%	74%	30
L. lenta	10	15°C	--	76	87%	71%	--
	17	25°C	56	138	62%	49%	7
	10	33°C	70	170	85%	86%	> 30

[a]Heart rate after 5 or 10 minutes of recovery divided by the maximum observed rate times 100.

[b]The time in minutes required for the heart rate to decrease from maximum to a value that is 133% greater than the alert heart rate.

Figure IV-4. The effect of temperature on the heart rates of active and recovering *F. hibernalis* (acclimated to 25°C). The curves, from top to bottom, are for 33°, 25°, and 15°C.


HEART RATE
(BEATS/MIN.)
200
150
100
50
0
0
2
+10
+20
+30
TIME
(MIN.)

interspecific differences in lactate in the working muscles of the prosma and legs (Table II-3). Partition into the prosoma (and legs) and opisthosoma of the same individuals as shown in Figure IV-5C points out interspecific differences.

In the prosoma and legs (Fig. IV-5, top panel) the resting and exhaustion (120 sec) lactate concentrations and also the maximum rate of lactate production (anaerobic scope, Bennett 1978) are different in all three species, being distinctly higher in *F. hibernalis*. By contrast, the highly active jumping spider *P. audax* was the lowest in all of these categories. A detailed look at the changes in lactate concentration that occur during the first 30 sec of activity in *L. lenta* is shown in Figure IV-8. In all species, recovery is characterized by a steady decrease in lactate concentration over 15 to 25 minutes (Fig. IV-5; the initial rise in concentration in *L. lenta* during the recovery period is not statistically significant.)

The handling of lactate by the opisthosoma (Fig. IV-5) is different than in the prosoma. Lactate accumulations during activity are much smaller than in the prosoma and are negatively correlated with opisthosomal size. Lactate concentrations during the recovery period were generally significantly higher than the pre-exercise concentrations. However, there are no statistically significant differences between opisthosomal lactate concentrations after 15 to 25 min of recovery compared to the concentration at the end of exercise. While prosomal lactate concentrations dropped in all species during this time, opisthosomal concentrations remained unchanged and at an elevated level.

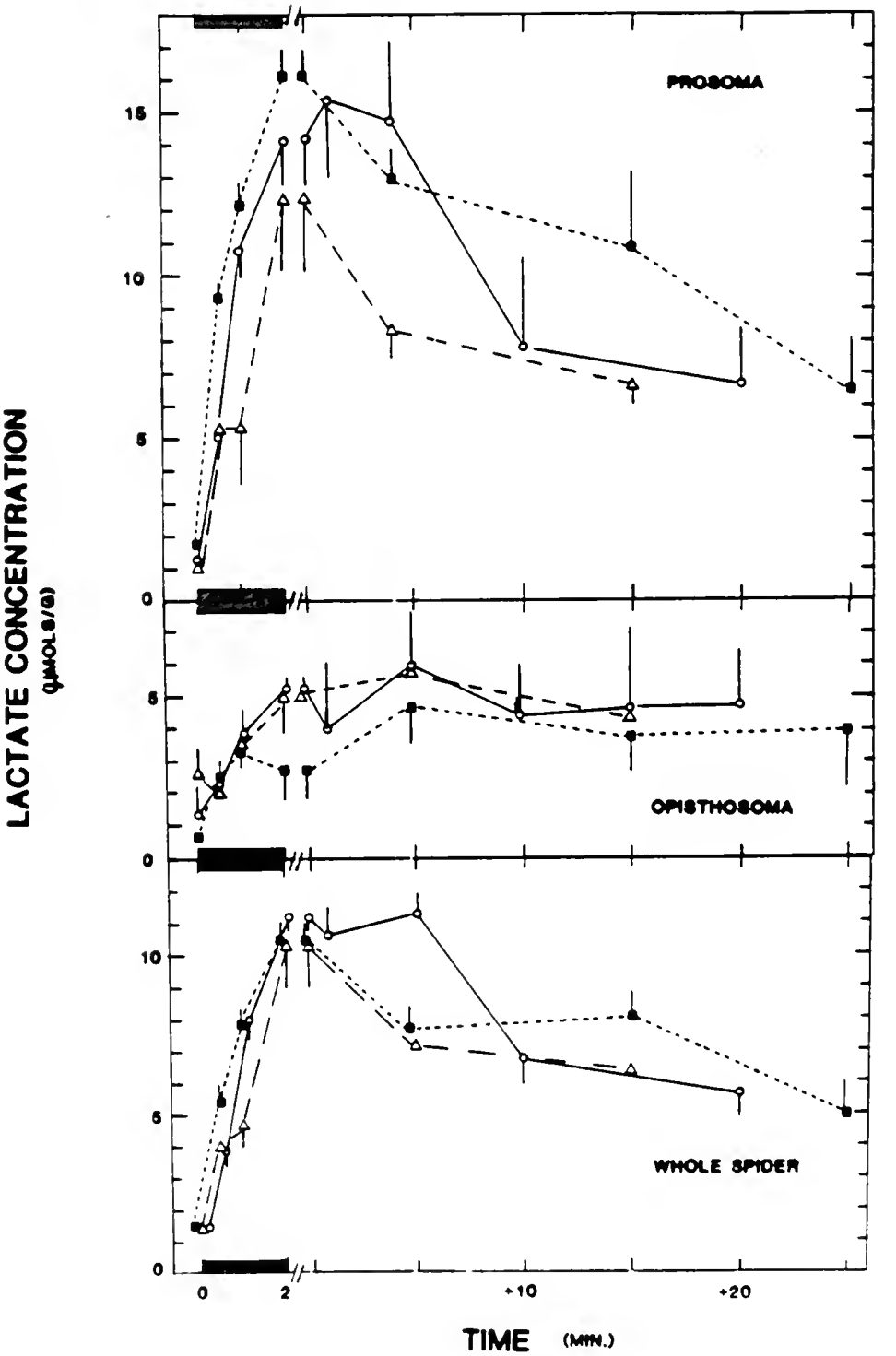
Figure IV-5. The accumulation and removal of lactate during exercise and recovery in three species of spiders at 25°C. Note that during recovery, prosomal lactate concentrations drop while those of the opisthosoma remain elevated or increase. Standard errors are shown as bars. (N values are for each point.)

Key: ■ *F. hibernalis* N = 9

○ *L. lenta* N = 12

△ *P. audax* N = 6

All spiders were acclimated at 25°C.


LACTATE CONCENTRATION
(μMOLS/G)
PROSOMA
OPISTHOSOMA
WHOLE SPIDER
15
10
5
0
5
0
10
5
0
0
2
+10
+20
TIME (MIN.)

The effect of temperature on lactate accumulation is shown in Figures IV-6 and 7 for *F. hibernalis* and *L. lenta*, respectively. A summary of the most pertinent data on lactate concentrations in activity and recovery is shown given in Tables IV-3, 4, and 5. The resting lactate concentrations increase with T_A in all tagamata and in both species. However, maximum lactate concentrations are the greatest at the acclimation temperature. Temperature affects the rate of accumulation of lactate differently in different species. In *Filistata*, the maximum rate of lactate accumulation (anaerobic scope) is highest at the acclimation temperature of 25°C and is lower at both 15 and 33°C, while in *Lycosa* the anaerobic scope increases with T_A between 15 and 33°C. Recovery in both species is most rapid at the acclimation temperature. At 15 and 33°C the whole spider lactate concentration increases or remains constant for the first ten minutes of the recovery period. However, the increases are not significant statistically. (During this time, prosomal lactate concentrations decrease.)

A different picture is presented in the opisthosoma (Table IV-4) where there is an increase in lactate concentration over the first five minutes of recovery. At temperatures other than 25°C, opisthosomal lactate concentrations continue to increase for at least 15 min (Figs. IV-6 and 7, middle panels). Thus, the total body lactate concentration remains constant since as the prosomal lactate concentration decreases, the opisthosomal concentration increases.

Table IV-5 contains data for the scorpion *C. hentzi*. Although lactate concentrations seem low compared to the spiders, this is partially due to the large mass of non-motion generating tissues.

Figure IV-6. The accumulation and removal of lactate in *F. hibernalis* at three different temperatures. The spiders were all acclimated at 25°C. Note the very slow recoveries at non-acclimation temperatures.

Key: ——— 33°C

····· 25°C

— — 15°C

Standard errors are not shown but were consistently less than 12% of any mean value except for the opisthosomal data at 15 and 33°C. Here SE approached 20%.

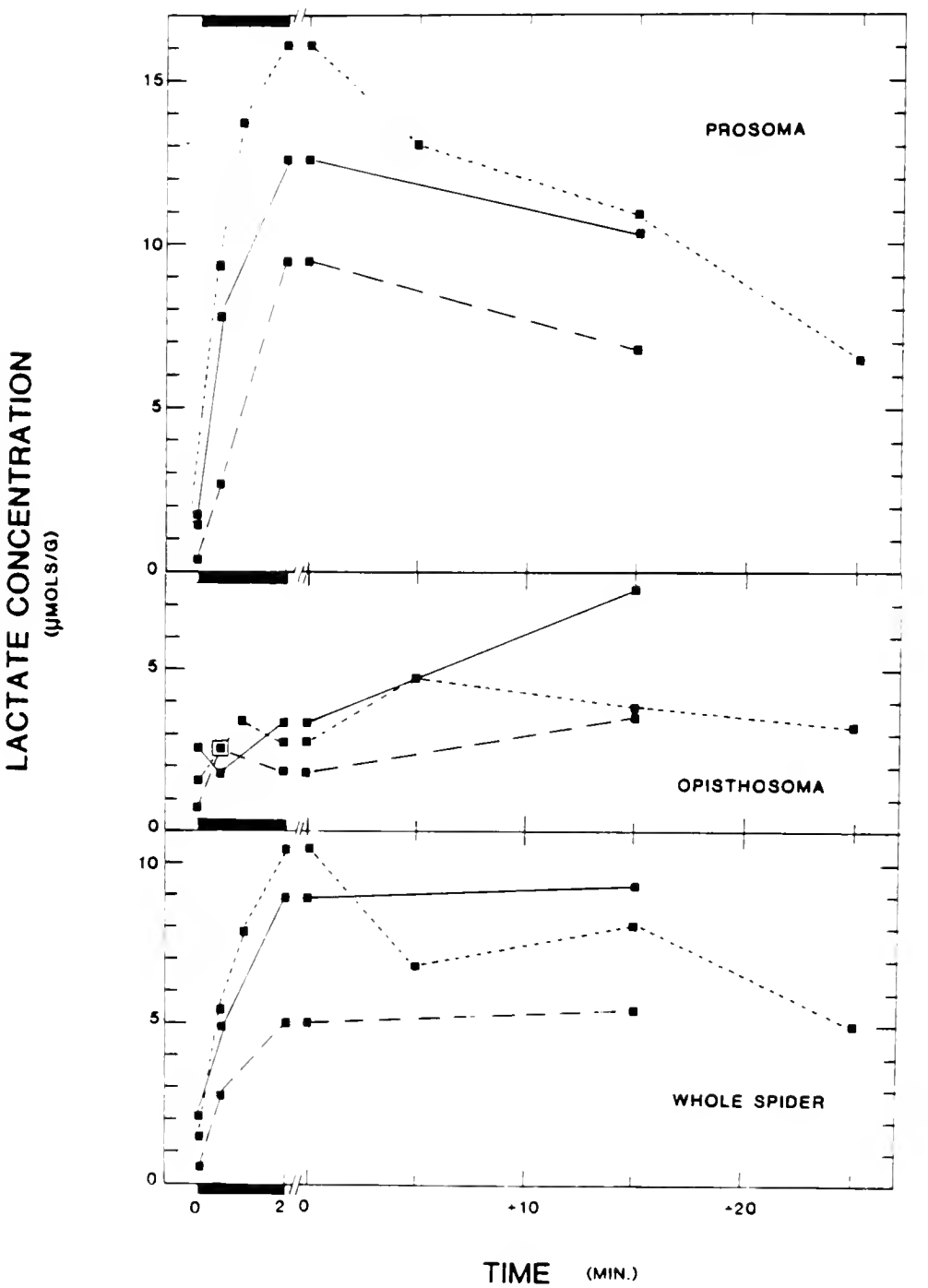

LACTATE CONCENTRATION
(μMOLS/G)
PROSOMA
OPISTHOSOMA
WHOLE SPIDER
15
10
5
0
5
0
10
5
0
0
2
0
+10
+20
TIME (MIN.)

Figure IV-7. The accumulation and removal of lactate in *L. lenta* at three different temperatures. The spiders were all acclimated at 25°C. Note the very slow recoveries at non-acclimation temperatures.

Key: ——— 33°C

····· 25°C

— — 15°C

Standard errors are not shown but were consistently less than 14% of each mean value with most less than 10% of the mean.

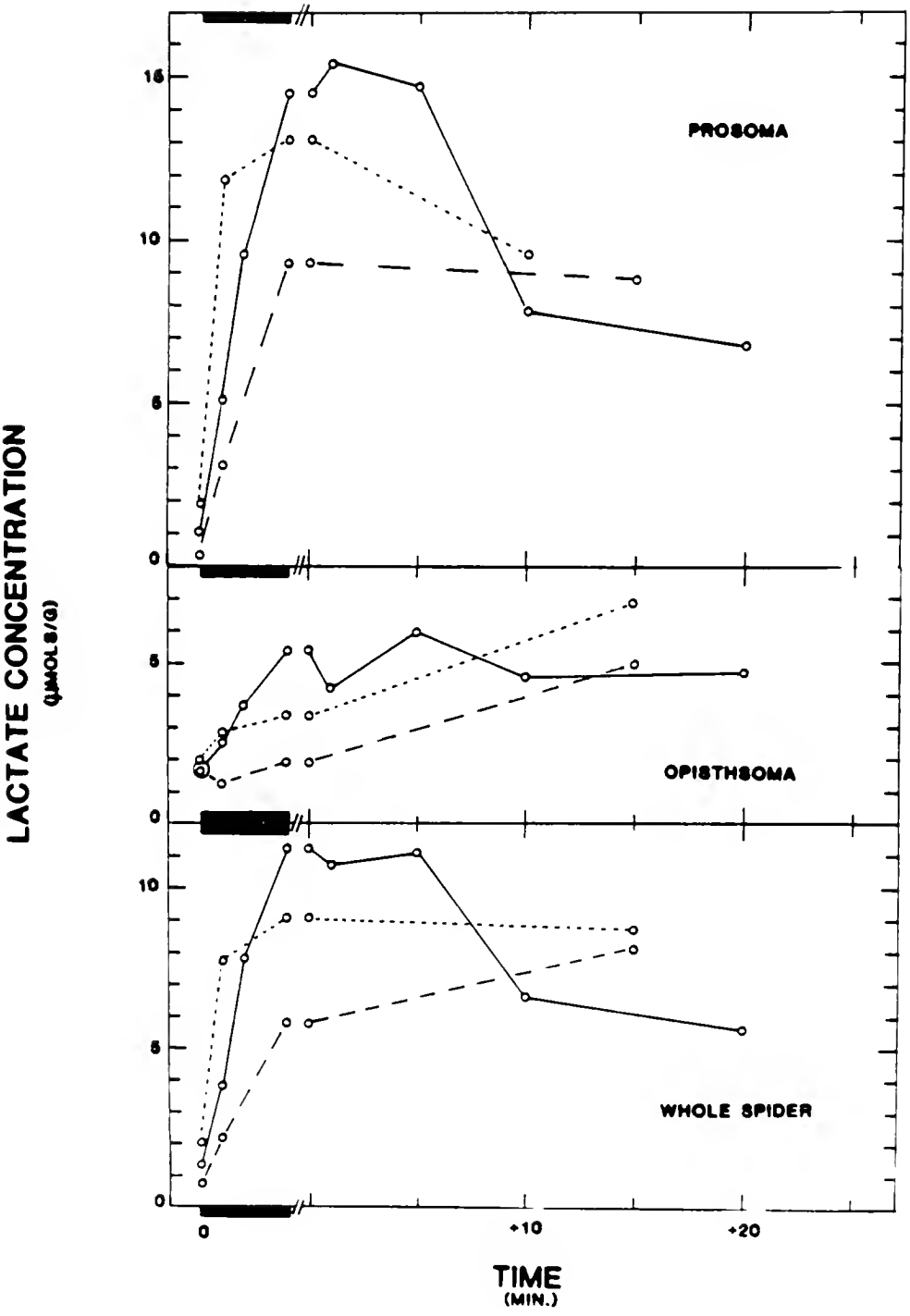

LACTATE CONCENTRATION
(μMOLS/G)
15
10
5
0
PROSOMA
5
0
OPISTHSOMA
10
5
0
WHOLE SPIDER
0
+10
+20
TIME
(MIN.)

Table IV-3. Lactate concentrations and accumulations as a function of activity, recovery and T_a for prosomas plus legs.

Species (mass, g)	N	T_a	Lactate Concentration (µmols/g) Rest	Maximum	Anaerobic[a] Scope	Recovery[b] + 5	+10	Time[c] to 33%
F. hibernalis (0.183±0.021)	6	15°	0.33	9.45	4.68	91%	80%	> 30
	9	25°	1.40	16.00	15.86	79%	72%	25
	6	33°	1.70	12.61	12.10	93%	85%	> 30
L. lenta (0.343±0.021)	10	15°	0.33	9.32	5.54	98%	96%	> 30
	12	25°	1.05	14.50	8.10	95%	47%	19
	10	33°	1.91	13.05	19.98	84%	70%	22
P. audax (0.068±0.004)	6	25°	1.00	12.33	5.7[d]	65%	56%	16

[a] Based on first 30 sec lactate accumulation µmols/(g·min) Bennett 1978.

[b] From data points defined as $\left(\frac{\text{lactate at time t}}{\text{lactate (max) - lactate (rest)}}\right) \times 100$, where t is for 5 or 10 minutes of rest.

[c] Defined as $[\text{lactate}_{max} - \text{lactate}_{min})(0.33) + (\text{lactate min})]$ using a best fit of the recovery points. Thus, this measures the time for accumulated lactate to drop to 1/3 of the anaerobic capacity.

[d] Interpolated between the t = 30s and t = 60s points.

Table IV-4. Lactate concentrations as a function of activity in the opisthosomas of spiders.

Species (mass, g)	N	T_a	Lactate Concentration (μmols/g) Rest	Max.	Anaerobic[a] Scope	Recovery[b] +5	+10	+15
F. hibernalis (0.225±0.02)	6	15°	0.70	1.85	1.98	---	---	221%
	9	25°	1.55	2.73	3.66	267%	---	140%
	6	33°	2.55	3.33	- 1.50	---	---	189%
L. lenta (0.247±0.06)	10	15°	1.45	1.80	- 0.78	---	---	279%
	12	25°	1.45	5.10	1.91	116%	79%	---
	10	33°	1.85	3.10	1.62	---	---	210%
P. audax (0.027±0.002)	6	25°	2.67	5.05	- 1.34	126%	---	85%

[a]Maximum rate of change in opisthosomal lactate between t = 0 and 30 sec. Note how small these values are compared to those for the prosoma (Table IV-3). Negative values indicate removal of lactate; these are not significant statistically.

[b]All percentages are based on the measured values of opisthosomal lactate after either 5, 10, or 15 min of recovery divided by the lactate concentrations at exhaustion (2 minutes) times 100.

Table IV-5. Lactate concentrations during activity and recovery in whole spiders and a scorpion. All indices of recovery and the anaerobic scopes (last four columns) are defined as in Table IV-3.

	N	T_a	Lactate Concentration (μmols/g) Rest	Max.	Anaerobic Scope	Recovery +5	+10	Time to 33%
F. hibernalis (0.408±0.033) (56% ± 3%)	6	15°	0.53	5.00	4.34	108%	109%	?
	9	25°	1.47	10.40	7.86	60%	67%	26 min
	6	33°	2.16	8.85	5.48	103%	104%	?
L. lenta (0.590±0.09) (41% ± 2%)	10	15°	0.70	5.72	2.80	118%	133%	?
	12	25°	1.21	11.15	6.60	99%	54%	22 min
	10	33°	1.88	9.03	11.18	99%	97%	>> 30
P. audax (0.095±0.015) (28% ± 8%)	6	25°	1.47	10.20	4.90	65%	60%	18 min
C. hentzi (6; 0.185±0.012 (> 60%)	10	25°	0.59	4.15	---	---	---	---

The non-prosomal mass (abdomen and telson) of the scorpion averages 63% of the total body size.

Discussion

Running Speeds

One problem with the previous discussions of exhaustion in spiders is the lack of a quantitative description of fatigue in running spiders (Millot 1949; Wilson and Bullock 1973; Linzen and Gallowitz 1975). The data presented in Figures IV-1 and 2 partially remedy this situation. However, they are somewhat misleading in terms of their description of the earliest moments of activity. During the initial 15 to 20 sec of struggle, spiders jump about alot, especially *L. lenta* and *P. audax*. Thus, the data reported in these figures and table represent minimal estimates of the work the spiders are doing. Given this underestimate, the figures do show that spiders generally slow to *ca.* one-third of their initial speeds before 30 sec of activity are complete. Thus it is reasonable to divide activity into two phases: an early, rapid-fatigue period (phase I) and the period that follows, which is characterized by a slower decrease in speed (phase II). Mathematically, I have demarked these periods as being before and after the moment that a spider has slowed to a speed that is 133% of its final speed. This mathematical definition corresponds well with the cessation of any jumping behavior in *Filistata* and *Lycosa*. Thus, phase I corresponds to the first 15 (*Lycosa*) to 40 sec (*Phidippus*) of the two minute activity period.

The fact that about 10% of *L. lenta* and *F. hibernalis* drag their IVth pair of legs during late phase I is significant. This is consistent with

the hydrostatic insufficiency hypothesis of Wilson and Bullock (1973). Forward motion is produced in the first through third pair of legs through either flexion (pair I) or rotation (pairs II and III) while in pair IV it is due to extension (Parry and Brown 1959; Wilson 1970). Since much or all of extension of pairs I-III is done while the legs are off the substratum, the work required is relatively small. Extension of these legs could be accomplished by a low hemolymph pressure (Manton 1958). However the IVth pair of legs require hemolymph under high pressure in order to extend rapidly while in contact with the substratum. Furthermore, these rear legs would be expected to be crucial in providing much of the forward thrust in spider locomotion (Parry and Brown 1959b). The spiders that were dragging their legs during late phase I could be suffering from inadequate pressures and/or amounts of prosomal hemolymph. This possibility and its relationship to fatigue will be specifically discussed in Chapter V.

Phase II is characterized by relatively slow movements of all limbs and a gradual decrease in speed (compared to phase I). After one minute of activity, many spiders refuse to move unless constantly prodded. This corresponds to a time when the prosomas contain considerable amounts of lactate (Figs. IV-5 through 7). The final running speeds shown in Figures IV-1 and 2 are biased towards high speeds since spiders that had refused to move for much of the previous 30 seconds (by assuming a ball-like posture) often burst into activity.

Suggestive similarities exist between fatigue in spiders and scorpions. Scorpions do not use a hydrostatic skeleton for leg extension (Manton 1958). However, their pattern of running when subject to

continuous stimulation resembles that of spiders (Fig. IV-1). This argues against the notion that fatigue in spiders might be largely related to hydrostatic insufficiency (Wilson and Bullock 1973).

Recovery of running ability in spiders is a slow process. I used a measure of recovery that applied only to the first five seconds of phase I activity. Spiders forced to run longer than 15 sec, even after 10 min of recovery, exhausted within 45 sec. This is strong evidence that complete (ultimate?) "locomotory collapse" (Wilson and Bullock 1973; Linzen and Gallowitz 1975) has nothing to do with hydrostatic insufficiency. Given the high heart rates of spiders during recovery (Figs. IV-3, 4; Table IV-2) hemolymph would seemingly be adequately redistributed after 10 min of recovery. If hydrostatics are the main limit to activity, running after recovery would resemble the running of completely rested animals. Instead, this limitation on activity is probably due to continued large concentrations of anaerobic byproducts (Figs. IV-5, 6, 7; Tables IV-3, 4, and 5).

The effect of temperature on locomotion in *L. lenta* and *F. hibernalis* can be evaluated in two ways: comparison of maximum speeds and total distance traveled. Maximum speeds are synonomous with initial speeds while total distances traveled are obtained by integrating each speed versus time curve. In terms of these two measures, temperature effects are presented in Tables IV-6 and 7. The Q_{10} values (Table IV-7) are nearly all below two indicating that the spiders' locomotory patterns are somewhat independent of T_A. As such, an 18°C increase in T_A results in a 210% increase in distance traveled over 2 min and a 315% increase in maximum speed in *Lycosa* and respective increases of 170 and 195% in

Table IV-6. Total distance traveled in two minutes of maximal activity as a function of temperature. Distances (total prosomal length) for *Lycosa* and *Filistata* were converted to percentages by defining the distance *Lycosa* traveled at 25°C as 100%.

	Relative Distance Traveled		
Species	15°C	25°C	33°C
F. hibernalis	68%	96%	115%
L. lenta	68%	100%	160%

Table IV-7. The effect of temperature on locomotion in *F. hibernalis* and *L. lenta*. Spiders were acclimated at 25°C; the temperature range of 15°C to 33°C is possible during a spring day in Gainesville, Florida.

Species	Temperature Range (°C)	Q_{10} Initial Speed	Q_{10} Total Distance
F. hibernalis	15-25	1.49	1.23
	25-33	1.40	1.52
	15-33	1.45	1.35
L. lenta	15-25	1.75	1.38
	25-33	2.1	1.67
	15-33	1.9	1.50

Filistata. Increases of 350% would be expected over this range if the Q_{10} were equal to 2. Thus, a spider threatened at temperatures below those to which it is accustomed can move faster than would be expected based on a Q_{10} of between 2 and 2.5 calculated from its resting $\dot{V}O_2$ (Anderson 1970).

Heart Rates

The change of heart rate as a function of exercise and recovery varies between species in spiders. Angersbach (1978) reported peak heart rates in a tarantula, *Dugesiella californicum*, within 2 min after the completion of a struggle of one to two minutes. Wilson (1967) reported a similar phenomenon in *Heteropoda*. These observations agree with mine for *L. lenta* (Fig. IV-3). However, in *F. hibernalis* the rate rapidly approaches the maximum and remains at an elevated value longer than in *L. lenta* at the same exercise level. This continued, elevated rate could be associated with the equal or larger lactate accumulation coupled with its smaller respiratory exchange capacity (Anderson 1970; Anderson and Prestwich 1982; Fig. II-2).

Temperature affects heart rate in the same manner as it affects running speed and total distance, and lactate production and removal. The Q_{10} for alert and maximal heart rates varies between 1.3 and 1.8 (Table IV-2) indicating these processes are less temperature dependent than are most chemical reactions. As a result, the spider gains a small degree of thermal independence.

Heart rate is not a totally adequate measurement of the ability of the circulatory system to deliver O_2, remove lactate, or redistribute

hemolymph after exercise. Total cardiac output, the product of stroke volume and heart rate, is the preferred measurement. However, I was not able to quantitatively measure stroke volume in either *Filistata* or *Lycosa*. Visual observations were inconsistent although there did appear to be a tendency for maximum stroke volumes to come after the completion of activity (based on the intensity of the transmitted light fluctuations).

Lacking direct data on stroke volume in this species, it is nonetheless possible to calculate the stroke volume for both alert and active tarantulas from published data. These calculations are given in Appendix I. Inasmuch as tarantulas can serve as a general model for spiders (see problems with this approach discussed previously in this section) the calculations indicate that while the stroke volume may increase slightly after activity, the heart rate is the major determining factor in changes in cardiac output. Estimated factorial increases in cardiac output range from about 4 to 6 fold for the tarantula. This corresponds to up to a ten fold increase in $\dot{V}O_2$ (Anderson pers. comm.). The difference is due to an increased loading and unloading of O_2 per volume hemolymph (Angersbach 1978). The speculative nature of these calculations cannot be over-emphasized.

Lactate Production

The colorimetrically determined lactate concentrations reported in this chapter are maximal concentrations for the conditions of time of exercise and T_A under which they were taken. Anaerobic scopes at 25°C (maximum rate of lactate production) and anaerobic capacities (net lactate

accumulations) are on the low end of the range of values reported for terrestrial ectotherms (Bennett 1978). However, if only the prosoma is considered, the anaerobic scopes and capacities are comparable to those of vertebrates with highly developed anaerobic abilities. Thus, maximum lactate concentrations in the motion-generating portion of the spider, the prosoma, are consistent with levels that are associated with fatigue in other animals. Ultimately, phase II fatigue is therefore related to anaerobic accumulations. Phase I fatigue is probably not related to lactate accumulation. In *Lycosa lenta* the two-thirds running speed reduction of phase I takes *ca.* 20 sec (Fig. IV-1; Table IV-1); however, prosoma lactate accumulations are only *ca.* 20% of maximum during this period (Fig. IV-8). Furthermore, during the first 10 sec of phase I when most of the speed decrease occurs, only very slight lactate increases occur. Thus, a non-lactate factor(s) is responsible for phase I fatigue.

In Chapter II, the hypothesis was advanced that the anaerobic capacities of spiders are directly related to the intensity of activity and inversely related to the book lung surface area (Fig. II-2). This hypothesis can be further examined using this chapter's data for the effect of T_A on anaerobic accumulations and running speeds. Maximum running speed and total distance traveled increase with T_A in both *Lycosa* and *Filistata*. These parameters increase faster in *Lycosa*: at 15°C both species' activity patterns are nearly identical while at 33°C *Lycosa* is much more active (Tables IV-1, 6, and 7). However, anaerobic capacities for both species follow a different pattern being largest at the acclimation temperature of 25°C (Fig. IV-9). Moreover, unlike 25°C where *Filistata* has a larger anaerobic accumulation, the

Figure IV-9. Anaerobic capacities in 25°C acclimated *F. hibernalis* (— — ■ — —) and *L. lenta* (— o —). Anaerobic capacity is largest at the acclimation temperature.

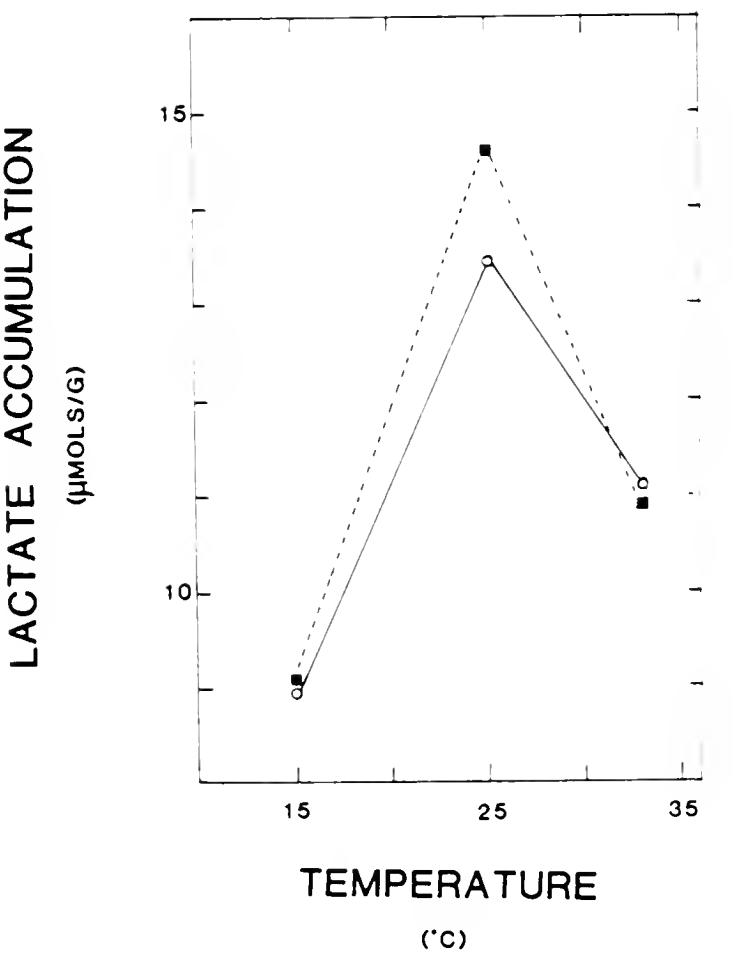
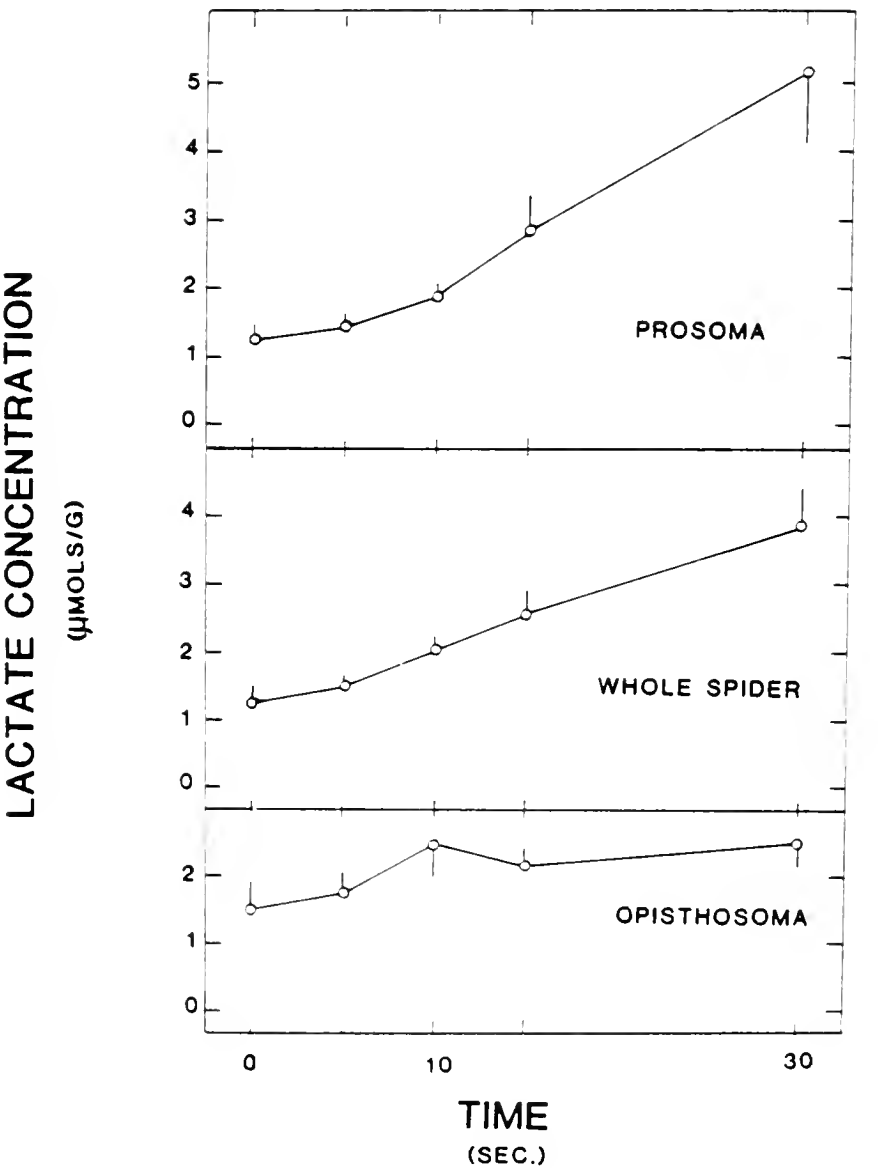

LACTATE ACCUMULATION
(µMOLS/G)
15
10
15
25
35
TEMPERATURE
(°C)

Figure IV-8. The accumulations of lactate at 25°C during the first 30 sec of activity in *L. lenta*. Note that during the first 15 sec when the spider slows greatly (Fig. IV-1) very little lactate accumulates. Standard errors are bars, N for all points = 12.


LACTATE CONCENTRATION
(μMOLS/G)
5
4
3
2
1
0
PROSOMA
4
3
2
1
0
WHOLE SPIDER
2
1
0
OPISTHOSOMA
0
10
30
TIME
(SEC.)

anaerobic capacities of both species are nearly identical at 15 and 33°C. While the 33°C data are not inconsistent with a relationship between book lung surface area and anaerobic accumulation (since *Lycosa* runs much further than *Filistata* at this temperature), the 15°C data do present some difficulties for the proposed correlation. At this temperature, both species' activity patterns are nearly identical and therefore, lactate accumulations are expected to be much greater in *Filistata*. This inconsistency points out the need for further testing of the surface area--anaerobic accumulation hypothesis. It does serve to remind one that other factors may also be important and that their importance may vary at different temperatures in different species, e.g., different use of stored forms of high energy phosphate such as arginine phosphate (Di Jeso *et al*. 1967).

Lactate Removal (Recovery)

For whole spiders, removal of lactate (recovery) occurs most rapidly in *P. audax* and *L. lenta* at 25°C. Fifteen minutes after exercise, lactate concentrations drop by over 50% while in *Filistata* they hover near 75% of the maximal value (Figs. IV-5-7; Table IV-3-5). At 15 and 33°C recovery is a very slow process, there being essentially no change in whole spider lactate after 15 min of recovery.

Examination of the changes in prosomal and opisthosomal lactate concentrations suggest how the lactate is subsequently handled. In the prosoma (Table IV-3; Figs. IV-5 through 7), lactate drops more rapidly than in the whole animal. This removal is probably not entirely the result of oxidation of lactate or gluconeogenesis. Instead, the lactate

appears to be moving to the opisthosoma as lactate concentrations in this compartment either remain relatively constant or increase while the spider is inactive and recovering (Table IV-4; Figs. IV-5 through 7).

The exact fate of the lactate in the opisthosoma can only be guessed. Evidence was presented earlier suggesting that most lactate is used as a substrate for gluconeogenesis (Table III-3). There are two likely locations for lactate metabolism: the heart and digestive diverticulum. The heart is probably always exposed to high PO_2 (Angersbach 1978). If spiders, like the horseshoe crab *Limulus*, possess an LDH that favors oxidation of lactate (Long and Kaplan 1968), then the heart could be a net oxidizer of muscle-produced lactate, much as in the chordate heart (Hochachka and Somero 1973). However, ratios of recovery oxygen to lactate removed suggest that most lactate is used for gluconeogenesis (Table III-3). The most likely site for this process is the digestive diverticula and associated cells (Millot 1949). Evidence for their involvement will be presented later (see Ch. VI).

The slow decrease in lactate at 15 and 33°C implies a slower recovery at these temperatures. However, recovery of the ability to run is faster than might be expected based on whole spider lactate concentrations. This is because a considerable amount of lactate has been shifted from the prosomal musculature to the opisthosoma. Additionally, the ability to locomote at maximal speeds for brief periods of time returns nearly as quickly at 15 and 33° as it does at 25°C (Table IV-1; Fig. IV-2). Replenishment of phosphagen stores may be a major factor in the recovery of the ability to run at maximum rates (see Ch. VI). The ability to run quickly, even if only for brief periods, doubtlessly has high survival

value for a spider facing danger. Transport of lactate from the prosoma and legs to the opisthosoma helps in this process. Although it does not result in an immediate decrease in whole animal lactate, transport removes the substance from the muscles where it contributes to fatigue and relocates the lactate in the tissues that may convert it to circulating carbohydrate stores.

CHAPTER V
THE HYDROSTATIC FATIGUE HYPOTHESIS

Summary

1. Leg pressures were monitored in maximally struggling, restrained *Filistata hibernalis*.
2. During the first few seconds of activity, pressures increased rapidly to 100-200 mm Hg. Peak pressures of 450 mmHg are not reached until after 20 sec of struggle (Fig. V-3).
3. In spiders with tight ligatures around their pedicels, pressures reached high values sooner than in non-ligatured spiders.
4. The maintenance of high pressure throughout a period of time when free-running *Filistata* rapidly slow down (Ch. IV) is contrary to the idea that defects in the hydrostatic leg extension mechanism of spiders results in fatigue (Wilson and Bullock 1973).
5. The slower development of peak pressures in spiders without ligatured pedicels supports the idea that prosomal pressures are partially dependent upon the degree of filling of the opisthosomal venous system (Stewart and Martin 1974).

Introduction

Maximal activity in spiders can be divided into two phases (Ch. IV). The second phase, which leads to nearly complete exhaustion, is probably terminated by high lactate concentrations in the prosoma and legs. This

does not appear to be the case with phase I. Over the 15 to 30 sec duration of phase I, lactate accumulations in *Filistata* and *Lycosa* are not large but these spiders lose about two thirds of their original speed. Thus some other causative factor for the fatigue must be sought.

One possible explanation is "hydrostatic or fluid insufficiency." Wilson (1970) and Wilson and Bullock (1973) showed there is a net loss of hemolymph from the prosoma to the opisthosoma during the first eight to ten seconds of vigorous struggles. They argued that insufficient fluid would be available to force extension of the legs if too much fluid was lost from the prosoma. Since spider legs are sealed, non-distensible tubes, legs would not be expected to run out of fluid if the hydrostatic insufficiency hypothesis is correct. Instead, insufficient prosomal fluid would result in low pressures for leg extension, possibly as a result of the prosomal muscles being forced to operate at lengths that are increasingly shorter than optimal (< Lo). Thus, rapidly decreasing prosomal or leg pressure recordings (resulting from lowered contractile force of the prosoma muscles) would support the hydrostatic insufficiency hypothesis. To test this hypothesis, I simultaneously measured leg hemolymph pressures and the movements of the major pressure-generating muscle groups of the prosoma and opisthosoma.

Methods

Pressure and Muscle Movements

A saline filled catheter was connected by a length of polyethylene tubing to a Sandborn Physiological Pressure Transducer, Model 267B. The saline was 440 mOSM consisting of 215 mM NaCl and 5 mM KCl (based on

Anderson pers. comm.). A series of valves allowed the entire system to be flushed with fresh saline. The pressure transducer was connected to a Hewlett-Packard 311A Transducer-Amplifier-Indicator which was in turn connected to a Sanborn Model 320 strip recorder. A Narco pressure gauge and mercury manometer were used for standardizing the pressure recordings after each run. Recordings of muscle group movements were made using myographs connected to a Narco Physiograph.

Preparation

Only large (mass $\geq$ 0.45 g) *Filistata hibernalis* were used in these experiments. The spiders were anaesthesized with CO_2 and then glued onto glass rods using quick-setting epoxy cement. One day later the spiders were again anaesthesized. A leg was severed near the middle of the femur and a catheter was inserted into the center of the stub and glued into place with epoxy. To avoid the introduction of air bubbles, the catheter was introduced into the spider while it had a small drop of saline on its tip. This drop merged with the hemolymph on the spider's leg and a good liquid bridge was maintained. The spider was kept anaesthesized for the half hour necessary to complete this procedure. A thread was attached (using epoxy) to both the prosomal carapace and to the opisthosoma above the anterior pericardium. These attachments permitted monitoring of muscle group movements (Anderson and Prestwich 1975). No obvious ill effects were produced by these procedures. Many of the spiders were successfully removed from the apparatus at the conclusion of the experiments and released several weeks later.

Recordings

Experiments were preceded by at least 20 min of baseline pressure and myograph recordings. Occasionally it was necessary to break clots in the catheter by gently squeezing the plastic tubing connecting the catheter to the transducer. Activity was initiated and maintained by lightly scratching the spider's legs or chelicerae with a thin metal wire. This produced vigorous struggles similar to those seen in the runway (see Ch. IV). The main difference was that the spiders' legs were not fully supported. Therefore, a smaller work load was involved in these struggles.

Lactate Concentrations

To evaluate the actual work done by the spider, I analyzed individuals for lactate. For non-ligatured spiders this was done using different, smaller individuals than were used in the pressure recording experiments. (I felt that this was permissible since none of my previous experiments had suggested any scaling relationship between lactate accumulation and body size.) These spiders were mounted exactly as described for pressure recordings and were stimulated in the same manner. At the end of two minutes of struggle they were frozen by immersion in liquid N_2. The ligatured spiders were killed in a similar manner except in this case the same individuals that were used for pressure recording experiments were analyzed for lactate. In both cases, the frozen spiders were quickly weighed and them homogenized in 4°C TCA and later analyzed for lactate using the colorimetric method (Ch. II).

Results

Hemolymph pressures in the legs of resting *Filistata* varied between 10 and 30 mm Hg and were independent of the leg measured. Also, since I cannulated the leg in a manner that reduced its ability to move, pressure changes were not recorded in response to flexion of the leg itself (Stewart and Martin 1974). Pressures during walking and the first few seconds of vigorous activity seldom exceeded 70 mm Hg. Maximum peak pressures occurred during violent struggles and were as high as 475 mm Hg and usually were not achieved until 15 or 20 sec after the initiation of maximal struggling activity.

Although both the prosoma and the opisthosoma appear to be involved in the generation and maintenance of pressure (Figs. V-1 and 2) the pressure pulses are most clearly associated with the prosomal carapace depressions (Fig. V-1). However, not all equally forceful carapace depressions result in equally large pressure changes. This is obvious in Fig. V-1. The first two large carapace depressions are associated with large but not maximal hemolymph pressure changes on the order of 100-200 mmHg (vs. 450 mmHg). The third carapace depression of force roughly equal to the first two, resulted in a much larger pressure pulse of 450 mmHg. Opisthosomal contractions are increasing slightly in force throughout this period of time.

The role of the opisthosomal musculature contractions are clearly shown in Fig. V-2. A general correspondence between both opisthosomal and prosomal contractions and leg pressure is evident. The large shifts in the baseline of this record occurring at the start of exercise and about 40 sec later are due to slight jarring of the apparatus.

Figure V-1. Pressure generation and muscle group movements in *F. hibernalis*. Note that maximum leg hemolymph pressures are not reached until after nearly 30 sec of activity. Also note the correspondence between contractions of prosomal musculature and pressure peaks.

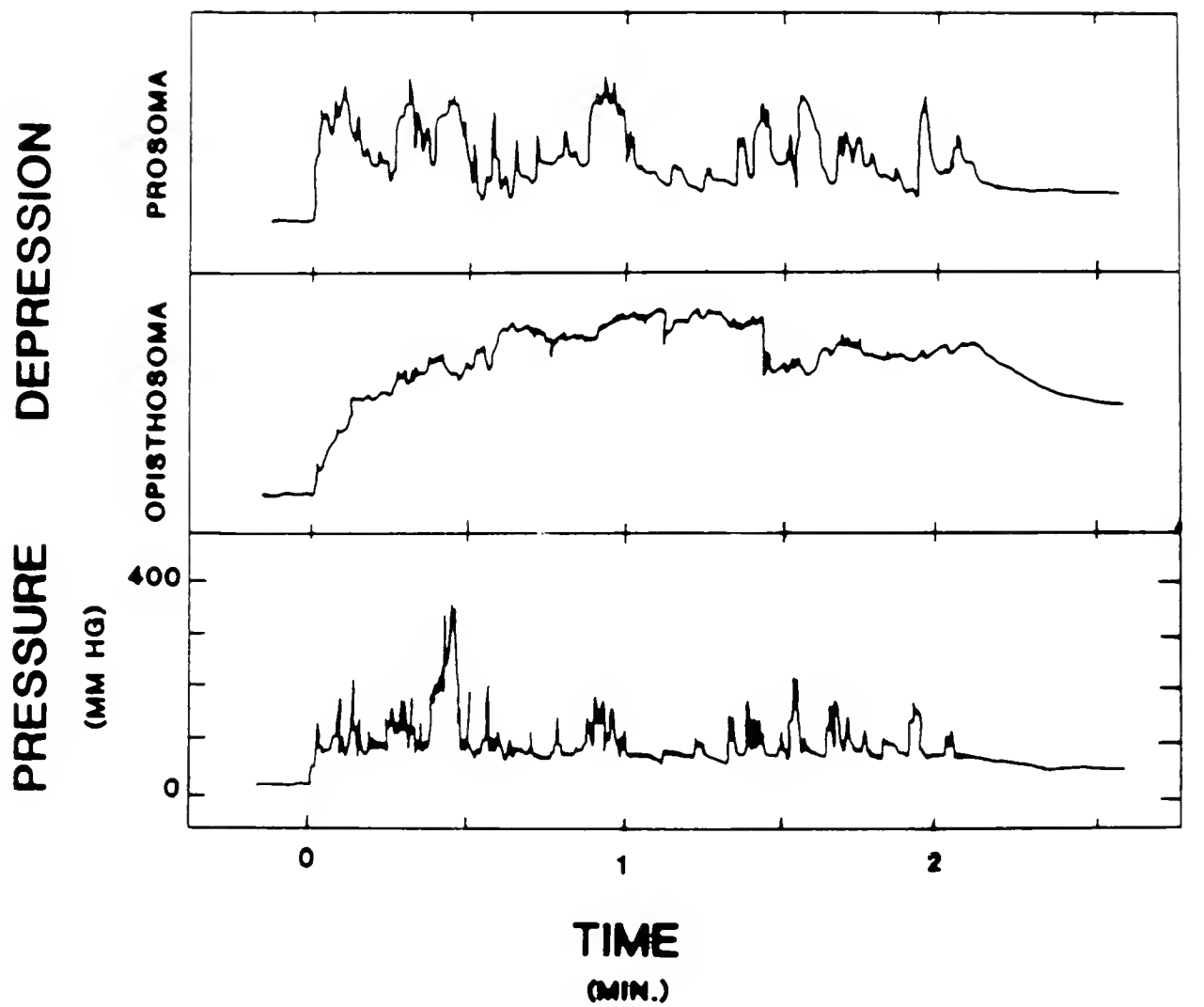
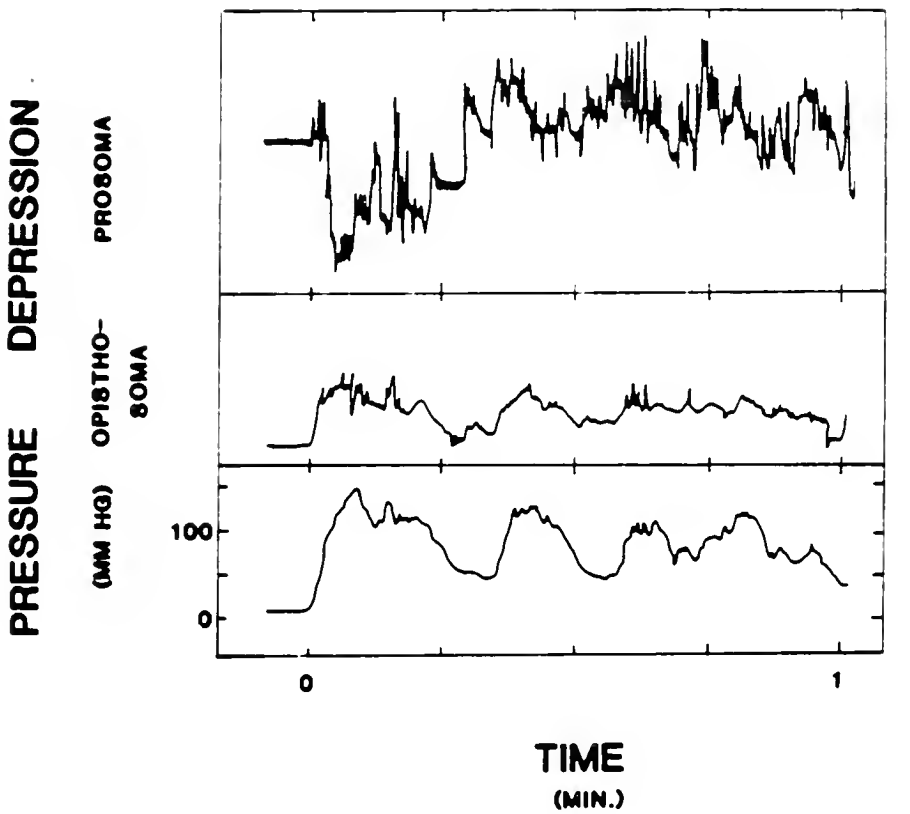

PRESSURE
DEPRESSION
(MM HG)
PROSOMA
OPISTHOSOMA
400
0
0
1
2
TIME
(MIN.)

Figure V-2. Same as Figure V-1. However, the figure more clearly shows the role the opisthosomal musculature can have in the generation of high prosomal pressures.


PRESSURE
DEPRESSION
(MM HG)
OPISTHO-
SOMA
PROSOMA
100
0
0
1
TIME
(MIN.)

Measurement of pressure and carapace depression under conditions of maximal struggle over a two minute period and then after differing periods of recovery are shown in Fig. V-3. The record is typical for eight spiders. Peak pressures are not reached until about 30 sec into activity. In the face of continued stimulation, maximum pressures dropped below 200 mm Hg over the next minute and a half and by the end of the activity period were near 70 mm Hg.

After five minutes of rest, four of the spiders were again stimulated for 30 seconds. Typical results are shown in Fig. V-3; peak pressures were 100-200 mm Hg. In general, neither the pressure curve nor the force of carapace contraction appear as high as in rested spiders. The final trace represents leg pressures after ten minutes of rest (N = 4). Pressures now peak between 250 and 300 mm Hg and average pressures are higher than those obtained after only five minutes of rest.

The final experiment involved tying a tight ligature around the pedicel and thereby preventing any movement of hemolymph from the prosoma to the opisthosoma. This was done on two spiders and the results for one spider are shown in Fig. V-4 (results for the other individual were essentially identical).

Upon tightening the ligature, leg pressures went to near 250 mm Hg. [This is in contrast to spiders without the ligature where lower (*ca.* 100 mm Hg) pressures were found during the initial moments of activity.] Peak pressures occurred between 20 and 40 sec after tying the ligature and were near 400 mm Hg. During the first minute, pressures seldom dropped below 100 mm Hg. After the first minute, movements by the spider became very feeble in response to vigorous stimulation. At the end of the

Figure V-3. Prosomal carapace movements and leg hemolymph pressures in *Filistata* during exercise and after five and ten minutes of recovery. Note that during the initial exercise period maximum pressures were not reached until nearly 30 sec into the activity period. Peak pressures after only five minutes of recovery were lower than after ten minutes. This agrees well with the running speed data in Figure IV-1.

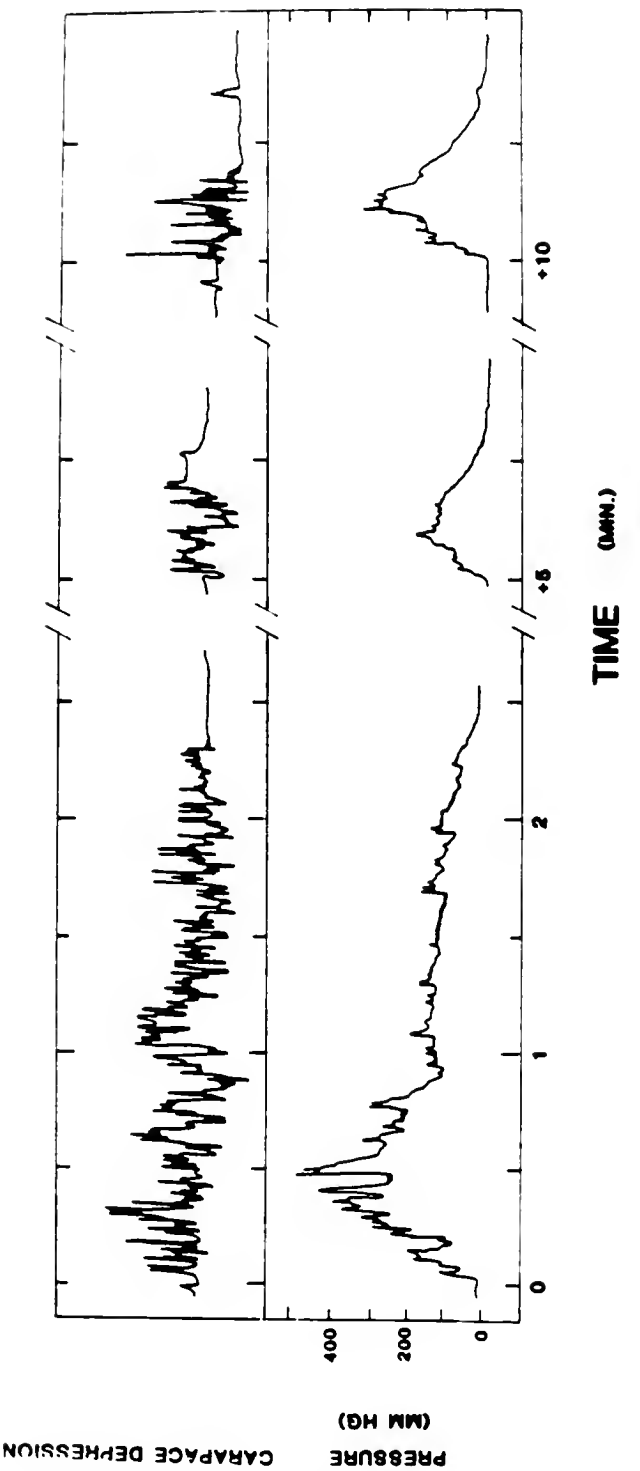
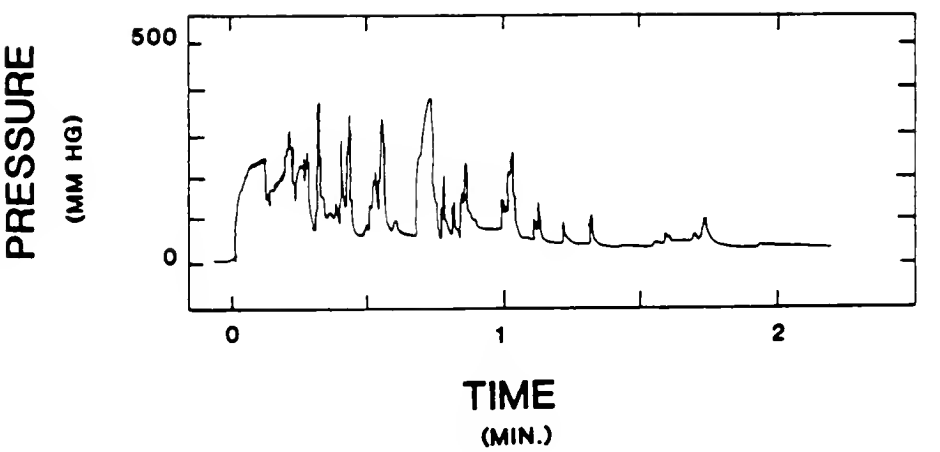

CARAPACE DEPRESSION
PRESSURE
(MM HG)
400
200
0
0
1
2
+5
+10
TIME (MIN.)

Figure V-4. Leg hemolymph pressures in a *F. hibernalis* with a ligatured pedicel. Note the faster initial rise of pressure. This is consistent with the idea proposed by Stewart and Martin (1974) that peak pressures cannot be achieved until resistance to flow into the opisthosoma is maximal. Note the lack of much pressure generation after one minute. This is probably the result of severe hypoxemia due to a complete block of circulation. Prosomal lactate concentrations after two minutes were high but not maximal (Chs. II, IV). This might be due to the lack of a load on the leg muscles.


PRESSURE
(MM HG)
500
0
0
1
2
TIME
(MIN.)

two minute stimulation period the spiders legs were extended and pressures of near 80 mm Hg were recorded. A gentle squeeze on the catheter showed a normal pressure response indicating that the preparation was still functioning normally. The catheter was then cut away by removing the leg at the coxa and the recorded pressure dropped to zero (not depicted). This showed that the high pressure recorded at the end of the two minute runs was not an equipment artifact.

Lactate Concentrations

In non-ligatured spiders, the mean whole body lactate concentrations after 120 sec of activity was 3.63 $\pm$ 0.22 µmols/g, N = 4, mass = 0.248 $\pm$ 0.03. All of these spiders possessed large abdomens like those in the *Filistata* used in Ch. II that certainly accounted for over half the total body mass (Table II-3). The two ligatured spiders had an average total body lactate of 3.2 µmols/g (mass = 0.567 g) with a mean prosomal concentration of 6.0 µmols/g (mass = 0.256 g) and opisthosomal concentrations of 0.9 µmols/g (mass = 0.311).

Discussion

The hemolymph pressures agree with values obtained using different techniques on *Filistata* (Anderson and Prestwich 1975) and other species (Parry and Brown 1959a and b; Stewart and Martin 1974). Walking movements occurred with pressures as low as 30 to 100 mm Hg; struggles were accompanied by higher pressures of 80 to nearly 500 mm Hg. I could not relate the intensity of the activity with the hemolymph pressure. In fact, the highest pressures usually occurred well after the period of most vigorous activity. In general, the myograph recordings (*e.g.*,

Figs. V-1, 3) suggest that muscle tension was relatively constant throughout this period of increasing hemolymph pressure.

The reason for obtaining the pressure data was to test the fluid insufficiency hypothesis. Pressure is the most important index of power for leg extension. The cross-sectional area of a spider leg is constant (a slight increase in volume of the leg of an active spider occurs mainly as the result of expansion of the arthritic membranes at each leg joint (Parry and Brown 1959b). Also, the lever system determined by the sizes of the leg elements and the joints are fixed. Therefore, greater pressures acting on the same area equate with greater force and result in more forceful and/or rapid movements. The fluid insufficiency hypothesis predicts that fluid loss from the prosoma to the opisthosoma leads to insufficient hemolymph to extend the legs. For the reasons mentioned above, any hydrostatic insufficiency must be reflected in low prosoma-leg fluid pressures. If pressure remains high (even in the face of a reduction of prosomal hemolymph volume), then there is no fluid insufficiency.

The data presented in the first three figures of this chapter show that leg pressures remain high (above 100 mmHg) for the majority of the two minute activity period. Furthermore, there was a marked tendency for all individuals not to achieve highest hemolymph pressures until after 20 or 30 sec of vigorous struggles. During this time, pressures never got low enough (below 100 mmHg) that recharge of prosomal fluid via the heart would have been possible (Stewart and Martin 1974). Thus, prosomal hemolymph loss is not a constraint in *Filistata* during phase I of activity.

A possible criticism to this conclusion arises from the fact that the spiders were mounted in such a way that they did not have to make contact with the substratum. Therefore, the experimental situation was not comparable to that of free running spiders. This was reflected in the low lactate concentration found in both ligatured and non-ligatured spiders (relative to free running spiders, Chs. II and IV). The lower load on the spiders legs actually allows for a more convincing demonstration that fluid dynamics are not limiting in spiders. Direct muscle fatigue resulting from biochemical factors was lessened due to the decreased load on the legs. The fact that the hemolymph pressures remained high for long periods of time with accompaniment of low lactate accumulations showed that hydrostatic collapse was not the cause of fatigue since maximum volumes of prosomal hemolymph should have been lost under these conditions.

The results of the ligature experiments are also not consistent with the hydrostatic insufficiency hypothesis. If fluid loss is an important constraint, then under the same experimental conditions the hemolymph pressures found in the non-ligatured spiders would be lower than in the ligatured spiders. There was no difference between the pressures found in these two groups (Figs. V-1 to V-4). Since the intensity of struggling appeared to be the same in both groups, the equal concentrations of lactate found in each group indicates there was no flow of freshly oxygenated hemolymph from the opisthosoma to the prosoma and therefore no recharging of the prosomal fluid levels.

Behavioral data is of significance in this context. The running pattern of the scorpion, *Centruroides hentzi*, is identical to the three

species of spiders (Fig. IV-1). Scorpions do not, however, use fluid pressure for leg extension (Manton 1958). Since rapid fatigue (phase I) occurs in both hydraulic (spiders) and non-hydraulic (scorpions) arachnids, it suggests that fluid constraints are not responsible for fatigue.

As mentioned in the Results of Chapter IV (see running speeds), about 10% of the *Lycosa* and *Filistata* dragged their IVth pair of legs after 20 sec of rapid running. This observation is the only support I can offer for the hydraulic insufficiency hypothesis. The fourth pair of legs are the only legs in spiders that relay mainly upon extension to propel the animal. The other three pairs mainly use flexion (pair I), rotation-flexion (pair II), or rotation extension (pair III) (Manton 1958; Parry and Brown 1959b). Insufficient fluid volume and/or pressure is a likely explanation for the behavior of these spiders.

Given the high pressure gradient that exists between the prosoma and opisthosoma in active spiders, why is fluid loss not so large that it becomes limiting? Stewart and Martin (1974) suggested that the prosomal hemolymph loss recorded by Wilson and Bullock (1973) was mainly due to filling of venous sacs upstream from the heart (Comstock 1948). Stewart and Martin (1974) reasoned that the plateauing of the fluid exchange between prosoma and opisthosoma that occurs in the first few seconds of activity was a result of increased resistence to flow as the opisthosomal venous sinuses filled to capacity. This explains several observations. In both my records and those of Stewart and Martin (1974), the highest pressures occur well after the start of activity (Figs. V-1, 2, 3) even though the spiders are no longer running rapidly (Figs. IV-1, 2). This

agrees with their suggestion that maximal pressure can not be generated until resistance to fluid loss is maximal. Furthermore, very high pressures may have a retardant effect on locomotion since the muscles must work antagonistically to the large forces resulting from these pressures (Stewart and Martin 1974). Finally, it is known that dehydrated spiders quickly end up walking on their "knees" when forced to run (Anderson and Prestwich 1975). Spiders with low hemolymph volumes would have insufficient fluid to both fill the opisthosomal and prosomal sinuses and also their legs. In a dehydrated spider, hydraulic insufficiency is a very real constraint.

In most cases hydraulics is not limiting to the activity of healthy spiders. As Wilson (1970) pointed out, there is a positive correlation between the degree of development of the prosomal pressure generating musculature and the subcuticular opisthosomal muscle sheet (which is probably responsible for the development of much of the opisthosomal pressure). This parameter also correlates with a spider's habits: more active spiders have thicker musculature. Spiders are designed to minimize any hydrostatic inhibition of their activity patterns. Their unusual locomotory system does not directly constrain spiders although it may exacerbate the hypoxemia found in the prosomal musculature of active spiders (Wilson and Bullock 1973). Nevertheless, the primary constraints of activity in spiders are biochemical, as in many other species of animals (Ch. IV and VI).

CHAPTER VI
THE METABOLISM OF PHOSPHAGENS, ADENOSINE PHOSPHATES, AND SOME GLYCOLYTIC INTERMEDIATES AND SUBSTRATES

Summary

1. The changes in concentration during exercise of high-energy phosphate compounds [arginine phosphate (AP), ATP, ADP, and AMP], glycolytic intermediates, and carbohydrate substrates were measured in the prosomas of *Filistata hibernalis* and *Lycosa lenta*.
2. The energy charge of resting spiders is high, being above 0.9. However, during the first 20 sec of maximal activity there is a near complete depletion of phosphagen stores (AP) and a lowering of the energy charge to *ca*. 0.6 to 0.8. After this time, a steady-state is reached between high-energy phosphate use and production. This is reflected in a constant energy charge for the remainder of the activity period.
3. Since the reactions involving the use of high-energy phosphate compounds are potentially faster than those that supply such compounds (such as glycolysis) the quick depletion of high-energy phosphate stores seems to be the cause of the rapid phase I fatigue of spiders (Ch. IV).
4. The cause of phase II (Ch. IV) fatigue is probably related to the effects of lactate accumulation.
5. Finally, the data suggest that carbohydrate availability is not limiting during two minutes of activity. The results also suggest

that carbohydrates enter the prosoma from the opisthosoma during moments of struggle when hemolymph can be pumped into the prosoma.

Introduction

Results presented earlier suggest that the ultimate cause of fatigue in active spiders is related to accumulation of anaerobic metabolites. However, the rapid fatigue that occurs early in exercise (phase I fatigue) is one of the most characteristic features of vigorous activity in spiders. In Chapter V, I showed that phase I fatigue is not normally the result of hydrostatic insufficiency.

Four factors could contribute to phase I fatigue: (a) aerobic limitations; (b) anaerobic accumulations; (c) phosphagen and adenosine phosphate depletion; and (d) substrate depletion. The first two factors are not important. Aerobic and anaerobic metabolism require time to become fully activated as various metabolites are required for both activation and de-inhibition of their rate controlling enzymes. This takes about five seconds in insects (Sacktor and Wormser-Shavitt 1966; Sacktor and Hurbut 1966). In *L. lenta*, maximum anaerobic scopes are not attained until after 10 sec of running (Fig. IV-8). While aerobic and anaerobic metabolism are activated, the spider is slowing down (Figs. IV-1 and 2). Secondly, anaerobic accumulations are probably too small during phase I to result in fatigue. In 15 sec of running, *L. lenta* and *F. hibernalis* show respectively 64 and 54% reductions in speed and yet their lactate accumulations are only 12 and 23% of their respective anaerobic capacities. Thus, it is other factors that are responsible for phase I exhaustion.

In mammals and in flying insects the main energy sources for the first 10 sec of activity come from stores phosphagens, ATP, and ADP (McArdle 1981; Sacktor and Hurlbut 1966). A 100 meter human sprinter relies almost exclusively on these substances for energy during a race. Near the finish they are depleted and the sprinter may actually be slowing down as he comes to rely on slower processes for ATP synthesis (McArdle 1981). To test whether depletion of high energy phosphate compounds could be related to phase I fatigue, I measured the levels of arginine phosphate (AP; the phosphagen used in spiders, Di Jeso *et al.* 1967) and the levels of ATP, ADP, AMP, and inorganic phosphate (P_i) after different lengths of struggles.

Another potential cause of fatigue in spiders is carbohydrate depletion. Spiders do not possess high concentrations of glucose or other anthrone-reactive substances (Collatz and Speck 1970; Stewart and Martin 1970; Collatz and Mommsen 1975; Rakotovao 1975). I measured the changes that occurred during periods of maximal activity in anthrone-reactive substances, hexose-phosphates, and some glycolytic intermediates.

Materials and Methods

Preparation of Samples

Spiders were exercised in the runway described in Chapter IV. At various intervals during activity and recovery, the spiders were quickly frozen in liquid N_2, the prosoma and legs separated from the opisthosoma, and then the prosoma plus legs were stored frozen for several days at -80 °C. Five spiders were used at each interval of activity except for

the plus ten minute recovery value in *Filistata* where N = 2. Homogenates of frozen prosomas were prepared according to the methods of Lamprecht and Trautschold (1974) except that the ratio of tissue powder to frozen $HClO_4$ was maintained at 1 to 10 (Sacktor and Wormser-Shavitt 1966) and the samples were filtered to remove the proteinaceous precipitant (see Ch. II) prior to neutralization with 4M K_2CO_2. These extracts were immediately refrozen at -80°C. Analyses were made on these samples within seven days of refreezing (Walesby and Johnston 1980).

All biochemical analyses, except for anthrone-reactive substances and glucose, were carried out by coupling to indicator reactions based on changes in concentration of reduced pyridine nucleotides. The absorbance of these coenzymes was monitored at 340 nm using a Zeis M4QIII spectrophotometer with a Zeis PMI digital readout. The analyses were as listed in Table VI-1 except for arginine phosphate (AP), glucose, and glycogen. Arginine phosphate (AP) was analyzed by placing between 30 and 200 µl of sample in a cuvette containing the buffer, coenzymes, and enzymes needed for the ATP and G6P analyses of Lamprecht and Trautschold (1974). ADP was added to produce a concentration of *ca.* 0.15 mM ADP. This mixture was allowed to react at 4°C for 40 minutes to remove all G6P and ATP. This step is important because the ADP supplied by Sigma contained traces of ATP. At the end of the 40 minutes, the samples were quickly brought to room temperature and absorbance was read three times at two minute intervals to obtain a correction factor for "creep" (Lamprecht and Trautschold 1974). Then, I added 20 µl of arginine kinase (AK). The subsequent reaction is slow and takes up to 90 minutes to complete (depending on the sample). Efforts to speed up this reaction through the addition of more

Table VI-1. Methods of analysis (Analyses #6 and 7 were only made on a few samples due to insufficient volume of homogenates).

Substance(s)	Sample Volume (µl)	Total Reaction Volume (µl)	Method (all 1974 except as noted)
1) Glucose-6-phosphate (G6P) ATP Glucose-1-phosphate (G1P) Fructose-6-phosphate (F6P)	30-300	700-730	Combination of Lamprecht and Trautschold Bergmeyer and Michal Lang and Michal
2) Inorganic phosphate (P_i)	30	730	Guynn *et al.* (1972)
3) ADP AMP	200-500	810	Jaworek *et al.*
4) Oxaloacetate Dihydroxyacetone phosphate (DAP) Glyceraldehyde-3-phosphate (GAP) Fructose-1,6-diphosphate (FDP)	500	700	Sequentially according to: Wahlefeld Michal and Bentler
5) Glycerol-3-phosphate (G3P) Malate D-lactate	100	810 820 830	Sequentially according to: Michal and Lang Gutmann and Wahlefeld Gawehn and Bergmeyer
6) Pyruvate Phosphoenolpyruvate (PEP)	500	700	Czok and Lamprecht
7) Glycerate-3-phosphate	300	700	Czok

AK resulted in the formation of an interfering precipitant of magnesium ammonium phosphate. Future determinations of samples from individuals suspected of having high AP concentrations should be done on much smaller samples and/or following dialysis of commercial AK. The final volume of all AP analysis reactions was 0.7 to 0.973 ml.

Glucose was analyzed using glucostat obtained from Worthington Biochemical and total hexose was determined using anthrone reagent.

Biochemicals for all the above analyses were obtained from either Sigma or Boehringer. These and all of the inorganic reagents were of the highest purity available. All reactions were checked with appropriate standards and blanks. Coefficients of variation were generally less than 20%; this is usually considered adequate for these types of analyses (Sacktor and Wormser-Shavitt 1966). The samples having higher variation were usually those at the limit of detection (oxaloacetate, pyruvate, PEP, GAP, glycerate-3-phosphate, and GIP). Other substances with larger coefficients of variation were anthrone-reactive substances (which would be expected to show significant differences in concentration between individuals) and FDP (reasons unknown).

Results are all expressed as mean $\pm$ standard error. Tests of significance were based on Student's t-test; the level of significance was 0.05.

Results

Substrate and Hexose Phosphate Levels

Measurements of total anthrone reactive materials and glucose during activity are presented in Figure VI-1 for *L. lenta* and *F. hibernalis*.

Glucose shows a slight but non-significant increase over the 120 sec of activity in *Lycosa*. Over the same period glucose concentration doubles in the prosoma of *Filistata*, a significant increase. During recovery in *Filistata*, glucose levels are similar to those measured at 120 sec. A non-significant decrease occurs between five and ten minutes into the recovery period. The concentrations of glucose in *L. lenta* are about half those of *Filistata*.

Total anthrone reactive substances are also higher in *F. hibernalis* than in *L. lenta* (Fig. VI-1). Two different patterns are seen. In *Filistata*, an initial decline in prosomal anthrone reactive compounds is followed by a more than doubling in these substances. Large variation occurs in samples taken at 20, 30, and 60 seconds. However, by the 120 sec measurements the increase in total hexose over resting levels is significant. During recovery, a non-significant decrease occurs between minutes 0 and 10 of recovery.

In *L. lenta* total anthrone reactive substances decrease throughout the 120 sec activity period although the change is not significant. At 120 sec nearly all the anthrone reactive substance is apparently glucose.

Figures VI-2 and 3 show the changes in concentration of glucose-6-phosphate (G6P) and fructose-1,6-diphosphate (FDP). During the 120 sec activity period G6P increases over eight fold in both species, a highly significant change. In *Filistata*, the G6P concentration continues to increase during recovery. The concentration of FDP varied and the apparently increased levels of this substance are not statistically significant changes. Finally, the concentration of glucose-1-phosphate (G1P) was below the level of resolution for the assay conditions I used (0.01 µmols/g).

Figure VI-1. The metabolism of carbohydrates in active and recovering spiders at 25°C. Circles are for *F. hibernalis* and triangles are for *L. lenta*. In *Filistata*, significant increases in total anthrone-reactive substances occur by the end of two minutes activity while in *Lycosa* these substances decrease slightly in concentration. The results suggest that in *Filistata* carbohydrate enters the prosoma from the opisthosoma during exercise. Recovery values for glucose and anthrone-reactive substances show non-significant decreases in concentration over the ten minutes of recovery.

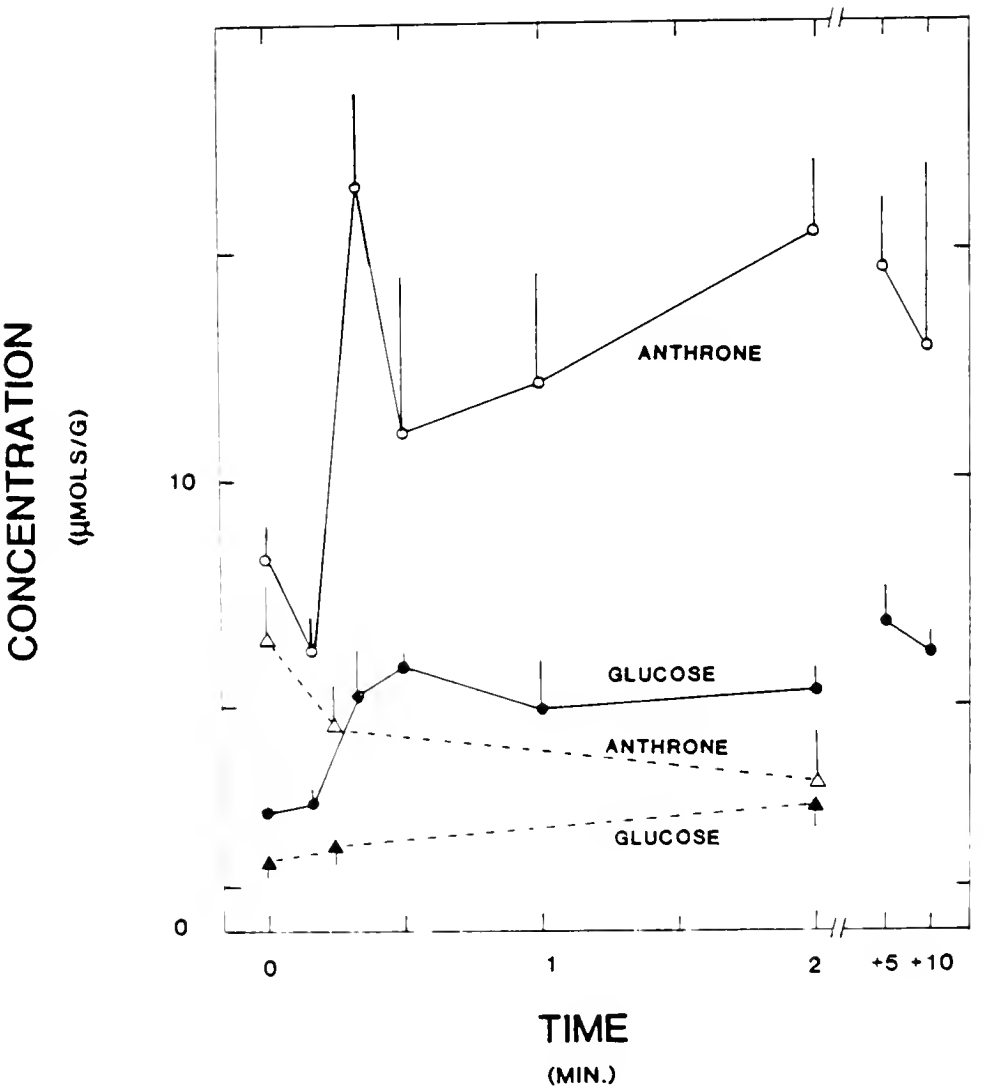
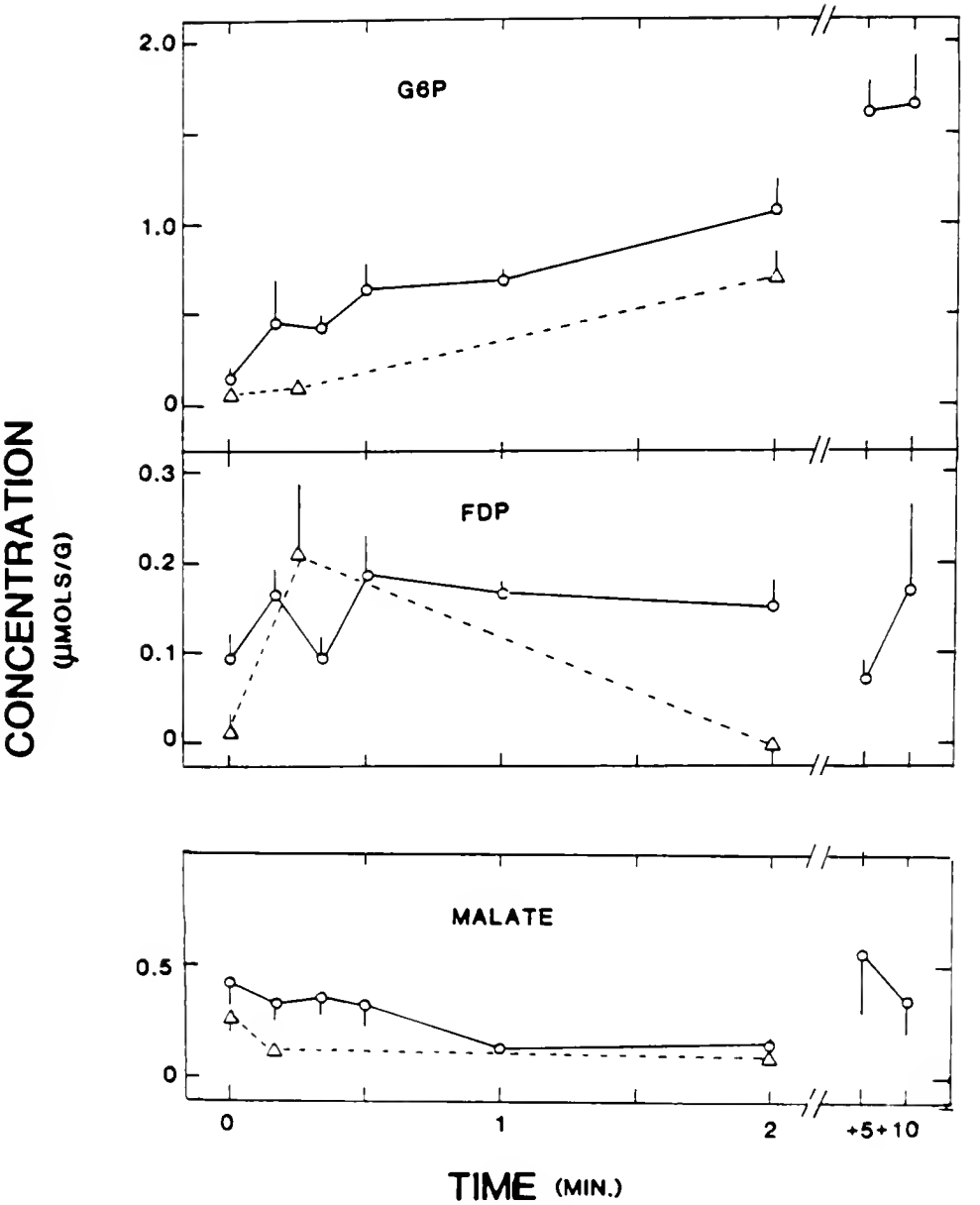

CONCENTRATION
(μMOLS/G)
10
0
ANTHRONE
GLUCOSE
ANTHRONE
GLUCOSE
0
1
2
+5
+10
TIME
(MIN.)

Figure VI-2 and 3. Metabolism of glucose-6-phosphate (G6P) and fructose-1, 6-diphosphate (FDP) during activity and recovery. Concentrations of G6P increase significantly, there are no significant changes in FDP concentration. Significantly higher concentrations of G6P in *Filistata* (circles) during recovery (compared to the end of activity) may be related to gluconeogenesis.

Figure VI-4. Malate metabolism. Significant decreases in malate concentration occur in *Filistata* (open circles). This is contrary to what would be expected under hypoxemic conditions such as prevail in exercise where an increase in malate is expected (see Ch. II). The results may be explainable by the conversion of malate to fumarate and/or succinate.


CONCENTRATION
(μMOLS/G)
G6P
2.0
1.0
0
FDP
0.3
0.2
0.1
0
MALATE
0.5
0
0
1
2
+5+10
TIME (MIN.)

Concentrations of Intermediates

The levels of glyceraldehyde-3-phosphate (GAP) varied between about 1 to 24% those of dihydroxyacetone phosphate (DAP, Fig. VI-6) averaging 12% of the concentration of DAP, or about 0.01 µmol/g. Values for glycerate-3-phosphate were near 0.7 µmol/g, phosphoenolpyruvate (PEP) was below the limit of detection (0.005 µmol/g) and pyruvate concentrations were at the level of detection of 0.05 µmols/g. However, for these three compounds, only 6 total samples were analyzed; 2 at t = 0 sec and 4 at t = 60 sec, all for *Filistata*. Finally, oxaloacetate levels averaged near 0.08 µmol/g. For all of these substances the standard errors were very large and approached 90% of the value of the mean in some cases. Thus, they were not graphed. More reliable data will require the use of more sensitive techniques involving enzyme cycling and/or flurorimetry (Lowry and Passonneau 1972).

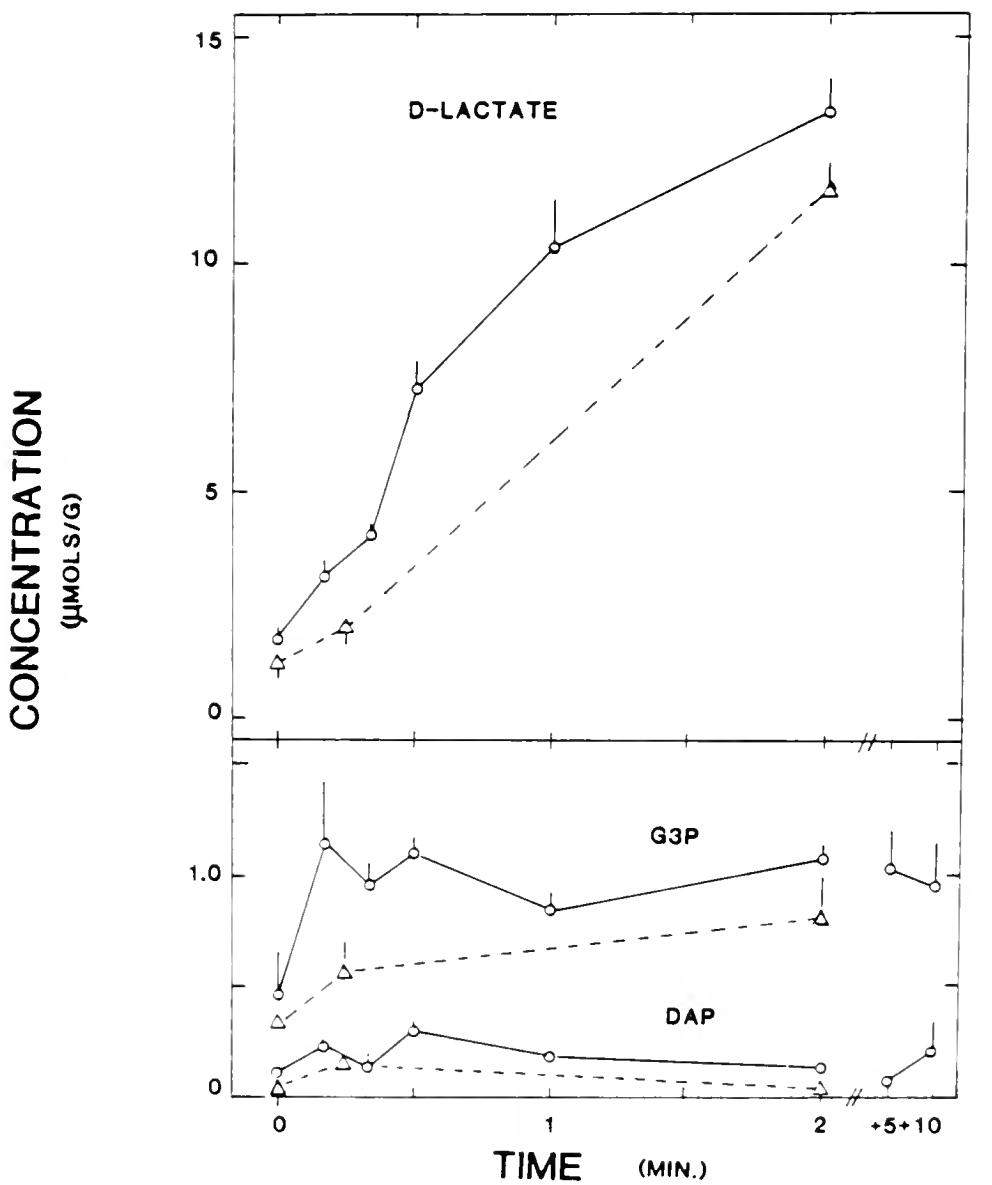
Other Intermediates

The concentration of L-malate (Fig. VI-4) declined significantly during the 120 sec activity period in both species. By the end of exercise malate concentrations had dropped to 1/3 of their initial values. In recovery in *Filistata* non-significant increases of malate occurred.

Enzymatically determined levels of D-lactate are shown in Fig. VI-5. Throughout 120 sec of activity, *Filistata* maintains higher concentrations of this substance than *Lycosa*. The same is true of the other known anaerobic by-product of spiders, glycerol-3-phosphate (G3P). Initial increases of G3P were not significant due to high variability but concentrations of this substance were significantly elevated after 120 sec of

Figure VI-5. D-lactate metabolism during exercise in *Filistata* (circles) and *Lycosa* (triangles). The results are consistent with those presented earlier (Chs. II and IV).

Figure VI-6. The metabolism of glycerol-3-phospate (G3P) and dihydroxyacetone phosphate (DAP). Increases in G3P concentration are significant but are only *ca.* 5% of those of lactate.


CONCENTRATION
(μMOLS/G)
15
10
5
0
D-LACTATE
1.0
0
G3P
DAP
0
1
2
+5+10
TIME
(MIN.)

activity (Fig. VI-6). The ratio of D-lactate to G3P was 19: 1 in *Filistata* and 21:1 in *Lycosa*. During recovery, there was a non-significant decrease in G3P coupled with a non-significant increase in DAP in *Filistata*.

High-Energy Phosphate Compounds

Changes in the concentrations of arginine phosphate (AP) and the adenosine phosphates (ATP, ADP, and AMP) are shown in Figures VI-7, 8, and 9 and fluctuations in inorganic phosphate (P_i) levels are depicted in Figure VI-10. The initiation of activity results in dramatic alterations in the concentrations of all of these substances with the changes being most extreme in *Filistata*. These changes will be carefully evaluated in the Discussion.

Discussion

Carbohydrate Metabolism

The prosomal carbohydrate concentrations of *Lycosa* and *Filistata* are similar to the range of concentrations reported for other spiders. Rakotovao (1975) reported whole spider trehalose concentrations ranged between 0.3 and 5.8 µmols/g with the range for glycogen being 12.2 to 62.7 µmols/g (glucose equivalents) in an orb weaver. Collatz and Speck (1970) reported a whole body glucose concentration of 1.8 and a sucrose concentration of 1.2 µmols/g; glycogen was 100 µmols/g in an Agelenid spider. Looking only at hemolymph, Stewart and Martin (1970) found a tarantula's glucose concentration to be 0.28 µmols/g with total anthrone

Figure VI-7. The metabolism of arginine phosphate (AP) in *Filistata* (circles) and *Lycosa* (triangles). Note the very rapid depletion over the first 15 sec or less of activity.

Figure VI-8. ATP metabolism in *Lycosa* and *Filistata*.

Figure VI-9. Changes in concentration of ADP (open symbols) and AMP (dark symbols) in *Lycosa* (triangles) and *Filistata* (circles). Especially important are the rapid increases in AMP which may have an important role in activating glycolysis (see Appendix II).

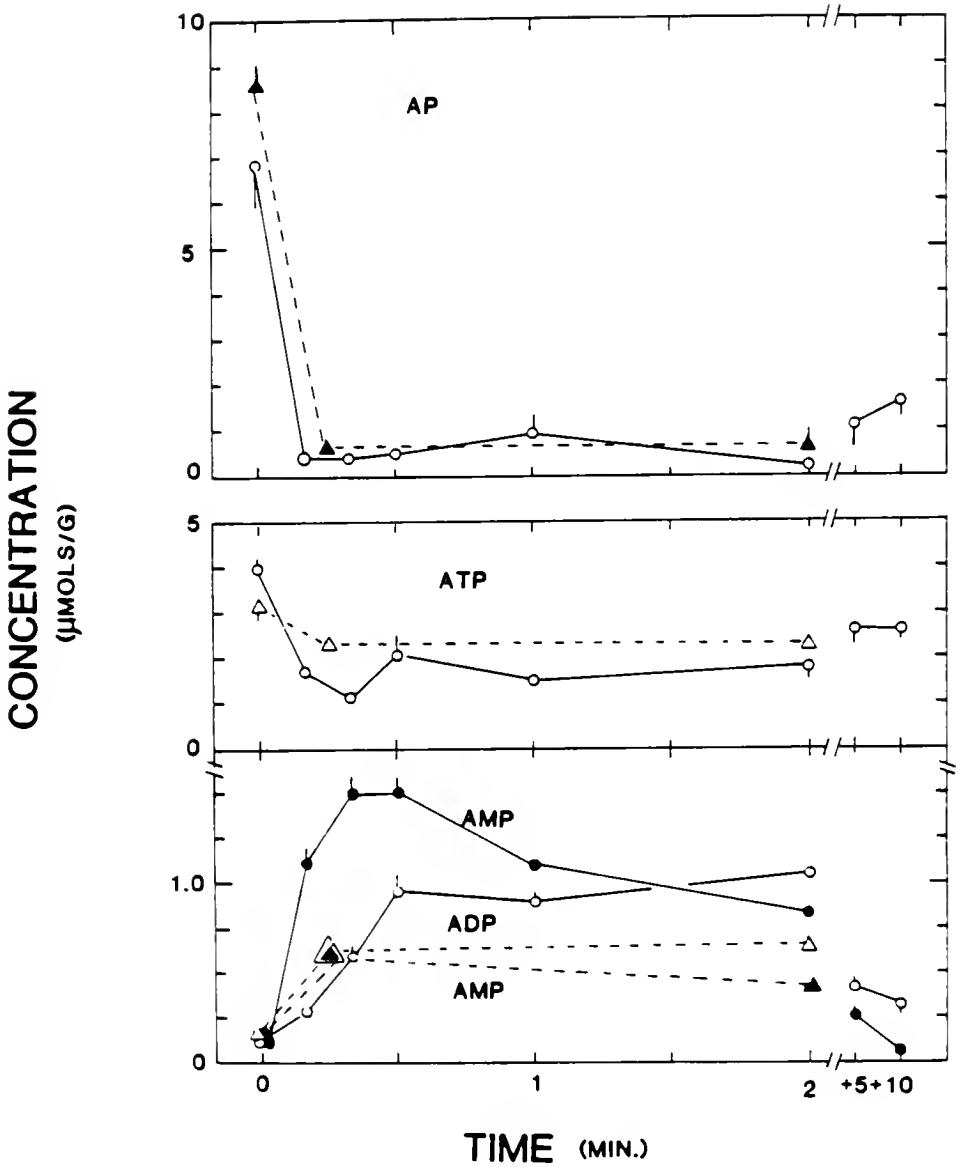
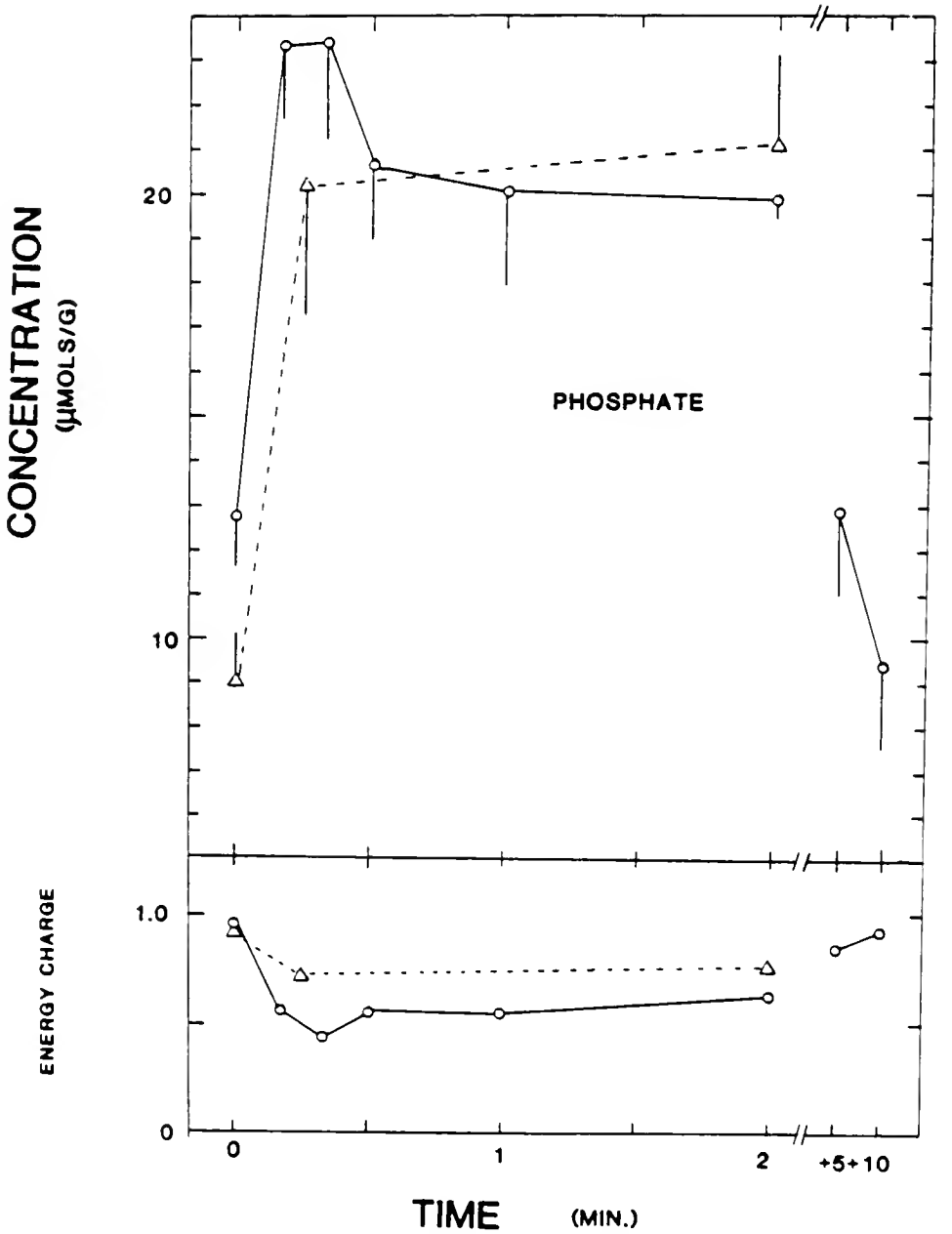

CONCENTRATION
(μMOLS/G)
10
5
0
AP
5
0
ATP
1.0
0
AMP
ADP
AMP
0
1
2
+5+10
TIME (MIN.)

Figure VI-10. Changes in the concentration of inorganic phosphate (P_i) during activity and recovery. Resting levels of P_i are higher than expected and suggestive of some hydrolysis of arginine phosphate (AP) during handling of the samples. Note the significant increases in P_i that occur simultaneously with decreases of AP (Fig. VI-7).

Figure VI-11. Energy charge during activity in *Filistata* (circles) and *Lycosa* (triangles). Energy charge is defined mathematically as:

$$\text{Energy charge} = \frac{(2ATP + ADP)}{2(ATP + ADP + AMP)}$$

An energy charge of 1.0 indicates all adenosine phosphates exist as ATP and a cell possesses a large amount of ~P potential energy. Conversely, an energy charge of 0 means all adenosine phosphate compounds are present as AMP and there are no ~P available in the adenosine pool. Note the high resting energy charge in both species, its rapid decrease and then attainment of steady-state after *ca.* 30 sec of activity.


CONCENTRATION
(μMOLS/G)
20
10
PHOSPHATE
ENERGY CHARGE
1.0
0
0
1
2
+5+10
TIME
(MIN.)

reactive substances of 0.72 µmols/g. They suggested the 0.44 µmols/g of non-glucose material was trehalose since this substance is found in scorpions (Bricteux-Gregoire *et al.* 1963). My data are for whole prosomas and although the glucose data agree well, the non-explained anthrone reactive materials (glycogen and trehalose?) are somewhat lower than those summarized above. This may indicate that the opisthosoma is richer in these materials than the prosoma.

In both *Filistata* and *Lycosa* the prosomal glucose concentration increases with the onset of activity (Fig. VI-1). The source of this glucose is not evident. The initial concentration of anthrone-reactive substances are not sufficient to explain the accumulations of different substances that occur over 120 sec of activity (Table VI-2) in both species. This conclusion is reached by subtraction of the t = 120 sec concentrations of glucose and total hexose phosphates from the initial total anthrone reactive substance concentration. The result must be at least twice the accumulation of lactate since 2 lactates are produced per hexose that enters glycolysis. Table VI-2 shows the initial hexose in *Filistata* is not even close to the required amount and that of *Lycosa* is also considerably less than needed.

Thus, it is reasonable that the opisthosomas of these spiders provide some form of hexose to the prosoma during exercise via the hemolymph. This is especially evident in Figure VI-1 for *Filistata*: the concentration of anthrone-reactive materials significantly increases during activity. The increase in glucose concentration in *Filistata* is about 2.5 µmols/g and is not sufficient to explain the 7 µmol/g increase in anthrone reactive substances. Based on the evidence from other species cited at the start of this section, I suggest that some opisthosomal tissues may be a

Table VI-2. The amount of carbohydrate present in spider prosomas at the start of exercise compared to the amount needed to produce all the intermediates and lactate found after two minutes of activity. The differences should be zero, because they are not, carbohydrate must be being added to the prosoma during exercise.

Species	Initial Anthrone-Reactive Substances	T - 120 Glucose Hexose-P Total	Needed For Lactate	Difference
F. hibernalis	8.3	6.8 1.8 -8.6	-5.8	-6.1
L. lenta	6.4	2.8 0.7 -3.5	-5.2	-2.2

Need: based on assuming that all carbohydrate is burned only to lactate. Therefore, the increase in lactate concentration ÷ 2 equals the required hexose. This figure tends to err in favor of less hexose needed that is really the case since it does not take into account the increases in concentrations of trioses and triose phosphates which in the case of DAP and G3P are significant (*ca.* 1 µmol/g total).

Difference: initial ([anthrone]-final (glucose]+[hexose-P]+(lactate/2).

Table VI-3. Total adenosine phosphate concentration in prosomas during rest, exercise, and recovery.

Species	time	Total Adenosine Phosphate Concentration[a] (μmols/g) 0s	10s	15s	20s	30s	60s	120s	+5m	+10m
F. hibernalis		4.12	2.53	---	2.60	3.82	3.02	3.26	3.07	2.93
		± (.275)	(.383)		(.548)	(.541)	(.413)	(.434)	(.437)	(.437)
L. lenta		3.32	---	3.31	---	---	---	3.25	---	---
		(.395)	---	(.377)	---	---	---	(.338)	---	---

[a]Total = [ATP] + [ADP] + [AMP]

± S.E. are the sum of the S.E. for each substance used to arrive at the total adenosine phosphate concentration

carbohydrate storage center, much like a vertebrate liver. During activity, glycogenolysis occurs and carbohydrates, such as glucose and trehalose (Rakotovao 1975) or sucrose (Collatz and Speck 1970) are released into the hemolymph. Given the low carbohydrate concentrations of tarantula hemolymph (Stewart and Martin 1970) compared to resting prosomal concentrations (this study) these materials would then be transported into the tissues. However, the problem remains as to how they are pumped to the prosoma when a large pressure gradient exists (Ch. V).

The data presented in Figure VI-1 suggest that *Lycosa* is more dependent on hexose already present in the prosoma than is *Filistata*. This is evident by the fact that the prosomal carbohydrate concentration decreases to nearly zero by the end of activity. Thus, it is possible that *Lycosa* may be ultimately constrained by loss of hexose substrate for glycolysis.

Finally the prosomal carbohydrate concentration remains high during recovery in *Filistata*. This may indicate a prosomal role in gluconeogenesis or merely indicate slow metabolism of accumulated G6P.

Intermediates

The resting levels of glycolytic and Krebs cycle intermediates are consistent with those reported for the blowfly (Sacktor and Wormser-Shavitt 1966). The only exceptions to this were G3P which is less than 2/3 as concentrated in the spiders. The low concentration of G3P may be related to the lower aerobic capacities of spiders (Anderson and Prestwich 1982). In insect flight muscles, G3P is the major vehicle for transfer of cytosol-produce/electrons to the mitochondrial electron transport system. Spiders possess a less active cytosol GPDH (Prestwich and Ing

in press) and many fewer mitochondria (Linzen and Gallowitz 1975) and therefore would not require or produce as large amounts of this substance as would insects. Similarly, the higher malate concentrations in insects may reflect the greater proportion of mitochondria and therefore higher cellular concentrations of Krebs intermediates. Finally, I was only able to set upper limits on the concentrations of several substances (G1P, F6P, GAP, 3PGA, PEP, pyruvate, and oxaloacetate). All of these substances except 3PGA and pyruvate are normally found in only trace amounts in flight muscle (Sacktor and Wormser-Shavitt 1966) and mammalian tissue such as brain (Lowry *et al.* 1964). The maximum possible concentrations I have found are consistent with other literature values.

Intermediates tend to increase in concentration early in activity. Some of these substances (that could be measured accurately), such as FDP and DAP, quickly level off or decrease by the end of activity. Another trend is found in the Krebs cycle intermediate malate which decreases significantly during activity. The cause of this reduction is not known but one possible explanation is that it is anaerobically metabolized to another substance(s) such as succinate and fumarate.

The intermediates that increased in concentration during activity were G6P, G3P, and lactate. The increases of G3P and lactate are related to the maintenace of cytosol redox (Ch. II). Lactate increases reported in Fig. VI-5 closely resembles those reported in Chapters II and IV. *Filistata* accumulates lactate faster than *Lycosa* and the rates of accumulation in both species are like those reported in Chapter IV. The accumulation of G3P suggests two possibilities. Either the pathway is inhibited after the initial phase of exercise or/and the mitochondria are better able to oxidize G3P to DAP later in activity. Given no

dramatic increase in DAP (even though earlier glycolytic intermediates are in high concentration) it is not likely that G3P oxidation is significantly enhanced later in the exercise period.

The continued increase of lactate throughout the activity bout coupled with constant [G3P] after the initial few seconds of activity suggest that in the presence of continued glycolysis GPDH must be inhibited (Guppy and Hochachka 1978). Generally, the rate of lactate production appears to increase between t = 0 and 30 sec, reaching a maximum value (anaerobic scope, Bennett 1978) between 10 and 30 sec and thereafter decreasing or remaining constant (Table VI-4). At the same time, G6P, the starting substrate continues to accumulate at a constant rate in both species. This suggests that regulation of the process is still limited by PFK since G6P (and therefore probably F6P, Newsholme and Start 1973) increases, FDP remains essentially constant or increases only slightly.

Arginine and Adenosine Phosphates and P_i

There is some evidence of partial hydrolysis of AP and possibly ATP from the initial, resting state, samples. First, the P_i values are somewhat higher than normally reported in resting muscular tissues where concentrations are on the order of 8 µmols/g or lower (Newsholme and Start 1973; Lehninger 1975; and see Table VI-5). Secondly, Di Jeso *et al.* (1967) report whole spider P_i values near 7.4 µmols/g and a ratio of AP to ATP of about 4:1. Since phosphagens are normally highest in muscle and nervous tissues (Lehninger 1975) such as those found mainly in the prosoma (Table I-2), I expected a ratio of at least 4:1 in the prosoma.

Table VI-4. Partition of phosphates during activity and recovery. The figures represent changes in concentration (μmols/g) during the given time interval: positive values represent increases, negative values are decreases. The total change in combined phosphate (III) should be equal to, but of opposite sign to the change in P_i (IV).

		Filistata hibernalis						*Lycosa lenta*	
Substances	time	0-10	10-20	20-30	30-60	60-120	120-+5	0-15	15-120
I. High-energy phosphates		-10.76	-1.64	+2.00	+0.61	+0.57	+3.20	-9.34	+0.08
II. Mobilized phosphates[a]									
A. G6P and FDP		+ 0.64	-0.36	+0.59	0	+0.34	+0.19	+0.45	+0.19
B. DAP and G3P		+ 0.80	-0.30	+0.33	-0.39	+0.21	-0.14	+0.38	+0.10
C. Total (A + B)[b]		+ 1.44	-0.66	+0.92	-0.39	+0.55	+0.05	+0.83	+0.29
III. Total change in combined phosphate (I & II, above)		- 9.32	-1.64	+2.92	+0.61	+1.12	+3.25	-8.51	+0.37
IV. Change in P_i		+10.62	+0.14	-2.82	-0.57	-0.10	-7.04	+11.3	+0.88

[a] GAP, PGAP, etc. through PEP are ignored due to their low concentrations; the same goes for G1P and F6P (although F6P is potentially significant).

[b] Decreases in hexose and triose bound phosphate should not be reflected in P_i increases. They are the result of either triose -P or ATP formation. Therefore, negative values in II-C are not added to I.

Table VI-5. The changes in high-energy phosphates, AMP, and P_i during exercise in spiders and a fly. Energy charge is also calculated.

		Concentrations (μmols/g)								
		Tegenaria domestica[a]	*F. hibernalis*[b]			*L. lenta*[b]			*Phormia regina*[c]	
Substance	time	0	0	10	120	0	10	120	0	10	120
AP		2.5	6.8	0.4	0.1	8.5	0.6	0.6	3.1	1.9	2.0
ATP		0.6	3.9	1.6	1.8	3.1	2.2	2.2	7.0	6.2	6.4
ADP		---	0.11	0.28	1.07	0.18	0.34	0.34	1.6	1.9	1.9
AMP		---	0.06	0.58	0.43	0.06	0.34	0.20	0.12	0.29	0.27
P_i		7.4	12.7	23.4	20.0	9.0	20.3	21.1	6.8	7.5	7.5
Energy Charge[d]		---	0.97	0.72	0.70	0.95	0.80	0.83	0.89	0.85	0.86
Phosphorylation Potential (Γ)[e]			2790	244	84	1915	520	310	640	435	450

[a] Di Jeso *et al.* 1967, whole spider

[b] This study, prosoma only, 10 sec value for *L. lenta* interpolated

[c] Sacktor and Hurlbut 1966; pterothorax

[d] Energy charge = $1/2\left(\frac{2ATP + ADP}{ATP + ADP + AMP}\right)$

[e] $\Gamma = \frac{[ATP]}{[ADP][P_i]}$ phosphorylation potential

However, my data show AP to ATP ratios of 1.75 to 1 and 2.75 to 1 with resting P_i concentrations of 12.7 and 9.0 in *Filistata* and *Lycosa* respectively. Given the lability of AP and the high energy charge of resting spiders (Fig. VI-11, suggesting little ATP hydrolysis), I assumed that the AP concentrations reported for rest (t = 0) are minimal estimates for resting conditions and that the elevated P_i is due to hydrolysis of this substance. There are two possible causes of low AP: (a) minor activity (less than one sec) occurred when some individuals were frozen and (b) the fact that the t = 0 samples had to be analyzed twice over a 1.5 day period due to problems in the AP assay encountered when high concentrations of AP were present (see Methods). (These difficulties were not experienced with any of the other samples.)

There is only one other discrepancy in the high-energy phosphate compound data. In Table VI-3, the total adenosine phosphate concentrations are summed for each interval during the activity period. These sums should all be roughly the same or decrease slightly with time due dephosphorylation of AMP to adenosine (Lowery *et al.* 1964) or deamination to inosine monophosphate (Lowenstein 1972). In *Lycosa*, the summed concentrations are constant. However, in *Filistata* the t = 0 and 30 sec summations are much greater than any of the others. Thus, a positive bias to the concentrations of one or more of the adenosine phosphates is possible. However, overall the agreement is good and the possibility of some of the fluctuations being due to conversion of adenosine needs investigation.

The general consistency of all of the data relating to the metabolism of phosphorylated compounds is shown in Table VI-4. Here, the changes in bonded phosphate concentrations (both in intermediates and in high-energy

compounds) are compared with the changes in P_i. Excellent correspondence is obtained in all intervals except early recovery in *Filistata* and the 0 to 15 sec interval in *Lycosa*. However, even these deviations are small in light of the large standard errors associated with some of the compounds used in obtaining these results (Figs. VI-3, 6, and 10).

One very important result shown in Table VI-4 is that the rate of phosphate cleavage reaches an equilibrium after 10 to 15 sec of activity. This is reflected in the relatively small changes in total AP, ATP, and ADP after this time (there may actually be a net ATP synthesis in *Filistata*, see Fig. VI-8). Another indication of equilibirum is shown on Fig. VI-11 which graphs the prosomal energy charge with respect to time. Energy charge is a measure of the ability to do chemical work. A value of 1.0 equates to maximal energization in the cell due to the presence of all adenosine compounds in the form of ATP. An energy charge of 0 indicates all adenosine compounds exist as AMP. In spiders, the energy charge drops precipitiously from 0.95 (an extremely high value) to a minimum of 0.56 or 0.8 within 20 sec. From then on, the level is constant. An even more sensitive measure of the ability of the cell to do work is the phosphorylation potential (Γ). It also drops rapidly at the start of exercise and thereafter changes very little. Both measures indicate an equilibrium between the use and production of ~P. Finally, the energy charge quickly returns to a higher value during recovery (0.80 and 0.93 after five and ten minutes of recovery).

It is evident that spiders are different from insects. This point was made most recently by Anderson and Prestwich (1982) in reference to the aerobic capacities of spiders. Another reflection of this is in

Table VI-5 where the metabolism of high energy phosphate compounds in running spiders is compared to a fly in flight.

Examination of the resting (t = 0) values in the table points up several interesting differences. First, ADP concentrations in spiders are quite low compared to *Phormia* (a fly) and also to other animal tissues where they normally are between 1 to 2 μmols/g (Newsholme and Start 1973; Lehninger 1975; Sacktor and Hurlbut 1966). The spider values are only 1/10 as much. This low concentration is correlated with the high energy charge ($\tilde{}$ 0.95) found in spiders compared to the ratio of 0.85 to 0.9 normally seen in other organisms (Lehninger 1975). Secondly, the ratio of AP to ATP in spiders is less like that of the fly, and more like the CP (creatine phosphate) to ATP ratio of mammals. In mammals the CP:ATP ratio of muscle is typically about 5 to 1 (Lehninger 1975) and in spiders it is at least 2 or 4 to 1. By contrast, *Phormia* has an AP to ATP ratio of 1 to 2.25. This indicates spiders and vertebrates have a much higher reliance on phosphagen stores during muscular activity than do insects.

The differences between insects and spiders are even more impressive when the changes that occur in the concentrations of metabolic and energy intermediates at the onset of flight and running are examined (Table IV-5). In spiders, within 15 sec of the onset of vigorous activity AP is completely depleted and ATP has dropped by 33 to 50%. By contrast, in *Phormia* AP concentrations drop 33% (a small absolute change) and ATP decreases 12%. Clearly, spiders are more dependent upon stored phosphate during the early phases of movement. This is analogous to the process as seen in a human sprinter where tremendous CP and ATP depletions occur during short runs (McArdle 1981).

The Relative Contributions of Stored High-Energy Phosphates and Glycolysis to Phase I of Activity

Glycolysis is completely over-shadowed as an energy source by the use of AP and ATP stores during the first 10 to 15 sec of activity. During this time, phosphate is cleaved from stores at a rate of 65 µmols/g min in *Filistata* and 37 µmols/g min in *Lycosa* (10 and 15 sec periods, respectively). These rates might be higher since it is possible the AP and ATP is used in a shorter time than the measurement intervals. During these same time periods, ~P is produced from anaerobic glycolysis at the rate of about 13 µmols/g min in *Filistata* and 10 µmols/g min in *Lycosa* (assuming 1.5 ~P per lactate and 1.0 per G3P; Ch. II). Thus the cleavage of stores occurs 4 to 5 times faster than its production via glycolysis. Finally, aerobic processes can probably be dismissed as a significant source of ~P due to the low number of spider muscle mitochondria (Linzen and Gallowitz 1975), low PO_2 (Angersbach 1978), and the time necessary to achieve full activation of the Krebs Cycle (See Appendix II, and Sacktor and Wormser-Shavitt 1966).

The relative roles of anaerobic glycolysis and ~P stores depletion are crucial to the understanding of the rapid slowing in running spiders. The summary presented above clearly shows that ~P stores are the major energy source during phase I. The depletion of these stores is mainly responsible for the rapid fatigue and change in gait (from jumping and running to walking) that occurs near the end of phase I (Ch. IV). In summary, I conclude that phase I slowing is the direct result of the spider being forced to rely upon processes (anaerobic and aerobic metabolism) that cannot deliver ~P to drive muscle contractions at as high

a rate as is possible by catabolzing AP and ATP stores (see previous paragraph). The reason for the slowness of delivery undoubtedly relates to: (a) these pathways large number of steps, (b) the low activities of their rate-limiting enzymes compared to that of arginine kinase (Prestwich preliminary data), and (c) poor availability of O_2 and poorly developed aerobic pathways (Angersbach 1978; Linzen and Gallowitz 1975; Prestwich and Ing in press).

CHAPTER VII
ACTIVITY IN SPIDERS: A REVIEW

Summary

1. The ranges of a number of important physiological parameters that change during exercise (hemolymph pressures, heart rate, $\dot{V}O_2$, lactate, phosphagen metabolism, pH, PO_2, and % saturation) are summarized (Table VII-1).
2. A model of exercise energetics in spiders suggests maximal struggles are limited first by phosphagen depletion and later by anaerobic accumulations. Overall, anaerobic metabolism produces most of the ~P used during activity; aerobic contributions are responsible for less than 10% of the total ~P used (Figs. VII-3 and 4).
3. The interrelationships between prey capture technique, resting $\dot{V}O_2$, and anaerobic metabolism are discussed. Spiders are characterized as animals of moderate power production capability; aerobic abilities are low while anaerobic capacities and phosphate stores are not unusually high. Use of silk and poison helps make it unnecessary for spiders to be able to generate ~P at high rates.

Introduction

This chapter will review the physiology of movement and recovery in spiders. As part of the review, a model of the energetics of spider locomotion in the prosoma is presented. The overall approach

comparable to the other results); (c) lactate, Chapter IV; (d) ~P from the sums of the concentrations of AP + ADP + 2X(ATP), Chapter VI; (e) pressure, the mean pressure from an integration over 15 sec intervals of the results of the experiments in Chapter V; (f) heart rates, Chapter IV. In Figures VII-5 and 6 the running speed percentages are based on the first ten seconds of running after five and ten minutes of recovery (see Methods, Ch. IV) and the hemolymph pressures during recovery are based on 30 sec activity periods like those shown in Figure V-3 (measurements integrated over 15 sec periods to give mean pressures).

Estimation of ~P Turnover

In Chapter III the relative importance of phosphagens, aerobic, and anaerobic metabolism to the energetics of struggling in spiders in respirometer flasks was estimated. However, there were several problems with these estimates. The most serious were that (a) the animals were performing at high but not maximal work loads and (b) assumptions were made as to the degree of phosphagen metabolism and aerobic metabolism during activity.

The data presented in Chapter VI allow an independent assessment of the relative importance of these three sources of ~P to maximal activity. However, Chapter VI data apply only to the prosoma. This is not a serious defect since the prosoma generates most of the power used during activity (Chapters II and IV). The only drawback to using the Chapter VI data deals with the necessity of estimating aerobic metabolism. To calculate aerobic power, I assumed that aerobic ~P synthesis could be estimated from knowledge of the spiders heart rate and resting $\dot{V}O_2$.

of this chapter will be comparative and deals with how the circulatory, respiratory, and locomotory systems have evolved in different species of spiders faced with different environmental pressures.

Methods

The ranges of values for several physiological functions that are important in activity are presented in Table VII-1. These values were obtained from the earlier chapters of this dissertation and from the literature. Also, the data presented in earlier chapters have been summarized in Figs. VII-1 through 4 and Tables VII-2 and 3. Figures VII-1, 2, 5, and 6 show changes in various physiological parameters during exercise in terms of percentages. The percentage scale is based on the maximum and minimum values observed for the variable in question. Percentages are expressed either as percent of maximum value (pressure, heart rate, lactate concentration, $\dot{V}O_2$) or minimum value (running speed, ~P concentration). Thus, all values are calculated according to an equation of the form:

$$\% = \frac{[V_t - M_{(1 \text{ or } 2)}]}{(\Delta M)} \times 100 \qquad (1)$$

where V_t is the value of the parameter in question at some time t, M_1 or M_2 is either the maximum or minimum value of that parameter and ΔM is the difference between the maximum and minimum value of the variable.

The calculation of all of the percentages in Figures VII-1, 2, 5, and 6 is from the following sources and uses 25°C data only: (a) running speed (fatigue), Chapter IV--relative speeds; (b) $\dot{V}O_2$, Chapter III (note: these involved submaximal activity and therefore are not strictly

Factorial increases in HR above resting were taken as factorial indicators of $\dot{V}O_2$ above resting (Anderson and Prestwich 1982). The estimated activity $\dot{V}O_2$ for some time period was converted to µmols ~P produced by assuming glucose-6-phosphate was the initial substrate and that 38 µmols of ~P were synthesized per mol of G6P used. Because 6 mols of O_2 are required to oxidize 1 mol of G6P and 38 mols of ~P are yielded per G6P oxidized, then 6.3 mols of ~P would be synthesized per mol of O_2 consumed. Thus, 1 µl O_2 (STPD) = 0.28125 µmols ~P synthesized. As an example of a calculation, if the resting $\dot{V}O_2$ = 22.4 µl O_2/g min at a HR of 20, then at a HR of 40 the estimated $\dot{V}O_2$ = 44.8 µl O_2/g min and estimated ~P production = 12.6 µmols/g min.

Calculation of ~P contributions from stores and anaerobic metabolism are straightforward: *Stores*: were calculated from the changes in the sum of AP + (ATP X 2) + ADP (Ch. VI); *Anaerobic Contributions*: are based on the differences in the concentration of lactate and G3P accumulations from one time to the next (Fig. VI-2) by assuming a net gain of 1.5 ~P per lactate and 1.0 ~P per G3P (Ch. II).

Discussion

Values for some physiological parameters under varying exercise conditions are given in Table VII-1. These values will be the basis for much of the following discussion.

Rest and Moderate Activity

It is well-documented that spiders have low resting $\dot{V}O_2$ (Dresco-Derouet 1969; Anderson 1970, 1974, 1978; Anderson and Prestwich 1982;

Table VII-1. Values for several physiological parameters in resting (alert) and active spiders.

Variable	Activity: Rest	Activity: Moderate	Activity: Maximal
1) $\dot{V}O_2$	20-120% Predicted, mean near 50%	1.5-4X[d,f,j,g]	up to 10X rest[b,k]
2) Hemolymph Pressure (mmHg)			
a) Prosoma or leg	38[a,e,h]	40-60[a,e,b,i]	~500
b) Opisthosoma	5-14[a,e,h]	17	~130[a]
c) Systolic-diastolic	4[e]		~30[h]
3) P_{O_2} (torr)[c]			
a) Leg (mixed venous)	16	--	25 to 0
b) Prosoma (mixed venous)	14	--	24 to 0
c) Heart (arterial)	28	--	60-92 ($\bar{x}$ = 74)
4) HR (beats/min)[b,c,j]	--	up to max.	~70-200
5) Lactate (μmols/g)[j]			
a) Legs only	1.1	none to max.	12.4
b) Prosoma only	0.7	none to max.	9.3
c) Legs and prosoma	1.0-1.8	none to max.	9-16
d) Opisthosoma	0.3-2.6	none to max.	2.0-5.5
e) Whole spider	0.5-2.0	none to max.	5-12

Table VII-1 continued

Variable	Activity: Rest	Moderate	Maximal
6) pH (hemolymph)[c]			
a) Heart (arterial)	7.49	--	0.26 decrease
b) Ventral sinus (venous)	7.45	--	0.50 decrease
7) % Saturation[c] ($C_{O_2} \cong 1.7$ vol %)	52% (95% bound)		up to 100% (89% bound)

[a] Anderson and Prestwich 1975 (araneomorphs)

[b] Anderson and Prestwich 1982 (all types)

[c] Angersbach 1978 (tarantula)

[d] Ford 1977a, b

[e] Parry and Brown 1959a, b (araneomorph)

[f] Prestwich 1977 (araneomorph)

[g] Seymour and Vinegar 1973 (tarantula)

[h] Stewart and Martin 1974 (tarantula)

[i] Wilson 1962 (araneomorphs)

[j] This study (araneomorphs)

[k] Anderson pers. comm. (tarantula)

Greenstone and Bennett 1980). Rates of oxygen consumption in spiders correlate strongly with book lung surface areas (Anderson 1970; Anderson and Prestwich 1982). This implies that resting $\dot{V}O_2$ must also correlate with resting cardiac output ($\dot{Q}$) since large lungs by themselves cannot deliver O_2 to the tissues. Thus, species with relatively high $\dot{V}O_2$ must have large book lungs and $\dot{Q}$ (assuming that hemolymph O_2 capacity is roughly the same in all species).

Cardiac output is determined by both stroke volume and heart rate (HR). Greenstone and Bennett (1980) and Anderson and Prestwich (1982) have shown that no good interspecific relationship exists between resting HR and $\dot{V}O_2$. Therefore, different species must rely on different stroke volumes. However, within a species, HR probably is a good indicator of $\dot{V}O_2$ since available data and calculations suggest that stroke volume changes little at different HR and, thus the main determinant of changes in $\dot{Q}$ is the HR (Appendix I; Ch. IV; Stewart and Martin 1974; Anderson and Prestwich 1982).

Besides the cardiac output and respiratory surface area, several other factors affect the amount of O_2 available to spider tissues. One very important factor appears to be ventilation of the lungs. Assuming tarantulas are a good model for all spiders, then it appears that in resting spiders there is little ventilation of the lungs. As a result, the P_{aO_2} (measured in the heart) is only about 30 torr and the hemolymph is only half saturated, containing about 1 vol % O_2 (Angersbach 1978). There is a time lag between the initiation of activity and an increase in ventilation. After ventilation begins, the P_{aO_2} may soar to 80 mm Hg and the hemolymph will completely saturate and contain about 2 vol % O_2

(Angersbach 1978). Additionally, other factors may operate to increase the availability of O_2 to active tissues. Spider hemolymph shows a Bohr effect and the A-V PO_2 difference may increase (Angersbach 1978). Also, spiders that possess extensive trachea in addition to their lungs may be able to use these structures to boost O_2 availability to their tissues (Anderson 1970).

Taking HR and ventilation as the main factors that can influence increases in $\dot{V}O_2$ during activity, then maximum rates of $\dot{V}O_2$ may be calculated. In mygalomorph spiders such as tarantulas, the maximum increase should be about 8X resting $\dot{V}O_2$ since these spiders can increase their heart rates a maximum of four times (Stewart and Martin 1974; Angersbach 1978; Anderson and Prestwich 1982) and the saturation of their hemocyanin can double (Angersbach 1978). This estimate agrees with observed $\dot{V}O_2$ for active tarantulas by Anderson (pers. comm.). For araneomorphs, the maximum increase in $\dot{V}O_2$ should be about 18X resting since HR can increase about 9X resting (Anderson and Prestwich 1982) and assuming that saturation of hemocyanin can double.

While the maximum $\dot{V}O_2$ of spiders may be nearly 18 times resting, the data that have been obtained on spiders performing routine activities suggest that normally $\dot{V}O_2$ during these activities seldom exceeds 3 or 4 times resting (Seymour and Vinegar 1973; Peakall and Witt 1976; Ford 1977a, b; Prestwich 1977). Working at levels far below their aerobic maxima helps spiders avoid the necessity of reliance on anaerobic metabolism. They preserve the ability to increase their aerobic metabolism and to begin to rely on anaerobic metabolism.

The metabolism of stored ~P in moderately active spiders is not known. However, it is not likely that these compounds are greatly

depleted during such activities: if a moderately active spider is threatened or presented with prey it can still move rapidly. Data presented in Chapter VI suggests that if phosphagens were depleted, this rapid movement would be impossible. It can be concluded that abundant ~P compounds are present in sub-maximally active spiders. However, this observation needs biochemical confirmation.

Thus, spiders working at moderate rates do not utilize anaerobic metabolism and phosphate stores to the large degree that these are relied upon during maximal activity (see Chs. IV and VI and also see below). This is not to say that some utilization of ~P from these sources does not occur. At the start of any activity, stores are almost surely used until aerobic processes can be fully activated. Furthermore, there are good reasons to believe that regions of reliance on anaerobic metabolism will exist even in moderately active spiders. First, even at its maximum O_2 carrying capacity, hemocyanin carries relatively little O_2 compared to other circulating pigments (Angersbach 1978). This, coupled with an open circulatory system, implies that some hypoxemic areas should develop where tissues are most active metabolically. Secondly, even when adequate O_2 is present, ~P demands may exceed the ability of the small number of muscle mitochondria to produce these compounds. Therefore, more alternative pathways must be used to supplement the production of ~P even in the presence of O_2. An example of this may be orb-web construction where slightly elevated levels of lactate are found in the prosoma after an hour of steady activity by the spider (Ch. III).

In summary, the picture of a spider engaged in moderate behaviors such as grooming, searching, and web-building is one of aerobic

steady-state activity. Reliance on aerobic metabolism allows for both maximum energy extraction and the use of the full range of energy substrates (fats, protein, and carbohydrates). It also allows the spider to retain the capacity for bursting into higher levels of activity (via anaerobiosis and the use of phosphagen stores) when circumstances require.

Previous discussion indicated that one of the two determinants of cardiac output (and therefore O_2 availability) was stroke volume but that normally stroke volumes are relatively constant. However, there is an important exception to this. To a large degree, stroke volume is determined by the sum of the pressures generated by the opisthosoma and heart compared to that of the prosoma. In resting and moderately active spiders, the slightly higher pressures found in the prosoma are easily overcome during systole and hemolymph is pumped to the prosoma (Table VII-1). However, during vigorous exercise this is no longer the case and the cardiac output into the anterior aorta suddenly drops to zero. Prior to this point, increased levels of exercise could still be fueled through aerobic processes as a result of increased HR and ventilation. However, once prosomal pressures force the stroke volume to zero, then the only possible sources of ~P in the prosoma become stored phosphagens and anaerobic metabolism. This event physiologically demarks the difference between moderate and maximal activity in spiders.

Maximal Activity

The physiological changes that occur in maximal struggles of *ca.* two minutes or less are shown in Table VII-1 and specifically for *F. hibernalis* and *L. lenta* respectively in Figures VII-1 and 2. The top

Figure VII-1. Changes in running speed, lactate, ~P stores, prosomal pressure and heart rate during a two minute maximal struggle in *F. hibernalis* at 25°C. All changes are expressed as percentages (see Methods and equation VII-1). A full explanation of this figure is given in the text.

Key for lactate panel:

■ prosoma

○ opisthosoma

⬡ whole spider.

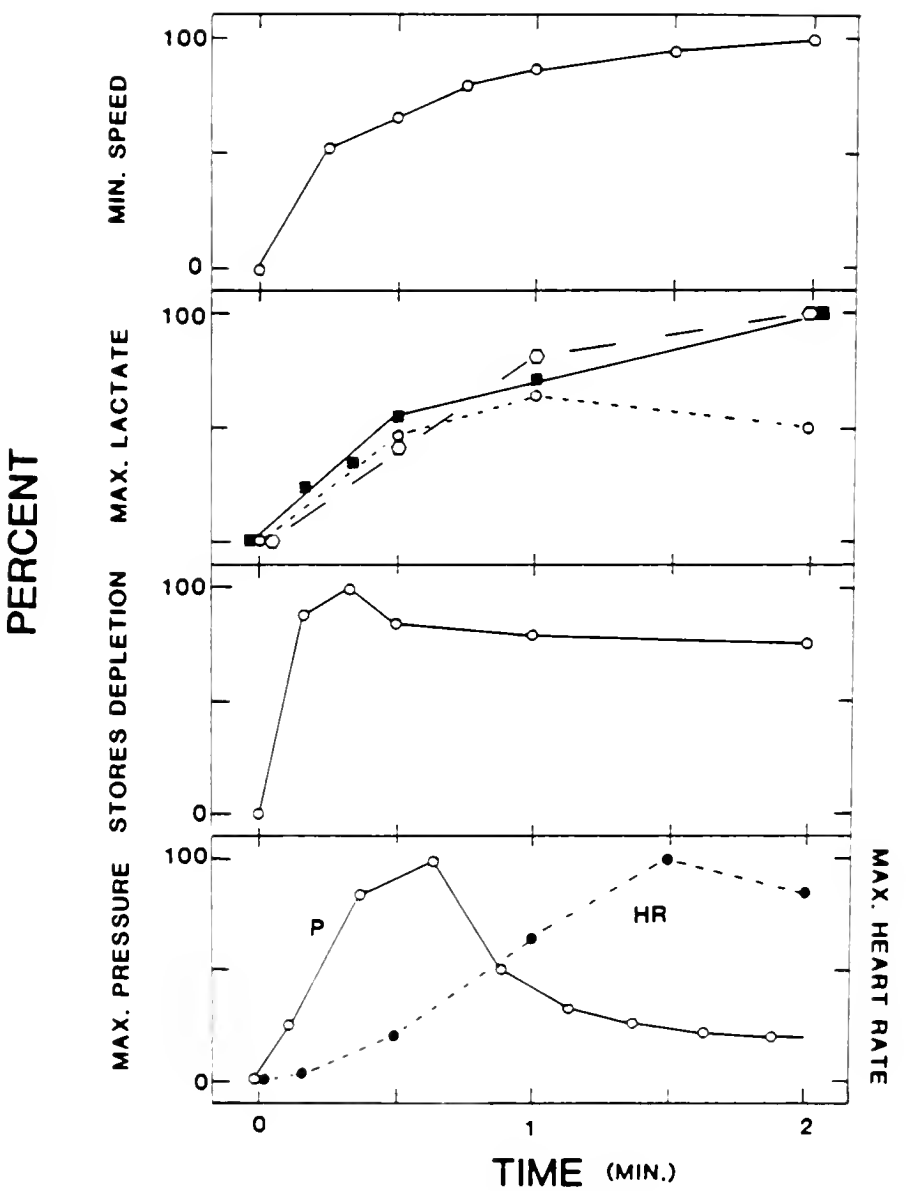

PERCENT
MIN. SPEED
MAX. LACTATE
STORES DEPLETION
MAX. PRESSURE
MAX. HEART RATE
100
0
P
HR
0
1
2
TIME (MIN.)

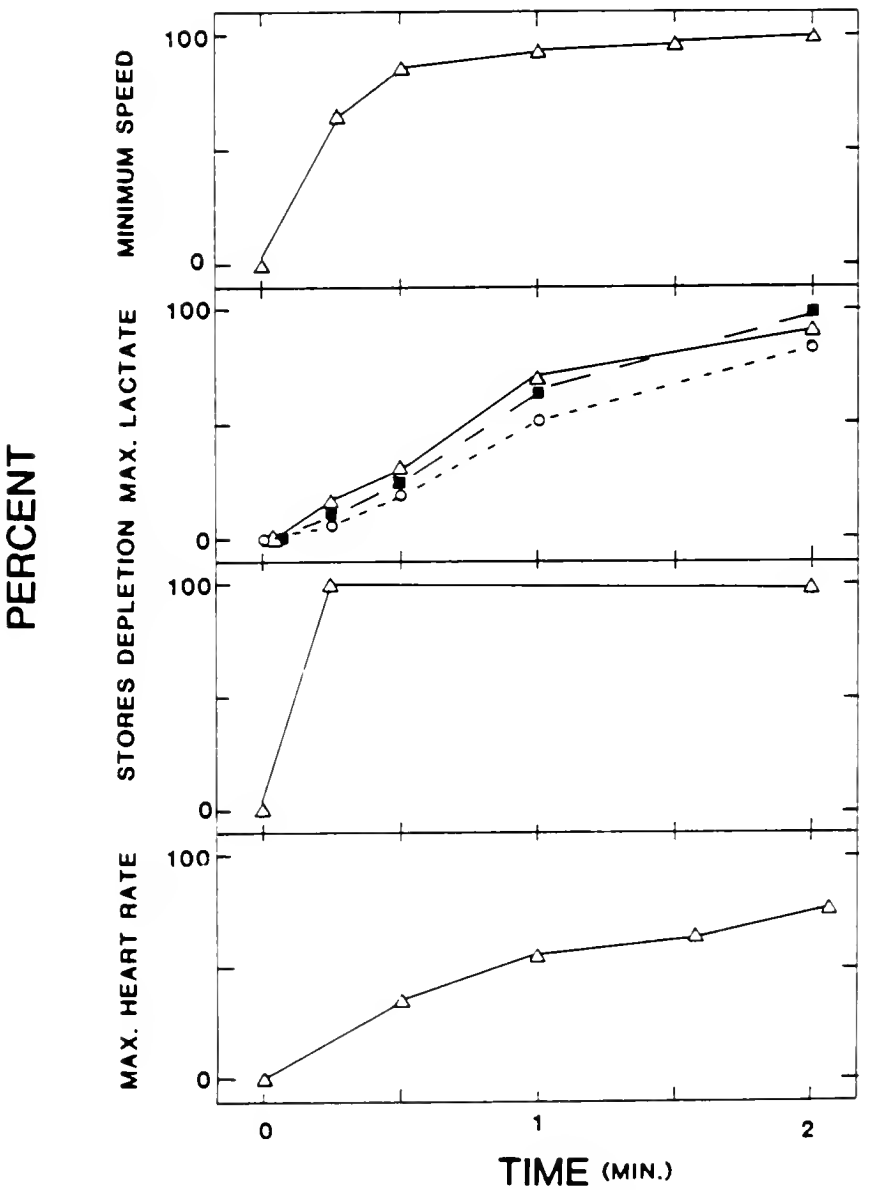
Figure VII-2. Changes in running speed, lactate, ~P stores, and heart rate during a two minute maximal struggle in *L. lenta* at 25°C. All changes are expressed as percentages (see Methods and equation VII-1). A full explanation of this figure is given in the text.

Key for lactate panel:

△ prosoma

○ opisthosoma

■ whole spider


PERCENT
MINIMUM SPEED
STORES DEPLETION MAX. LACTATE
MAX. HEART RATE
100
0
0
1
2
TIME (MIN.)

panel of each of these figures shows that during the first 15 to 30 sec of forced runs (referred to as phase I in Ch. IV), measured speed decreases by 50 to 85%. Also, the spiders' gait changes from a jumping, bounding run to a slow run or walk. Simultaneously, heart rate (HR) doubles, but this rate is still only 20 to 30% of the HR found near the end of activity or during early recovery. It has not been possible to measure $\dot{V}O_2$ during phase I. The inability to measure $\dot{V}O_2$ is not surprising. The oxygen that the spider will use during phase I must already be in the prosoma since little if any hemolymph flows to the prosoma during the first minute of activity (Stewart and Martin 1974; Anderson and Prestwich 1975; Ch. V this study). The low numbers of mitochondria and low activities of Krebs cycle enzymes in spiders (*e.g.* NAD-isocitrate dehydrogenase, malate dehydrogenase, and citrate synthatase; Linzen and Gallowitz 1975; Prestwich and Ing in press) may be the result of selection both for a low resting aerobic metabolism and in response to the frequent denial of O_2 to the prosoma when the spider is active.

The pressure generated by the prosoma obviously helps determine both the force and speed of a spider's movements. It does this by allowing for more rapid and forceful extension at higher pressures. High pressures also present the leg muscles with a load they must overcome and could potentially slow the spider's movements (Parry and Brown 1959a, b; Stewart and Martin 1974; Anderson and Prestwich 1975).

However, there are three lines of evidence that suggest that direct hydrostatic effects (such as turgidity and fluid loss) do not normally limit the degree activity in spiders. First, the only legs that mainly rely on hydrostatics to produce forward motion are the

fourth pair (Parry and Brown 1959b). The other legs produce much of their effect via flexion (pair I) or various mixes of rotation, flexion, and extension (pairs II and III, Manton 1958). Since leg pairs I-III are not always in contact with the substratum when they extend, then lower pressures are necessary for their rapid extension than in pair IV. Much of the spider's propulsion can be supplied by legs I-III with little necessity for high pressure. Second, maximum prosomal pressures are not reached until 30 to 45 sec into an activity bout (if no activity has recently occurred) (Fig. VII-1). By this time, running speed has decreased by 70 to 80 percent. Finally, as was shown in Fig. IV-1, scorpions (which lack a hydrostatic skeleton) show nearly identical patterns of fatigue development to spiders.

The relatively low pressures of phase I (100 to 350 mm Hg vs. the 450+ mm Hg of early phase II) may logically be ascribed to a high rate of prosomal hemolymph loss (Wilson and Bullock 1973) leading to the filling of the anterior opisthosomal venous sinuses. As these are filled to capacity for the high pressure regime found during activity, higher pressures are measured in the prosoma (Stewart and Martin 1974) because of the distended sinuses' higher resistance to additional hemolymph flow from the prosoma.

The most important constraints engendered by a spider's hydrostatic skeleton are the limitations placed on the delivery of O_2 to the prosoma and legs (Wilson and Bullock 1973). Given limited O_2 availability to the prosoma and legs during exercise, it is not surprising that spiders have well-developed anaerobic capabilities. The generation of ATP via anaerobic glycolysis is comparable to that of reptiles and amphibians

(Bennett 1978), the vertebrates that seem most analogous ecologically to spiders. The accumulation of lactate is shown in the second panel of both Figures VII-1 and 2. Maximum rates of lactate production occur during the first 30 sec to minute. However, glycolysis takes time to be fully activated. Figure IV-8 showed that the rate of glycolysis in *Lycosa* does not reach maximum until after the first 10 to 15 sec of activity.

Neural-hormonal mechanisms for activation of glycolysis in spiders are not known. However, one of the most important activators of the glycolytic pathway is AMP (Appendix II). Increases in its concentration are probably slowed by the action of arginine phosphate (AP) in decreasing the rate at which ATP and ADP depletion occurs. Arginine phosphate slows the depletion of these compounds through the transfer of ~P to ADP formed from the hydrolysis of ATP by the myosin-ATPase. This transfer of ~P from AP to ADP becomes thermodynamically favorable as soon as ATP stores are slightly depleted. Figures VII-1 and 2 show the rapid depletion of phosphate stores, principally AP, during the first 20 sec of running coincident with the full activation of glycolysis (Table VI-2, Figure IV-8) and phase I fatigue (Chs. IV, VI; Appendix II). Thus, AP performs a function analogous to creatine phosphate in the white muscles of vertebrates by maintaining the levels of ATP and fueling the first few seconds of muscular contractions. Furthermore, the rate at which glycolysis is activated by AMP may be slower than would be the case if little AP were present.

Energy Usage

A model of the use of ~P stores, anaerobic, and aerobic metabolisms in the prosoma and legs of *Lycosa* and *Filistata* for a two minute struggle

is given in Figures VII-3 and 4. These estimates indicate that anaerobic glycolysis accounts for over half of the total ~P used during a struggle with only a slight dependence on aerobic metabolism. The results agree with those in Chapter III that were for whole spiders and were obtained from an entirely independent data base.

Earlier results (Ch. VI) indicated that the relative importance of the different sources of ~P change with time. Figure VII-4 models these changes in the prosomas of *Filistata* and *Lycosa* (see Methods). During the first 10 to 15 sec of activity ~P stores accounted for 77% of the total ~P used in *Lycosa* and 55% in *Filistata*. Over the same time period, anaerobic glycolysis was of greater importance in *Filistata*, accounting for 43% of the total ~P versus 21% in *Lycosa*.

Total energy use/production declines during exercise for two reasons: rate limits on ~P availability and the results of the accumulation of anaerobic by-products. Based simply on the activities of rate-limiting enzymes, the availability of ~P from AP is much greater than from anaerobic glycolysis which is in turn a potentially much faster producer of ~P than is possible from the mitochondria of spiders (Prestwich unpublished; Prestwich and Ing in press; Linzen and Gallowtiz 1975). Therefore, during the first 10 to 15 sec of activity (when AP is available), ~P cleavage rates of 30 to 40 µmols/g min are found. Thereafter, energy use trails off rapidly and maximum rates of ~P cleavage drop to 6-10 µmols/g min in *Filistata* and 6.5 in *Lycosa*. During this time period (phase II), anaerobic metabolism becomes the dominant souce of ~P, being responsible for 2 to 6 times more ~P production than aerobic metabolism. Stores of ~P are not depleted any more during the approximately 90 sec of phase II, in fact, they are actually replenished

Figure VII-3. Total ~P use during two minutes of maximal exercise in *Filistata* and *Lycosa*. These totals represent the sums of ~P obtained from stores of ~P and aerobic and anaerobic metabolism (see Methods). The lower panel shows each species' relative distance traveled (prosomas/sec) as integrated for various time periods. Note the general correspondence between the shape of each species ~P use graph and distance traveled. The main disagreement is for the first 15 sec of activity, ~P use is higher per distance traveled than later. This is an artifact of measurement: the spiders jump a great deal during this time and this was not quantified (Ch. IV).

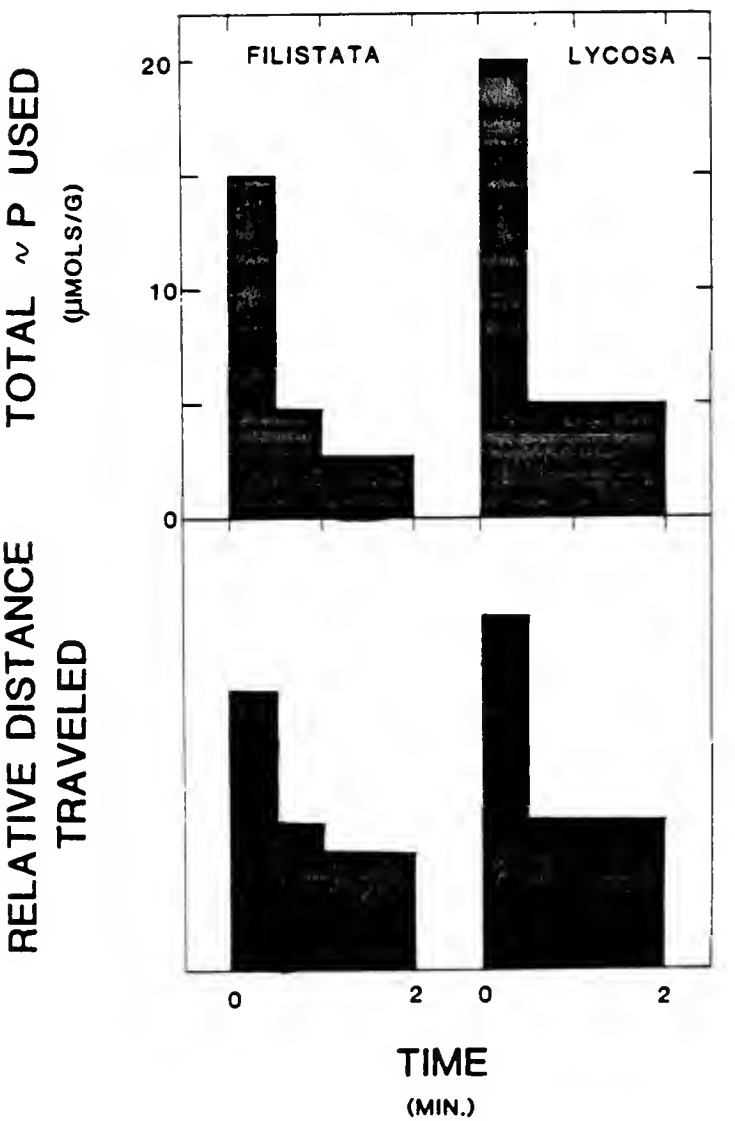
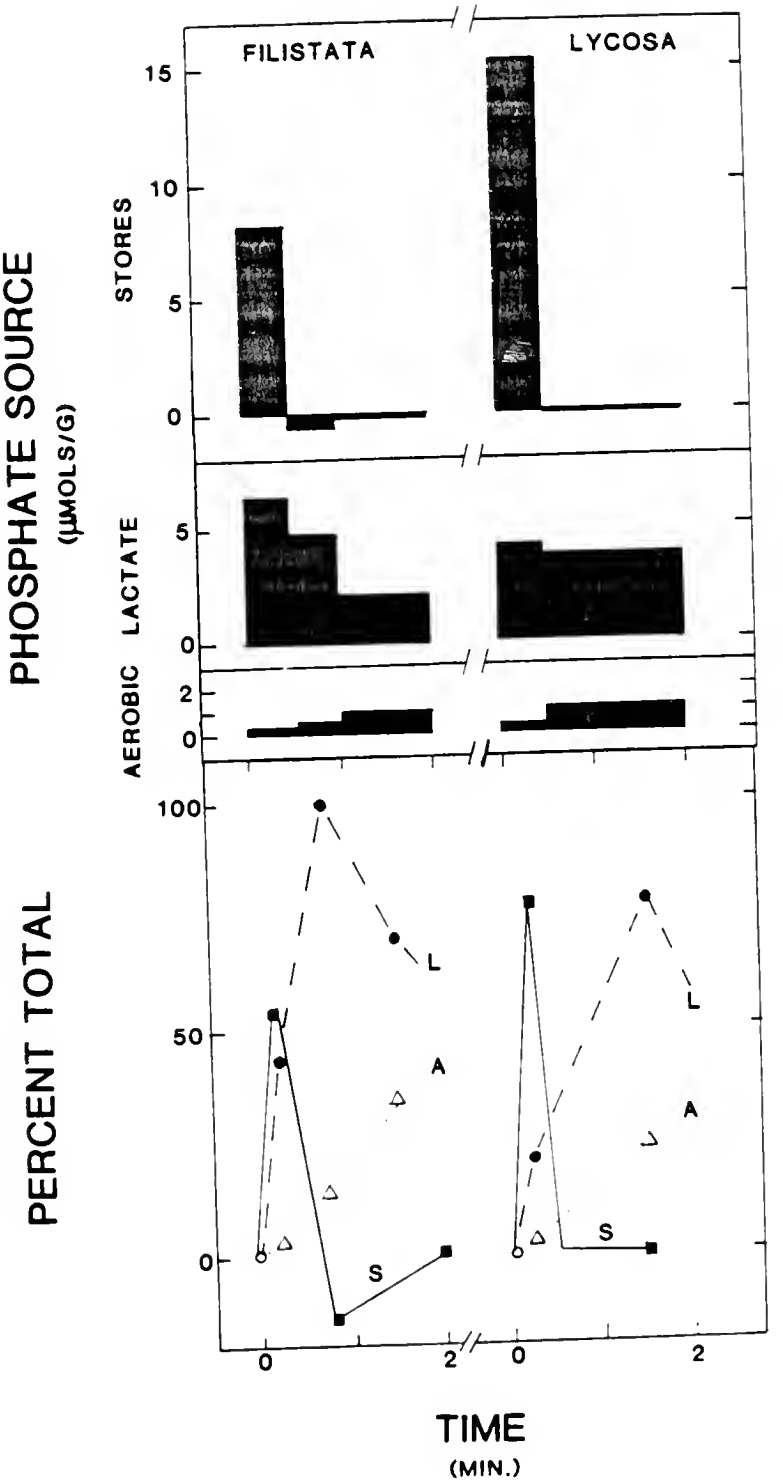

FILISTATA
LYCOSA
20
10
0
TOTAL ~P USED
(μMOLS/G)
RELATIVE DISTANCE
TRAVELED
0
2
0
2
TIME
(MIN.)

Figure VII-4. The changes in utilization of ~P from stores and aerobic and anaerobic metabolism during a two minute maximal struggle. Note that stores are quickly depleted as a ~P source and rapidly replaced by anaerobic metabolism. According to the model used (see Methods), aerobic metabolism becomes more important during late exercise because prosomal pressures are lower (Ch. V) and book lung ventilation has begun (Angersbach 1978). The proportional amounts of ~P used during exercise from each of the three energy sources are shown in Table VII-2.


FILISTATA
LYCOSA
PHOSPHATE SOURCE
(μMOLS/G)
STORES
AEROBIC LACTATE
PERCENT TOTAL
TIME
(MIN.)
15
10
5
0
2
100
50
L
A
S
0
2

slightly. Thus, during late phase I and early phase II, steady-state production/use of ~P is achieved. By the time this steady-state is reached, maximum activation of the glycolytic pathway has occurred and doubtlessly, the potential for maximum aerobic metabolism has been reached biochemically. Final decreases in the rate of ~P production from these sources comes when anaerobic accumulations inhibit the ability of muscles to contract and therefore ~P demand drops.

Support for the model in Figures VII-3 and 4 comes from agreement between the use of ~P and the distances traveled during the exercise. Distances traveled over 30 sec intervals are shown in Figure VII-3. However, inspection of parts A and B of Figures VII-3 and 4 show that changes in ~P usage and distance traveled do not exactly parallel each other. The ratio of distance traveled (arbitrary units) to ~P (μmols/g) can be calculated for two intervals: 0 to 15 and 15 to 120 sec. The resultant ratios are measures of efficiency (power output in terms of linear distance divided by power input in terms of ~P). For *Lycosa*, the ratio increases from 0.33 to 0.86; and in *Filistata* 0.46 to 0.86. Thus, the ratio is higher, that is, the efficiency is greater in phase II. This result is at least partially artifact: jumping behavior, that I was unable to quantify, occurs during phase I of activity (see Results, Ch. IV). However, this result may also reflect a real difference in efficiency of movement at different speeds.

In summary, the information presented in Figures VII-1 through 4 support the hypotheses that (a) spiders are ultimately limited by fatigue that is biochemical in nature due initially to phosphagen depletion and later to lactate accumulation and (b) that spiders are mainly dependent

on anaerobic metabolism and phosphagen stores as energy sources during maximal activity. Any locomotory constraints that might be expected to arise as a result of a spider's hydrostatic leg extension system do not occur because of the design of the respiratory-circulatory systems (where the book lungs provide resistence against prosomal hemolymph loss) and the thickness of the opisthosomal musculature (which has coevolved with the prosomal musculatures such that it is thicker in more active spiders) (Wilson 1970; Wilson and Bullock 1973; Stewart and Martin 1974; Ch. V).

Recovery

Fatigue in spiders is related to depletion of ~P stores and to lactate accumulation. Thus, the ability to run at maximum speeds is dependent on both the resynthesis of phosphagens and removal of lactate from the prosoma and legs. Figure VII-5 shows partial recovery of phase I speed in *Filistata* during a time when prosomal lactate is reduced to about 60% of the peak values and about half of the ~P stores have been recovered. Average prosomal pressures also correlate approximately with recovery of the above mentioned parameters.

In a spider with large anaerobic accumulations, removal of lactate and resynthesis of ~P compounds is dependent upon aerobic processes. For example, lactate may be removed from the prosoma by either physical processes (that are ultimately aerobic process-dependent such as the circulation) and/or biochemical processes (such as gluconeogenesis and oxidation to CO_2 and water).

The results presented earlier in Chapters III and IV suggested that the rate of recovery is related to the respiratory surface area (which is a direct reflection of the spiders aerobic abilities:

Figure VII-5. Recovery in *F. hibernalis* at 25°C after a two minute bout of maximal activity. All physiological values are presented as percentages (see equation VII-1, Methods). Pressures are given with solid lines (bottom panel).

Key to lactate percentages:

■ prosoma

○ opisthosoma

⬡ whole spider

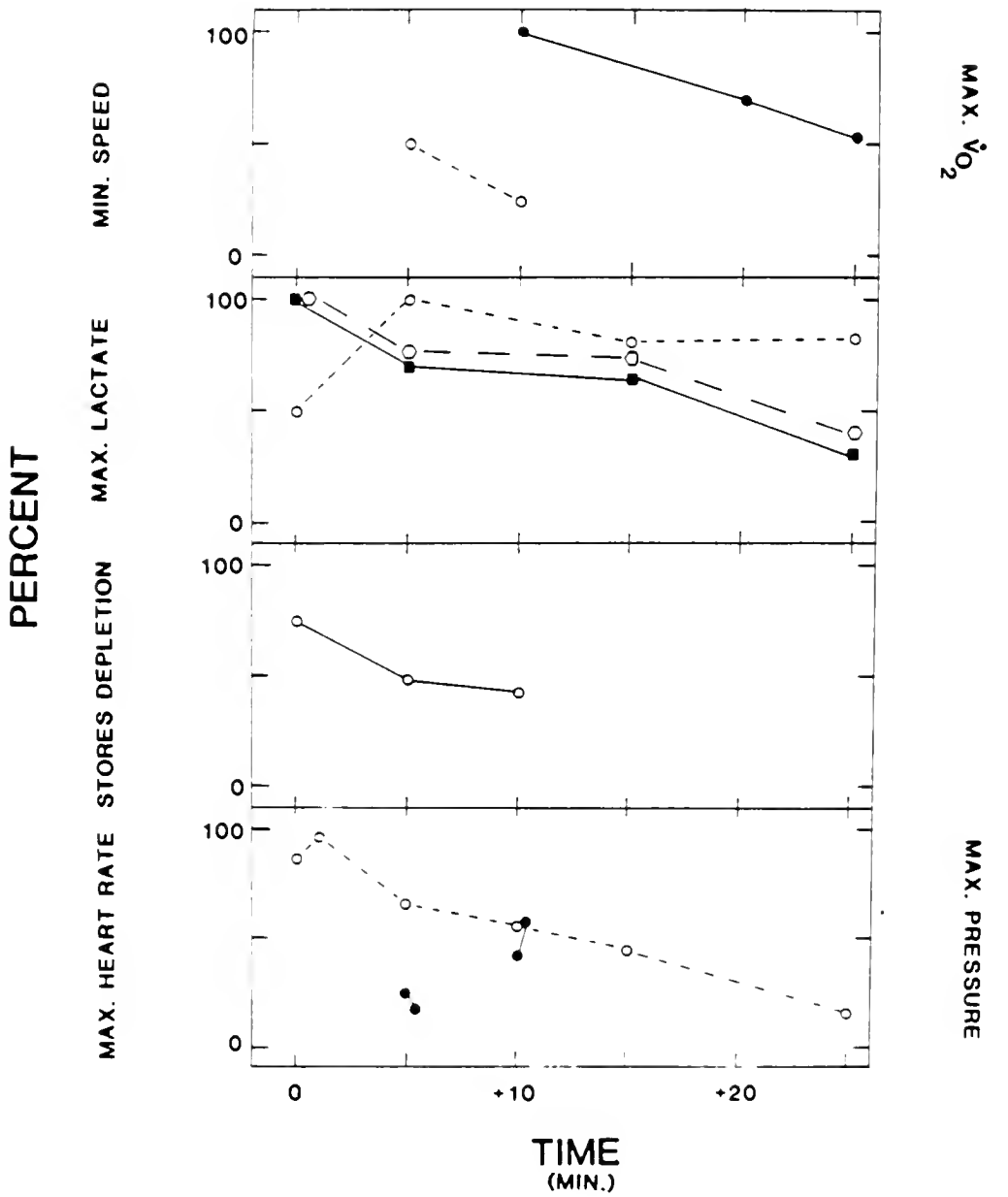

PERCENT
MAX. HEART RATE
STORES DEPLETION
MAX. LACTATE
MIN. SPEED
MAX. $\dot{V}O_2$
MAX. PRESSURE
100
0
0
+10
+20
TIME
(MIN.)

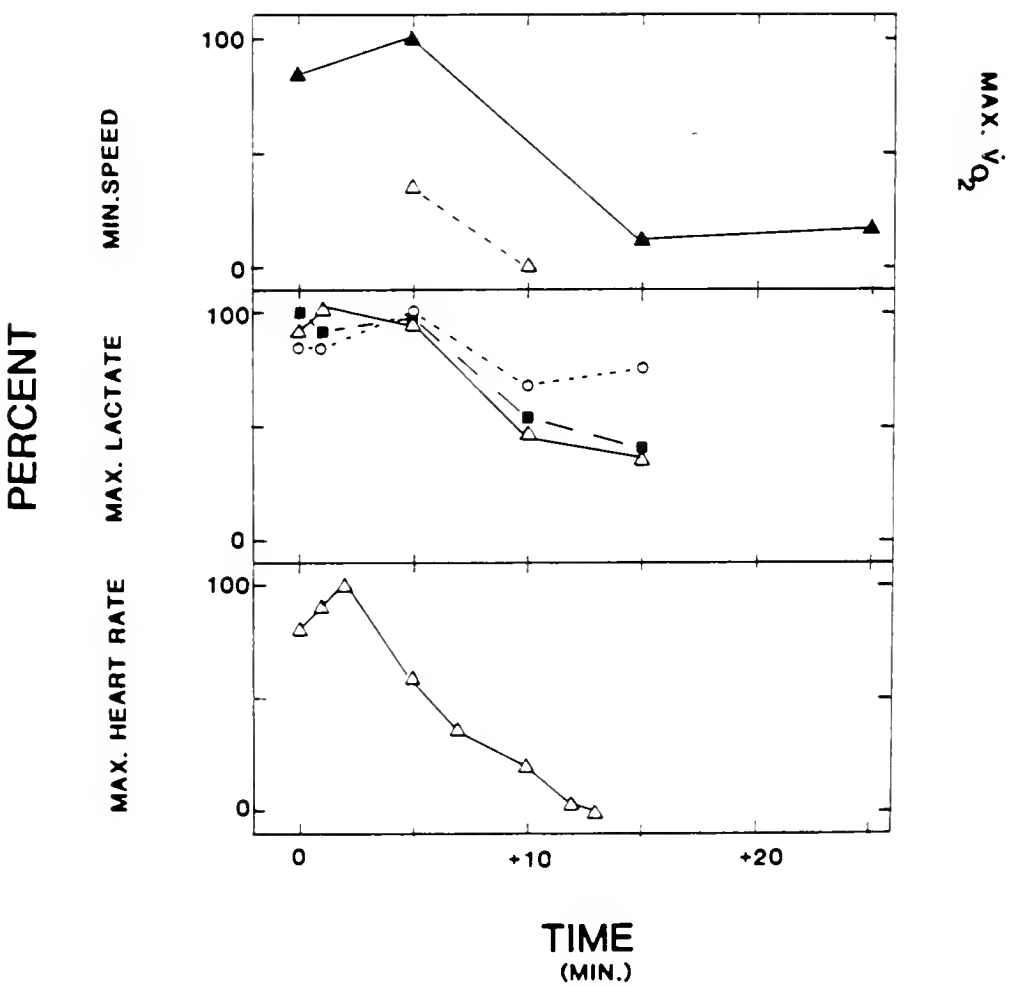
Figure VII-6. Recovery in *L. lenta* at 25°C after a two minute bout of maximal activity. All physiological values are presented as percentages (see equation VII-1, Methods).

Key to lactate percentages:

△ prosoma

○ opisthosoma

■ whole spider


PERCENT
MIN.SPEED
MAX. LACTATE
MAX. HEART RATE
100
0
100
0
100
0
0
+10
+20
TIME
(MIN.)
MAX. $\dot{V}O_2$

Anderson 1970; Anderson and Prestwich 1982). Recovery occurs more quickly in *P. audax* (which possess a large book lung surface area and tracheal system, Ch. I) and slowest in *F. hibernalis* (with small lungs and vestigal trachea). Comparison of *Filistata* (Fig. VII-5) and *Lycosa* (Fig. VII-6) again shows a rate of recovery is correlated with exchange surface area. Every physiological parameter ($\dot{V}O_2$, HR, lactate concentration, speed) returns to resting values at a rate that is 10 to 20% greater in *Lycosa* than in *Filistata*.

Given a slower recovery in *Filistata*, nevertheless, similarities exist in recovery between these two species and with other spiders. These similarities deal with the overall pattern of recovery, especially as pertains to the circulation. Peak heart rates are reached in both species after the completion of exercise. This seems to be a characteristic of spiders in general, having been reported for mygalomorphs (tarantulas: Stewart and Martin 1974; Angersbach 1978; *Atypus* Anderson and Prestwich 1982) and araneomorphs (Heteropodidae, Wilson 1967; *Filistata* and *Lycosa*, Anderson and Prestwich 1982, and this study). The highest rates appear to correspond to the peak $\dot{V}O_2$ (Ch. III) and are probably associated with the removal of anaerobic metabolites by both physical (circulation) and biochemical (oxidation) means.

The exact mechanism of removal of lactate from the prosoma is speculative. However, the data strongly suggest that it diffuses from prosomal muscles to hemolymph and then is transported to the opisthosoma where some is oxidized completely to CO_2 and H_2O but most is oxidized to pyruvate and then converted into hexose (Ch. III). The evidence is reviewed as follows: (A) Heart rates are high and prosomal pressure is

is low (Ch. V: Stewart and Martin 1974; Angersbach 1978; Anderson and Prestwich 1982). Therefore, maximum movement of hemolymph occurs. (B) Prosomal lactate drops during a time when hemolymph pH falls suggesting diffusion of lactate from the muscles (Ch. IV: pH data for a tarantula, Angersbach 1978), while meanwhile opisthosomal lactate either remains constant or rises, suggesting that at least some prosomal lactate journeys to the opisthosoma (Figs. IV-5 through 7). Removal of lactate from the prosomal muscles by circulation is an important part of recovery because it ameliorates the effects of high lactate more quickly than is possible by direct biochemical oxidation of this by-product. Thus, the muscles are able to work again even though the lactate has not been removed from the spiders body. (C) High $\dot{V}O_2$ is maintained while the spider is inactive (Ch. III; Seymour and Vinegar 1973; Anderson pers. comm.). The elevated $\dot{V}O_2$ is made possible by the high heart rates and possibly increased stroke volume (Stewart and Martin 1974; Appendix I) coupled with maximal ventilation of the book lungs and a significant Bohr effect (Angersbach 1978). (D) The amount of O_2 used during recovery corresponds to the amount used for gluconeogenesis of similar quantities of lactate in vertebrates (Ch. III, Bennett 1978). (E) The primary biosynthetic tissues associated with the opisthosomal digestive diverticulum appear to be well-suited to perform this task (Millot 1949; Prestwich and Ing in press). (F) The opisthosoma may be a source of glucose compounds that slip into the prosoma during activity (Fig. VI-1). The concept of spiders being organized into biosynthetic and locomotive regions that is envisioned in the above scheme seems logical and is found in other animals. However, it is well to remember that the above scheme is only circumstantial and needs extensive validation.

Temperature

The effects of temperature (15° to 33°C) on activity in 25°C acclimated *Filistata* and *Lycosa* are summarized in Table VII-4. These spiders have a degree of thermal independence in terms of the rate at which they can perform maximal activity. Most processes driven by chemical reactions show a Q_{10} of between 2 and 3; resting $\dot{V}O_2$ in spiders usually has a Q_{10} of between 2.0 and 2.5 (Anderson 1970). However, the Q_{10} for maximum running speed and total distance traveled in 2 min is less than 2 in both species. Maximum rates of struggle (which are important in prey capture and escape) are performed at a rate that changes less with temperature than would be expected based on spiders' Q_{10} value for aerobic metabolism.

Data on the effects of temperature on spider activity are incomplete because there are no data for phosphagen metabolism at temperatures other than 25°C. Earlier results have shown the critical role played by these compounds during maximal struggles. Nevertheless, the data for 15°C appear consistent and explainable. At this temperature, HR, rate of lactate production ($\dot{L}$), and $\dot{V}O_2$ are all lower than at 25°C. These observations all agree with the shorter distances traveled and slower maximum speeds at this temperature. Likewise, the slower whole animal removal of lactate can be ascribed to the slow rate of oxidative processes at lower temperatures (Anderson 1970).

The 33°C results are not so consistent. The spiders ran faster and further than at 25°C. However, $\dot{L}$ was greater than at 25° in *Lycosa* and less than at 25° in *Filistata*. Heart rate and $\dot{V}O_2$ were both greater than

Table VII-2. The effect of temperature on 25°C acclimated *Filistata* and *Lycosa*.

Temperature (°C)	Species	Maximum Speed (prosomas/sec)	Lactate Accumulation (µmols/g) P	Lactate Accumulation (µmols/g) Σ[a]	Recovery (min) Lactate[b]	Recovery (min) HR[c]	Max. $\dot{V}O_2$ (µl O_2/g h)	Aerobic Scope[d]
15°	*F. h.*	10.8	9.12	4.5	> 30	> 30	100	4.5X
	L. l.	10.8	9.32	5.0	> 30	--	275	3.7X
25°	*F. h.*	15.3	14.60	8.9	25	19	195	2.6X
	L. l.	15.9	13.45	9.9	15	7	425	3.4X
33°	*F. h.*	18.2	10.90	6.7	> 30	30	350	2.3
	L. l.	25.3	11.14	7.2	> 30	> 30	370	2.6X

[a] P = prosoma; Σ = whole animal

[b] Time to 50% reduction from peak values

[c] Time to reduce HR to 133% of resting

[d] Maximum $\dot{V}O_2$: resting $\dot{V}O_2$

at 25°C while recovery took longer. Perhaps the longer recovery is caused by temperature effects on gluconeogenesis-specific enzymes. The inconsistencies between lactate production and distance traveled may be explained by two factors in *Lycosa*. First, much of the extra distance covered was in phase I. Since phase I is mainly fueled by ~P stores, it is possible that *Lycosa* relies more on ~P stores at this temperature. Second, the very high $\dot{L}$ in *Lycosa* at this temperature could have led to fatigue due to lactate accumulations within specific muscles before high concentrations in the prosoma as a whole could occur. This could help explain the rapid fatigue at 33°C and the refusal of most animals to run after one and a half minutes. However, neither of these speculative explanations can be invoked in the case of *Filistata* at 33°C. Clarification of the effect of temperature on locomotion will probably have to wait until data on the use of ~P stores at non-acclimation temperatures is in hand.

Interrelationships Between the Ecology, Behaviors, and Aerobic and Anaerobic Abilities of Spiders

A priori, it seems that an interrelationship should exist between a spider's behavior, its aerobic metabolism ($\dot{V}O_2$), and anaerobic metabolism. The bases for this supposition deal with the ways that resting $\dot{V}O_2$ on one hand, and anaerobic metabolic abilities coupled with the intensity of activity, on the other, correlate with the book lung surface area. A review of these interrelationships is necessary prior to discussion of the factors that determine the mix of aerobic and anaerobic capabilities possessed by a given species of spider.

Convincing evidence has been presented that indicates that resting $\dot{V}O_2$ is largely determined by ecological factors; principly the quality, abundance, and pattern of food availability (Anderson 1970, 1974, 1978; Anderson and Prestwich 1982; McNab 1980). Thus, spiders that are long-lived and/or may commonly experience extended periods of low prey availability tend to have low resting $\dot{V}O_2$ and the ability to survive long periods of starvation. Examples of such spiders are *Lycosa* and *Filistata* (Anderson 1974). Other spiders, *e.g.* the orb weavers, have life cycles that are tightly interfaced with the availability of prey. These spiders possess relatively high $\dot{V}O_2$ (Anderson and Prestwich 1982).

Anderson (1970) and Anderson and Prestwich (1982) have shown that a strong direct correlation exists between book lung surface area and resting $\dot{V}O_2$. In a general sense, it is also reasonable that maximal $\dot{V}O_2$ should correlate directly with respiratory surface area assuming ventilatory and circulatory adjustments are similar in all species. Data suggesting that this is in fact the case in spiders is presented in Chapter III; peak $\dot{V}O_2$ was highest in *P. audax*, intermediate in *L. lenta*, and lowest in *F. hibernalis*. The same ordering is found with both resting $\dot{V}O_2$ and book lung surface area (Table I-1).

In the earlier chapters evidence was presented that the magnitude of anaerobic metabolism for any activity correlates directly with the intensity of activity and inversely with the book lung surface area (Fig. II-2; Chs. II, III, IV). Thus, at some common intensity of activity a spider with small respiratory surface area shows a larger anaerobic accumulation and a smaller aerobic dependence than a spider with large lungs. The remainder of this section deals with the actual

mix of behavior and aerobic and anaerobic metabolisms found in spiders and speculates on the forces that shape these interrelationships.

Web-Builders

Orb-weavers possess high resting $\dot{V}O_2$ and large book lung surface areas (Anderson and Prestwich 1982). Their aerobic capacities are large compared to other spiders since even a small factorial increase in $\dot{V}O_2$ over resting levels will result in a large total VO_2. Their major daily activity is web-building which may take one hour to complete. It is performed without rest periods and requires an approximate doubling in $\dot{V}O_2$ over resting levels (Peakall and Witt 1976). Anaerobic contributions amount to less than one percent of the total cost when measured at the moment the web is complete (see Ch. III).

The completed orb web simultaneously increases the area the spider is able to sample for prey, minimizes the necessity for the spider to move about to locate and capture prey (since it both ensnarls the victim and informs the spider of its exact location), and enables the spider to capture two or more prey at the same time. The necessity for active searching and intense activity is further reduced by the use of silken swathing bands which the spider throws on its victim from a distance. The enshrouded prey may then be easily killed with a poisonous bite. Furthermore, escape from predators by orb-weavers does not require extended vigorous activity since these spiders either go to an off-the-web retreat, or shake the web, or drop to the ground, form a ball, and hide in the vegetation. Thus, orb-weavers can avoid the requirement for anaerobic activity by possession of relatively large aerobic capacities,

an efficient trapping web, a separation of activity into preparation for prey capture (web-construction) and capture itself, and by using relatively passive predator escape techniques.

Filistata hibernalis offers an interesting contrast to the orb weavers. Like the orb-weavers, it makes a trapping web. However, this web is not suspended but instead forms a sheet over the substratum. Unlike the orb-weavers, *Filistata* lives for many years and may frequently face times of low prey availability (such as winter). These spiders have very low $\dot{V}O_2$ and small book lungs (Anderson and Prestwich 1982) and high anaerobic capacities (Chs. II and IV). The large anaerobic capacity is probably not related to web-making since it involves minimal activity in this species. (*Filistata* stands in one place and combs out silk to form its web. To enlarge its snare it occasionally moves to another place and combs silk.)

Filistata probably requires its well-developed anaerobic abilities for struggles with its prey. Unlike orb-weavers, it does not throw swathing bands and instead timidly battles with its trapped victim. Also, its poison does not seem especially potent (based on the time it takes prey to succumb after being bitten). The total time required by *Filistata* to completely subdue its prey may be several minutes. Lacking the ability to perform these attacks aerobically, it must rely on anaerobic capabilities.

Finally, other spiders with low rates of $\dot{V}O_2$ that make webs such as Agelenid spiders (Prestwich unpublished) require large anaerobic capacities since their webs are often non-viscid and these spiders in effect must hunt on their webs much like spiders that lack webs (Prestwich 1977; Prestwich and Ing in press).

Hunting Spiders

Jumping spiders such as *P. audax* do not make webs. Like orb-weavers they possess high aerobic capacities but they achieve these capacities differently. Jumping spiders have a moderately large book lung surface area and moderate resting $\dot{V}O_2$ (Anderson 1970). They are able to increase their rate of oxygen consumption many times, possibly through the use of trachea (Anderson 1970; Ch. III). Their large aerobic capacity is vital to their characteristically active prey pursuit. They commonly either patrol vegetation or wait and then pursue any potential victims (Hill 1979). Unlike most spiders they are frequently in motion for extended periods and this activity is undoubtedly aerobic. Escape is also highly aerobic. These spiders spot predators and actively flee them. They also possess excellent anaerobic capacity. This is vital in prey capture (especially of larger items or in cases where the spider had been chasing its victim for some time) because they lack a sticky web to hold their prey and therefore cannot attack incapacitated prey at their leisure.

The more common pattern in hunting spiders is seen in *Lycosa lenta*. This is a "sit and wait" predator that spends most of its time motionless--short activities of more than minimal intensity result in lactate accumulations (Ch. IV). Given their low aerobic abilities and the intense activity that accompanies capture of large prey (Rovner 1980) it is not surprising that they have such high anaerobic capacities.

In summary, spiders like other animals, periodically rely on stores and anaerobic metabolism to fuel their activities. This permits them to accomplish feats that would be impossible were they forced to rely on

their feeble aerobic abilities. However, even with their low aerobic capability, they do not possess unusually well-developed anaerobic capacities (Bennett 1978) or phosphagen stores (Lehninger 1975). These non-exceptional metabolic abilities are probably related to the extensive use of silk and poison by spiders. Used both offensively and defensively, these materials lessen the demand for high peak rates of ~P generation. In sum, these adaptations show spiders can be characterized as having reached an "apex" in low energy life style (Anderson 1970, 1974, 1978; Anderson and Prestwich 1982).

APPENDICES

APPENDIX I
THE ESTIMATION OF CARDIAC OUTPUT AND STROKE VOLUME IN SPIDERS

Summary

A calculation shows that in active tarantulas over 90% of the increase in cardiac output over resting conditions is due to increased heart rate. Stroke volume is roughly constant or may drop.

Introduction

The most complete data on spiders internal and external gas exchange and hemolymph distribution exists for tarantulas. Using data on oxygen consumption ($\dot{V}O_2$) and A-V PO_2 differences, it is possible to calculate cardiac output by the Fick Equation:

$$\dot{Q} = \dot{V}O_2/(C_{aO_2} - C_{vO_2})$$

where $\dot{Q}$ is the cardiac output, $\dot{V}O_2$ the rate of oxygen consumption and C_{aO_2} and C_{vO_2} are the arterial and venous O_2 contents respectively. Potential problems with the use of this equation to find $\dot{Q}$ arise from two sources: (a) correct determination of the A-V difference and (b) the assumption of steady state conditions. Correct A-V differences depend upon having representative, fully mixed arterial and venous C_{O_2} values. In the spider, C_{aO_2} can be determined from the pericardial sac or heart itself since the hemolymph has just passed through the book lungs. The C_{vO_2} is more difficult to find since most opisthosomal

hemolymph does not combine with hemolymph from the prosoma until after passing through the lungs. However, if one assumes roughly constant A-V differences throughout the resting spider and insignificant opisthosomal $\dot{V}O_2$ during maximal activity, then estimates of C_{vO_2} can be made based on the PO_2 of the ventral sinus of the prosoma. Prosomal venous hemolymph flows through this structure just before returning to the book lungs. Steady-state assumptions can be met based on the pattern of the animal's activity. Animals at rest or in long-term activity are assumed to be in steady state.

Once $\dot{Q}$ has been calculated, it is a simple matter to calculate an average stroke volume ($\overline{SV}$) because:

$$\dot{Q} = \overline{SV}\ (HR)$$

where HR is the heart rate.

Calculation of Resting $\dot{Q}$ and $\overline{SV}$ in Tarantulas

Assuming a mass of 30 g, then resting $\dot{V}O_2$ = 840 µl O_2/h at 23°C (estimated from Anderson 1970). Data from Angersbach (1978) and Stewart and Martin (1974) indicate a resting heart rate of 30/min. According to Angersbach (1978) the ΔC_{O_2} = 8.542 µl O_2/ml hemolymph (if P_{aO_2} = 27.8, P_{vO_2} = 5 torr, then 7.94 µl O_2/ml hemolymph are released from hemocyanin and 0.6017 µl from physical solution).

Therefore:

$$\dot{Q} = (840\ \mu l\ O_2/h) \div (8.542\ \mu l\ O_2/ml)$$

$$= 98.3\ ml/h$$

or $$\dot{Q} = 1.64\ ml/min$$

The mean stroke volume can now be calculated,

$$\overline{SV} = (1.64 \text{ ml/min}) \div (30 \text{ b/min})$$

$$= 55 \text{ µl/b}$$

If the total hemolymph volume in a 30 g tarantula is *ca.* 6 ml (Stewart and Martin 1974), then *ca* 0.9% of the total hemolymph volume is ejected per beat.

Calculation of Maximal $\dot{Q}$ and $\overline{SV}$ Under Steady-State Conditions

Using a flow through system, Anderson (pers. comm.) has data indicating the peak sustainable $\dot{V}O_2$ in tarantulas exercised continuously for ten minutes is *ca.* 4600 µl O_2/h, a value that is between 5 and 9X resting $\dot{V}O_2$ (resting $\dot{V}O_2$ varies greatly in tarantulas). Anderson's data suggests the ventilation of the book lungs reaches a maximum after several minutes of activity. This is consistent with Angersbach's (1978) PO_2 measurements that showed maximal A-V differences were not reached until after at least a minute of activity. Angersbach (1978) also reported lowest hemolymph pH values (and largest Bohr effects) several minutes after the start of activity, consistent with observations of lactic acid accumulation presented in Chapter IV. Because Anderson's tarantulas exhibited an O_2 debt after the completion of 10 min exercise that was far greater than the total O_2 capacity of the spider's hemolymph, therefore, it is reasonable to assume that anaerobic pathways were utilized. Thus, exercise was maximal. Under these conditions, ΔC_{O_2} is *ca.* 15.6 µl O_2/ml hemolymph (Angersbach 1978). Heart rate maxima for tarantulas are given by Angersbach (1978) and Stewart and Martin (1975) to be 85 b/min; for

argument's sake I will calculate $\overline{SV}$ based on a rate of 120 b/min, a figure consistent with measurements on other spiders (Ch. IV; and Anderson and Prestwich 1982).

Thus,

$$\dot{Q} = (4600\ \mu l\ O_2) \div (15.6\ \mu l\ O_2/min)$$

$$= 294.9\ ml/h \text{ or } ca.\ 4.9\ ml/min.$$

This represents a 3X increase in $\dot{Q}$

--If the heart rate is 85 b/min;

$$\overline{SV} = 58\ \mu l/b$$

--If the heart rate is 120 b/min;

$$\overline{SV} = 40\ \mu l/b.$$

Thus, average stroke volume remains constant or may actually decrease by 28% while the heart rate increases by 2.8X and 4X. Therefore, heart rate probably accounts for nearly all of the increase in cardiac output.

Estimation of Maximum $\overline{SV}$ Based on Non-Steady State Conditions

During short bouts of activity, it appears that no increase in ventilation and therefore no increase in C_{aO_2} occurs (the resting arterial hemolymph is usually only about 50% saturated with O_2). The C_{vO_2} does decrease to nearly 0, however, this is only a slight change from resting conditions since the ΔC_{O_2} is now 9.557 (*vs.* 8.542). Heart rates are slightly elevated (Angersbach 1978). If anything, it is likely that $\overline{SV}$ is low during maximal running (up to 1 min) since the maximal systolic pressure of the tarantula heart (with help from the opisthosomal muscles) is *ca.* 100 mmHg while prosomal pressures during activity peaks may exceed

450 mmHg and be above 100 mmHg for much of the activity period (Stewart and Martin 1974; also see Ch. V). Thus, during most of this time, pumping of hemolymph through the anterior aorta is impossible. Hemolymph may still exit through the posterior aorta (to the opisthosoma); however, this is a much smaller vessel and doubtlessly can transport less hemolymph.

APPENDIX II
THE REGULATION OF GLYCOLYSIS IN SPIDERS

Summary

1. Relative activities of enzymes associated with glycolysis in Lycosid spiders were calculated from literature sources. The results suggest that hexokinase (HK), phosphofructokinase (PFK), aldolase, and glycerol-3-phosphate dehydrogenase may catalyze non-equilibrium reactions (Table AII-1).
2. Calculations of mass-action ratios (Γ) for resting and active spiders compared to K_{eq} values suggest that control points for glycolysis in spiders involve (a) PFK and (b) the feed-in reactions responsible for providing glucose-6-phosphate (Table AII-2).
3. The role of AMP (Ch. VI) in glycolytic regulation is discussed.

Introduction

The study of the regulation of biochemical pathways can be approached many ways. Two relatively simple but valuable approaches involve first, the characterization of the relative activities of different enzymes used in a pathway and second, the calculation of the mass-action ratios (Γ) of the individual steps of the pathway compared to the equilibrium constants (K_{eq}) for those reactions.

Comparison of relative activities of pathway enzymes is useful because it points out the places in a pathway where rate-limiting

steps occur--*i.e.* the reactions catalyzed by enzymes present only in low activity (note: the activity of an enzyme takes into account many factors including titer, thermodynamics of the reaction, "catalytic efficiency" of an enzyme, and modulation of the enzyme). Activities of an enzyme are measured *in vitro* under conditions that are thermodynamically optimal and in the presence of optimal amounts of substrates and activators (Lehninger 1975).

Measurement of mass-action ratios (Γ) is done under conditions of both activity and inactivity of a given pathway. Thus, the resting and active Γ values for a given reaction can be compared. Regulatory reactions are usually far from equilibrium (K_{eq}) at rest and move towards K_{eq} when the pathway is activated (Newsholme and Start 1973).

Enough data are available for Lycosid spiders to permit a preliminary study of the regulation of glycolysis in spiders. In most animals the control points for glycolysis are at the feed-in points for substrates (*e.g.* glycogen $\xrightarrow{\text{phosphorylase}}$G1P and glucose $\xrightarrow{\text{hexokinase}}$G6P) and the activation of fructose-6-phosphate [F6P $\xrightarrow{\text{phosphofructokinase (PFK)}}$ FDP]). Other minor control points may exist at the cleavage of FDP to triose phosphates by aldolase and the ~P transfer to form ATP as phosphoenolpyruvate is converted to pyruvate in a reaction catalyzed by pyruvate kinase (see Fig. II-1 to locate all of these steps). It is the purpose of this section to compare regulation of glycolysis in other animals, especially insects, with the available data for spiders.

Methods and Results

Relative Enzyme Activities

The activities of nearly all of the glycolytic enzymes have been measured in spiders. Linzen and Gallowitz (1975) measured activities from prosomal, leg and heart muscle homogenates from the wolf spider *Cupiennius salei*. Prestwich and Ing (in press) measured activities of some of the same enzymes as did Linzen and Gallowitz; however, Prestwich and Ing also measured the activities of several other enzymes. One of the species studied by Prestwich and Ing was the wolf spider *Lycosa lenta*. There are important methodological differences between these studies: Linzen and Gallowitz (1975) studied isolated muscle not whole tagmata and expressed their results as activity/g fresh weight of muscle (vs. activity/g protein). However, the legs and prosomas of spiders are mainly muscle (Table I-2) and because it is unlikely that other tissues make as large contributions to glycolysis as does muscle, then it is reasonable to combine the data for *Cupiennius* and *Lycosa* to obtain a rough overview of the relative activities of the glycolytic enzymes of wolf spiders.

Combining the data from these two different species was accomplished by comparisons of activities of enzymes used in both studies [hexokinase, (HK)--Prestwich and Ing, unpublished; glycerol-3-phosphate dehydrogenase (GPDH); lactate dehydrogenase (LDH); malate dehydrogenase (MDH); glutamate-pyruvate transaminase (GPT); glutamate-oxaloacetate transaminase (GOT)]. The ratios of activities of these enzymes in *Cupiennius* and *Lycosa* were similar. Therefore, a compilation of activities was made using the activity of HK as the index to which the other enzymes were

Table AII-1. Relative activities of glycolytic enzymes and 3 Krebs cycle enzymes. All are based on hexokinase activity of 1.0.[a]

	Legs		Prosoma	
Enzyme	*C. salei*	*L. lenta*	*C. salei*	*L. lenta*
Hexokinase	1	1	1	1
Phosphoglucose isomerase	90	--	52	--
Phosphofructokinase	--	0.5[b]	--	0.5[b]
Aldolase	34	--	15	--
Triose phosphate isomerase	1716	--	2000	--
Glycerol-3-phosphate DH	1	9[b]	2.4	4.4[b]
Glyceraldehyde DH	161	--	107	--
Phosphoglycerate kinase	88	--	38	--
Phosphoglycerate mutase	90	--	48	--
Enolase	64	--	48	--
Pyruvate kinase	75	--	49	--
Lactate DH	54	49[b]	37	34[b]
Citrate synthetase	0.07	--	0.05	--
Isocitrate DH (NAD)	--	0.4[b]	--	--
Malate DH[c]	134	134[b]	95	95[b]

[a]Data for *C. salei* are calculated from Linzen and Gallowitz (1975); *L. lenta* from Prestwich and Ing (in press).

[b]Range of activities from 11 species of spiders are PFK, 0.161 to 4; G3PDH 1.5 to 10; LDH 16 to 77; ICDH-NAD 0.06-1.0; MDH 25-135 (Prestwich and Ing, in press)

[c]Probably over 80% of all MDH activity is cytosolic in nature (Linzen and Gallowitz 1975).

Table AII-2. Equilibrium constants and mass action ratios [Γ] for four reactions. Γ is calculated as a function of both exercise and recovery for *F. hibernalis* (top numbers) and *L. lenta* (beneath). Numbers in parenthesis by resting Γ are for the blowfly *Phormia regia* and are calculated from Sacktor and Wormser Shavit (1966) and Sacktor and Hurlbut (1966). K_{eq} values were taken from Newsholme and Start (1973). Calculations for PFK assume equilibrium between G6P and F6P (Newsholme and Start 1973).

Reaction Catalysed by	K_{eq}	Mass Action Ratios (Γ) Rest	60 sec	120 sec	+5 min Recovery	Maximum Change
Phosphorylase	0.07[a]	0.0015		0.11	0.036	~ 100X
and			0.130			
Hexokinase (HK)	$3.9\text{-}5.5 \times 10^{3}$[b]	0.0016		0.07	--	~ 44X
Phosphofructokinase	$0.9\text{-}1.2 \times 10^{3}$	0.072	0.780	0.291	0.039	~ 11X
(PFK)		0.051	--	--	--	--
Aldolase (ALD)	$6.8\text{-}13 \times 10^{-5}$M	1.27×10^{-8}M	1.7×10^{-8}	5.76×10^{-8}	7.0×10^{-8}	~ 4.5X
		3.78×10^{-8}M	--	--	--	--
Triosephosphate	0.036-0.045	0.216	0.162	--	0.26	1.3X
isomerase (TIM)		0.196	--	0.29	--	1.5X

[a] Phosphorylase

[b] HK only

Resting Γ for *Phormia regia* flight muscle: (HK 0.008; PFK 0.052; ALD 3.09×10^{-8}M; TIM 0.212).

compared. Because HK was assigned the activity of 1.0, then an enzyme that had 10 times the activity of HK was given a relative activity of 10. The results of this compilation are given on Table AII-1. The only enzymes closely associated with glycolysis that have not been measured in wolf spiders and are therefore not included in the table are phosphorylase (phosphate cleavage of glycogen) and phosphoglucomutase (G1P to G6P).

Mass Action Ratios

Using the data for *Lycosa* presented in Chapter VI, mass action ratios for glycolytic reactions were calculated whenever possible. The data in Chapter VI are given as µmols/g fresh weight; for the purpose of calculation these were converted to molar concentrations by assuming a water content of 78%. For comparison, the blowfly data of Sacktor and Hurlbut (1966) and Sacktor and Wormser-Shavitt (1966) were converted to molarity using the same assumptions as for the spider data. All calculations were done using the equations presented in Newsholme and Start (1973). The results of these calculations are given as Table AII-2.

Discussion

The activities of all of the enzymes associated with glycolysis are quite high except for HK, PFK, and GPDH. The first two enzymes have activities that are at best only 0.07 those of any other glycolytic enzyme. Depending on the source of the sample, GPDH occupies a middle ground between PFK and HK and the other enzymes.

Generally, enzymes that are integral parts of a pathway but that occur with activities that are *ca.* 0.1 to 0.001 those of the other pathway enzymes catalyze non-equilibrium reactions (Newsholme and Start 1973). Their low activities indicate that there are simply not enough active sites available for the reaction to obtain equilibrium in the face of the catalytic actions of the other enzymes that are in much higher activities. Thus, by this criterion, HK, PFK, and GPDH are all good candidates for controllers for non-equilibrium reactions.

Non-equilibrium reactions are often the result of either low enzyme titers (*e.g.* in reactions that are not important in certain cells) or where control points exist. In the latter case, the activity of the enzyme may be modulated in accordance with the physiological needs of the cell. Identification of reactions that correspond to control points is made on the basis of two criteria. First, the reaction must at times be non-equilibrium. Secondly, when the pathway is perturbed such as to increase the flux through it, the mass-action ratio of the control reactions must increase. This second criterion eliminates the possibility of simple thermodynamic control where with increased flux through a pathway the mass action ratio will remain roughly constant (Newsholme and Start 1973).

Calculations of Γ for resting and exercising spiders are presented in Table AII-2. Large discrepancies ($> 10^3$) between reaction K_{eq} and resting Γ exist for HK, PFK, and aldolase controlled reactions. It was not possible to calculate Γ for GPDH since the concentrations of NAD and NADH were not known. The K_{eq} and resting Γ for the triose isomerase (TIM) reaction differ only by a factor of 10. This difference could easily be due to

poor measurements of glyceraldehyde-3-phosphate since its concentration was at the limit of resolution (Ch. VI). Overall, the pattern of resting Γ values calculated for *Lycosa* and *Filistata* agree well with those for the blowfly.

During exercise $\dot{V}O_2$ increases 2 to perhaps 18 times above resting (see Ch. VII). Based on the initial disappearance of anthrone-reactive substances and accumulation of lactate (Figs. VI-4 and 11) it is obvious that there is a large increase in glycolytic flux during exercise. High-flux Γs were calculated for 15, 60 and 120 sec into activity; for comparison Γ was also calculated during recovery for *Filistata*. Table AII-2 shows insignificant changes in Γ for the aldolase- and TIM-catalyzed reactions. However, for PFK the Γ increased by a factor of 11X, this is normally regarded as significant and indicative of a control point reaction (Newsholme and Start 1973).

The increases in the Γ for the PFK reaction parallels the increase in AMP (Fig. VI-8). During the first 15 sec of activity, AMP increases by a factor of 10X. It is the factorial increase of this substance and its absolute concentration that are important in understanding its role as a regulator of various enzymes (Newsholme and Start 1973). Thus, the situation in spiders appears to be like other animals; as AMP increases the PFK reaction moves towards equilibrium, as it decreases (assuming AMP changes in recovering *Lycosa* are like *Filistata*), the PFK reaction moves away from equilibrium. Other substances probably also help de-inhibit the PFK. One of these is phosphate (P_i, see Fig. VI-9). After 15 sec of activity, P_i has more than doubled in *Lycosa* and thus it may also be important in the regulation of PFK (Lehninger 1975). There are several

other substances that are commonly thought of as activators of PFK; especially important are increased Ca^{2+} and 3'5' cyclic AMP (Newsholme and Start 1973). However, the regulatory roles of these substances in spiders are not known.

Finally, the status of the HK-catalyzed reaction cannot be interpreted unambiguously. A large shift of the Γ toward K_{eq} may be misleading since spiders can obtain G6P (used in calculation of Γ for HK), from other reactions, especially the pathway leading from glycogen to G1P to G6P (catalyzed by glycogen phosphorylase and phosphoglucomutase). Another possible source of G6P is via the metabolism of trehalose.

Given the decrease of anthrone-reactive substances during exercise (even after correction is made for glucose), it is likely that the concentration of G6P is determined by several reactions. Thus it is impossible to comment on the importance of HK as a regulatory enzyme with the present data. However, the increase of G6P does indicate that the sum of the reactions leading to the production of G6P are probably regulated and therefore important in the overall regulation of glycolysis as in other animals. The presence, activity, and regulation of glycogen phosphorylase in particular needs investigation due to the major role this enzyme plays in other species and because of insights it may give into the uses of Ca^{2+}, cyclic AMP, and AMP in metabolic regulation in spiders.

In summary, the results in this section should only be regarded as preliminary. They suggest the major regulatory points of glycoylsis in spiders are the same as in other animals. These results should be used to guide future studies of the regulation of glycolysis in spiders.

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BIOGRAPHICAL SKETCH

Kenneth Neal Prestwich was born on March 8, 1949, in Inglewood, California. He spent his youth moving about the country with his parents and was especially intrigued as a child with the animals and plants of north Florida. Upon graduating from Robert E. Peary High School in Rockville, Maryland, in 1967, he attended Davidson College in North Carolina where he received his B.S. in biology in 1971. In 1974, he interrupted his studies to ride across the United States on a bicycle with two friends. He received his M.S. in zoology from the University of Florida in 1975. Since then he has continued his work on spiders and crickets and has actively pursued hobbies of bicycling, running, canoeing, and beekeeping.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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