A STUDY OF THE EFFICIENCY OF CONVERTING FREE ENERGY INTO MECHANICAL WORK IN MOUSE SOLEUS MUSCLES

CHANG YIN

Department of Physiology

University College

University of London

A thesis presented for the degree of Doctor of Philosophy in the University of London

July 1990

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ABSTRACT

1. A study has been made of energetic cost of contraction in mouse soleus muscle by recording tension, length change and the heat production from contracting muscles. A new and rigorous method has been introduced in this study to assess the efficiency of converting free energy into mechanical work in mouse muscles.

2. The "energy balance" in mouse soleus muscle has been tested by comparing the ratio of recovery energy to initial energy. It has been found that this ratio is bigger in working contractions than that in isometric tetani.

3. The energetic cost of work production in mouse soleus muscles has been assessed by comparing the work/enthalpy ratio with the work/free energy ratio. It is found that the efficiency value in mouse soleus muscle is lower compared with that in frog and in tortoise muscle.

4. It is striking and totally unexpected to find that the recovery ratio in working contractions is not the same as that in isometric contractions. A series of control experiments has been designed and conducted in order to clarify the existence of this phenomenon.

5. Experiments have also been done on mouse soleus muscles which are stretched during tetani. The ratio of recovery energy to initial energy has been studied and compared with that in shortening.

6. This study indicates that there may be an unknown but energetically important process in mouse soleus muscles. The possible existence of such an unidentified process has been critically discussed.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 INTRODUCTION

The most important function of muscles is their capacity to produce mechanical work. It is this work that makes most vital life processes possible, such as circulation, respiration and, of course, body movement. However, muscles cannot provide this mechanical work for nothing. Like other converters, muscles have to consume energy of another form in order to provide mechanical work. In fact, it has been generally assumed that the mechanical work produced by muscles is derived from the chemical energy available from adenosine triphosphate (ATP) splitting. From this point of view, muscles can be considered as a living device whose task is to transform chemical energy into work. It is for this reason that muscles' capacity to produce work is ultimately decided by the energetics of this energy transformation process.

Naturally, a muscle which can produce work while consuming little energy is an ideal one. The study of efficiency, which investigates the effectiveness of the energy transformation process, is therefore one of the important issues in muscle biology.

1.1 Energy transduction in muscular contraction

Muscular contraction is associated with a chain of chemical reactions which can be divided into two parts: initial (during contraction) and recovery (following contraction). Although energy transformation only takes place during the initial phase, the recovery phase restores the muscles to their pre-contraction state and therefore is necessary for sustained energy transformation. Within each phase, there are several reactions. For example, the net reaction for the initial phase of contraction is often considered to be phosphocreatine (PCr) splitting and the net reaction for recovery phase to be the breakdown of glycogen units into carbon dioxide and water coupled to the resynthesis of PCr. Accordingly, the efficiency associated with either initial phase or with recovery phase is called initial or recovery efficiency, respectively. This division is artificial but is potentially useful for the study of the relation between these two parts of muscular contraction. If the contraction and the recovery processes are considered as a whole, the efficiency for the entire process is called overall or total efficiency.

1.2 Definition of efficiency of muscular contraction

By definition, efficiency is simply the ratio of work done by the muscle to the free energy change of the reaction(s) which provide(s) the work. However, because ideas about what these reactions are has changed over the years, the literature of muscle efficiency research contains various operational definitions of efficiency as will be described. For a better understanding of the meaning of efficiency, some knowledge of thermodynamics is useful.

Work is a form of energy. In order to obtain any kind of work, mechanical work, electrical work, osmotic work or whatever, some other sort of energy has to be consumed. This is the first law of thermodynamics, which shows that energy cannot be created or destroyed. In muscle energetics, this simply means that muscles have to use the chemical energy available from ATP splitting or other processes in order to provide mechanical work. For example a muscle in the rigor state (without ATP) cannot contract and produce work until ATP is supplied.

However, the first law of thermodynamics only states the

possibility of energy conversion, it states neither the direction of this conversion nor the limits. For example, the first law does not forbid the conversion of a certain amount of heat into the same amount of work. However, it is well known that heat can only be converted into work by its passage down a temperature gradient. Thus thermal energy can never be transferred into work in muscular contraction because, in muscle, there are no significant temperature gradients.

If not all forms of energy can be transferred into work, what kind of energy can be converted into work? The second law of thermodynamics states that only free energy can be transferred into work. Therefore, in order to assess efficiency of muscular contraction, it is the free energy that should be considered, not the enthalpy. Take an example, for one mole of ATP splitting (Benzinger & Hems, 1956), the free energy change is -7730 calories, and the enthalpy change -4800 calories. Suppose that the corresponding work production is -1000 calories, the efficiency value should be 0.13 (-1000/-7730), not 0.21 (-1000/-4800). This becomes even clearer if we consider the case when the work is -7000 calories. In such a case, the efficiency is 0.91 (-7000/-7730), not 1.46 (-7000/-4800). The thermodynamic efficiency can never be greater than 1.

From the above discussion, it is clear that, in order to correctly assess the efficiency of muscular contraction, the ratio of mechanical work done by a contracting muscle to the free energy change due to the reaction(s) which produce(s) the work should be used. This ratio is called the thermodynamic efficiency and its value is always between 0 and 1. For more detail, see Wilkie (1960, 1974); Woledge (1985, 1989).

1.3 Experimental approaches to measuring efficiency

Although it is very simple to define efficiency in theory, in practice, there is no easy way to measure it. Existing techniques allow the measurement of the work and heat produced by muscles with sufficient accuracy and with fairly good time resolution. The experimental difficulties arise when the corresponding free energy change is to be assessed. For this, one needs to know the chemical nature of the driving reaction.

In muscle there are many reactions which may provide free energy at various stages of muscular contraction. It has been shown that ATP splitting is a direct free energy source for muscular contraction (Cain & Davies 1962; Infante & Davies 1965). The depletion of ATP from muscles will cause rigor and a muscle in a rigor state cannot contract unless ATP is provided. It is now

widely accepted that ATP is the only direct source of free energy for muscular contraction. However, biochemical studies show that ATP splitting may not be the direct free energy source for contraction, the free energy from ATP splitting may be used to "recharge" crossbridges. The free energy in the "charged" crossbridges may be the direct energy source for contraction.

PCr splitting is another reaction associated with muscle contraction. This reaction can provide a large amount of free energy and is an important reaction in muscular contraction. However, PCr splitting is not the direct source of free energy for contraction. Without PCr a muscle can still contract. The role of PCr is to buffer the change of ATP concentration in the muscle and thus to maintain contractions. Therefore if PCr is available, ATP concentration in a contracting muscle can be maintained virtually unchanged, the net result of this "buffering" is PCr splitting. The role of PCr has been demonstrated using dinitrofluorobenzene (DNFB) which can block the creatine phosphokinase (CPK) reaction (Cain & Davies 1962; Infante & Davies 1965).

Glycolysis can provide free energy for contraction. But this is a relatively slow process and the free energy from glycolysis can only be used during a long contraction. A brief contraction will

be over before glycolysis is activated. The same is true for oxidative phosphorylation of ATP by the breakdown of carbohydrates. This oxidative process is even slower than glycolysis and is important only in steady state contractions. The molar free energy change from the oxidation of carbohydrates is far more than that from the other reactions mentioned and this reaction is thus most effective in terms of free energy production.

It has been found in frog muscle that the movements of calcium ions can produce energy (Smith & Woledge 1982). The heat production from the calcium movements can be quite large, about 25 kJ/mol calcium bound. However, because there is no obvious mechanism of coupling the calcium movements to crossbridge interaction, this energy probably cannot be utilized for contraction.

It is not only reactions of small molecules in muscle that can produce energy, crossbridges themselves can also produce energy by changing from one state to another. During rapid shortening, less ATP is split but more heat is produced (Homsher et al. 1981). The extra energy production exceeding that from ATP splitting probably come from crossbridges themselves. However, with our available knowledge, it is not known whether or not this is free energy which could be used to drive contraction. This possibility

needs to be tested.

As discussed above, in muscle there are many processes which may be sources of free energy for contraction. It is very difficult to identify the reaction(s) which produce free energy for contraction under most circumstances. In fact the nature of the driving reaction(s) cannot be found out by any single direct experiment. In order to identify the driving reaction a more comprehensive approach is needed.

A "energy balance" study is one approach to finding out the reaction which serves as the source of free energy for contraction. This method is to measure the energy produced as heat and work during a contraction, and to compare this with the amount of energy that can be explained by certain observed chemical reactions. For this purpose the following equation is used:

$$h + W = \sum_{i} \Delta H_m \xi_i$$

where the left side of the equation is the energy produced as heat (h) and work (W). The right side of the equation is the sum of all the reactions (i) that occur to the extent of each reaction ξ multiplied by its molar enthalpy change ΔH_m. When a particular

chemical reaction occurs under specific conditions of pH, temperature, and ionic strength, etc., an energy change occurs (ΔH_m in J/mol reaction). The important feature of this equation is that the ΔH_m (energy per mole of reaction) is the same when the reaction occurs *in vitro* as when it occurs *in vivo*.

If the energy from measured chemical reactions (right side of the equation) is equal to the energy measured as heat plus work, all the energy appearing as heat and work during contraction comes from the measured chemical reactions and thus is "explained" by the energy from these reactions. If the energy appearing as heat and work is larger than the energy from measured chemical reactions, there must be "missing" chemical reactions or other processes which produce the extra amount of energy. Because the measurements of heat and work can be achieved with reasonably high precision, this quantitative method is very powerful for detecting any possible driving reaction which may not otherwise be detected.

"Energy balance" studies are essential not only for muscle energetics in general, but also for muscular efficiency studies. Only when there is an energy balance prevailing in a muscle, i.e. all the driving reactions have been identified, is it then possible to assess the true thermodynamic efficiency properly.

In practice, the following experimental approaches are usually used to estimate the efficiency of muscular contraction.

1.3.1 Hill's method (Efficiency = $W/(h_i+W)$, Hill 1939)

As it is fairly easy to measure work and heat production, since A. V. Hill's work, it has been traditional to use the ratio of $W/(h_i+W)$ as an indicator of efficiency. Hill called this ratio "mechanical efficiency". The advantage of this method is that the measurements of heat and work are accurate and can be repeated many times on the same muscle. This gives reliable experimental results. The weak point of this method is that free energy change is not used. Therefore, it is impossible to assess the thermodynamic efficiency of the muscle contraction in this way without extra information. However, heat and work measurements are very important for muscle energetics, in particular these measurements are very powerful because even previously unknown reaction(s) which have not been detected by chemical analysis cannot generally escape from these measurements.

Hill's method can be made to give a true thermodynamic efficiency if the assumption is made that all the energy comes from PCr

splitting. For this reaction the molar enthalpy change (ΔH) and the molar free energy change (ΔF) values are available. More will be said about this below.

1.3.2 Davies' method (Kushmerick & Davies 1969)

Kushmerick and Davies used chemical analyses to investigate the efficiency of muscular contraction. In their experiments, work production was calculated as the product of tension and length change. The free energy change from ATP splitting was worked out by measuring the changes in the concentrations of ATP, ADP and Pi. The strong point of this method is that a free energy change is calculated, so the true thermodynamic efficiency can be assessed. The weak points are that this method is destructive to the muscle and is also less precise, compared with heat measurements. In addition the chemical reactions which are the source of free energy have to be specified, i.e. an assumption has to be made. This method is based on the *assumption* that ATP splitting during contraction is the only reaction producing free energy. It should be realised that this method can only give the right answer when this is true.

1.3.3 Wilkie's method (Wilkie 1960)

This method takes the whole contraction-recovery process into account. For this whole process, studies *in-vivo* have established that the net process is largely the oxidation of glycogen (also including some fats) to carbon dioxide and water. It is also known that for the oxidation of glycogen, the free energy change is 1.03 times the enthalpy change ($W + h_t$). So this method combines the advantages of Hill's and Davies' methods, i.e. h_t and W can be measured directly and then converted to the free energy equivalent. However, this method can only assess the overall efficiency for the whole contraction-recovery process, *not* the efficiency corresponding to the contractile process itself, which is of more interest from a crossbridge theory point of view.

1.3.4 Oxygen consumption method

This method assesses overall efficiency by measuring work production and oxygen consumption. Basically, this is the same method as that of Wilkie mentioned above. The only difference is that, in this method, oxygen is measured while in Wilkie's method,

heat is measured. Therefore, Wilkie's method is a direct one while this method is an indirect one in terms of energy measurement. If it is true that all the h_t + W comes from oxidation these methods will give the same results.

As can be seen from the above description, all the experimental methods for efficiency assessment are based on an assumption of one kind or another. For example, if an assumption is made that all the energy produced during contraction is from ATP splitting only, the measurements of heat and work (Hill's method) can be used to estimate the thermodynamic efficiency as can the measurement of chemicals levels (Davies' method). Similarly if it is assumed that all the energy ultimately comes from the aerobic breakdown of carbohydrates, the measurements of work and heat for the overall process (Wilkie's method) can be used to assess the thermodynamic efficiency, as can the oxygen consumption method. Therefore, these methods are equally valid. It is wrong to think one method is more "powerful" or "superior" than the other. They are all based on an assumption. It is important to remember this when assessing muscle efficiency by these methods.

1.4 Efficiency values found in previous studies

Due to the different experimental approaches used, there are

various types of "efficiency". The following are efficiency values from previous studies.

Tab. 1.1 lists the efficiency values found in different species. From this table, it is clear that efficiency varies between different species. Among the species that have been studied so far, tortoise has the highest efficiency and frog has the second highest. Mammalian muscles generally have the lowest efficiency. From this table it seems that the efficiency values obtained using Davies' method are generally high, compared to those obtained by other methods. As mentioned before, Davies' method is based on the assumption that ATP splitting during shortening is the only reaction producing free energy. Therefore, consistently high efficiency values obtained by Davies' method seems to suggest that the assumption may be wrong, because other reaction(s) which may produce free energy have been excluded according to the assumption. A final point that can be drawn from Tab.1.1 is that there seems a fairly constant quantitative relation between the initial and the overall efficiency value. In general the overall efficiency value seems to be about 0.6 times the initial efficiency (Woledge 1989b).

Tab. 1.1 Efficiency values of isolated muscle from different species found by other workers (a: Hill 1964; b: Hill 1939; c: Nwoye & Golspink, 1981; d: Kushmerick & Davies, 1969; e: Heglund & Cavagna, 1987; f: Woledge, 1968; g: Woledge, 1989; h: Gibbs & Gibson, 1972; i: Norman et al, 1985). For the details of the techniques used by the workers, see the text.

		Technique							
		Hill's		Wilkie's		Davies'		O2 consmp	
Species	Muscle	%	ref	%	ref	%	ref	%	ref
Frog	sartorius	45	a	18	b	56	c	25	e
						54	d		
Tortoise	rectus femoris	72	f	36	f	88	c		
Dogish	myotomal	30	g						
Chicken	post l d					25	c		
Mouse	soleus					79	c		
	biceps					45	c		
Rat	soleus			19	h			15	e
				15	i				
	edl							19	e
Hamster	soleus					68	c		
	biceps					40	c		

1.5 Previous studies in recovery process

In order to assess the thermodynamic efficiency in mouse soleus muscle by the method to be used here, it is essential to know whether the relation between initial and recovery process is the same as that predicted from PCr splitting and subsequent oxidative resynthesis of PCr. Without this information, it is impossible to assess the thermodynamic efficiency correctly. This relation is usually described by the recovery ratio. This is the ratio of recovery energy to initial energy. It is very important because it reflects the relation between the chemical processes occurring during the initial and the recovery periods(details in 1.6).

However, little work has been done in this area, and of this work, most was on frog muscles and very little on mammalian muscles. Particularly, in the past, recovery heat measurements have been made almost exclusively with isometric contractions. So far, there has been no report on the recovery ratio in mammalian muscles when performing working contractions.

Available results of recovery ratios are listed in Tab. 1.2. In this table, all contractions are isometric, except those marked by double stars (**). Values in this list are means and standard errors. In the experiments of Crow and Kushmerick, the recovery ratio

Tab. 1.2 Recovery ratios of isolated muscle in different species.

** : working contraction, otherwise isometric contraction.

	mean	sem	n		
Frog sartorius	1.03	0.05	5	**	(0°C, Hill 1939)
	0.89	0.08	15		(0°C, Hill 1939)
	1.28	0.07	6		(18°C, Hartree 1928)
	1.27	0.05	6	**	(18°C, Hartree 1928)
	1.77	0.27			(20°C, Dawson 1975)
	1.29	0.03	44		(20°C, Godfraind-De Becker 1989)
Frog semitendinosus	1.22	0.14	7		(25°C, this study)
	1.22	0.14	7	**	(25°C, this study)
Toad sartorius	1.65	0.07	9		(20°C, Godfraind-De Becker 1971)
Tortoise biceps cruris	1.22	0.06	8		(19°C, Hartee & Liljestrand 1926)
	1.5		24		(19°C, Hartee & Liljestrand 1926)
Tortoise rectus femoris	1	0.05	3		(18°C, Woledge 1966)
	0.99	0.06	3	**	(18°C, Woledge 1966)

Tab. 1.2 Recovery ratios of isolated muscle in different species.

** working contraction, otherwise isometric contraction.

	mean	sem	n	
Mouse EDL	0.8	0.09	16	(20°C, Crow & Kushmerick 1982)
	0.95	0.14	6	(20°C, Elzinga & Leijendekker 1988)
Mouse soleus	1.54	0.22	7	(20°C, Elzinga & Leijendekker 1988)
	1.01	0.06	31	(22°C, Wendt& Gibbs 1976)
	1.19	0.06	4	(27°C, Wendt& Gibbs 1976)
	1	0.05	12	(12°C, Wendt& Gibbs 1976)
	0.92	0.08	22	(20°C, Crow & Kushmerick 1982)
Rat EDL	0.61	0.05	31	(22°C, Wendt& Gibbs 1976)
	0.28	0.04	4	(27°C, Wendt& Gibbs 1976)
	0.55	0.1	12	(12°C, Wendt& Gibbs 1976)
Rabbit papillary	1.1	0.04	27	(20°C, Mast et al 1988)

listed here was calculated according to their data, using P/O=6.3, pH_i=7.23. Their experiments were originally made by using an oxygen consumption method. All the other experiments are heat experiments. In Godfraind-De Becker's experiments, pH values in this list are the values for Ringer solution.

From Tab. 1.2 it can be seen that (a) the recovery ratio may be species dependent, e.g. this ratio seems different between frog and mouse; (b) even in the same species, the recovery ratio may be dependent on muscle type. e.g. in mouse this ratio is higher in soleus than in extensor digitorum longus (EDL).

There is some evidence that the initial energy is derived from ATP (PCr) splitting and the recovery heat from oxidative resynthesis of ATP (PCr); if so, the recovery ratio would have a value which depends only on the intracellular pH (Fig. 1, Woledge, 1989b). For example the value should be 1.0 in frog muscle and about 0.9 in mouse muscle. The value for frog is greater than that predicted. This adds to the evidence that reactions other than PCr splitting contribute to the initial energy output and therefore reactions other than PCr resynthesis occur during recovery. In mouse EDL muscle the value is as expected though probably a bit higher than expected in mouse soleus, especially in one study (see Tab. 1.1). Therefore, this classic view cannot satisfactorily explain

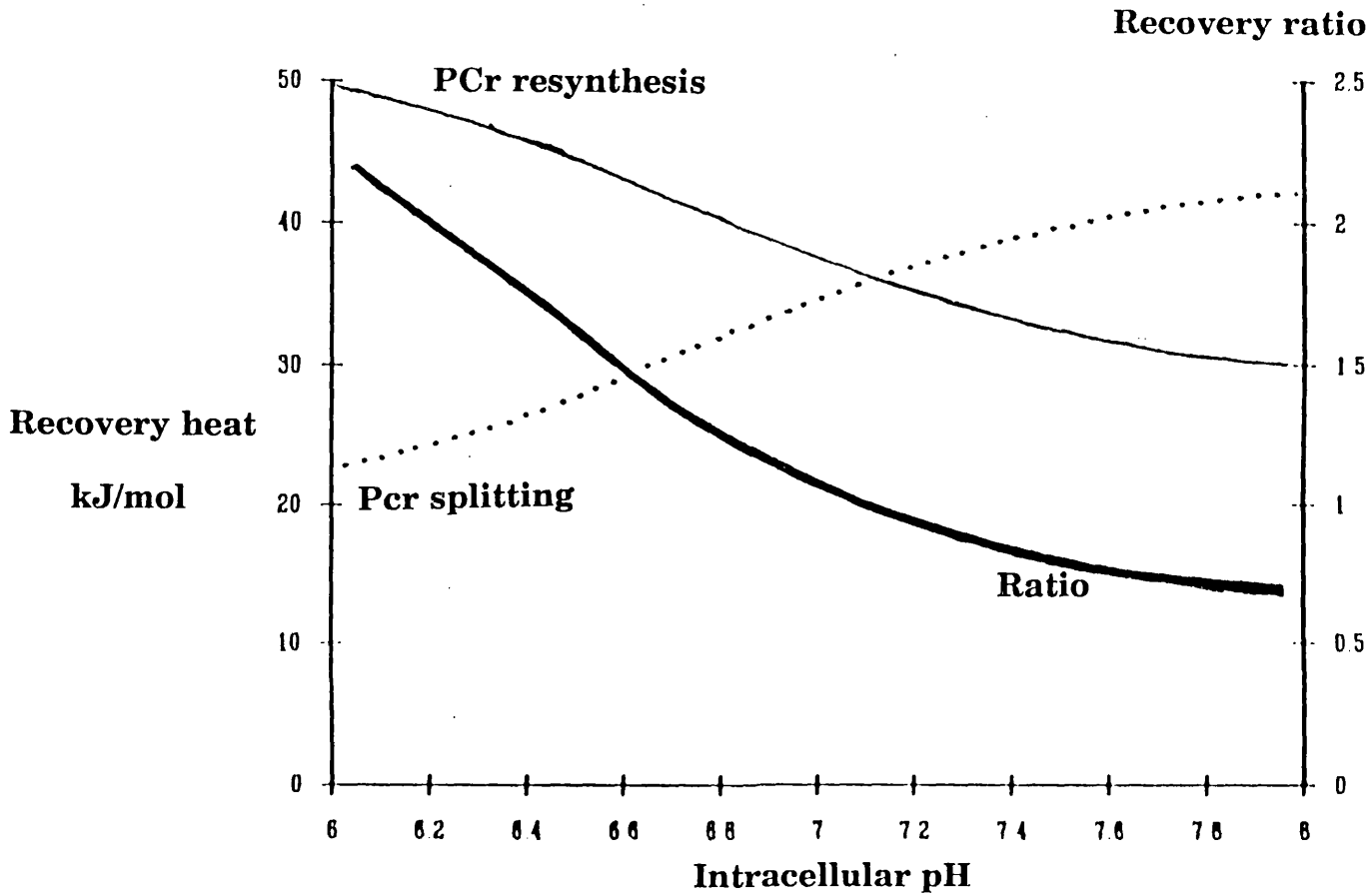


Fig. 1 The effect of intracellular pH on the heats of reaction for the initial and recovery processes, and on their ratio. The dotted line shows the heat of splitting of PCr from Woledge and Reilly (1988). The thin line shows the heat of PCr resynthesis. The heavy line is the recovery ratio expected if these two processes are the only significant sources of energy during contraction and during recovery respectively (From Woledge 1989a).

either the initial heat and the recovery heat or the relation between the initial and recovery process.

Obviously, the processes underlying the initial and the recovery periods are more complex than they were previously thought. Therefore, in order to understand these processes and their relation, a more comprehensive study is needed.

1.6 Outline of this project

1.6.1 Purpose

This project aims at: a) testing whether there is an "energy balance" in mammalian muscle (mouse soleus), particularly in mouse soleus muscles doing working contractions, b) assessing the thermodynamic efficiency of mammalian muscle, c) estimating thermodynamic efficiency for both the initial process and the overall process.

Little work has been done in the area of mammalian muscle efficiency. The available experimental information remains scarce and incomplete. Therefore, more information would obviously be beneficial. Moreover, some important differences between frog

muscle and mouse muscle have been reported as will be described next.

1.6.2 The choice of muscle

There were two reasons for choosing mouse soleus muscle as the preparation in this project. Firstly, classic experiments are usually carried out using frog muscles; it has been assumed that the muscles of other species behave as frog muscles do. However, this assumption is being challenged by increasing reports which have shown that mouse soleus muscles (probably other mammalian muscles as well) exhibit some unique phenomena.

In frog muscles, it has been well established that there is an unexplained energy production occurring at the beginning of a contraction. Only if this part of contraction is excluded, can an energy balance be found in frog muscles. In contrast Crow and Kushmerick (1982) have reported that there is an "biochemical energy balance" prevailing in both soleus and extensor digitorum longus muscles of mouse. This is a very significant discovery in muscle energetics because this is the first time a biochemical energy balance has been detected for a whole contraction in muscles of any species. This finding is striking because it is opposite to what has been found in frog muscles, the classic

muscle favoured by muscle researchers for a long time. However,as we have seen, recovery ratio observations do not support this view.

As presented in 1.6.1, there are a few reports about efficiency of mouse soleus muscle. However, due to the possible chemical complexity of the reactions in this muscle, the "efficiency" obtained so far cannot be trusted until a myothermal energy balance has been confirmed, preferably in working contractions from which mechanical work is produced and efficiency is, therefore, assessed. Crow and Kushmerick's results suggest that there is a "chemical energy balance" in isometric contraction in mouse soleus muscle. It seemed likely therefore that there is a true energy balance in mouse soleus muscle when the muscle undergoes working contractions. However, this possibility needs to be tested. If there is indeed an energy balance in working contractions, then the thermodynamic efficiency of this contraction could be properly assessed using established knowledge. Therefore, the key point is to find a convenient way to test whether there is an energy balance when the muscle undergoes working contractions.

1.6.3 An new experimental approach

A new and rigorous method is suggested to estimate the

thermodynamic efficiency of mouse soleus muscle.

In this project, the way to test whether there is an energy balance in mouse soleus muscle in a working contraction is to compare the recovery ratio obtained from a working contraction to that obtained from an isometric contraction. Recovery ratio is the ratio of recovery energy to initial energy. If the two ratios are the same and if the values are similar to that predicted from PCr splitting at the known pH value, it is very likely that there is an energy balance in both types of contraction. The reason is that, if the same reaction provides energy for both isometric and working contractions, the relation between initial energy and recovery energy should be the same in a working contraction as in an isometric contraction. So long as PCr splitting is the only energy source, the stoichiometric factor and, thus, the recovery ratio will be the same, no matter whether a muscle contracts isometrically or shortens. Conversely, if recovery ratio is the same under different conditions, the underlying reaction should be the same. Therefore, the recovery ratio is an indicator of the underlying reaction, just as a rate constant is a marker of reaction.

If the recovery ratio in working contractions is found to be the same as that in isometric contractions and equal to the predicted value, taken together with Crow and Kushmerick's results, it

would seem certain that PCr splitting is the only energetically significant process in both isometric and working contractions. Then the next step would be to work out the molar enthalpy change and molar free energy change of the PCr splitting reaction using established knowledge.

From Woledge's work (Fig. 1.1), it is now possible to estimate the molar enthalpy change of PCr splitting (ΔH_{PCr}) from the recovery ratio (r) under known pH values because the relation between molar enthalpy change and recovery ratio is given by the equation below (from Woledge 1985):

$$r = (72/\Delta H_{PCr})-1$$

The molar free energy change of PCr splitting (ΔF_{PCr}) can be considered as the same as that of ATP because of the effectiveness of the creatine kinase equilibrium.

So far, all the values needed for calculating thermodynamic efficiency are available. And the final step is to work out the thermodynamic efficiency of the contractile process itself by using the equation:

$$\varepsilon_i = (\Delta H_{PCr}/\Delta F_{PCr}) \times (W/(h_i+W))$$

or

$$\varepsilon_t = (\Delta H_{PCr}/\Delta F_{PCr}) \times (W/(h_t+W))$$

where ε_i is initial efficiency and ε_t total efficiency.

In summary, a new method suggested in this project consists of the following procedures:

a) recording heat and work in both isometric and working contractions.
b) obtaining recovery ratio for both contractions (see 3.1.2)
c) comparing this ratio for working and for isometric contractions
if the ratio is the same, then
d) calculating the molar enthalpy for PCr splitting according to recovery ratio obtained
e) finally, working out the thermodynamic efficiency from
the work, heat, the molar enthalpy change and molar free energy change.

This method has the following advantages:

a) it assesses thermodynamic efficiency rather than just the ratio

of (work/enthalpy)

b) it estimates not only the efficiency of the overall process but also the efficiency of the contractile process, which is of great interest from chemomechanical coupling point of view;

c) heat and work measurement can be done directly and precisely;

d) this method is not destructive and can be repeated to get reliable results and to compare different conditions on the same muscle.

CHAPTER 2 THEORETICAL CONSIDERATIONS OF EFFICIENCY

This chapter deals with the theoretical analysis of muscle efficiency. It is interesting and informative to consider factors which may affect efficiency in theory. Some practical aspects will be involved where necessary.

2.1 Efficiency of a coupled process in general

In muscular contraction, ATP splitting is coupled to work production, and, at the same time, heat is produced. As with other coupled process, there are general rules which can be applied.

2.1.1 The efficiency of a coupled process

Spontaneous and antispontaneous processes

In Nature, some processes can take place naturally, without the need of any help provided by other processes. These processes take place of their own nature and are called spontaneous.

Examples include the falling of water from a high place to a low place, the heat flow from a hot place to a cooler place. On the other hand, there are processes which cannot happen by themselves. They can take place only when they get "pushed" or powered by some other process(es). Examples of these are heat flow from low temperature to high temperature, raising water from a low place to a high place. These are just the opposite to the examples of spontaneous processes illustrated above. In order to raise water to a high place, a water pump is needed and electrical energy is used to power the process. Similarly, in the case of transferring heat to a hotter place, a refrigerator is used and again, electrical energy is needed to "push" the heat flow from cooler part to hotter part. Therefore, in order for a antispontaneous process to proceed, a spontaneous process is needed to provide the driving force. Thus, only with the help of the spontaneous process, can an antispontaeous process be driven. Therefore, theses two processes are said to be "coupled" together. The spontaneous process is called the "driving process" because it provides free energy which propels or pushes the antispontaneous process which is called the "driven process". In the process of a coupled reaction, the "driving process" goes towards its uncoupled equilibrium and the "driven process" is pushed away from its uncoupled equilibrium.

Definition of efficiency of coupled processes

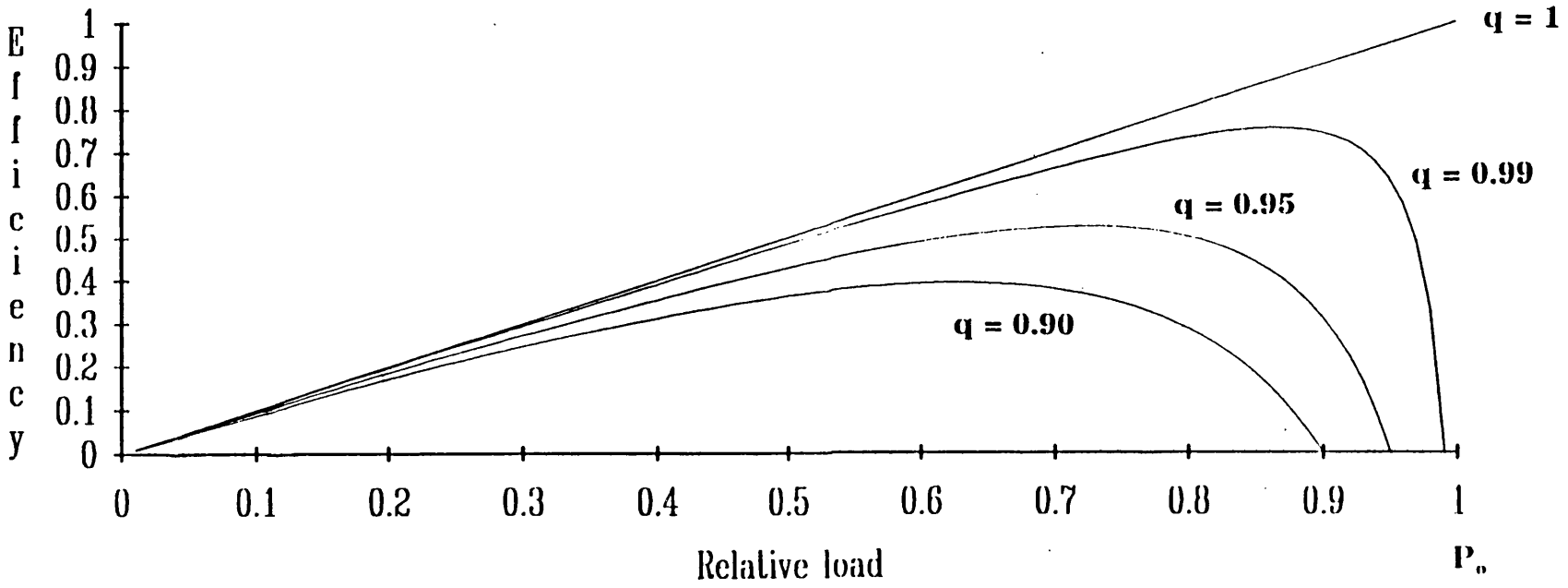
The efficiency of such a coupled system is defined as the ratio of the free energy gained by the "driven process" (work) to that lost from the "driving process". If all the free energy input was wasted, there would be no work production. Therefore, the efficiency would be zero. At the other extreme, if the coupling is perfect, there is no free energy input wasted and the efficiency would be 100%. However, in practice, the efficiency value can never be quite 100%, simply because the coupling can never be quite perfect.

2.1.2 Factors causing inefficiency in general

Generally speaking, for any coupled system, the following factors can cause inefficiency.

Firstly, inefficiency can be caused by the fact that the coupled system is not close to its equilibrium position (Fig. 2.1, from Kedem & Caplan 1965). Just like a single, uncoupled process, the closer to its equilibrium position, the more reversible the process is; the more reversible a process is, the less energy is wasted and the higher the efficiency that can be expected. So, in order to achieve a higher efficiency, a coupled system should not be too far

Fig. 2.1 The relation between efficiency and the degree (q) of coupling (modified from Keden & Caplan 1965) at different loads. Four q values are used to show that the maximum efficiency is ultimately decided by q. P_o is the equiibrium position at which the maximum load (corresponding to the isometric force in muscle) can be obtained.



away from its "constrained" equilibrium (P_o on the diagram). For example, in terms of muscle contraction, this means that a high efficiency can be expected only when the load is close to muscle's isometric load (P_o). This can be seen in Fig. 2.1.

Secondly, inefficiency can be caused by the degree of coupling being less than unity, the coupling is not tight enough. Suppose there are the following reactions:

$$A \xrightarrow{L_{11}} B \qquad (1)$$

$$X \xrightarrow{L_{22}} Y \qquad (2)$$

$$A + X \xrightarrow{L_{12}} B + Y \qquad (3)$$

where L_{11}, L_{22} and L_{12} are rate constant for reaction (1), (2) and (3) respectively. Reaction (1) and (2) are called uncoupled reactions because they are independent of each other. Reaction (3) is called the coupled reaction. The ratio $(L_{12}/\sqrt{(L_{11} \times L_{22})})$ is called "degree of coupling" (q) which is a quantitative measure of the "tightness" of the coupling. The higher the q value, the tighter the coupling. Fig. 2.1 also shows the relation between efficiency and the degree

of coupling at different loads. This figure illustrates that the maximum efficiency is ultimately decided by the degree of coupling (q in Fig. 2.1), the larger the q value, the higher the maximum efficiency.

Thirdly, there is another type of inefficiency which comes from the system containing several stages for energy transformation. In this case, the overall efficiency of transformation is the product of efficiencies of each individual stage. Because of this, transduction involving more stages incurs more inefficiency. This is particularly true when efficiency at one stage of the long chain is very low. This "bottle neck" effect will limit the overall efficiency. Generally speaking, the more stages the transduction involves, the less the overall efficiency will be. For example, in frog muscle, initial efficiency is about 0.45 (Hill 1964) and the efficiency of oxidative phosphorylation is about 0.80 (Wilkie 1960). Therefore, the overall efficiency is about 0.36 (0.45x0.80).

Fourthly, any side reaction accompanying the energy transduction process is another source of inefficiency. Fig. 2.2 shows the energy transduction and its side reaction in living muscle. In this figure, the energy transduction process is the chain of the reactions including crossbridge cycling, ATP splitting and oxidative resynthesis of ATP. The energy transduction, in terms of force and

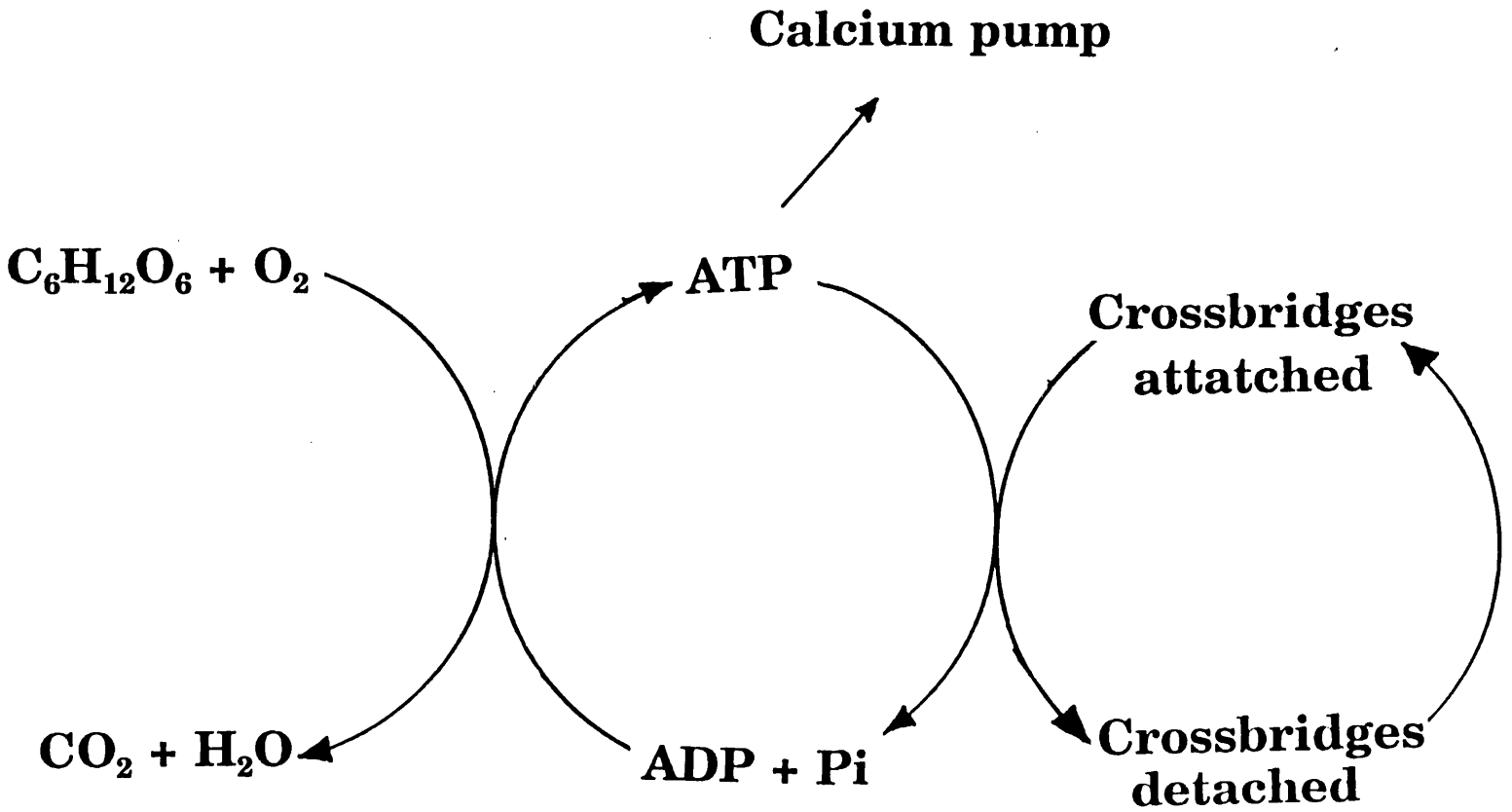


Fig. 2.2 Processes underlying muscular contraction. Calcium movements is considered as a side reaction for chemomechanical transduction in muscle. ATP split by calcium pump is thus a source of inefficiency.

work production, is completed within this process. But some ATP is split outside the process, i.e. not for force or work production but for the calcium pump in the process of excitation-contraction coupling. Because there is no force or work production from excitation-contraction coupling, the amount of ATP split by the calcium pump, which can, in principle, produce work, is wasted in terms of work production. This is another reason for inefficiency.

Finally, the speed of the energy transduction process would affect the efficiency of the transduction. In general the faster the transduction is, the lower the efficiency. The reason for this is that, in order to achieve a high rate of transduction, a larger "driving force" is necessary. Only by setting a system far enough away from its equilibrium, can an adequate free energy (acting as the "driving force") be available to overcome "chemical friction". Fortunately in muscle the "chemical friction" is probably small.

2.2 Efficiency of muscular contraction

Having considered efficiency and the factors affecting it in general, now attention is switched onto the efficiency of muscular

contraction. A theoretical discussion is based on A. F. Huxley's 1957 theory (Huxley 1957).

2.2.1 Huxley's model

In 1957, Huxley presented his model to explain the mechanism of muscular contraction. Because his model successfully predicted many important properties of muscle, it has been very influential.

Some major points of Huxley's model:

Firstly, Huxley assumed that there are two states in a crossbridge cycle, attached and detached (Fig. 2.3). A crossbridge can only exist in one of these two states. It was assumed also that an ATP is split when a crossbridge passes in sequence through these two states. Therefore, the energy from ATP splitting is utilised to drive the cyclic attachment and detachment of the crossbridge. Here, crossbridge denotes a myosin head of a thick filament, no matter whether it is attached or detached.

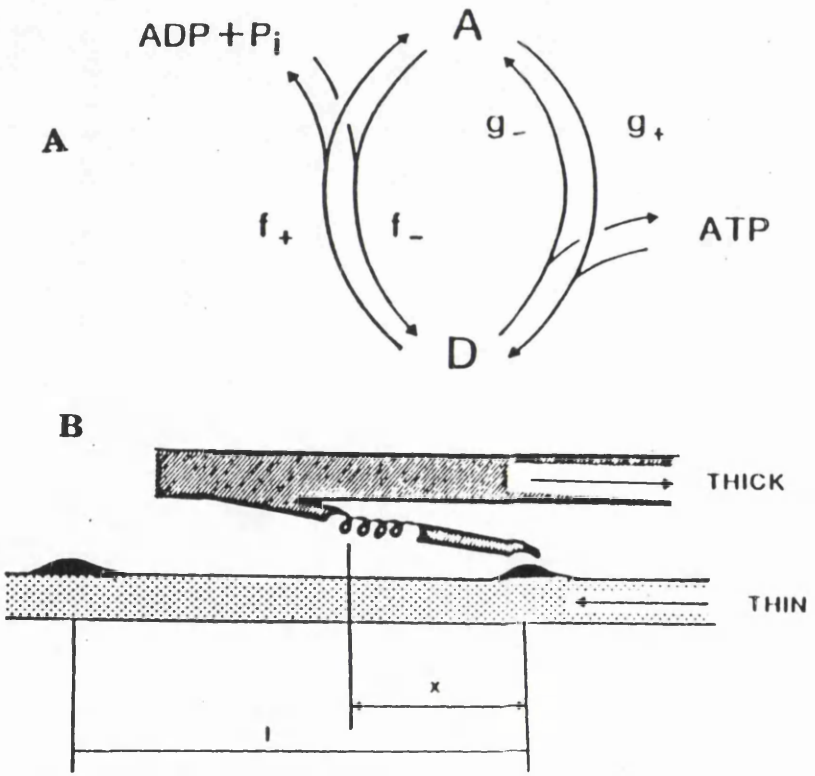


Fig. 2.3 **Huxley's 1957 theory postulates the existence of two crossbridge states: attached (A) and detached (D). The cyclic attachment and detachment is driven by ATP splitting. f_+ and g_+ are the rate constants for the forward (clockwise) processes; f_- and g_- are those for the reverse processes. (B) The 1957 theory supposes that the thin filament has sites spaced at a distance l from one another to which a crossbridge can attach. The crossbridge contains a spring so that the attachment site can move with respect to the thin filament. The variable x describes this movement, and is zero when the force in the spring is zero. The arrows show the direction of the relative movement of the filaments during shortening.**

Secondly, it was assumed that a crossbridge can only exert force when it is in the state of attachment. The amount of force exerted by an attached crossbridge varies with the degree of displacement between thick and thin filaments. This displacement is indicated by the term x (Fig. 2.3). By definition, x value is zero if a crossbridge is in the position where it exerts zero force.

Thirdly, Huxley assumed that the rate constants for the transitions between the crossbridge states are functions of x (Fig. 2.4). He chooses the functions such that the crossbridges attach more rapidly at positive values of x than at negative values of x. On the other hand, the crossbridges detach more quickly at negative values of x than at positive values. It is this asymmetry that causes shortening and the development of force.

2.2.2 Muscular efficiency

Fig. 2.5 shows the path (the dashed line) of a crossbridge follows during shortening when it does the maximum possible amount of

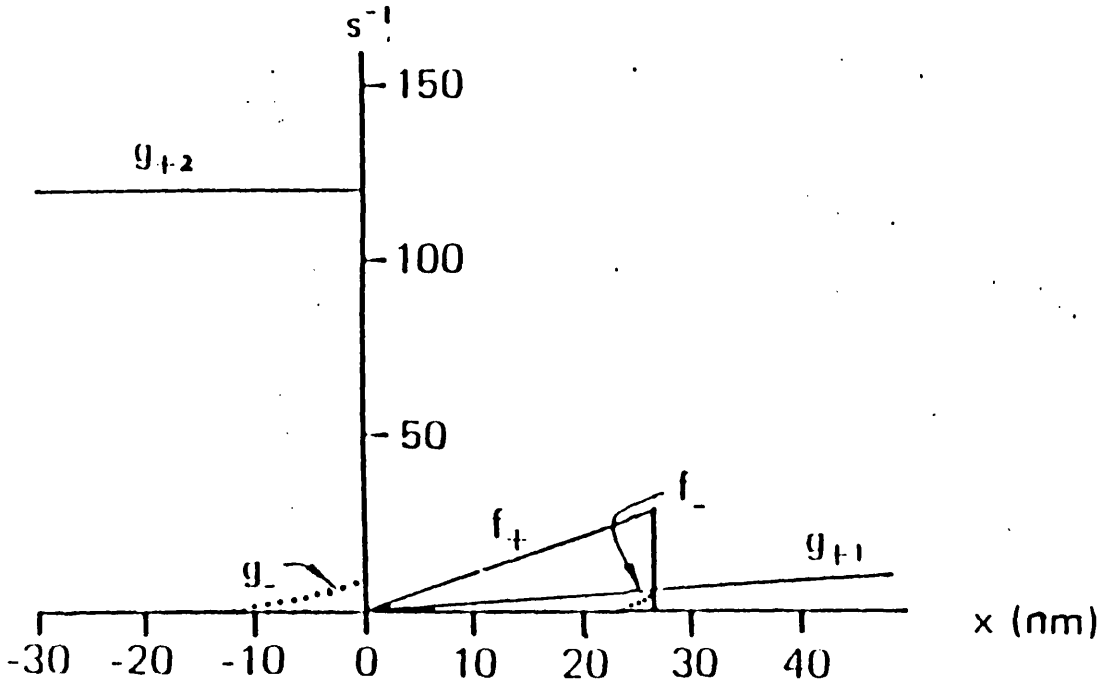


Fig. 2.4 The rate constants for attachment (f_+) and detachment (g_-) of crossbridge are assumed to be functions of x as shown here. The dotted lines are the values (f_- and g_-) of the rate constants for the reverse reactions (from Woledge, 1985).

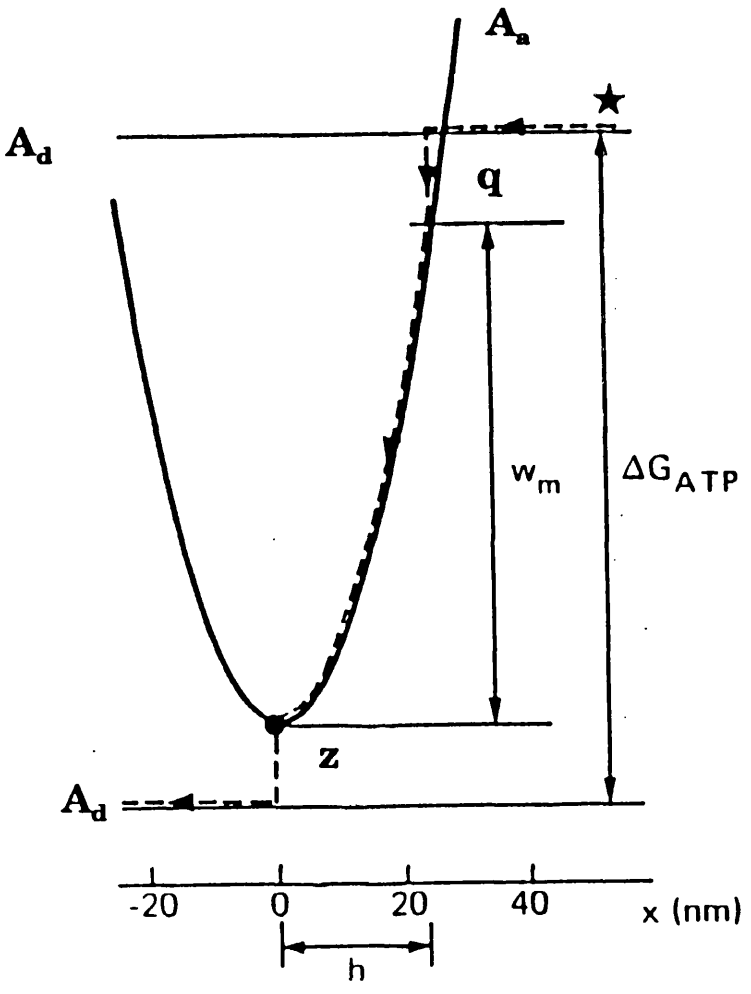


Fig. 2.5 The path (dashed line) a crossbridge follows during shortening when it does the maximum possible amount of work. A_d is the free energywith the bridge detached; A_a with it attached. q and z are the free energy changes for the attachment and detachment process. h is the maximum range of x over which attachment is possible.

work. As can be seen in the figure, in order to achieve high efficiency, the maximum work (W_m in the figure) needs to be high. Therefore, the free energy change for both attachment (q in the figure) and detachment (z in the figure) needs to be small. This means that a crossbridge needs to join immediately at x=h and break just at x=0.

(a) Can muscular efficiency be 100%?

If a muscle is to be 100% efficient, the following condition needs to be met: (1) its f_+ value would be very large at X=h and zero elsewhere in order to make q=0; (2) its g_+ value would be very large at X=0 and zero elsewhere in order to make z=0; and (3) $W_{max}=\Delta F_{ATP}$ (see Fig. 2.5). If the muscle can satisfy the three conditions, it can be 100% efficient; because condition (1) and (2) ensure tight coupling (q=1) and condition (3) brings the reaction close to its equilibrium position. Therefore the muscle would, in principle, be 100% efficient.

(b) Real muscle cannot be 100% efficient

As just mentioned, a "theoretical muscle" (like the one in (a) above)

can be 100% efficient. However, a theoretical muscle incurs the following consequences (Fig. 2.6): (1) muscle force is independent of shortening velocity; (2) power output is linear with shortening velocity; and (3) muscle efficiency is independent of shortening velocity. As can be seen in Fig. 2.6, these properties of a theoretical muscle are very different from what have been observed from real muscle. This renders such a theoretical muscle very unrealistic. Therefore, real muscle cannot be 100% efficient. For more details, see Wilkie & Woledge (1967).

(c) What makes real muscle less than 100% efficient?

Firstly, in real muscle crossbridges join at $x<h$. This is particularly obvious at rapid shortening (Fig. 2.7). As the consequence of this, the free energy change for attachment (q in Fig. 2.5) is high, i.e. more free energy is wasted for attachment. Secondly, in real muscle some crossbridges break at $x>0$, as happens in slow shortening. The free energy change for detachment is lowest only at $x=0$ but higher elsewhere. Therefore crossbridges breaking at $x>0$ use more free energy for their detachment and leave less free energy for work production. Thirdly, in real muscle during rapid shortening, some crossbridges break at $x<0$ (Fig. 2.7). These crossbridges thus exert negative force (pushing instead of pulling)

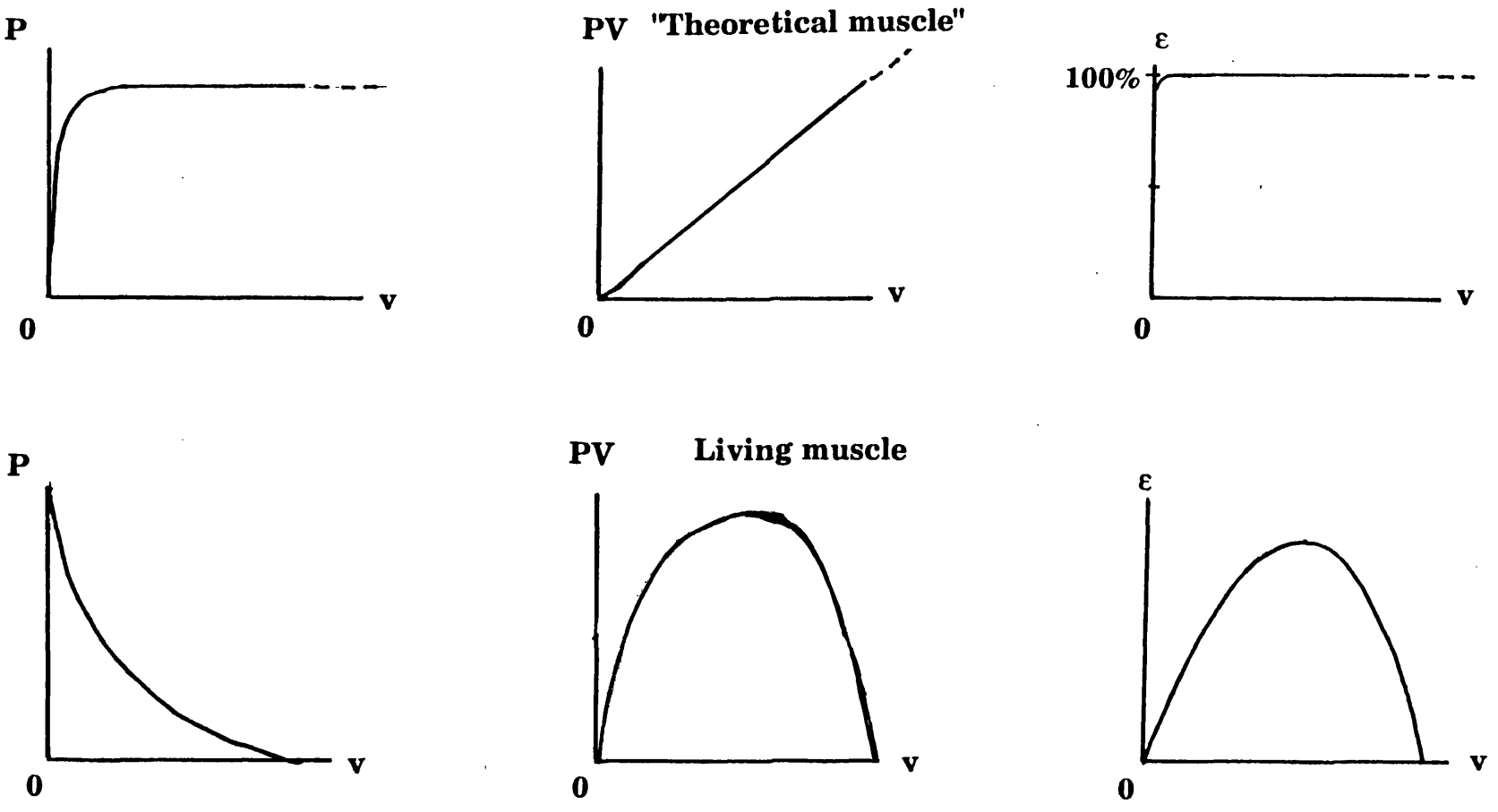


Fig. 2.6 Relation between force (P), power output (PV), efficiency (ε) and shortening velocity (v) predicted from a "theoretic" muscle (top) with 100% efficiency, compared with those observed from living muscle (bottom).

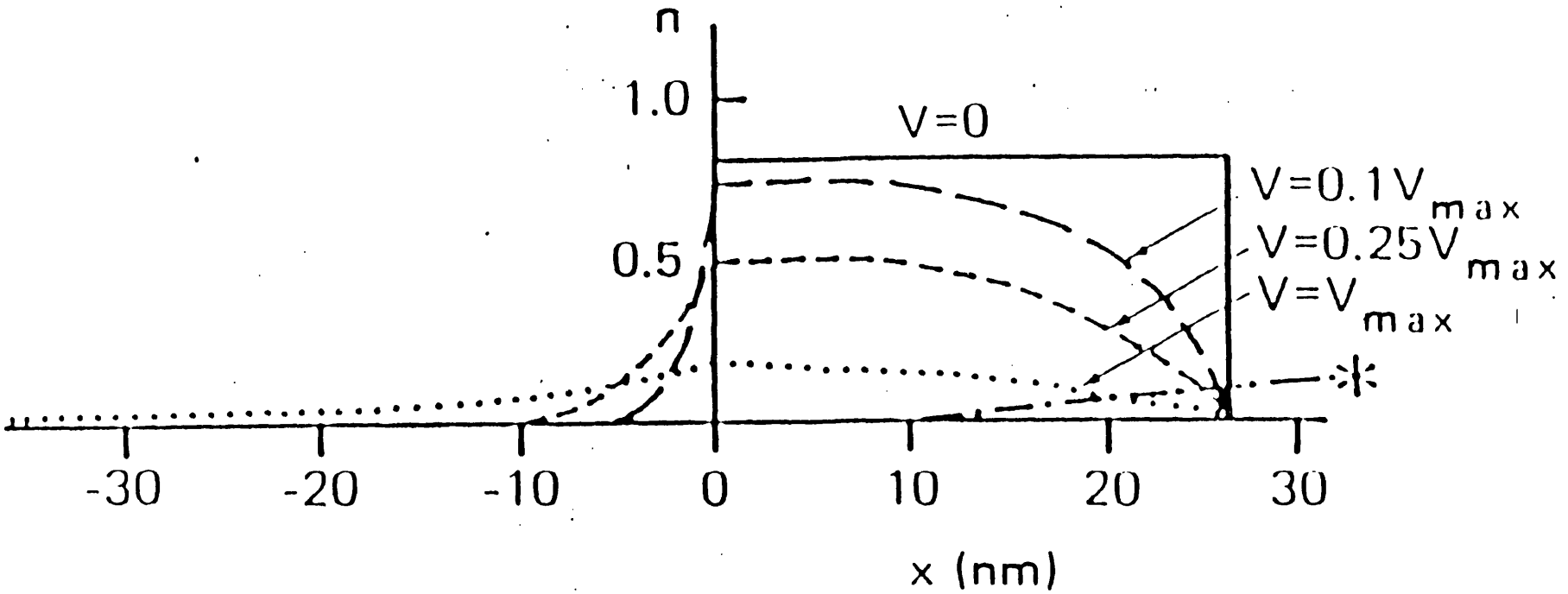


Fig. 2.7 The proportion of crossbridges attached (n) as a function of x for four different velocities of shortening (v). The line marked with the asterisk shows for v = V_{max} the proportion of crossbrisges that might be still attached to the previous site.

and counteract with other crossbridges which are doing positive work. The net consequence is low efficiency. Finally, crossbridges may join if g (the reverse of the detachment process) at $x \approx 0$ is large because the energy barrier (z in Fig. 2.5) for attachment is small. If crossbridges join instead of break at x=0, more free energy would be wasted. This is another reason for inefficiency.

The reasons given above not only explain why efficiency in real muscle is less than 100%, but also explain the "characteristic" force-velocity curve. When there is no shortening (isometric contraction), a muscle produces maximum force because crossbridges have plenty of time for attachment. This gives the intercept (P_o). At a low velocity of shortening, less crossbridges join at or closer to x=h and break at x=0; at the same time some attached crossbridges are brought into the region of x<0. The net effect is less positive force and more negative force. Therefore, at low shortening velocities, the force is lower than P_o. As shortening velocity increasing, the positive force is getting less and the negative force more. So the net effect is a decrease in force as velocity of shortening increases. When shortening at V_{max}, the positive force is completely balanced by the negative force. The net force is thus zero and this gives the intercept (V_{max}).

(d) Why muscle efficiency is not 100%?

One reason may be that the cost of shortening is very high in muscle which is tightly coupled. In order to shorten for a distance which is much larger than h value, all the crossbridges have to interact cyclically at each site. This cyclic interaction is costly because for each cycle one ATP is split to power the cycle. The more the cycles, the more ATP is split. An example will demonstrate this. If the crossbridges are spaced 35 nm apart, then when the tightly coupled muscle shortens, each crossbridge splits an ATP at every 35 nm shortening per half sarcomere. Muscle contains 0.3 μmol/g of crossbridges and thus would split 0.3 μmol/g of ATP in each 3% of shortening, i.e. 0.1 μmol/g ATP per 1% shortening. Actually the energetic cost of shortening is much less, about 1/4 of this.

Another reason for an efficiency less than 100% is that enzymatic control of muscle contraction may not be good enough. There is a chain of reactions underlying muscle's contractile and recovery process. Muscular contraction is possible not only because the reactions are thermodynamically possible, but also because the reactions are rendered possible biologically, i.e. channelled by enzymes. Without relevant enzymes in muscle, even

thermodynamically possible reactions cannot react in living muscle. Moreover, enzymic regulation can affect efficiency. An example is the Lohmann reaction which is nearly 100% efficient. This reaction is at equilibrium because the enzyme is not subject to control. If Nature can create enzymes of this sort to catalyse all the reactions in energy transduction processes in muscle, the muscle would be 100% efficient. In theory it is possible to change the route of reactions in order to improve efficiency. In a muscle or a biological system, this can only be achieved by creating new enzymes which catalyse and regulate the reactions via a new route. Therefore, the lack of high degree of enzymic control may be another reason which limits the maximum muscular efficiency.

CHAPTER 3 METHODS

3.1 General design of experiments

3.1.1 Procedures for working out thermodynamic efficiency

Firstly, obtain the recovery ratio for both isometric and working contractions and compare them. This includes the following steps:

a) measuring work, initial heat and total heat from experimental recording (see Fig. 4.3 for example)

b) adding work to initial and total heat to obtain the initial energy and total energy

c) obtaining recovery energy by subtracting initial heat from total heat

d) working out the recovery ratio by dividing recovery energy by initial energy

e) comparing the recovery ratio obtained from isometric contractions with that obtained from working contractions

If the two ratios are the same and close to the theoretical value, the reactions corresponding to the initial process and the recovery process could be assumed to be PCr splitting and the oxidative resynthesis of PCr, respectively.

Secondly, calculate or estimate the following parameters of the PCr splitting reaction by using established thermodynamic and biochemical knowledge:

f) calculate the molar enthalpy change for the initial phase by using the equation (Woledge et al 1985)

$$r = (72/\Delta H_{PCr}) - 1$$

thus,

$$\Delta H_{PCr} = 72/(r + 1)$$

where r is the recovery ratio and ΔH_{PCr} is the molar enthalpy change for PCr splitting in kJ/mol.

g) using the molar free energy change for ATP splitting calculated by Kushmerick and Davies (1969) as molar free energy change

(ΔF_{PCr}) for PCr splitting i.e. assume the creatine kinase reaction is in equilibrium.

And finally, calculate the thermodynamic efficiency for the initial process (ε_i) by using the equality (Wilkie 1960).

$$\varepsilon_i = (\Delta H_{PCR}/\Delta F_{PCR}) \times (w/(h_i+w))$$

3.1.2 Experimental procedure

The following experimental routine was followed in each experiment.

Pre-observation:

Temperature equilibrium: The temperature used in the experiments was 25°C. After dissection, the muscles were mounted on the thermopile. The thermopile with the muscle was then covered by a chamber containing Ringer's solution, but not enough to cover the muscle, i.e. the muscle was in moist air over Ringer solution. The chamber was immersed into the water bath. The water bath was stirred to avoid temperature gradients. The muscles were gassed with 95% O_2 and 5% CO_2 which bubbled

through the solution in the chamber. Usually, after 30 minutes, temperature equilibrium was reached and then the gas flow was turned off.

Force-stimulation strength curve: This was established by varying the stimulation voltage and recording the resultant tetanic tension. In this way, a supramaximal voltage was found and it was used throughout the experiment (Fig. 3.1).

Force-frequency curve: Different stimulation frequencies were tried to establish the relation of tension and stimulation frequency (Fig. 3.2). As shown by the example in the figure, frequencies above 50 Hz did not increase force much, however they did seem to cause more rapid deterioration of the muscle. Therefore, 50 Hz was in fact used in the main part of each experiment.

Force-length curve: Having established the stimulation parameters, the relation between tension and muscle length was investigated by varying the resting length. Fig. 3.3 shows an example. The muscle length corresponding to the plateau of length-tension curve was identified, this plateau was about 2 mm wide. The starting length for shortening was chosen usually 0.5-1 mm beyond the plateau ("↑" in Fig. 3.3). The distance of shortening used was 2-3 mm. Under these conditions, the muscle should give

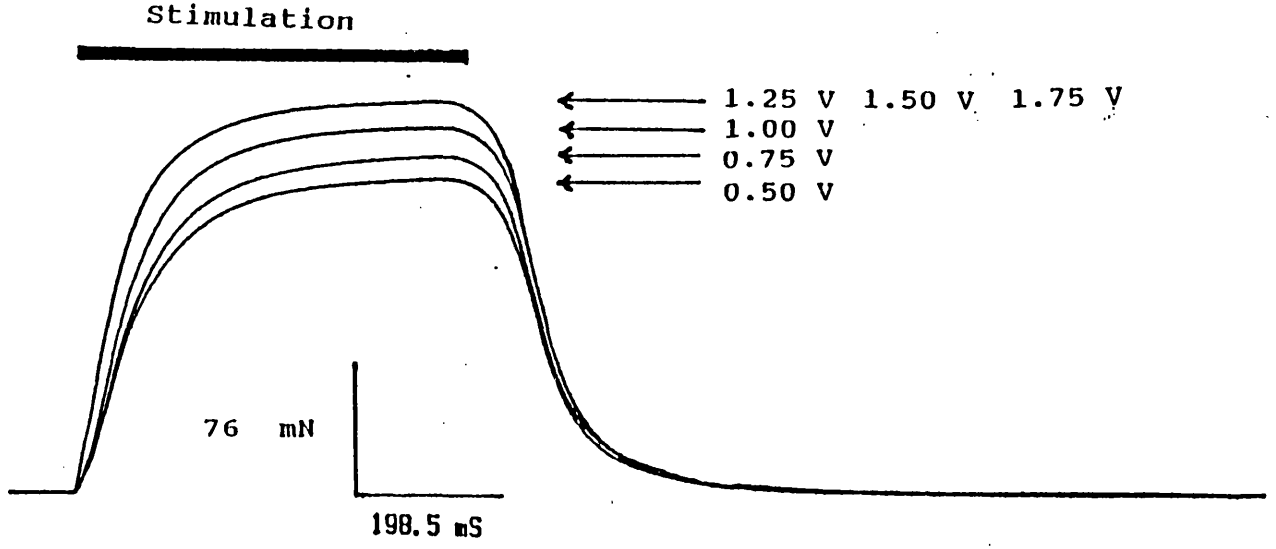


Fig. 3.1 Relation of muscle tension and stimulus voltage. Records of tetanic force with different stimulus voltage. Note that the records with the three highest voltages are superimposable.

Fig. 3.2 The force–stimulation frequency relation in one experiment (one point for onr observation, mouse soleus, 25 C).

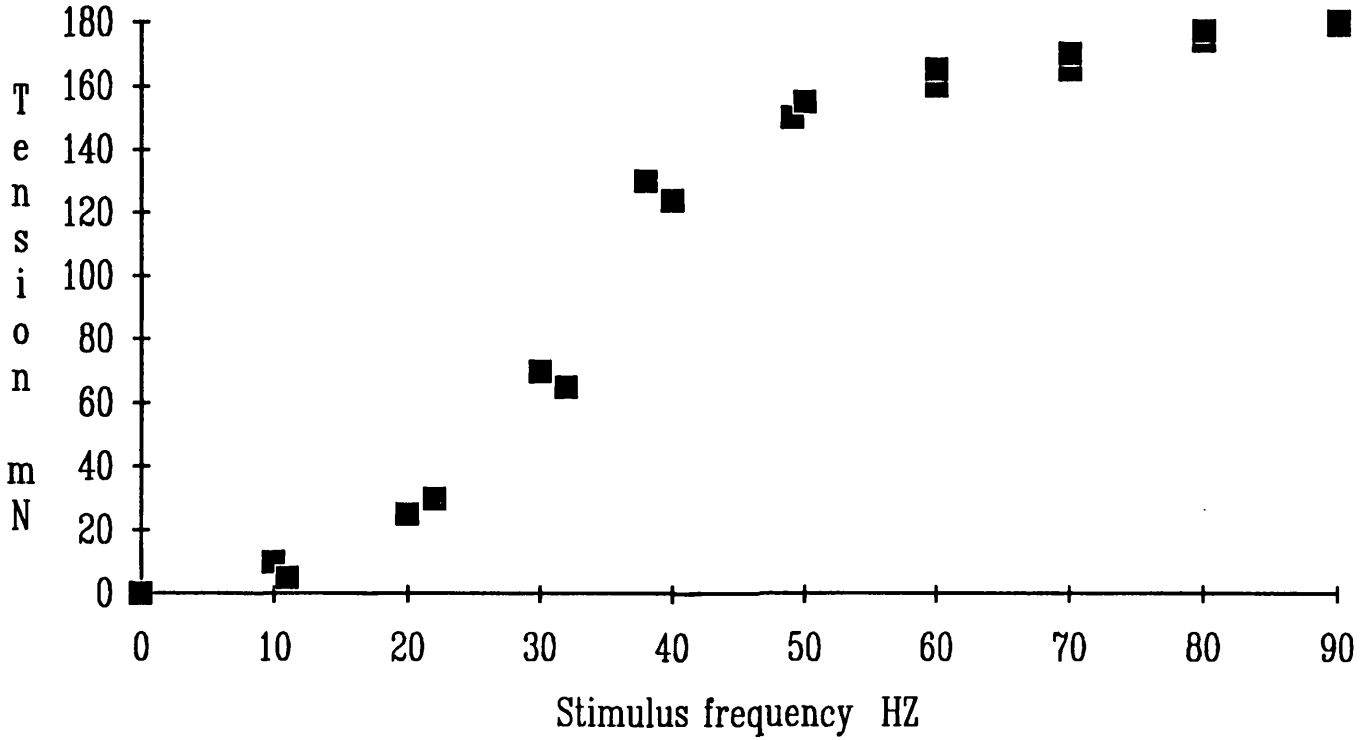
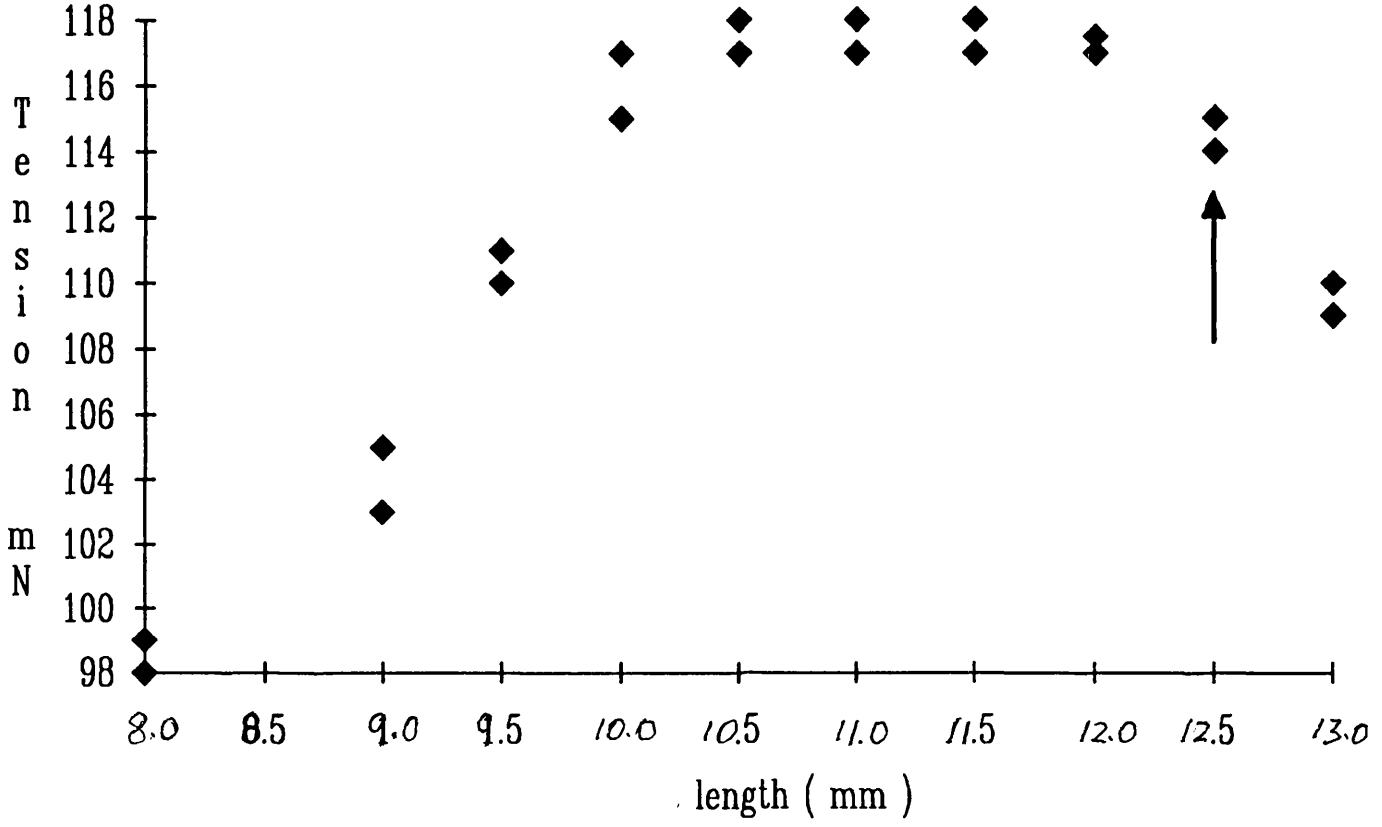


Fig. 3.3 An example of force-length relation of a muscle obtained in one experiment. The starting point of shortening is shown by the arrow. One point represents one observation (mouse soleus, 25 C).



optimal tension or work as most of shortening would occur within the plateau of the length-tension curve.

During observation:

During experimental observations, a particular pattern of observation was used to minimise any effect due to the order of observation. Where several conditions were to be compared, each was repeated several times within a systematic pattern, so that the average result for each treatment would be affected equally by any deterioration in the muscle. As a simple example, the pattern A-B-B-A-A-B-B-A was used. Here, the average of all A and of all B are equally affected by any progressive linear change in the muscle.

After the end of observations:

At the end of the experiment, the muscle length was measured before it was taken off from the thermopile. Then the muscle was set at the same length in a Sylgard coated dish and fixed by a solution of 2% gluteraldehyde in Ringer's solution. The part of the muscle which had covered the active region of the thermopile was cut out. Both this part and the remainder, free from tendon, were dried and weighed separately. Thus, the work done by this part of

the muscle, which had covered the active regions of the thermopile and, hence, produced the recorded heat production, can be worked out by the equality

$$w = W \times (m/M)$$

in this equality:

w: work done by the part of muscle which was covered the active region of the thermopile

W: work done by the whole muscle

m: dry weight of the part of muscle which covered the active region of the thermopile

M: dry weight of the whole muscle

This assumes that work done is in proportion to the dry weight of muscle.

3.2 Muscle preparation

Soleus muscles of Mus. musculus Strain C_3H were used. The ages of the mice were about 3 months. Both male and female mice were

used randomly. The mice weighed 20-28 g. An injection of pentobarbitone sodium was given to the mice. After anaesthesia was established, the mouse was killed by cervical dislocation. Dissection was done immediately after the killing. The isolated soleus muscles were 8-11 mm in length and 12-14 mg in wet weight. The fibre length of soleus muscles from these mice is 7.1 ± 0.16 mm (n=8, S. K. Philips, personal communication).

3.3 Solution

Modified Ringer's solution was used. The composition of the solution was the following (mMol/l):

NaCl 135 KCl 5 $CaCl_2$ 2.5 $MgCl_2$ 0.5 NaH_2PO_4 1.0

$NaHCO_3$ 24 Glucose 11 Calf serum 2 (in mg/l)

This solution was gassed with 5% CO_2 and 95% O_2 for 5 minutes in order to get a pH of 7.35.

3.4 Heat measurement

Fig. 3.4 shows the sketch of experimental equipment used. Details

about this equipment will be described in the relevant parts.

3.4.1 Thermopile

A long electroplated type thermopile (Constantan-silver, Bozler 1930, Ricchiuti & Mommaerts 1965, Woledge et al 1985) was used. Only part of the thermopile was used to record the temperature change due to muscle contraction. The active sections used for heat recording contained 20 thermocouples with a length of 3 mm. The sensitivity of this pile is 32.7 μV/K per couple. The thermopile output was connected to a Nicolet 4094 Digital oscilloscope via a high gain pre-amplifier (1,000,000 X, Ancom 3A modified to "chop" at 1 kHz with output filtered to 50 Hz).

In this thermopile, a "protecting region" was provided to minimise the artefact due to shortening (Fig. 3.5A). The "protecting region" is a part of the thermopile which is not connected to the voltage-measuring circuits. With this "protecting region", the heat loss from the muscle part which covers the "protecting region" is the same as the heat loss from the rest of the muscle. Without such an arrangement, temperature gradients can be caused by uneven heat loss and this will distort the time course of temperature change recorded during subsequent shortening and heat production in turn.

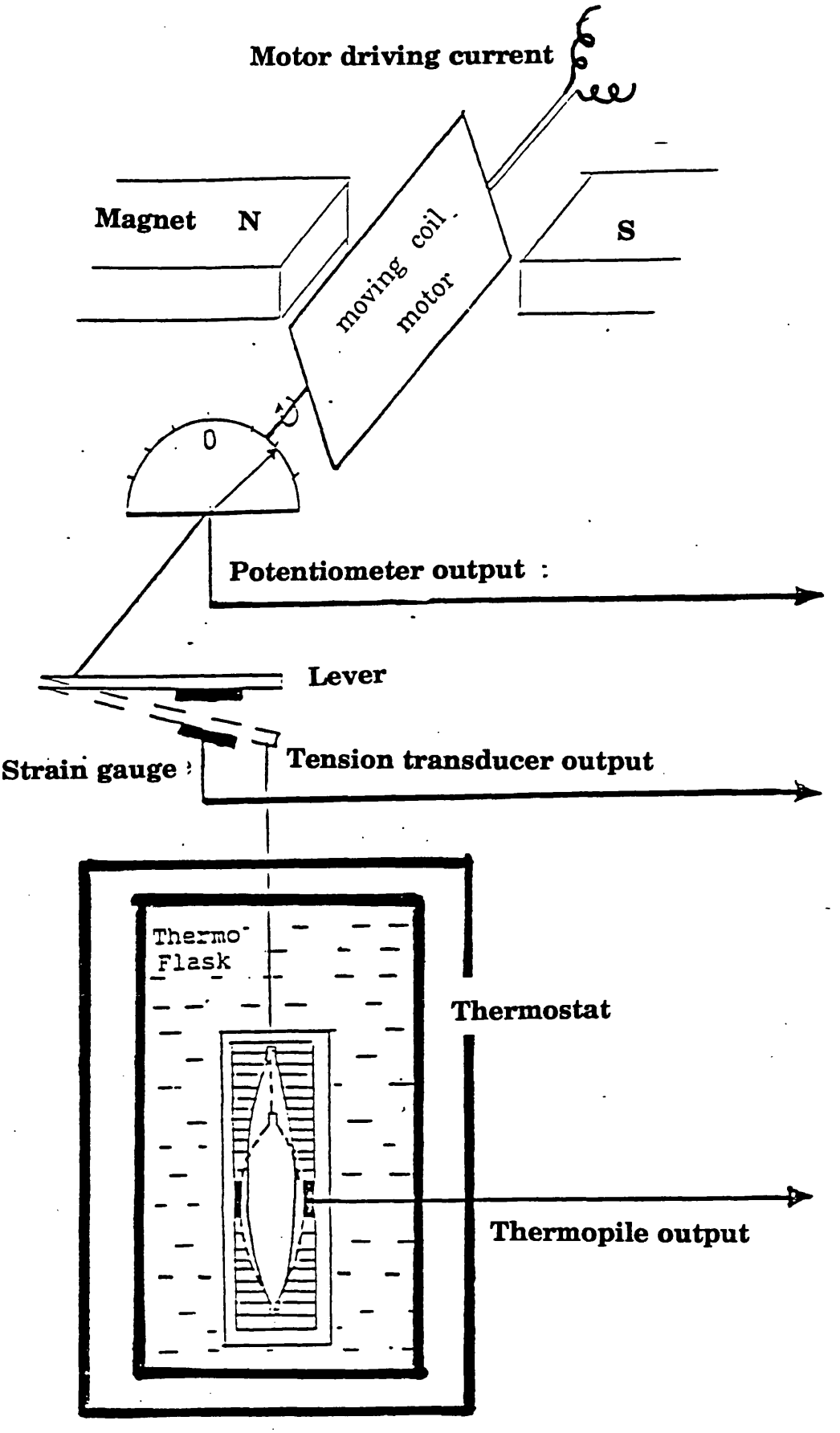


Fig. 3.4 A schematic drawing of the arrangement of experimental equipment.

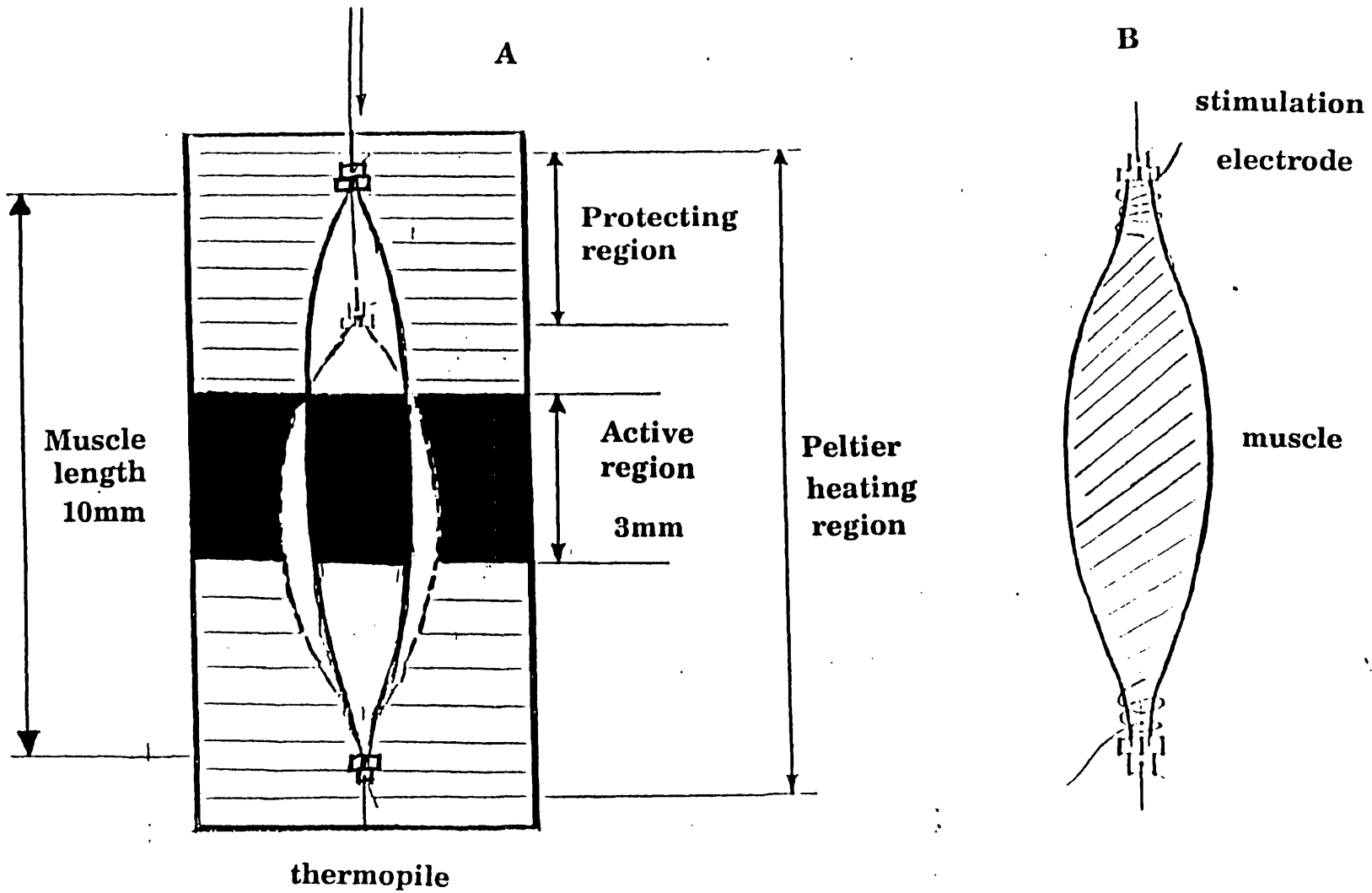


Fig. 3.5 (A) A schematic drawing of thermopile and the position of muscle on it. (B) The arrangement of stimulation electrodes.

3.4.2 Oscilloscope

A Nicolet 4094 Digital oscilloscope was used so that 4 signals, i.e. muscle temperature, heat, tension, length and bath temperature could be followed simultaneously. To follow the different signals which last for different lengths of time,two separate time bases on the oscilloscope were used. The fast time base was for mechanical changes (tension and length) and the slow one for the heat production which lasted for 2-3 minutes. These different time bases will be seen in the experimental records later.

3.4.3 Thermostat and water bath

As can be seen in Fig. 3.4, the thermopile with a muscle was immersed in a water bath contained in a large thermos flask. A thermostat was used to keep the bath temperature steady. The bath water was stirred by air bubbles to avoid temperature gradients in the water.

3.4.4 Heaters

There were 3 heaters in the water bath contained in the thermos flask (not drawn in Fig. 3.4). These heaters were controlled by a

negative feedback circuit, when temperature in the bath water dropped, this was sensed by the platinum resistance detector in the bath and the signal from the detector was fed back to a control device which, in turn, switched on the heaters. Due to this feedback loop, the stability of the bath water temperature and the stability of thermopile baseline was greatly improved. In fact, temperature variation of less than 0.001°C in the base line of the thermopile was achieved. This stability is vital for obtaining consistent and reliable recording of the recovery heat which lasts for about 2-3 minutes, several hundreds times longer than the mechanical events.

3.4.5 Peltier control heating

The output of the thermopile only reflects the temperature change due to heat production of muscular contraction, but is not proportional to heat production itself. In order to convert the temperature signal into heat, the heat capacity of muscle and its immediate surroundings should be known. Moreover, allowance needs to be made for heat loss from the muscle to its environment if the real time course of heat production is to be obtained. It is for these purposes that Peltier control heating is used.

The principle of Peltier control heating is to heat the muscle with

a small known current (usually less than 100 μA) passed through the thermopile. When such a small current is passing, the main effect is the transportation of heat from cold junctions to hot junctions, or vice versa (Peltier effect). Joule heating with such tiny current is negligible. Because the rate at which the heat transported by the Peltier effect can be known from the current (in amperes), the absolute temperature, the number of junctions and the Seebeck coefficient (volts/°C), and because the rate of temperature change can also be known (from the cooling curve after Peltier heating), the heat capacity of the muscle and its surroundings can be easily worked out. Knowing the heat capacity, the temperature signal, this time from muscle contraction, not from Peltier heating, can be converted into a time course of heat production. Details about this method are given by Kretschmar and Wilkie (1972, 1975) and Woledge et al (1985).

In this project, Peltier current was passed through the whole length of the thermopile for the purpose of calibration and for heat loss correction (Fig. 3.5A). The Peltier current used was 90 uA and the heating period was about 40 seconds which was sufficient for the muscle to reach a steady temperature. The cooling after the Peltier heating was recorded and was assumed to have the same time course as the heating. The main part of the cooling curve was nicely fitted by an single exponential curve

(Fig. 4.2).

The amount of heat loss was calculated by multiplying the reciprocal of the time constant obtained from the fitting curve in Fig. 4.2 and the area under the temperature curve in Fig. 4.1. Then, the amount of heat equal to this heat loss was added to obtain the true value of muscle heat production.

3.4.6 Stimulation heat

In order to minimise the heat due to the energy produced by the electric stimulation current, a special arrangement of stimulation electrodes was used (Fig. 3.5B). Two fine thin platinum wires (25 μm in diameter) were used as the stimulation electrodes, so that these wires could be easily wrapped loosely around the muscle-tendon junction region two or three times. This arrangement gave the muscle more surface area to contact with the electrode wires and was found to reduce considerably the electric energy needed for maximum activation. With these stimulation wires, the muscles were fully activated by using much less electrical energy (typically the pulses were 1.5 V, 50 Hz, 0.5 ms pulse width).

Some observations were made to measure the size of stimulation

heat by treating muscles with procaine at the end of experiment. The results confirmed that stimulation heat was indeed negligible.

3.5 Mechanical measurement

3.5.1 Tension

The muscle force was measured by a strain gauge which was attached to a lever (Fig. 3.4). The lever was connected to a fine steel tube with a hook, to which the muscle was connected. Therefore, when the muscle was stimulated, it contracted and pulled the lever via the tube. This deformed the force transducer and caused a change in the electric resistance of the strain gauge. This gave rise to the tension signal.

The tension calibration of the strain gauge was done by hanging known weights on the hook which was connected to the strain gauge via the rod and recording the resultant output from the strain gauge. The calibration factor was 19.2 mV/gm (Fig. 3.6A).

3.5.2 Length

A potentiometer was used to detect the length change of the muscle (Fig. 3.4). The lever was connected to a moving coil motor

via the potentiometer. When the motor was driven, the lever was moved, either stretching or releasing the muscle. At the same time, the distance of this movement was sensed by the potentiometer. Therefore, the output of the potentiometer reflects the distance moved.

The length calibration was performed by measuring the distances of the movement at the end of hook under microscope while recording the output of potentiometer. The calibration factor was 395 mV/mm (Fig. 3.6B).

Fig. 3.6A The force calibration. The slope is 19.2 mV/g.

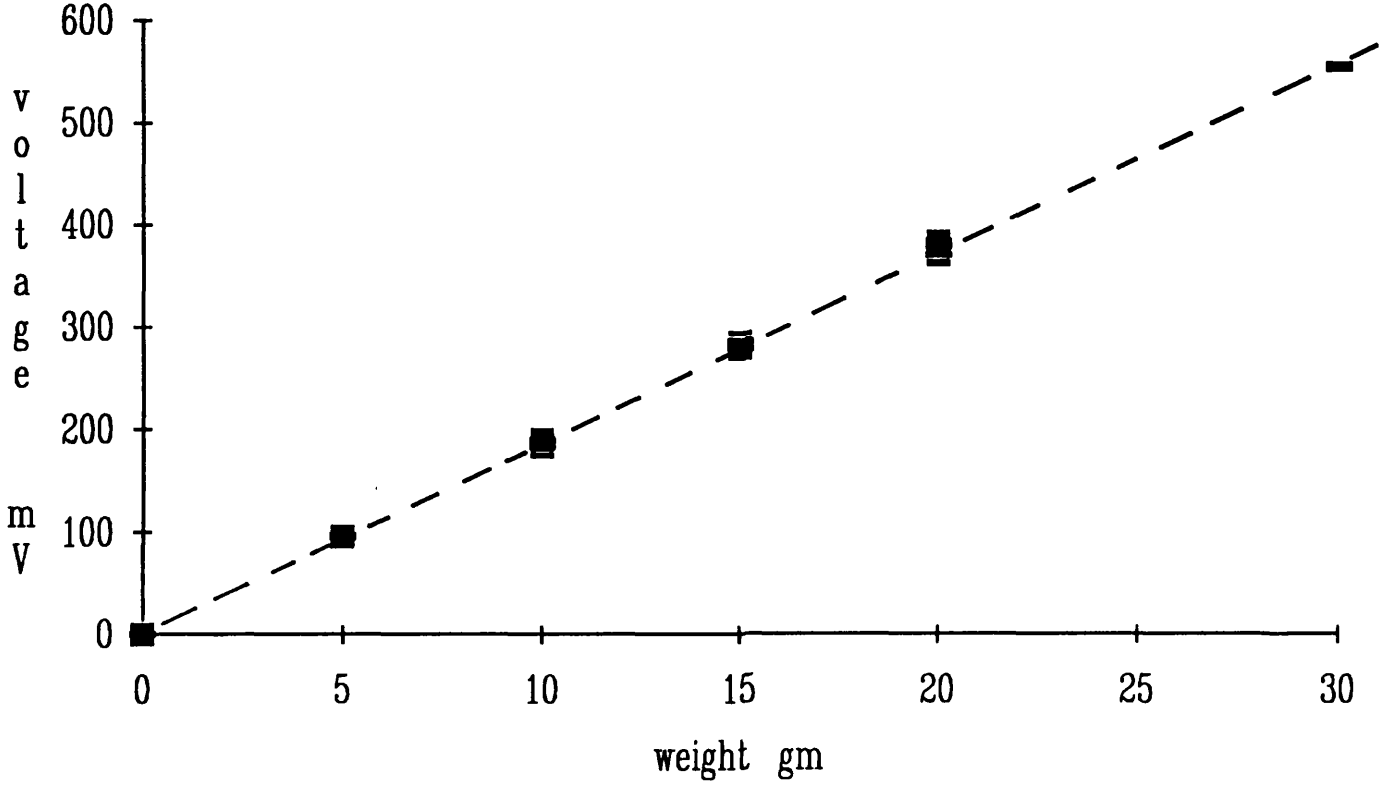
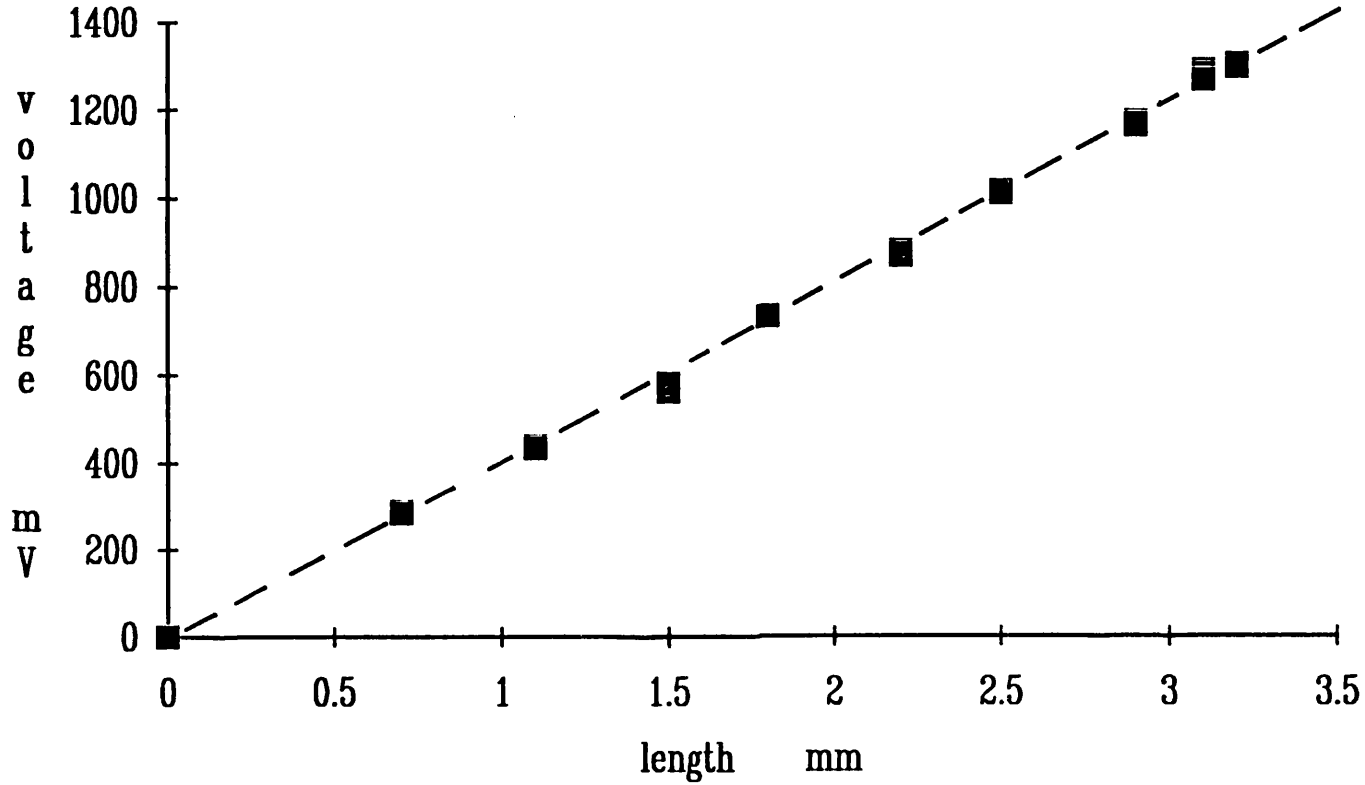


Fig. 3.6B Length calibration. The slope is 395 mV/mm.



CHAPTER 4 RESULTS OF THE FIRST SERIES OF EXPERIMENTS

Altogether, five successful experiments of this type were made on muscles from different mice and a total of 197 successful observations were obtained from these experiments.

4.1 A typical experiment

4.1.1 A Typical observation

Fig. 4.1 shows the data from one observation of a typical contraction during which mechanical work was produced. In this figure, the length and tension changes during the contraction are shown and the work production during the contraction was calculated from them. The amount of work was equal to the enclosed area shown in the inset of the figure. The temperature change during and after the same contraction is also recorded in this figure.

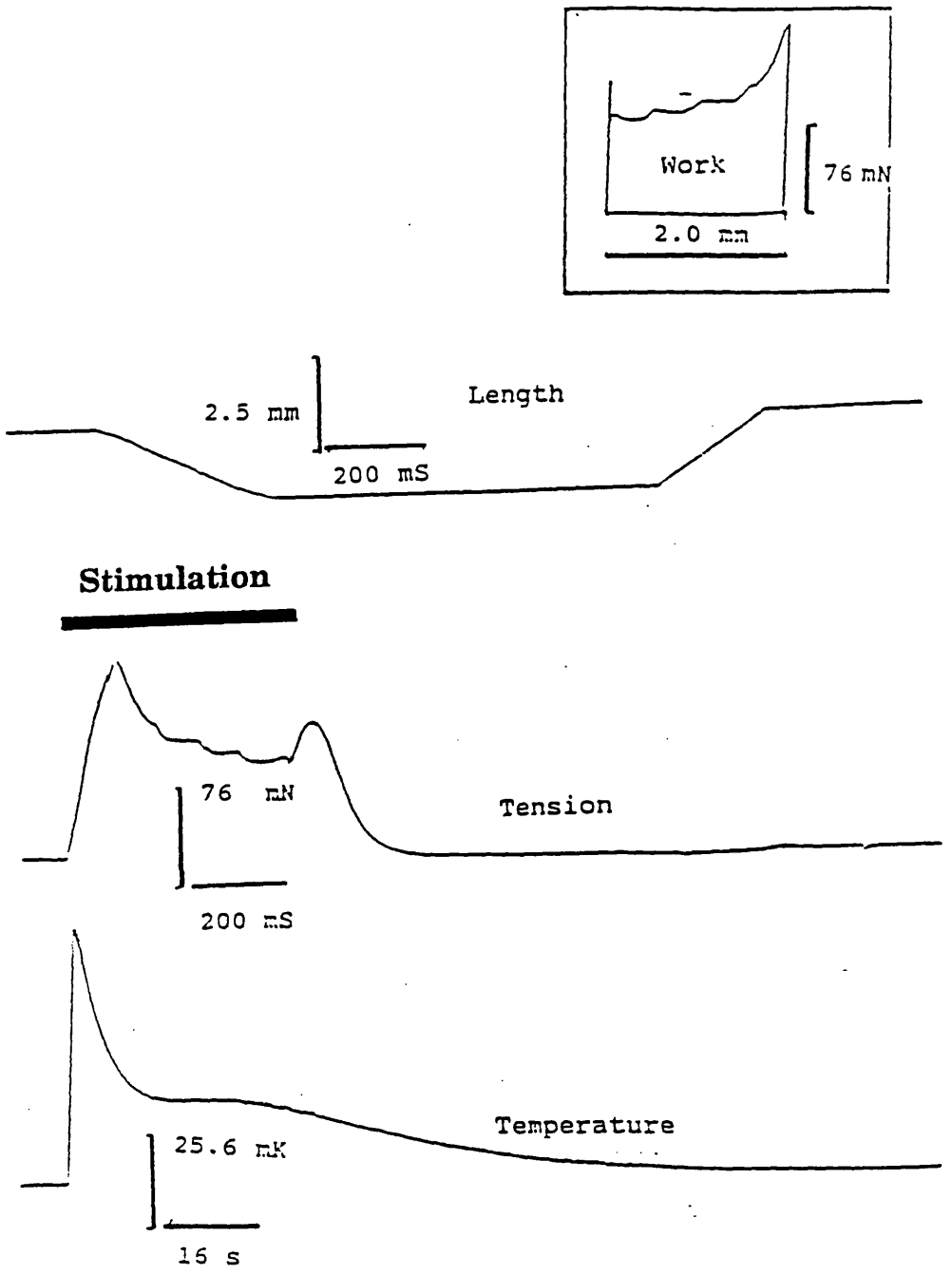


Fig. 4.1 **Experimental records of muscle length, tension and temperature change from a contractionof a mouse soleus at 25 C. during which work was produced. Two time bases were used, the fast one for length and tension records, the slow one for the temperature record. The amount of work is represented by the enclosed area in the inset. obtained by plotting tension against length change.**

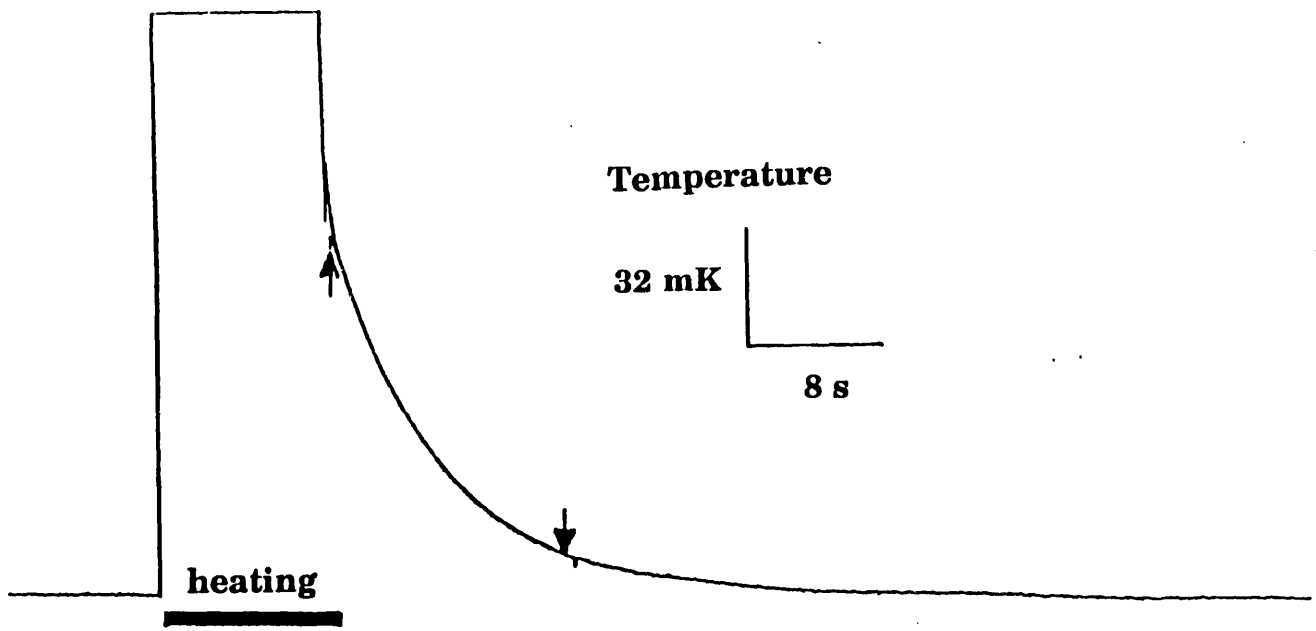


Fig. 4.2 Experimental record of Peltier control heating (90 μA, applied for 10 sec shown by the bar, average of 8 runs at 25°C). From the same muscle which produced the contraction in Fig. 4.1. Note that no recording is possible during the heating because the voltage drop across the pile caused by the Peltier current flow saturates the amplifier. The temperature change during cooling after Peltier heating was fitted (between the two arrows) by an single exponential curve, shown superimposed on the record. If the direction of the Peltier current is changed, a very similar output is observed but in the opposite direction i.e. cooling. The cooling curve was the same as, but in an opposite direction to the heating curve.

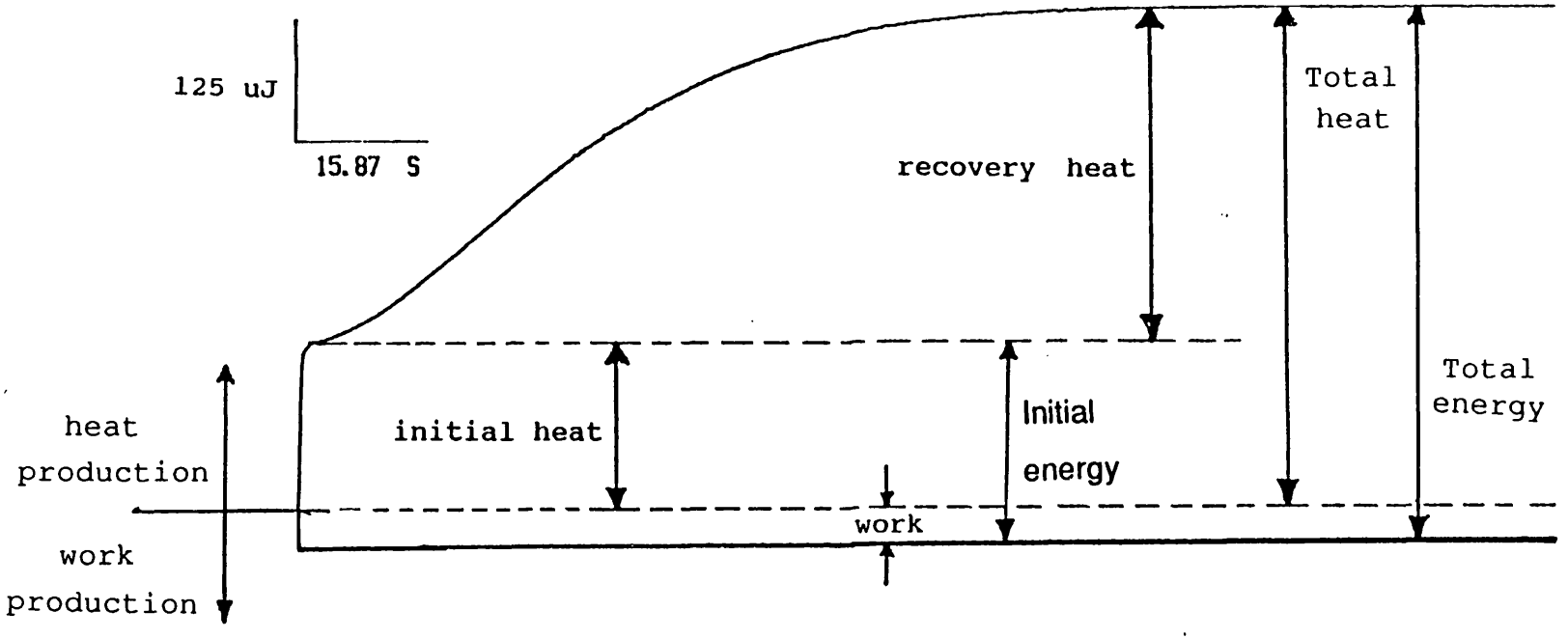


Fig. 4.3 Heat and work production obtained from the same contraction shown in Fig. 4.1. Heat production has been corrected for heat loss by using the time constant and amplitude of the Peltier control heating record in Fig. 4.2. The amount of work was that shown in the inset of Fig. 4.1.

Fig. 4.2 is a record of the Peltier control heating for the same contraction. As can be seen from this figure, the temperature change during the cooling part of the Peltier heating record is nicely fitted by a single exponential curve. The time constant and amplitude of this Peltier control heating is used for transforming temperature change into the corresponding heat production.

From the results in Fig. 4.1 and Fig. 4.2, the energy production, including heat and work production during and after the contraction, can be worked out. In Fig. 4.3 the measurements of initial heat, total heat, recovery heat, are shown. The amount of work equal to that in the inset of Fig. 4.1 is also shown in this figure.

In the case of isometric contractions, initial heat and total heat are the same as initial energy and total energy respectively, because there is no external work production. In fact, some internal work is still produced by a isometrically contracting muscle. However, this work is dissipated as heat during relaxation. Therefore, no "work correction" is needed. In the case of working contractions, initial and total energy are obtained by adding the work done to the initial and total heat, respectively as illustrated in Fig. 4.3. In both types of contraction, the recovery energy is the same as the recovery heat because there is no work production during the recovery period in either case.

4.1.2 The plan of the experiment

During the course of this experiment, 63 observations similar to the one shown above were made, some with isometric contraction and some with working contraction. From all these observations, several of the relations between the parameters of interest can be gained. These relations will be described in subsequent sections.

In order to study an alternative way of increasing the energetic cost of contraction, observations were also made with more than one contraction in the same initial period. As, in a long contraction, the muscle cannot be made to work at a high rate throughout, several short tetani rather than a single long contraction were used. In these cases, the initial period consisted of multiple short tetani which lasted 0.5s and were 3.0s apart from each other. An example is shown in Fig. 4.4. The term "total tetanic duration" is used to refer to the sum of all such 0.5 sec short tetani contained in an initial period. For example, the contraction shown in Fig. 4.4 contains three 0.5s short tetani and its total tetanus duration is 1.5 second.

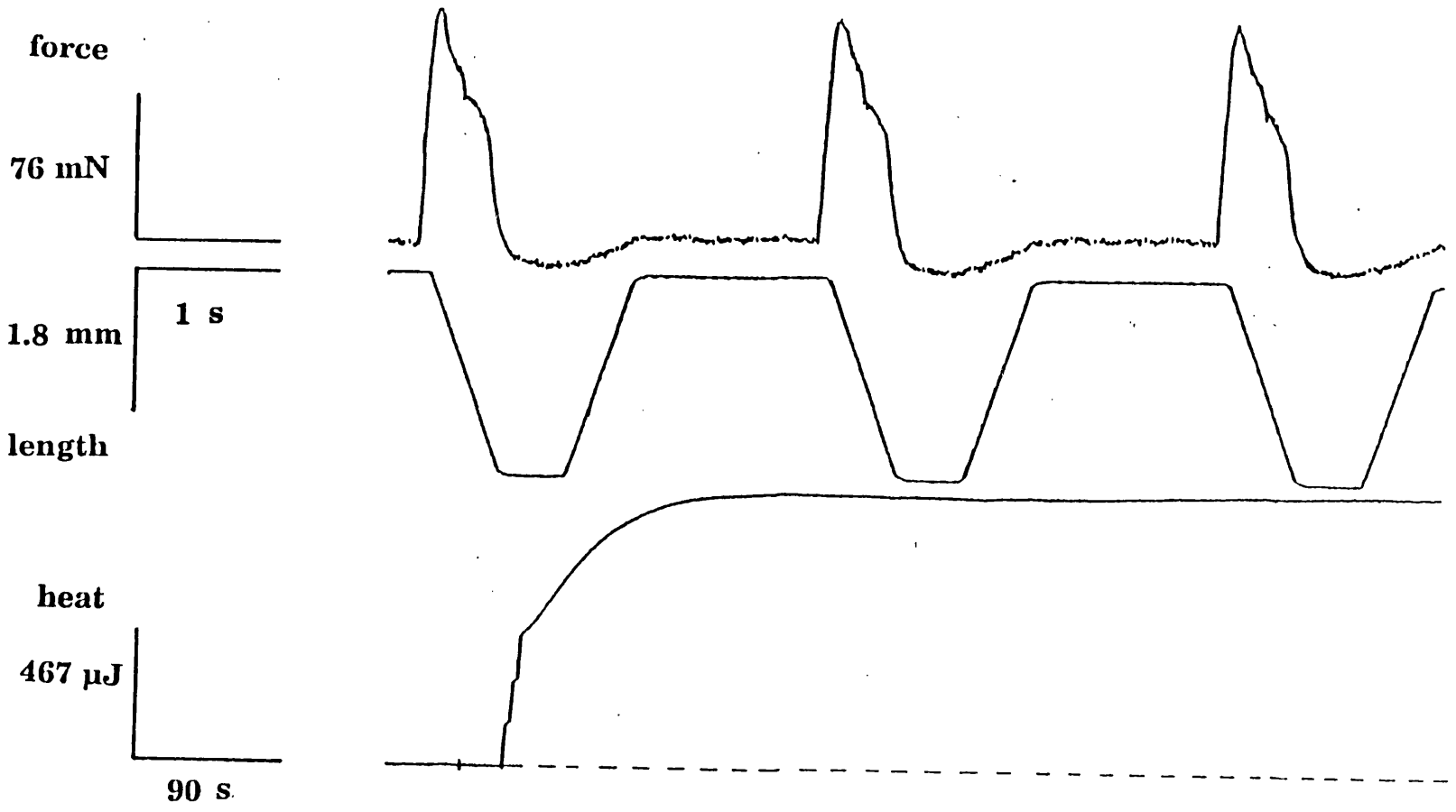


Fig. 4.4 The experimental record of a contraction containing three 0.5 s short tetani (top: force; middle: length; bottom: heat). The short tetani are 3 s apart. The total tetanus duration is 1.5 second. Two time bases were used, the fast one for length and tension records, the slow one for the temperature record.

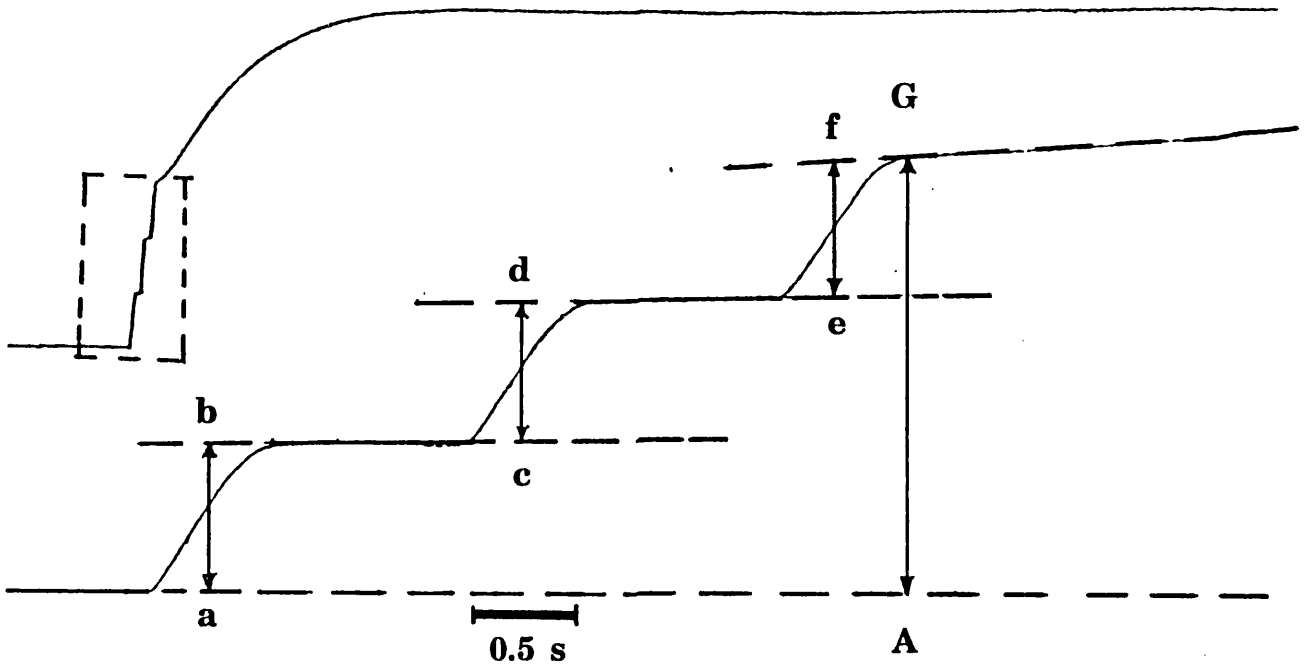


Fig. 4.5A The method for initial heat correction. The top trace is the heat record shown in Fig. 4.4. The trace is plotted at expanded time scale (the lower trace) in order to see details of the heat production in the initial phase. Line ab, cd and ef are drawn through the middle of each 0.5 s tetanus, i.e. at the time 0.25 s after the start of each tetanic stimulation. The "corrected" initial heat is the sum of (ab + cd + ef). The uncorrected "initial" heat is AG. For contractions containing multiple short tetani the corrected initial heat is smaller than the uncorrected "initial" heat (e.g. (ab+cd+ef) = 451 μJ, AG = 467 μJ here).

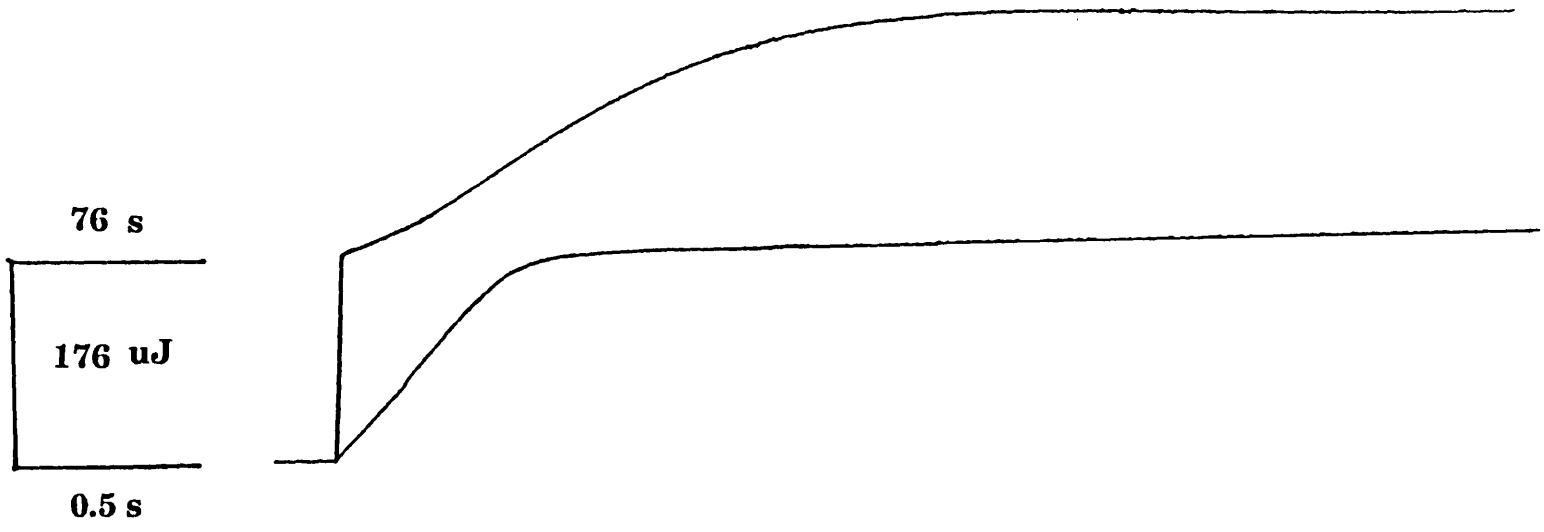
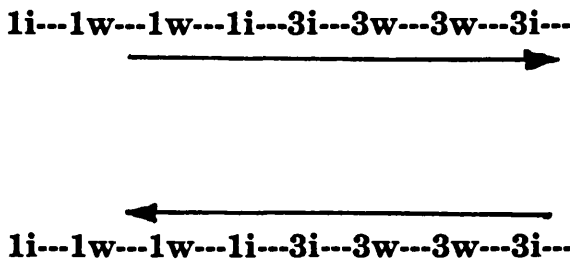


Fig. 4.5B A heat record for a contraction containing only one 0.5 s short tetanus (top trace). The record is plotted at expanded time scale (lower trace). It shows that there is a level part after the contraction in the record. Therefore, there is no overlap between the initial and recovery process in such brief contraction.

In these contractions containing multiple short tetani the recovery process and the initial process may overlap. Therefore, some allowance needs to be made for this in order to obtain the real initial heat. Fig. 4.5A illustrates the method used to obtain initial heat from the experimental heat record. In this figure, the record is the same as that in Fig. 4.4, but the part of the curve containing the initial period and the early recovery period, is expanded in order to show the details of the initial phase. The method is based on the assumption that recovery heat production starts at the middle of the 0.5s tetani. Therefore, in Fig. 4.5A the lines (ab, cd and ef) are through the middle point of each 0.5 s short tetanus and perpendicular to the base line the (bottom dashed line in the figure). The "uncorrected" initial heat (AG, 467 μJ) is slightly larger than the "corrected" initial heat (the sum of ab+cd+ef, 453 μJ). For contractions containing only one 0.5s short tetanus, no such correction was made, because there is virtually no recovery heat production during the initial period, as illustrated in Fig. 4.5B. In the figure there is a level part of the record in the earlier phase of the recovery, which is the clear evidence that there is no overlap between initial heat and recovery heat in such a brief contraction.

During the course of the experiment, the muscle deteriorated

gradually. In order to minimize any effects of this deterioration on the experimental measurements, the observations were made firstly in the direction of increasing the number of short tetani contained in each observation, and then in the reverse direction. In order to make working contractions and isometric contractions more comparable, paired observations (an isometric matching a working contraction) were made throughout the whole experiment. As an illustration, in this experiment, the order of the observations was



where, "1i" or "1w" stands for an isometric or working contraction containing one 0.5 s short tetanus; "3i" or "3w" for an isometric or working contraction containing three 0.5 s short tetani; and so on.

The experiment was stopped when the force had fallen to about 2/3 of that in the beginning of the experiment. The muscle was then cut off and the work and heat were calculated as described in 3.1.3.

4.1.3 Recovery ratio

The recovery ratio is the ratio of recovery energy to initial energy. The value of the ratio can be illustrated without losing sight of the two numbers forming the ratio by plotting the recovery energy against the initial energy. Fig. 4.6A is such a plot. In this figure, each observation is represented by one point. The slope of a line joining any point to the origin gives the value of recovery ratio. In fig. 4.6A a range of values for the recovery ratios are shown by the dotted lines.

From this figure, it can be seen very clearly that the recovery ratio is different between isometric and working contractions. It would be expected, if the ratio was the same, that points for isometric and working contractions should fall on the same dotted line. But the working contractions consistently lie on higher lines than the isometric contractions. This is particularly true when paired observations are considered, i.e. contractions adjacent to each other in the series of observations. In each pair, the ratio is always bigger in the working contraction than in the isometric contraction, this is illustrated by the fact that the full lines joining each pair always have a higher slope than the dotted lines and cross over them.

Fig. 4.6A A comparison of recovery ratio between isometric (open squares) and working (black squares) contraction. Each pair (an isometric contraction matching a working contraction) is connected by a full line. The dashed lines show where point having particular values of the recovery ratio would fall.

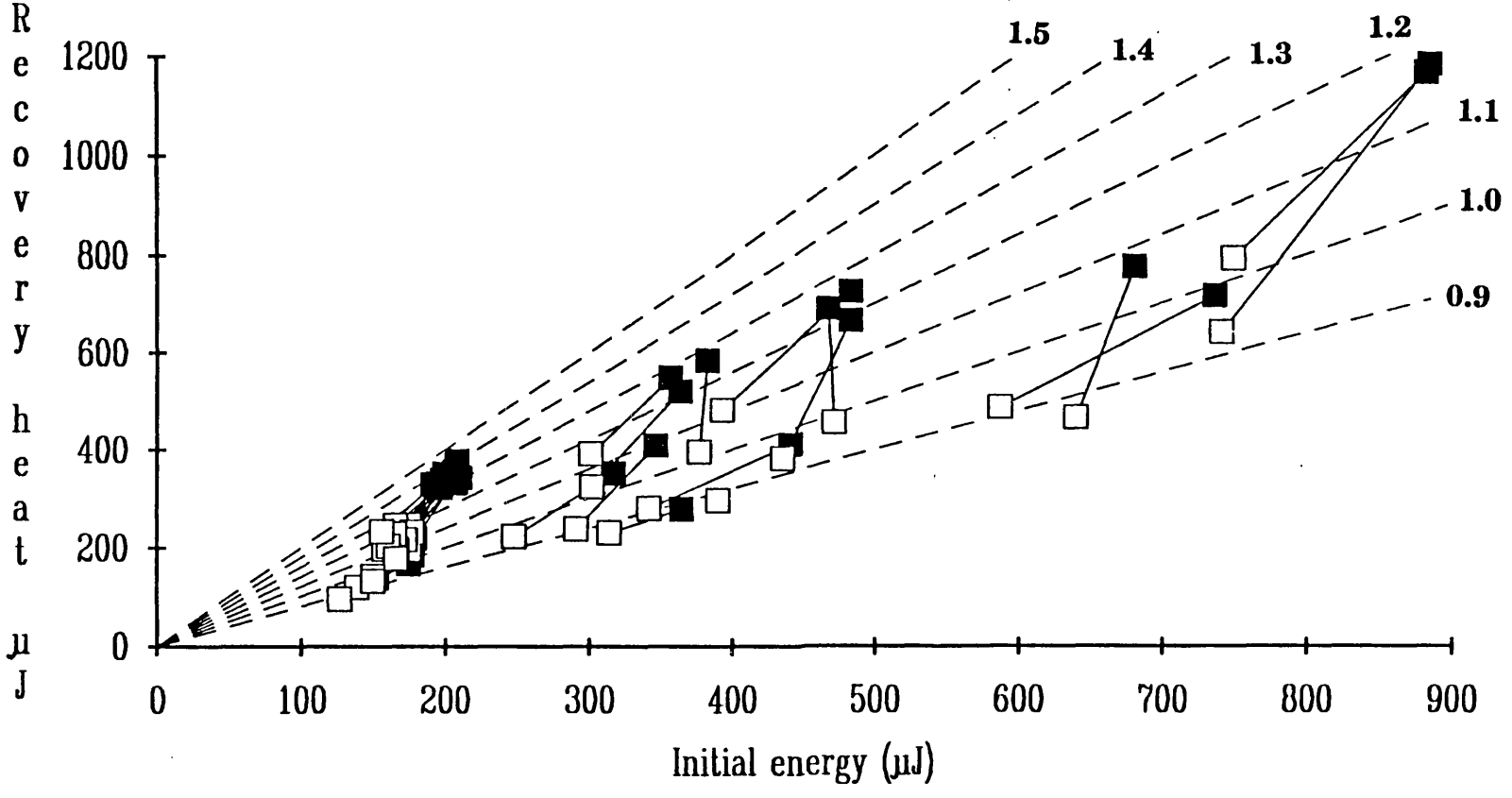


Fig. 4.6B A comparison of recovery ratio in working contraction (black squares) with that in isometric contraction (open squares). Each point represents for one observation (mouse soleus at 25°C). The data is a part of that shown in Fig. 4.6A but is plotted here on larger scale to see details.
The dash-point lines show the values of recovery ratio.

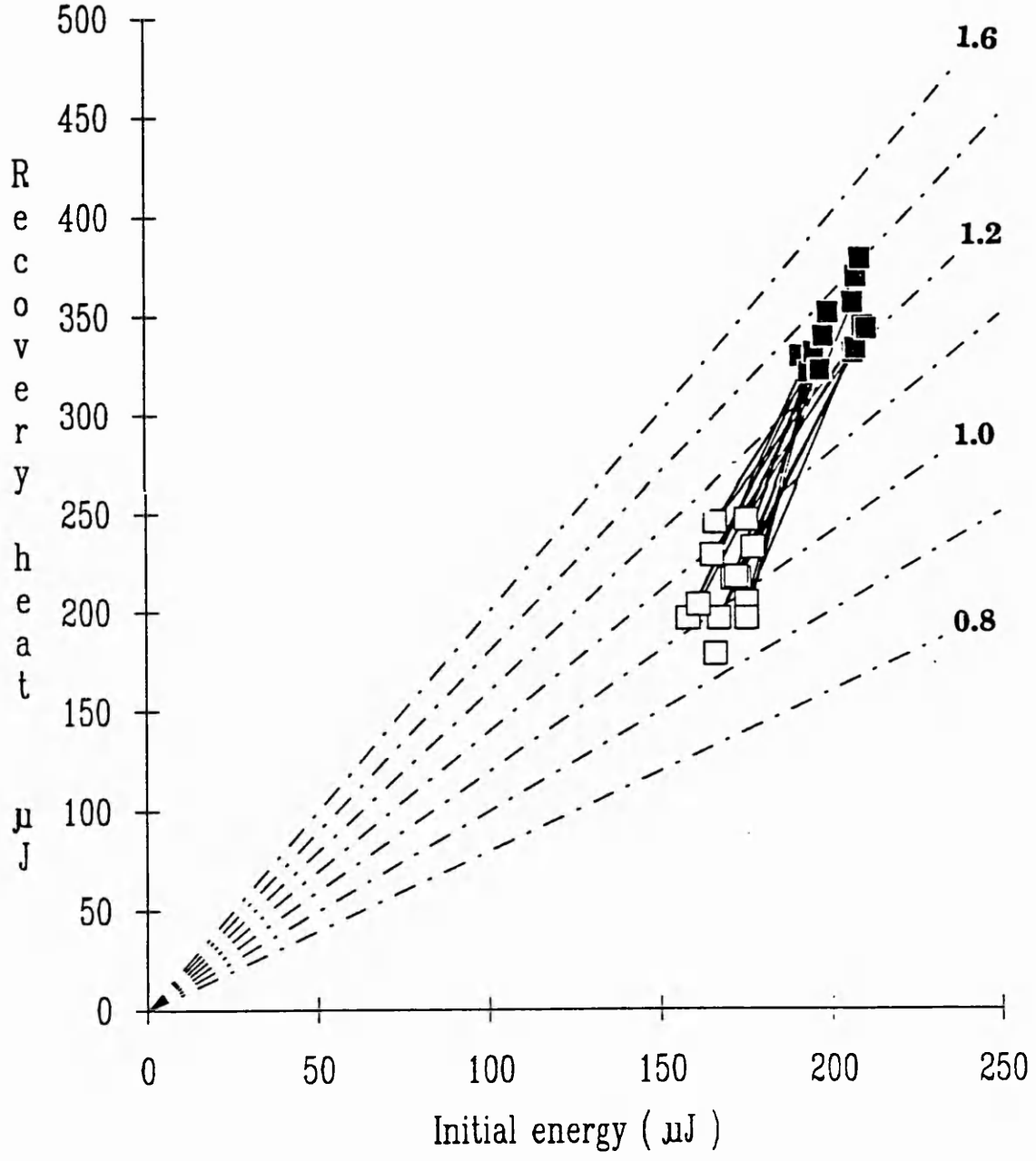


Fig. 4.6B shows part of the data whose details are difficult to see in Fig. 4.6A. As can be seen here more clearly, there is a significant difference in recovery ratio between isometric and working contractions.

Fig. 4.7 is obtained by using the same data as in Fig. 4.6A but is plotted in a different way. From this plot, it is easier to see the difference in recovery ratio between isometric and working contraction at different total tetanus durations used. There is a slight tendency for the recovery ratio to fall with tetanus duration. This is seen whether the contractions are isometric or working. This contrasts with the fact that recovery ratio is larger when work is done. Thus clearly the change in the recovery ratio with work is not due to an increase in the metabolic cost during the initial period, but must be a consequence of the mechanical conditions during the contraction.

It has been seen that working contraction did cause the recovery ratio to differ from that in isometric contraction. Is this due to shortening per se or due to the amount of work produced? It should be interesting to compare the recovery ratio of working contractions at different shortening velocities and with different amounts of work to try to answer this question.

Fig. 4.7 A comparison of recovery ratio between isometric and working contraction over a range of tetanus durations. Each observation is shown by an square (black for working and open for isometric). Mean and standard error of each group are shown by the bars. There are four groups of observations. In each group, the observations were made from contractions containing the same number of short tetani.

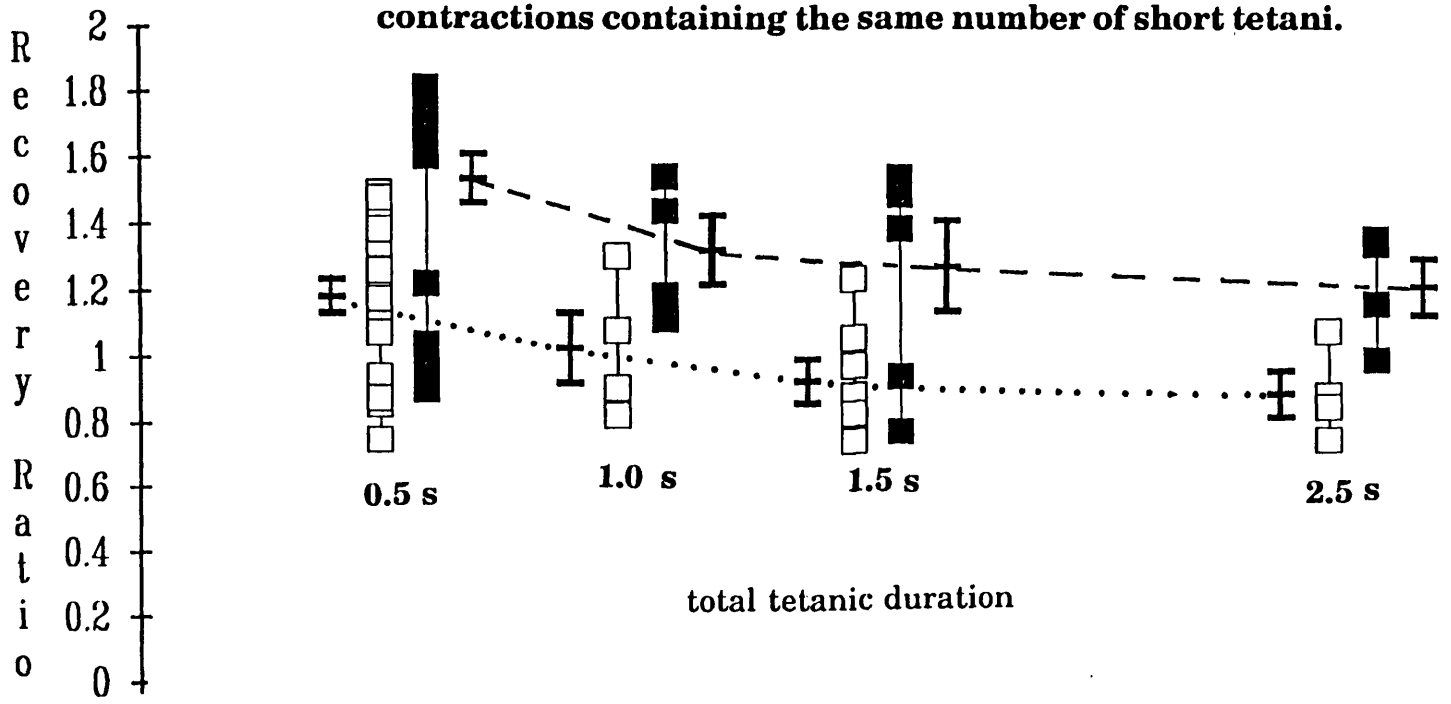


Fig. 4.8 is a plot of recovery ratio against shortening velocity. From this figure, it seems recovery ratio is independent of velocity of shortening over the range available. However, the fastest velocity used is only about 0.5 Vmax. So, it is not possible to study shortening without work, as would be seen near Vmax.

Fig. 4.9 shows recovery ratios in working contractions during which the amount of work production was varied. However, recovery ratio appears more or less the same regardless of changes in the amount of work produced during those contractions.

In summary, it seems that during or after a working contraction some process occurs in the muscle which is not occurring during isometric contraction. The amount of this process does not vary strongly with the speed of shortening, or the amount of work, within the limits of variation I was able to impose. Discussion of the nature of this process will be found in Chapter 8.

Fig. 4.8 The relation between recovery ratio and shortening velocity (mouse soleus, 25°C). One point represents one observation.

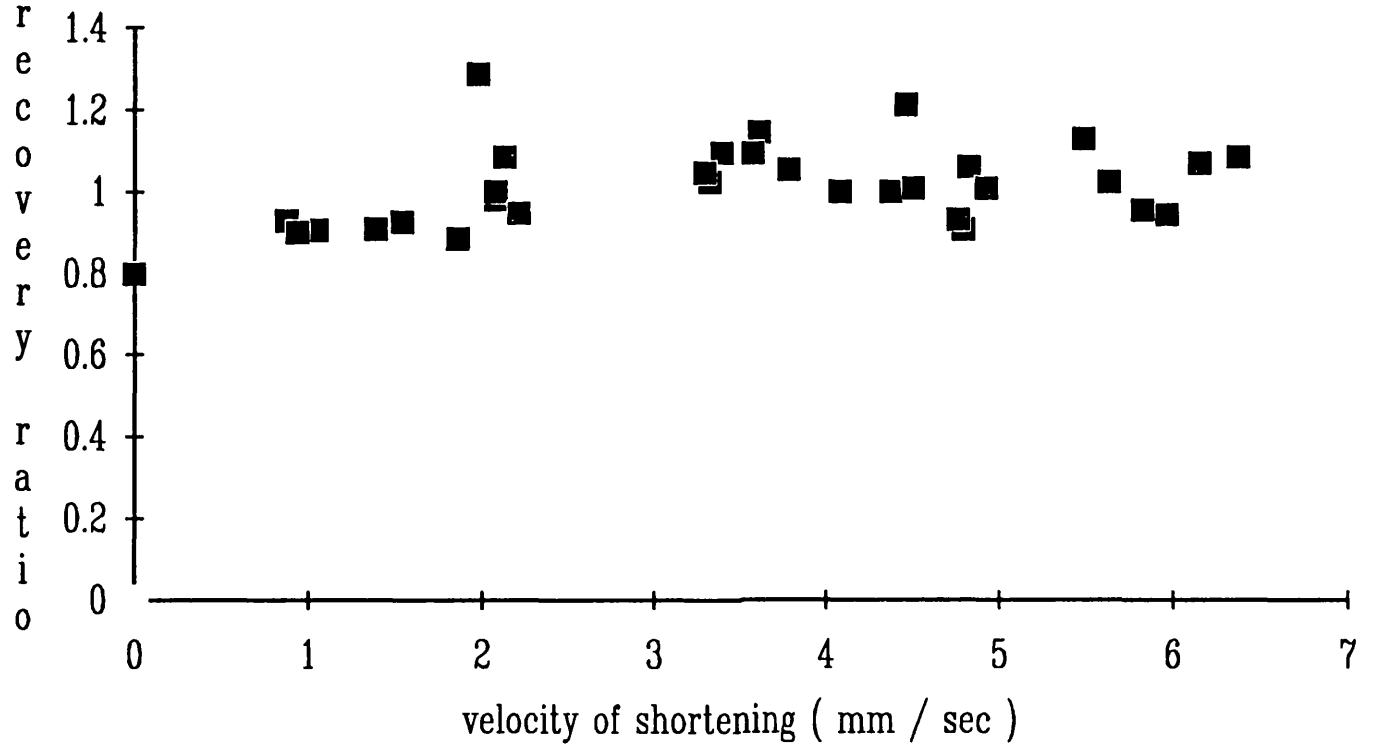


Fig. 4.9 relation between recovery ratio and work production. The data are those shown in Fig. 4.8 (one point for one observation, mouse soleus muscle at 25°C).

4.1.4 Efficiency

Because recovery ratio is not the same between isometric and working contractions, it cannot be true that PCr splitting is the only net reaction in both types of contraction. As explained in Section 1.4.3, the recovery ratio is a function of the ΔH_{PCr} when PCr splitting is the only reaction contributing to the initial heat and work, and PCr resynthesis from oxidation of carbohydrates is the only reaction responsible for the recovery heat production. Consequently, without further knowledge about the cause(s) of this difference, it is impossible to assess the thermodynamic efficiency for the initial process of muscular contraction in mouse soleus muscles used in this project, as had been planned in the design of the project (for reasoning see Section 1.4).

Nevertheless, as an approximation, the relative effectiveness of the initial processes can be estimated by using the (work/enthalpy) ratio, as traditionally done. However, for the overall contraction-recovery process, there is no reason to doubt that oxidation of carbohydrates is the only reaction for recovery heat and thus the thermodynamic efficiency can still be assessed properly, because for this process, it is known the free energy change is about 1.04 times the enthalpy change (Burk 1929). Therefore, for the purpose of simplicity, the ratio of (work/enthalpy) will be called "efficie-

ncy", for both the initial process and the overall process. However, this difference in the status of the two efficiency should be kept in mind.

Fig. 4.10 shows initial and overall efficiency values obtained from the specimen experiment. As can be seen the range of velocities used covers the velocity which gives the maximum efficiency value, which is about 0.26 for the initial process and 0.13 for overall processes.

4.1.5 Energy production

Fig. 4.11 shows the relation of energy production and velocity of shortening in the same experiment. The relation of different kinds of energy production: total energy, initial energy and work, and velocity of shortening follows the same pattern. In each case, rising to the maximum at a velocity of 3 to 5 mm/sec and then declining somewhat. In this figure, the energy production in the paired isometric contractions, including initial and total heat, are also shown (points on the ordinate) for the purpose of comparison.

Fig. 4.10 Efficiency values for the initial (diamonds) and overall (triangles) processes in mouse soleus muscle (data from one experiment, each point for one observation).

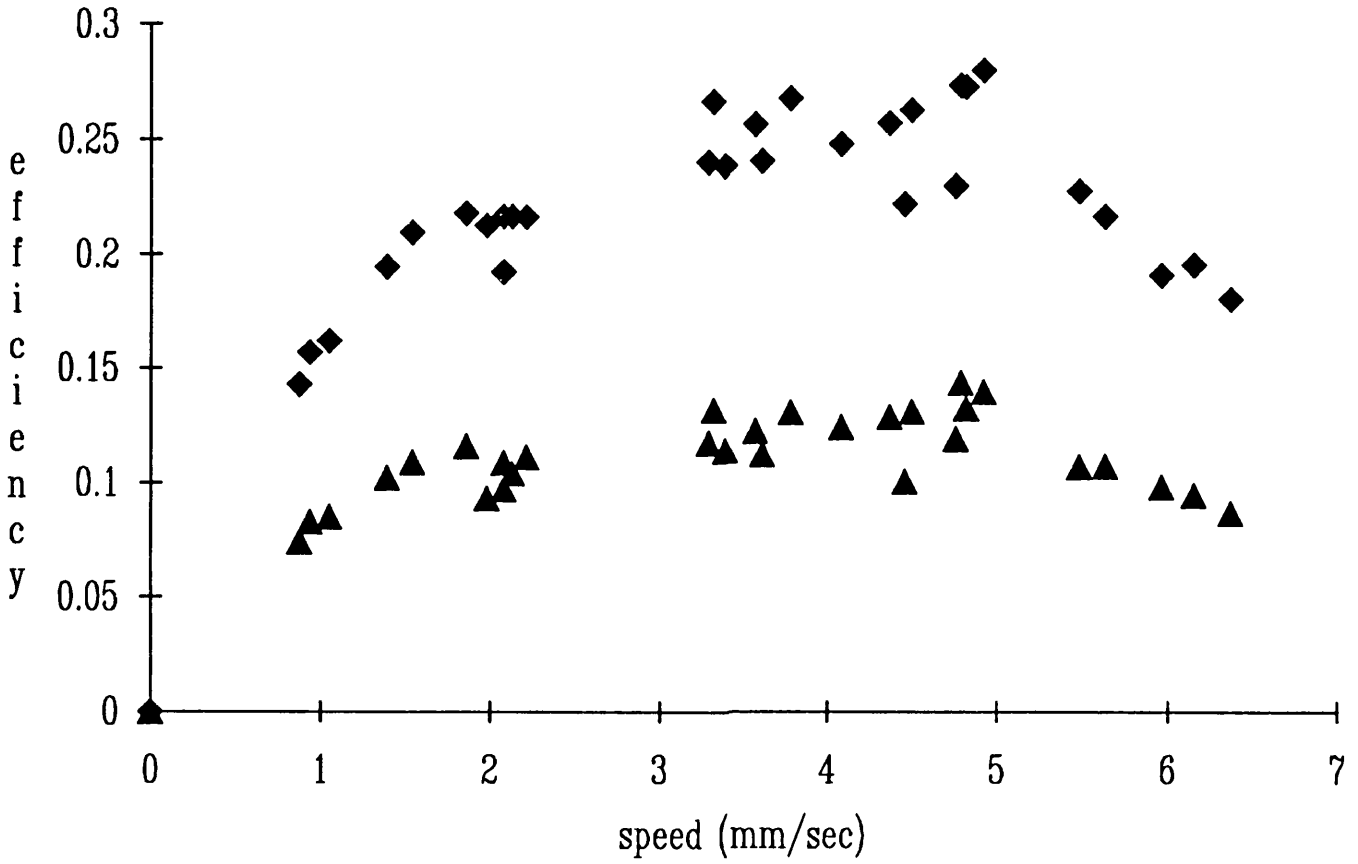
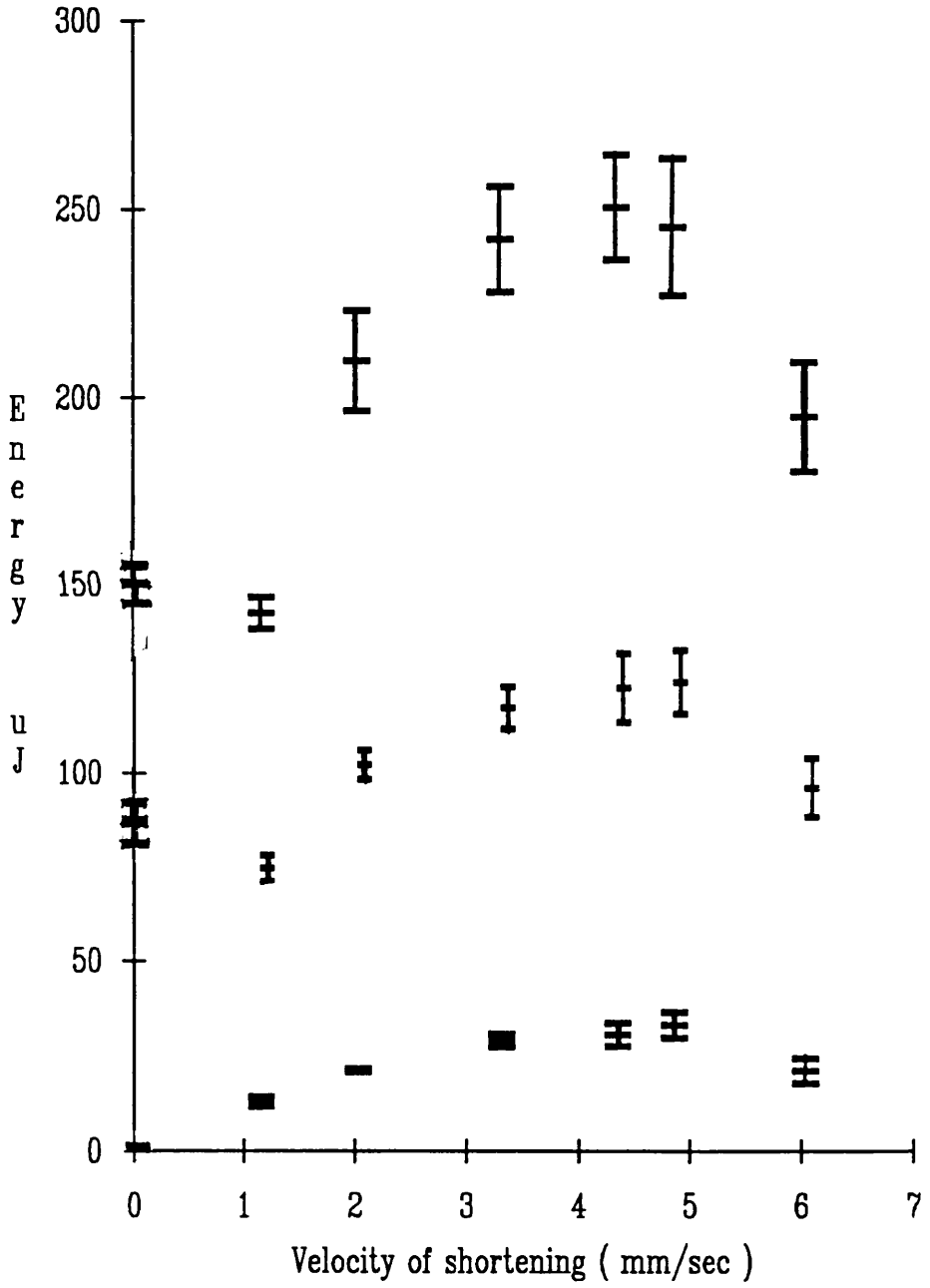


Fig. 4.11 Energy production and velocity of shortening(data from one experiment, mean & sem, n=6 at each velocity). Energy production in paired isometric contractions on the ordinate(n=30). Top-total energy, middle-initial energy, bottom-work.



4.2 Results from all the five experiments

4.2.1 Recovery ratio

Table 4.1 is a summary of all five experiments of this type. From this table, it is quite clear that there was a statistically significant difference between Recovery Ratio of Working contraction (RRW) and Recovery Ratio of Isometric contraction (RRI). In each experiment, this difference was clearly shown. In fact, as will be seen later, in the control series of experiments, the difference between RRW and RRI was seen also in further experiments; in fact in every single experiment.

As already seen in Fig. 4.7, which shows the results from one experiment, the recovery ratio (both RRW and RRI) seems to decline slightly when the total tetanus duration was increased. Interestingly, the decline in RRW and RRI seems to follow the same trend and this makes the difference between RRW and RRI fairly constant over the range of tetanus duration used, as shown in the same figure.

	isometric			working			difference			comparison	
expt.	mean	sem	n	mean	sem	n	mean	sem	n	paired t test	
1	1.01	0.05	9	1.25	0.05	13	0.269	0.065	9	t = 4.15	P < 0.01
2	1.07	0.04	33	1.4	0.06	31	0.34	0.03	30	t = 11.3	P < 0.001
3	1.1	0.03	20	1.27	0.04	20	0.17	0.04	20	t = 4.25	P < 0.001
4	0.94	0.05	23	1.14	0.05	24	0.229	0.06	23	t = 3.817	P < 0.01
5	0.9	0.06	10	1.19	0.04	14	0.308	0.073	10	t = 4.219	P < 0.01
5 expts	*1.00*	*0.04*	*5*	*1.25*	*0.04*	*5*	*0.246*	*0.03*	*5*	*t = 8.2*	*P < 0.001*

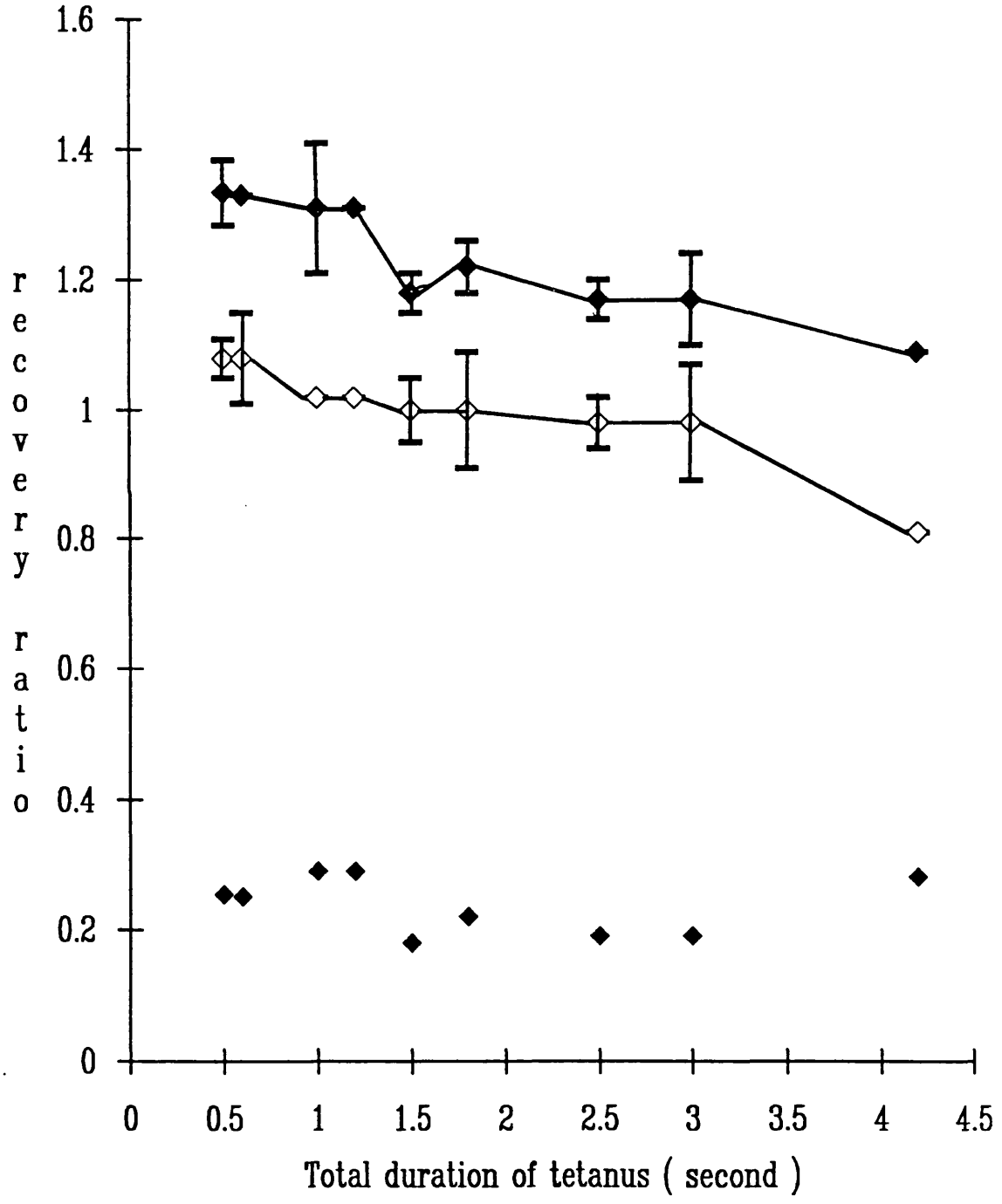
Tab. 4.1 Comparison of recovery ratio (summary of results of the first series of experiments)

Fig. 4.12 shows results from all five experiments. From Fig. 4.12, a similar pattern of change can be seen although there were some variations in the recovery ratio between individual mice. Therefore, it is clear that there was a tendency for recovery ratio (RRW and RRI) to decline when total tetanus duration was prolonged. Also, because RRW and RRI changed in the same pattern, the difference between them tended to be constant when total tetanus duration was varied. An increase in total tetanus duration in isometric contraction alone does not result in a higher recovery ratio. Thus, the increase in recovery ratio between RRW and RRI is not a consequence of increasing the metabolic cost.

It was very striking to find that results from all the experiments consistently showed that there was a significant difference in recovery ratio between shortening and isometric contractions.In frog muscles, these two ratios are the same, as shown by Hartree (1928) and also confirmed in this project (to be mentioned later).

The possible reasons for this phenomenon will be discussed in detail in Chapter 8.

Fig. 4.12 Recovery ratio and total tetanus duration. Results for five experiments(top: working; middle: isometric; bottom: the difference; mean and SEM; where no SEM is shown there is only a single experiment).



4.2.2 Efficiency

Table 4.2 gives the maximum efficiency values obtained in each experiment. As can be seen in this table, the initial efficiency is very constant and the average is 0.23 for the initial process and 0.11 for the overall process.

It is clear that the maximum efficiency values, both for the initial and overall process, are relatively low compared with these found in other species. For example, the two ratios are 0.45 (Hill 1964) and 0.18 (Hill 1939) in frog muscle, 0.72 and 0.36 in tortoise muscle (Woledge 1968).

The maximum efficiency

As the maximum efficiency values found are relatively low, the question is raised whether the maximum efficiency values are really "maximum". In this section, the experimental conditions used are examined in order to check this point.

In the experiments, the duration of tetanus was, in most cases, 0.5 second. The stimulation frequency was 50 Hz. Voltages used were supramaximal. The muscle length was adjusted to that corresponding to the plateau of the length-force curve. And the

Table 4.2 Maximum efficiencies from 5 experiments

****

(Temperature 25°C)

Efficiency	expt1	expt2	expt3	expt4	expt5	mean & S.E.
initial	0.23	0.22	0.21	0.22	0.26	0.23 ± 0.01
overall	0.12	0.09	0.10	0.11	0.12	0.11 ± 0.01

****

Table 4.3 Maximum efficiency at 15°C and 25°C

****

	25°C			15°C		
Efficiency	Mean	S.E.	n	Mean	S.E.	n
Initial	0.23	0.01	5	0.21	0.01	2
Overall	0.11	0.01	5	0.10	0.01	2

****

starting length was set just beyond the plateau to make sure that the range of shortening was confined almost within the plateau in order to avoid the possibility of encountering an "internal load" at short length. This should maximise efficiency. The velocity of shortening was varied over a range as wide as possible (with the equipment available) in order to cover the velocity which can result in maximum efficiency.

By using the above conditions, the biggest values of efficiency found in each experiment should be the real maximum values. Because, under these conditions, muscle fatigue, insufficient activation, acidification should not happen. Nevertheless, it is possible that higher efficiency value could be found under other conditions.

Efficiency and temperature

All five experiments in this series were conducted at 25°C. In another series of control experiments which will be presented later, two experiments were performed at a lower temperature (15°C) for the purpose of checking the oxygen supply. So, it may be worthwhile to present here the efficiency results from these experiments.

Table 4.3 gives maximum efficiency values obtained at these two different temperatures. All the experimental conditions were the same save for temperature.

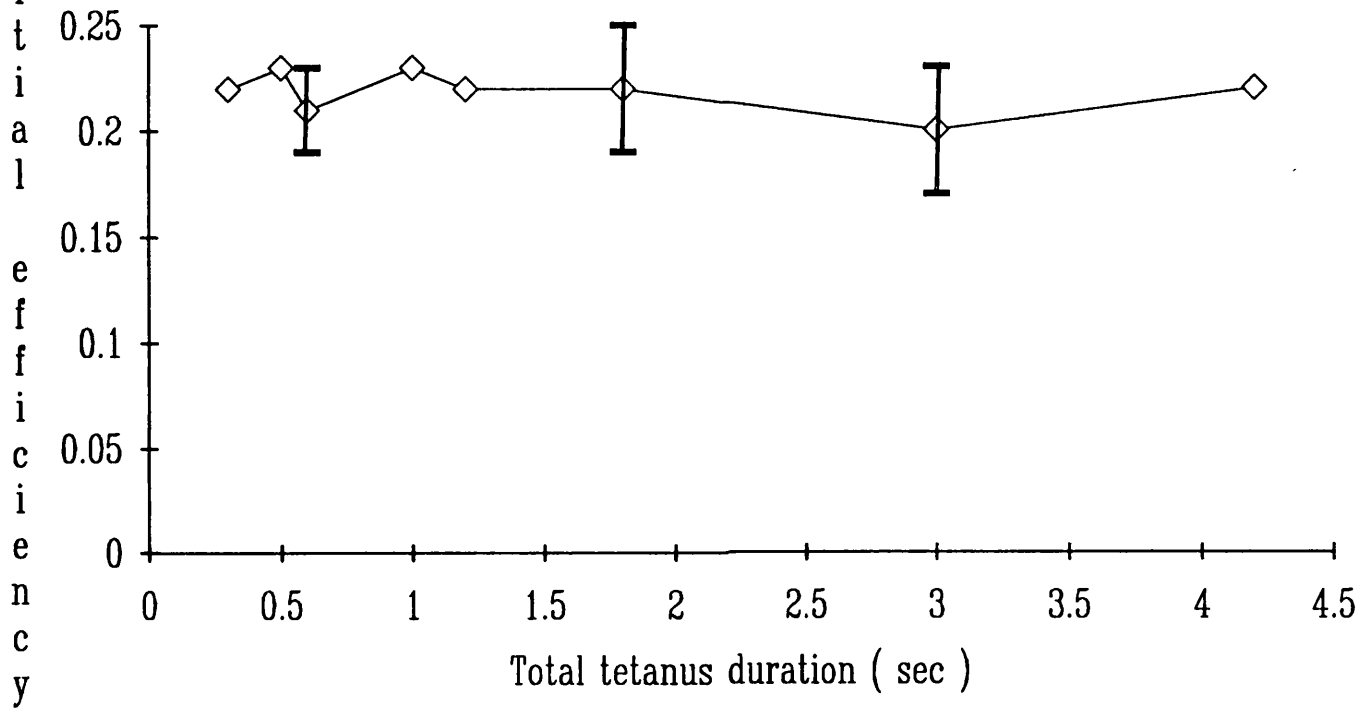
There is no evidence here that efficiency is strongly influenced by temperature.

Efficiency and total tetanic duration

In these experiments, total tetanic duration was also varied and the effect of this variation on maximum efficiency was studied. Fig. 4.13 presents the results of this type. Five experiments were included and all the observations were made at 25°C.

As shown in this figure, maximum efficiencies are fairly constant when total tetanus duration varies between 0.3 and 4.5 seconds in this project.

Fig. 4.13 Relation between maximum efficiency and total tetanus duration (data from 5 experiments, diamonds: means; bars: standard errors of mean, n=4; when there are no bars, n=1)



CHAPTER 5 CONTROL EXPERIMENTS

5.1 Introduction

It was totally unexpected to find a difference between RRW (Recovery Ratio for a working contraction) and RRI (Recovery Ratio for an Isometric contraction). The result is striking because it is the opposite to what has been found in frog muscles, in which, RRW and RRI have been shown to be the same (Hartree 1929, Hill 1932). Because this is an important issue in muscle energetics, the phenomenon needs to be checked carefully to clarify whether it is an artefact or a genuine physiological process. A series of control experiments were therefore designed and conducted for this purpose, consisting of three different types of experiments: (1) experiments with passive release, (2) experiments on small muscle bundles, and (3) experiments at lower temperature (15°C).

The general design of these control experiments aims at the following aspects:

a) Eliminating any artefact due to length displacement

It is possible for shortening to cause some artifacts. For example, the movement of muscle may cause artificial heat production, particularly if there is a temperature gradient along the muscle.

Another possible artefact is that, the movement of the muscle caused by shortening may stir the air surrounding the muscle and improve the oxygen supply. This could conceivably change the recovery metabolism and result in a bigger recovery ratio for working contraction.

b) Checking that the oxygen supply is adequate by taking steps to improve it

The possibility exists that a shortage of oxygen could cause the difference between RRW and RRI. If oxygen is short, then the recovery process could be incomplete. If the oxygen supply is then increased, extra recovery would occur to make up for previous "oxygen debt". Thus, a large recovery ratio after an increase in oxygen supply could be expected. One way to improve oxygen supply would be to make the muscle move.

Unless specially mentioned, all the conditions used in the control experiments were the same as those in the first series of experiments.

5.2 Experiments with passive release

5.2.1 The experiments.

The experiments with a passive release imposed immediately before, or just after, an isometric tetanic stimulation were designed to see the effect of a length change without active shortening on the recovery ratio. These experiments were essentially the same as those of the first series, except that an extra type of observation was added, in which the muscle contracts isometrically during stimulation, and an imposed length displacement (passive release) occurs outside the stimulation period, either immediately before or just after stimulation. Fig. 5.1 illustrates the timing of the latter type of passive release. In this figure, the release can be seen in the heat record as a dip. For simplicity, an isometric contraction with such a passive release will be referred as a control isometric contraction in order to distinguish it from a normal isometric contraction. RRC will be used to denote its recovery ratio (Recovery Ratio for Control isometric contraction).

If the difference between RRW and RRI is caused by a physiological change incurring during <u>active</u> shortening, there should be no difference between RRC and RRI. Because there is no active sliding. It is important to make another comparison in

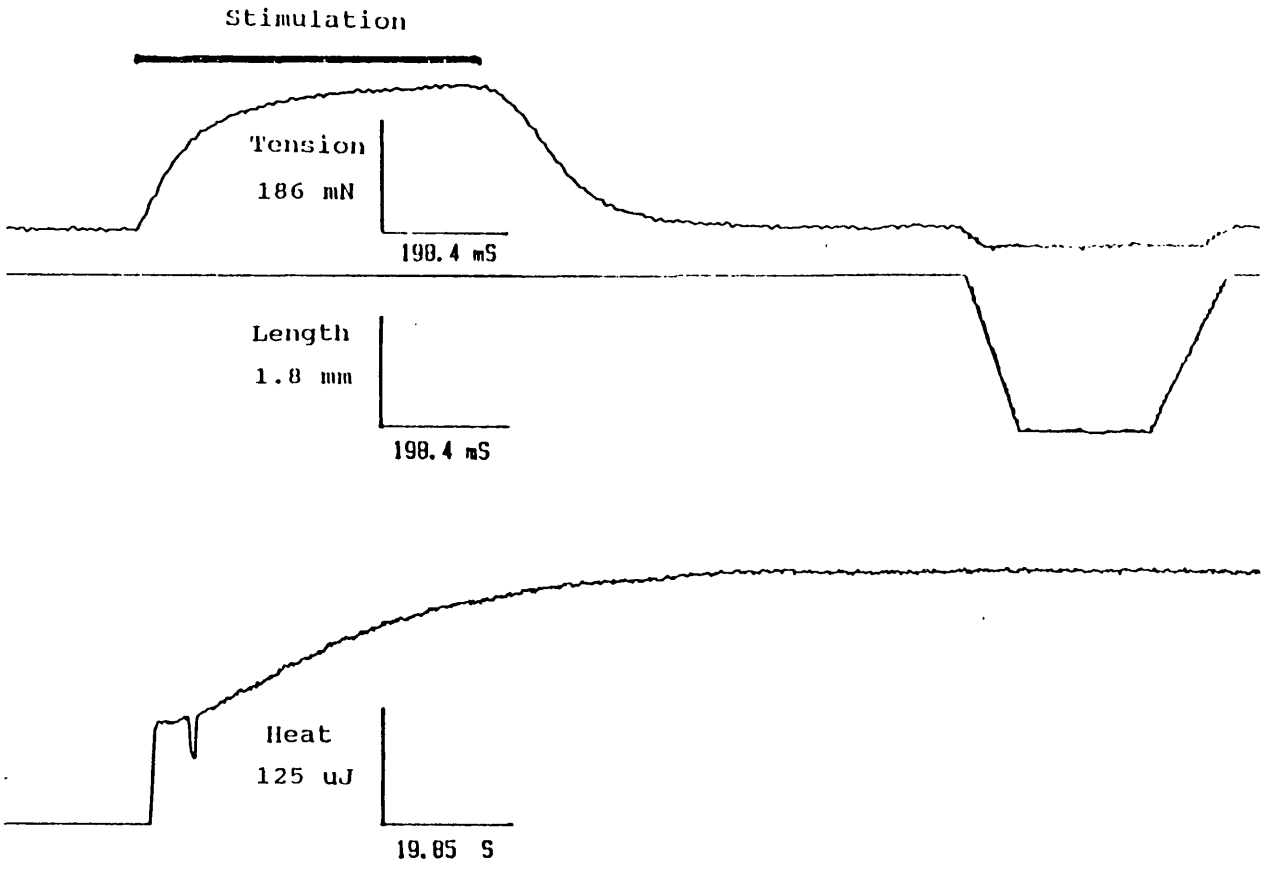


Fig. 5.1 Records of force, length change and heat production during and after a 0.5 s tetanus of mouse soleus muscle. This is a control observation in which a passive release was applied after relaxation. Note the different time scale of the heat record. Heat record has been corrected for heat loss. The dip on the heat record occurs during the movement and is probably a thermoelastic phenomenon in parallel elastic tissue.

the same experiment, that of RRW with RRI. If there is no difference between RRC and RRI, and if, in the same experiment, there is a difference between RRW and RRI, this difference must be due to changes in the underlying processes associated with active filament sliding, rather than to an artefact caused by the length change.

In this type of control experiment, muscles were stretched well beyond the plateau of their tension-length curve in order to make passive release effectively. Before making the heat observations, the movement of the muscles by passive release was measured under a microscope. The distance of the passive release was about 2 mm at the moving hook and about 1mm in the middle of the muscles. This check was essential. It was found that unless the muscles were well stretched the movement in the centre was much less than half of that applied at the free end. In such a case, the control observation would not be appropriate.

Because the muscles were stretched more than those in the first series of experiments, there is some variation in the force and heat production. However, this variation should not affect the validity of the comparison between different kinds of recovery ratio within each control experiment, because all the observations within each experiment were over the same range of muscle lengths.

5.2.2 Results

Four experiments were conducted, using four mice. A total of 191 successful observations were made from these experiments.

Results from one typical experiment:

Fig. 5.2 compares heat production from different types of contractions. These contractions are a working contraction (the top trace), a normal isometric contraction (the trace without a dip), a control isometric contraction with passive release before stimulation (the trace with a dip in the baseline) and a control isometric contraction with passive release after stimulation (the trace with a dip at a later time point). From this figure, it is clearly seen that there is no difference in time course between different isometric contractions. In fact, it is quite hard to distinguish one from the other among these three.

Fig. 5.3 shows all the observations from one experiment which includes the four types of contraction just mentioned. In this diagram, the number of each type of contraction and the value of recovery ratio (mean and standard error of mean) are shown. As can be seen clearly, the mean values for control isometric and normal isometric contractions are virtually the same. However,

Fig. 5.2 A comparison among heat records obtained from different types of contractions. The top trace is for shortening; the rest are for normal isometric contraction (without dip), control isometric contraction with passive release before stimulation (with dip ahead) and with passive release after stimulation (with dip behind). All the isometric heat records are virtually the same.

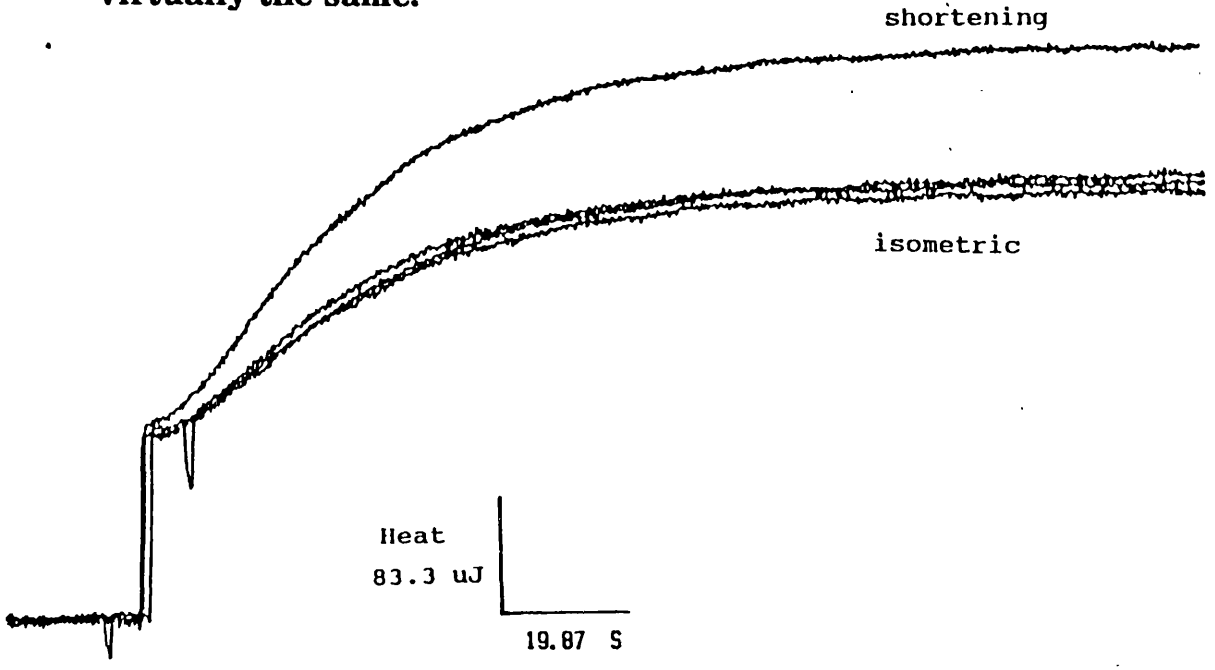
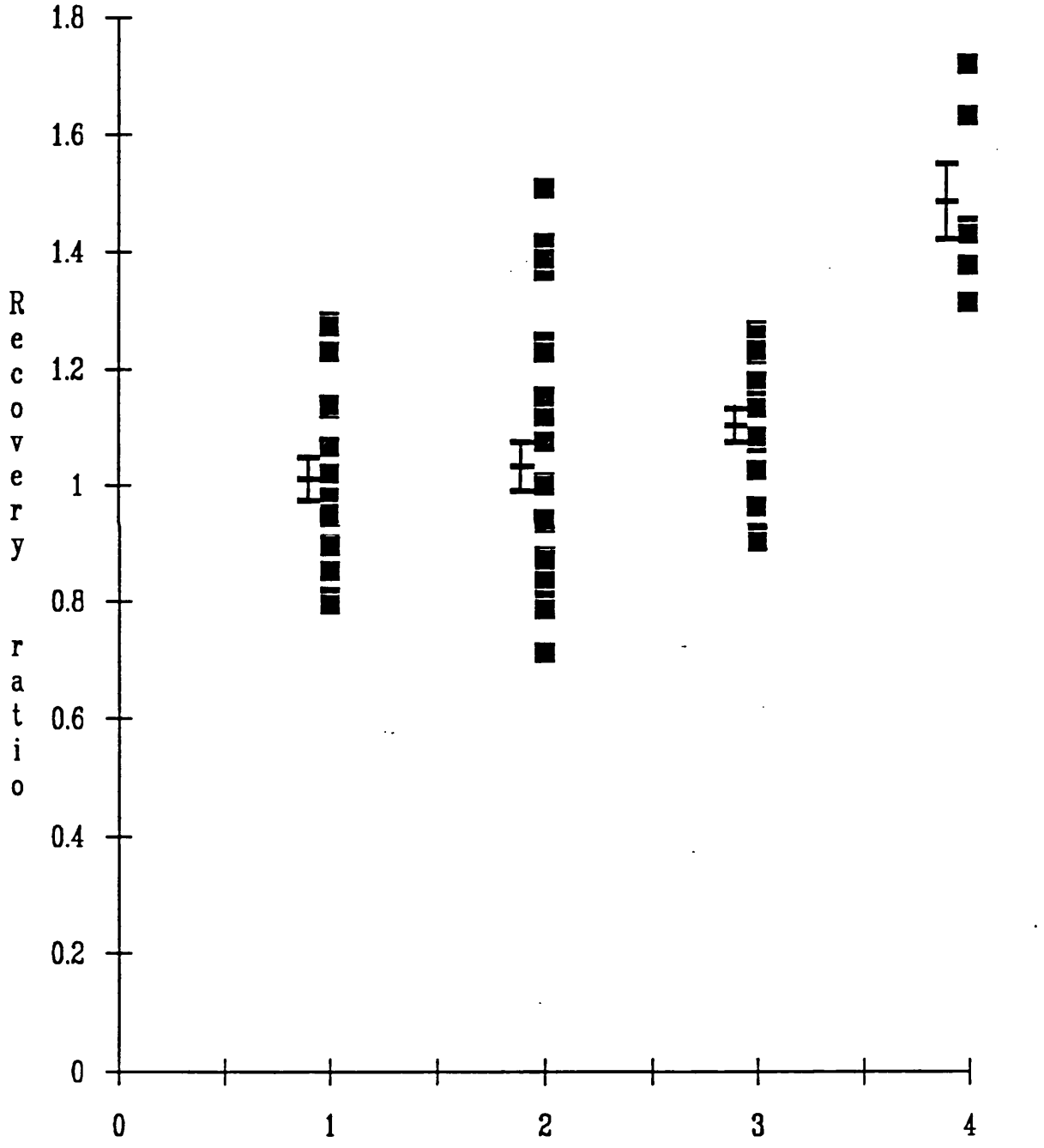


Fig. 5.3 Recovery ratio for different types of contractions (data from one experiment, each square for one observation). The horizontal bars are mean and standard errors of mean. N values are given in Tab. 5.1 in which this is Expt.1.



there is a clear difference in recovery ratio between isometric and working contractions.

Results for all experiments:

Table 5.1 is the summary of results from all the four experiments. In each experiment, three types of contractions were used, working contraction, normal isometric contraction and control isometric contraction. In the first experiment, passive release was imposed both before and after stimulation. For the remaining experiments, passive release was only imposed after stimulation. From Table 5.1, it is quite clear that there is no difference between RRC and RRI, but there is, as usual, a difference between RRW and RRI, and between RRW and RRC. These two comparisons clearly indicate that the difference between RRW and RRI is a consequence of active shortening, not the result of an artefact. It seems that when no crossbridge cycling is involved, there is no difference in recovery ratio caused by shortening.

Another way to check the effect of length displacement on the recovery heat production is to release the muscle and return it to its original length, without any stimulation and to observe the heat production afterwards. This type of observation was also made in some of the experiments. Fig. 5.4 shows the heat

Tab. 5.1 Summary of recovery ratios in passive release experiments.
P values given are for paired comparison with the normal isometric contraction.

	Control isometric contraction						Normal isometric			working		
	mean	sem	n	mean	sem		mean	sem	n	mean	sem	n
expt.1	1.07	0.04 P > 0.5	13	1.1	0.03 P > 0.5	17	1.06	0.05	16	1.42	0.06 P < 0.001	6
expt.2				0.75	0.02 P > 0.5	21	0.76	0.02	35	0.91	0.02 P < 0.001	32
expt.3				0.86	0.01 P > 0.5	8	0.86	0.02	8	1.02	0.04 P < 0.02	9
expt.4				0.91	0.03 P > 0.5	9	0.88	0.05	7	1.15	0.04 P < 0.01	6
4 expts.				0.91	0.07	4	0.89	0.06	4	1.1	0.08 P < 0.02	4
5 expts. (From Tab. 4.1)							*1*	*0.04*	*5*	*1.25*	*0.04* *P<0.01*	*5*

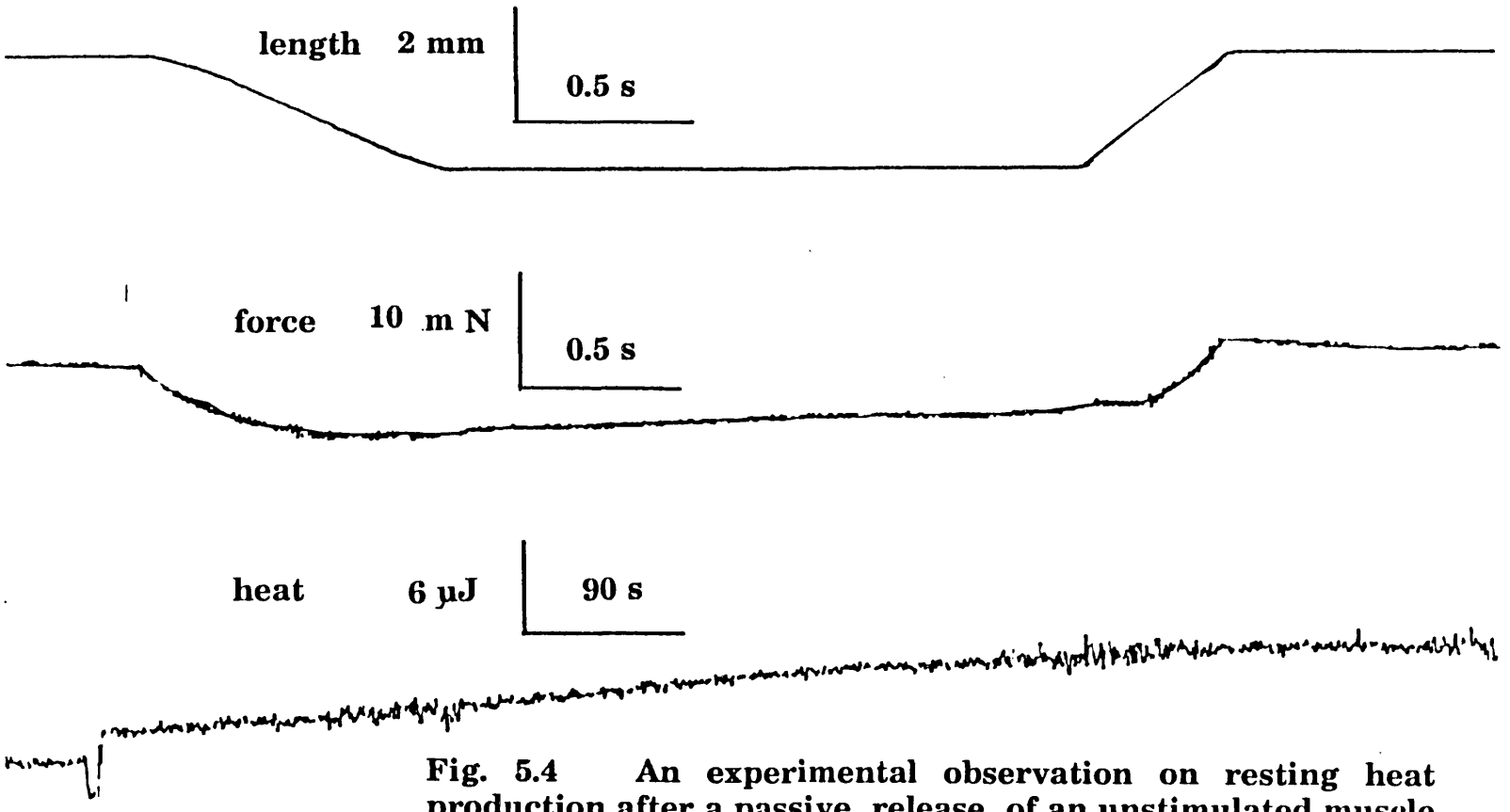


Fig. 5.4 An experimental observation on resting heat production after a passive release of an unstimulated muscle (top: length, middle: force, bottom: heat record). Note that the time scale for heat record is different. The heat record shows that the release causes very little artificial heat production (about 4 µJ) which is only 2% of the initial heat (167 µJ) produced by the same muscle for the same amount of release.

production after imposing a passive release on the muscle which did not contract at all. As can be seen from this figure, there is little initial and "recovery" heat production (about 2% of the initial heat in a 0.5 s tetanus) due to such release. This again indicates that if no active shortening is involved, passive release alone will not result in any significant difference in recovery ratio.

Altogether, the results of these experiments illustrate that the different recovery ratio is very unlikely to be due to the any artefact related to length displacement. Therefore, the results indicate that the different recovery ratio is a consequence of active shortening and probably therefore of different crossbridge cycles elicited by active shortening.

5.3 Experiments on muscle bundles

As discussed before, the recovery ratio could perhaps be altered if recovery metabolism is affected by an inadequate oxygen supply. This could be due to a diffusion problem, although the total oxygen supply in the chamber is sufficient. This control experiment aims at eliminating the diffusion problem by using small muscle bundles.

5.3.1 Experiments

Four experiments were conducted at 25℃.All the experimental conditions were the same as those mentioned in Chapter 3 except that smaller muscle bundles were used.

These bundles, about half the size of the whole muscle, were prepared by cutting down the centre line of the muscle carefully. Tension per milligram wet weight in these bundles was measured and compared with that in whole muscle preparations in order to make sure that the damage done by obtaining bundles was minimal. In fact, this value was
26±3mN/mg (wet weight, n=4) in bundles and 30±2 mN/mg (wet weight, n=5) in whole muscles. It is apparently easy to obtain a bundle of half muscle size with little damage. Typically, the dry weight of such bundles were about 1.1mg compared with 2.3 mg for the whole muscle.

The oxygen tension required to supply the needs of a muscle and to prevent an anoxic core depends on the square of the thickness of the muscle (Hill 1965), so a muscle bundle half as thick as a whole muscle needs only a quarter as much oxygen tension to prevent an anoxic core developing. The oxygen supply is therefore much better in these bundles. If the difference in recovery ratios

between isometric and working contractions is due to an inadequate oxygen supply, this difference should be greatly reduced or eliminated in this type of experiment.

5.3.2 Results

Four experiments were performed using soleus muscle bundles from four mice. From these experiments, a total of 180 successful observations were obtained.

Fig. 5.5A shows the results from one of these experiments. In this figure, the results for isometric contraction lie close to a recovery ratio of 0.8, the working contractions uniformly give a higher recovery ratio of 1.1. The slope for working contractions is clearly steeper than that for isometric contractions. Fig. 5.5B is the same results as those in Fig. 5.5A, plotted to show the values of mean and standard error of mean. These two figures illustrate the same point: the difference in recovery ratio between isometric and working contractions exists also in small muscle preparations.

Table 5.2 presents the results from all these experiments. From this table, it can be seen that, in each individual experiment, there is a difference between RRW and RRI and that this difference is

Fig. 5.5A Recovery ratios in muscle bundle experiment. One square for one observation (black for working and open for isometric contraction). The slopes of the lines show the recovery ratios for each type of contraction.

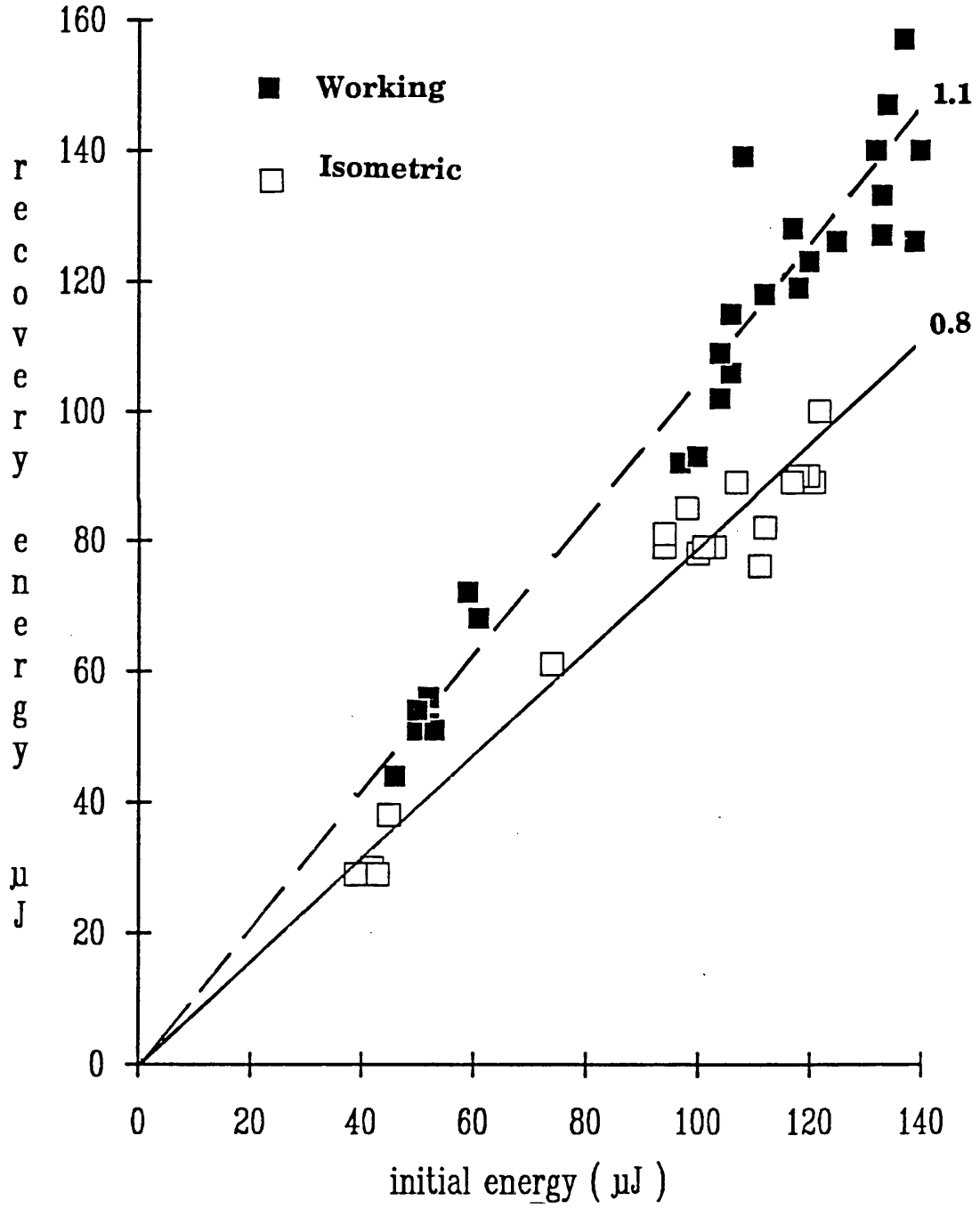
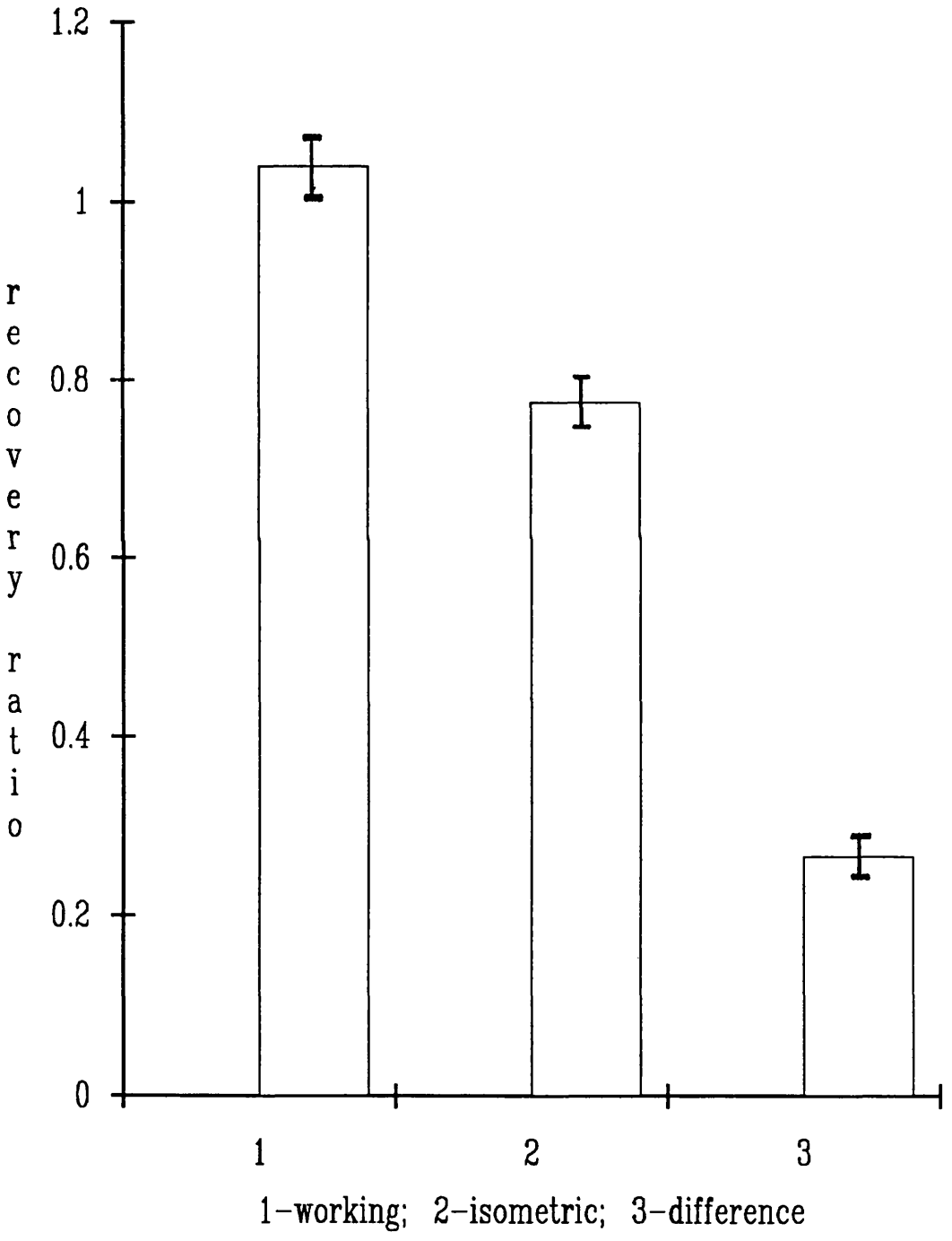


Fig. 5.5B A comparison in recovery ratio between working and isometric contraction (mean ± standard error of mean; same data as that shown in Fig. 5.5A but plotted in a different way).



Tab. 5.2 Control experiments on small muscle bundles of mouse soleus.

RRI: recovery ratio for isomrtic contraction.

RRW: recovery ratio for working contraction.

	isometric contraction			working contraction			difference			comparison		
	RRI			RRW			RRS-RRI			paired t test		
Expts.	mean	sem	n	mean	sem	n	mean	sem	n	t	P	n
1	0.86	0.01	20	1.03	0.02	19	0.17	0.02	19	8.916	0.001	19
2	0.76	0.03	20	1	0.02	20	0.24	0.02	20	14.23	0.001	20
3	0.77	0.02	26	0.96	0.01	34	0.19	0.02	26	9.836	0.001	26
4	0.84	0.02	18	1.02	0.03	23	0.18	0.03	18	5.571	0.001	18
4 Expts.	*0.81*	*0.03*	4	*1*	*0.02*	4	*0.2*	*0.02*	*4*	*10*	0.001	*4*

statistically significant. The size of difference is similar to that seen in whole muscle. The value of recovery ratio in these experiments were, for both isometric and working contraction, less than those found for whole muscle. This could be due to a higher internal pH in the bundles, perhaps due to better exposure to the Ringer solution, easier removal of CO_2 and lactate. Whatever the explanation this fact does not seem to alter the interpretation because both isometric and working contractions are affected.

In summary, the results of this type of control experiments still shows that there is a clear difference between RRW and RRI, even when small muscle bundles were used. This makes it very unlikely that the difference between RRW and RRI is due to an inadequate oxygen supply.

5.4 Experiments at lower temperature

Another way to check that the oxygen supply is adequate and if it is not, to improve it, is to conduct experiments at a lower temperature. Muscle resting metabolism has a Q_{10} value of about 3 (Hill 1965). So, at a temperature 10 degrees lower, the oxygen consumed by a muscle's resting metabolism would be cut down to about 1/3. This will have a strong effect on the recovery ratio, and

thus, eliminating or vastly reducing the difference between RRW and RRI if this difference is due to inadequate oxygen supply. Because at the lower temperature the muscle's resting metabolism is low, the "stored" or "unused" oxygen is thus high. Most importantly the "stored" oxygen in the core of the muscle is high, compared with that at high temperature. Next, the rate of oxygen consumption for recovery is also slowed down at low temperature because less ATP is split during contraction. Furthermore, because the rate of recovery metabolism is slowed down, more time is available for replenishment of oxygen supply during recovery. Taking all these factors together, the amount of oxygen consumption at 15°C is about 20 times less than that at 25°C. Therefore if the difference between RRW and RRI is due to an inadequate oxygen supply, this difference should disappear at 15°C.

5.4.1 Experiments

The experimental conditions were the same as those in the first series (Chapter 3), except the lower temperature of 15°C was used. Compared with other experiments in this project, the experiments done here should consume much less oxygen. However, a further step was taken to ensure that the oxygen supply was sufficient.

During the whole experimental period, the chamber containing the muscle was being bubbled continuously with oxygen. to stir the gas inside the chamber and to avoid an unstirred layer of gas surrounding the isometrically contracted muscles.

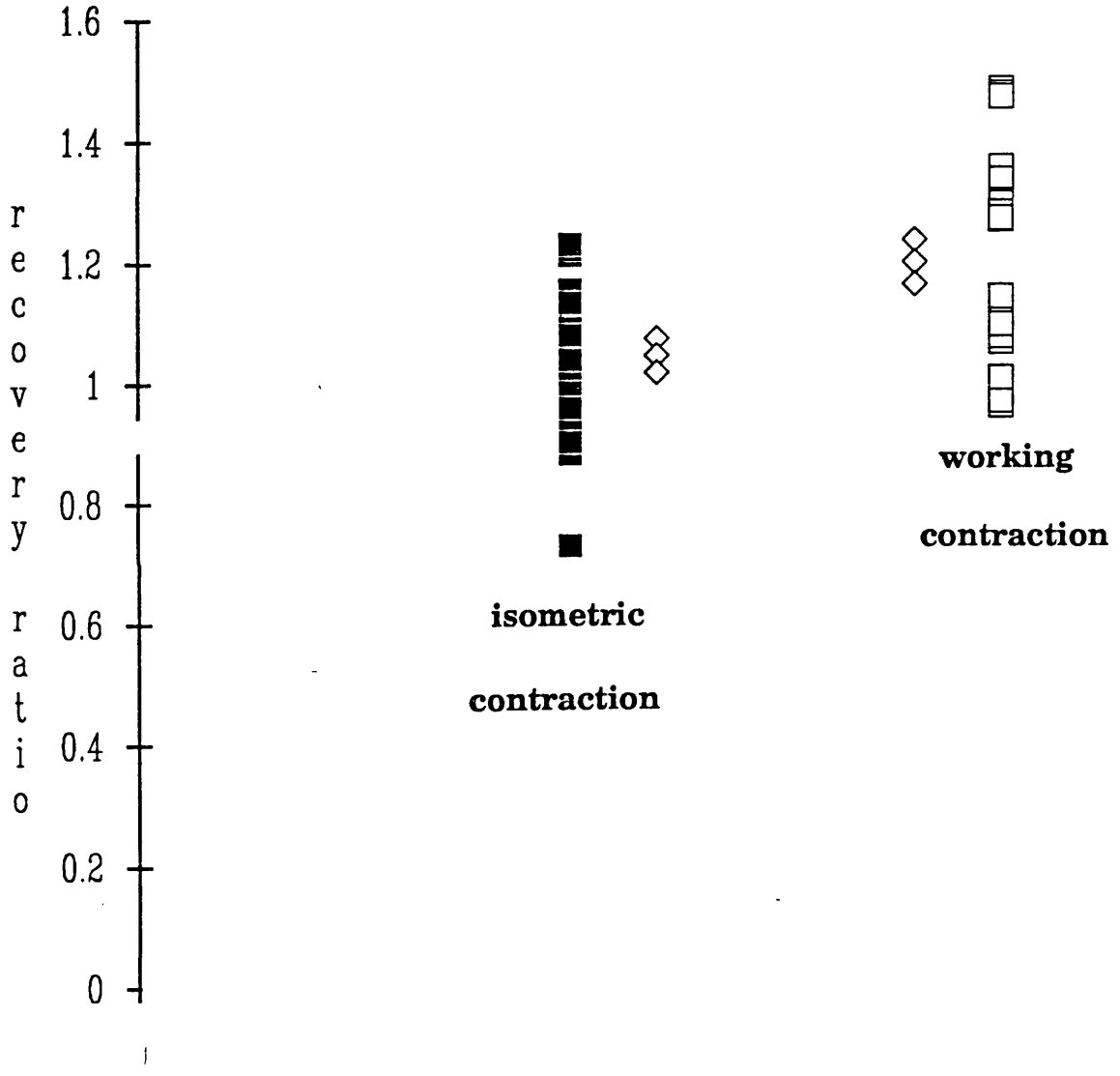
5.4.2 Results

Two experiments were conducted and 54 successful observations were made from them. At 15°C, the recovery process was slowed down and it lasted about 2 to 2.5 minutes, almost twice as long as at 25°C.

Fig. 5.6 shows the results from one experiment. In this figure, the individual observations and the mean values for recovery ratio are presented and compared. It can be seen that there is a difference between RRW and RRI.

Table 5.3 is a summary of these two experiments. The results are comparable with those obtained from the first series of experiments. Therefore, these two experiments again confirm that the difference in recovery ratio between working and isometric contractions is not due to an inadequate oxygen supply.

Fig. 5.6 Comparison of recovery ratio between working contraction (RRW) and isometric contraction (RRI). RRW is bigger than RRI (one square for one observation, diamond for mean and standard error of mean, mouse soleus, 15°C).



Tab. 5.3 Recovery ratios in low temperature experiment.

	isometric			working			comparison			
	mean	sem	Number of observations	mean	sem	Number of observations	Pared t test			n
Expt. 1	1.05	0.03	22	1.2	0.04	22	t = 2.951	P < 0.01		22
Expt. 2	0.98	0.08	5	1.21	0.06	5	t = 5.707	P < 0.01		5
			Number of experiments			Number of experiments				
15 C	*1.02*	*0.04*	*2*	*1.21*	*0.01*	*2*				
25 C	*1*	*0.04*	*5*	*1.25*	*0.04*	*5*	*t = 8.2*	*P < 0.01*		*5*

(From Tab. 4.1)

To summarize the results from all the three types of control experiments in this chapter, it has to be concluded that the difference in recovery ratio between working and isometric contractions is neither due to inadequate oxygen supply, nor due to artifacts caused by length displacement in working contractions.

CHAPTER 6 EXPERIMENTS ON FROG MUSCLE

In 1928, Hartree compared RRW with RRI in frog muscles (Hartree 1928). His conclusion was that RRW and RRI were the same in frog muscles. Hartree's results were later confirmed by Hill (1932) and were regarded as well established. In view of the results obtained with mouse soleus muscle in this study, it was sensible to repeat Hartree's experiment. If the same results as Hartree's (RRW=RRI) could be reproduced in frog muscle, this would be a demonstration that the mouse result is not an artefact due for example to a calibration error, which would affect results for frog and mouse equally.

6.1 Experiments

Muscle preparation

Frog semitendinosus muscle were isolated and the whole muscles were used. The choice of frog semitendinosus muscles, rather than

sartorius muscles as in Hartree's original experiments, is that semitendinosus is very similar to mouse soleus muscles in terms of muscle shape, size and thickness. For the purpose of checking technical aspects of the equipment, these similarities are important. For example, if the size and the shape of muscle preparations are the same or similar, they cover the same part of the thermopile. If there is an error due to the positioning of the muscle on the thermopile, this error should have the same effect on both species of muscle. Therefore, any error is more reproducible and can be detected more easily.

Solution

The composition of the frog Ringer's solution was the following (mM):

NaCl 96.6 KCl 2.2 $MgCl_2$ 1.0 $CaCl_2$ 1.8

$NaHCO_3$ 20 Ca-EGTA 1.0

The solution was bubbled with mixed gas (2% CO_2 and 98% O_2 in volume) for 30 minutes to get a pH approximately 7.35.

Stimulation

The stimulation parameters were: frequency 50 HZ, pulse width 0.5 mS, voltage 9 V, tetanus duration 0.3 Sec. The same stimulation electrodes arrangement as for mouse experiment (see Fig. 3.5B, Chapter 3) was used. Experiments were conducted at 25°C (Fig. 6.1A).

The other experimental conditions were the same as those in the mouse experiments. And, of course, the same experimental equipment was used.

6.2 Results

Seven experiments were conducted using semitendinosus muscle from different frogs. From these experiments, a total of 169 successful observations were made.

Results from one experiment:

Fig. 6.1B shows the results from one experiment. In this figure, it can be seen quite clearly that all the data points, no matter whether isometric or working contraction, are scattered about a

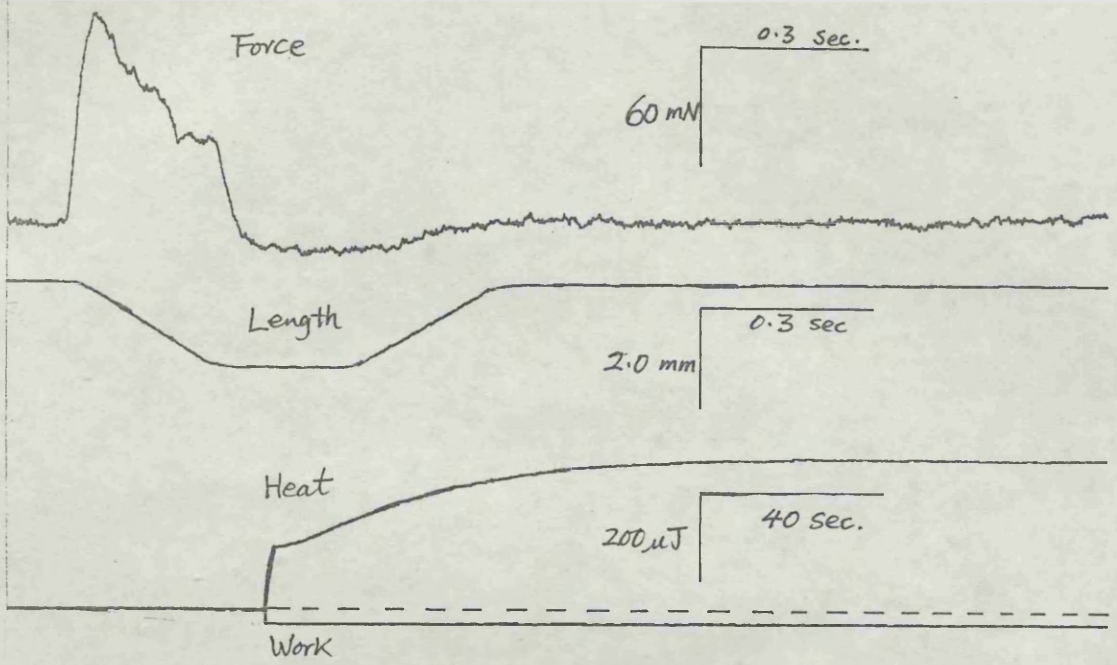


Fig. 6.1A Experimental records of muscle length, force and calculated heat and work productions for a working contraction (frog semitendinosus muscle, 25 C, stimulation: 50 Hz, 0.5 mS, 9 V, 0.3 S). Note that the time scale for heat and work production is different.

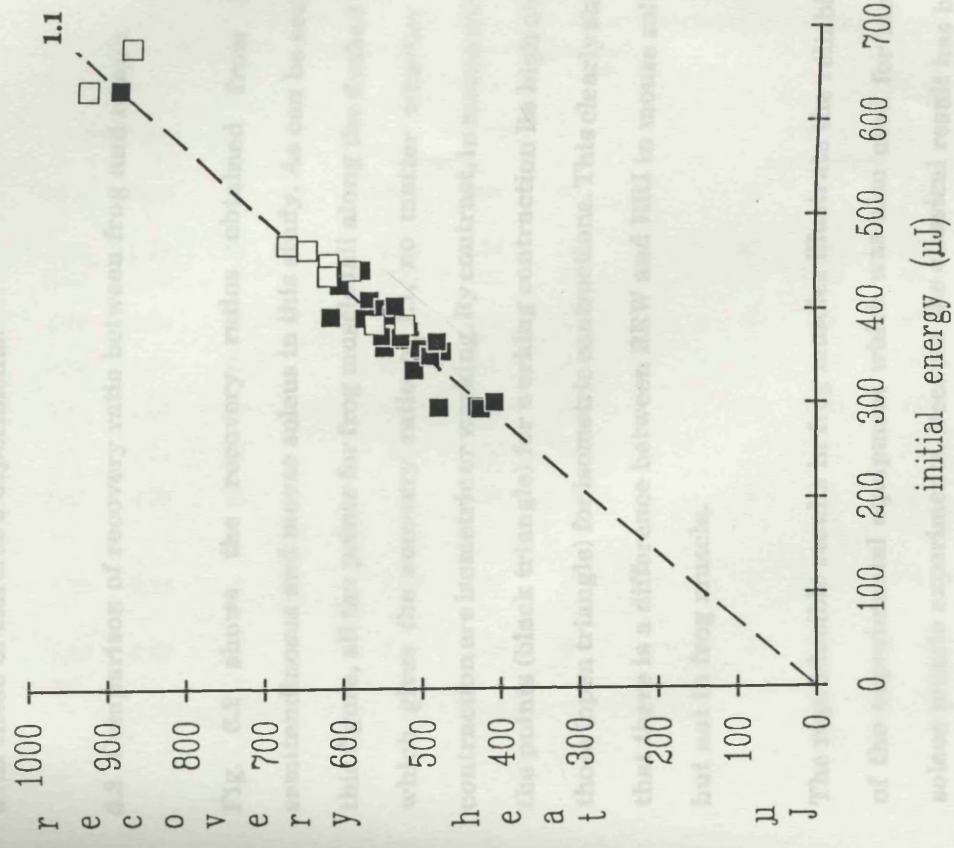


Fig. 6.1B Recovery ratio in frog semitendinosus (25 °C). The ratio is the same in isometric contraction (open squares) as in working contraction (black squares).

single line, representing a recovery ratio about 1.1.

Tab. 6.1 summarizes the results from all seven experiments. As can be seen there is no difference between RRW and RRI in any individual experiment. The results are thus in good agreement with those of Hartree's experiments.

6.3 Comparison of recovery ratio between frog and mouse

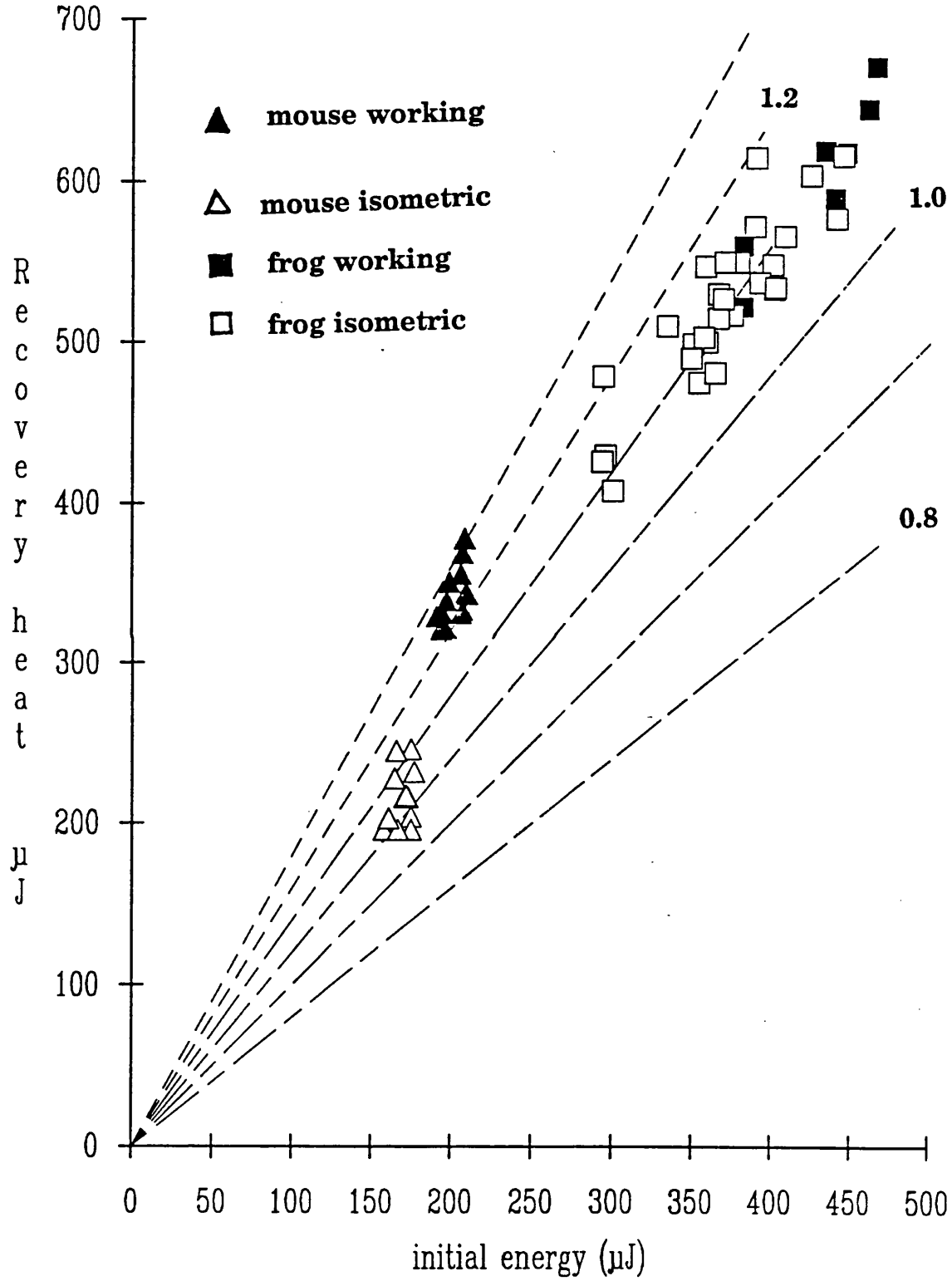
Fig. 6.2 shows the recovery ratios obtained from frog semitendinosus and mouse soleus in this study. As can be seen in this figure, all the points for frog muscle fall along the dashed line which gives the recovery ratio of 1.1, no matter whether the contraction are isometric or working. By contrast, in mouse soleus, the points (black triangle) for working contraction lie high above those (open triangle) for isometric contractions. This clearly shows that there is a difference between RRW and RRI in mouse soleus, but not in frog muscle.

The reproducible results in frog muscles illustrate the reliability of the experimental equipment which was also used for mouse soleus muscle experiments. Because the classical result has been reproduced, the possibility that the difference in recovery ratio in mouse soleus experiments might be due to a technical error relating to the experimental equipment can be ruled out.

Tab. 6.1 Recovery ratio in frog semitendinosus (whole muscle, 25°C).
There is no diffence in recovery ratio between working and isometric contraction in frog semitendinosus.
In Hartree's experiments, frog sartorius muscle was used.

	Isometric			Working			Pared comparison		
Expts.	mean	sem	n	mean	sem	n	n	t	p
1	1.03	0.09	10	1.04	0.04	16	10	1.1	>0.3
2	0.68	0.02	10	0.71	0.02	16	10	0.45	>0.7
3	1.16	0.04	6	1.18	0.03	6	6	0.5	>0.7
4	0.98	0.06	8	0.91	0.1	4	4	1.34	>0.3
5	1.76	0.03	12	1.74	0.03	16	12	0.25	>0.5
6	1.42	0.01	29	1.4	0.02	9	9	0.91	>0.2
7	1.53	0.03	13	1.54	0.04	14	13	0.9	>0.2
7 Expts.	*1.22*	*0.14*	*7*	*1.22*	*0.14*	*7*	*7*	*t = 0.443*	*P > 0.6*
Hartree's	*1.28*	*0.07*	*6*	*1.27*	*0.05*	*6*	*6*	*t = 1*	*P > 0.3*

Fig. 6.2 A comparison of recovery ratios in a frog muscle experiment. The figure shows clearly that, in frog semitendinosus muscle, the recovery ratio is the same in working contractions as in isometric contractions.



CHAPTER 7 STRETCH EXPERIMENTS ON MOUSE SOLEUS MUSCLES

7.1 Introduction

Although the main function of a muscle is to produce force and to shorten, there are some conditions under which a contracting muscle is forced to lengthen, for example, when one walks down stairs. In such a case, although the muscle involved contracts, the weight of the body is not lifted but the muscle is stretched by the weight.

In muscle research, experiments on stretching muscle have attracted considerable attention. Information in this area is very helpful for a better understanding of muscular function and hopefully for getting some insight into the mechanism of muscular contraction .

In the light of finding the phenomenon that there is a difference in recovery ratio between working and isometric contractions, naturally, it is interesting to make a comparison of the recovery ratio between working contractions and those with stretching. Such information is not available because there is no study of the recovery ratio in stretched muscles, not even in frog muscles. Therefore a preliminary study of this question has been undertaken in this project.

7.2 Method

For the purpose of comparison, the experimental conditions/methods were kept as close as possible to those in the shortening experiments. Unless mentioned below, the conditions were the same as before (see Chapter 3 for reference).

7.2.1 Stimulation

Mouse soleus muscles were stimulated and a stretch was imposed on the muscles during stimulation. A release was given immediately after the stretch in order to allow the muscle to

return to pre-stretch length. This will minimize the stretch response (Feng effect, Feng 1932) which, otherwise, will give rise to high resting metabolism and thus lead to high recovery ratio.

The stimulation parameters were: about 1.0 volt, 50 Hz, 0.5 ms pulse width and, in most cases, 0.5 s tetanus duration. The muscle was stretched by approximately 1 mm, 0.01 s after the start of stimulation. The duration of the stretch was about 0.24 sec. A release was applied immediately after the stretch and lasted for 0.24 sec (see Fig. 7.1). Note that the muscle was active during both stretching and subsequent shortening. The experiments were conducted at 25°C.

7.2.2 The procedures of working out recovery ratio

In isometric contraction

The procedures is the same as those in previous experiments:

a) measure initial heat (h_i) and total heat (h_t)

b) calculate recovery heat (h_r) by subtracting h_i from h_t

i.e. $h_r = h_t - h_i$

c) work out the ratio of h_r to h_i. This ratio is called

Recovery Ratio of Isometric contraction (RRI).

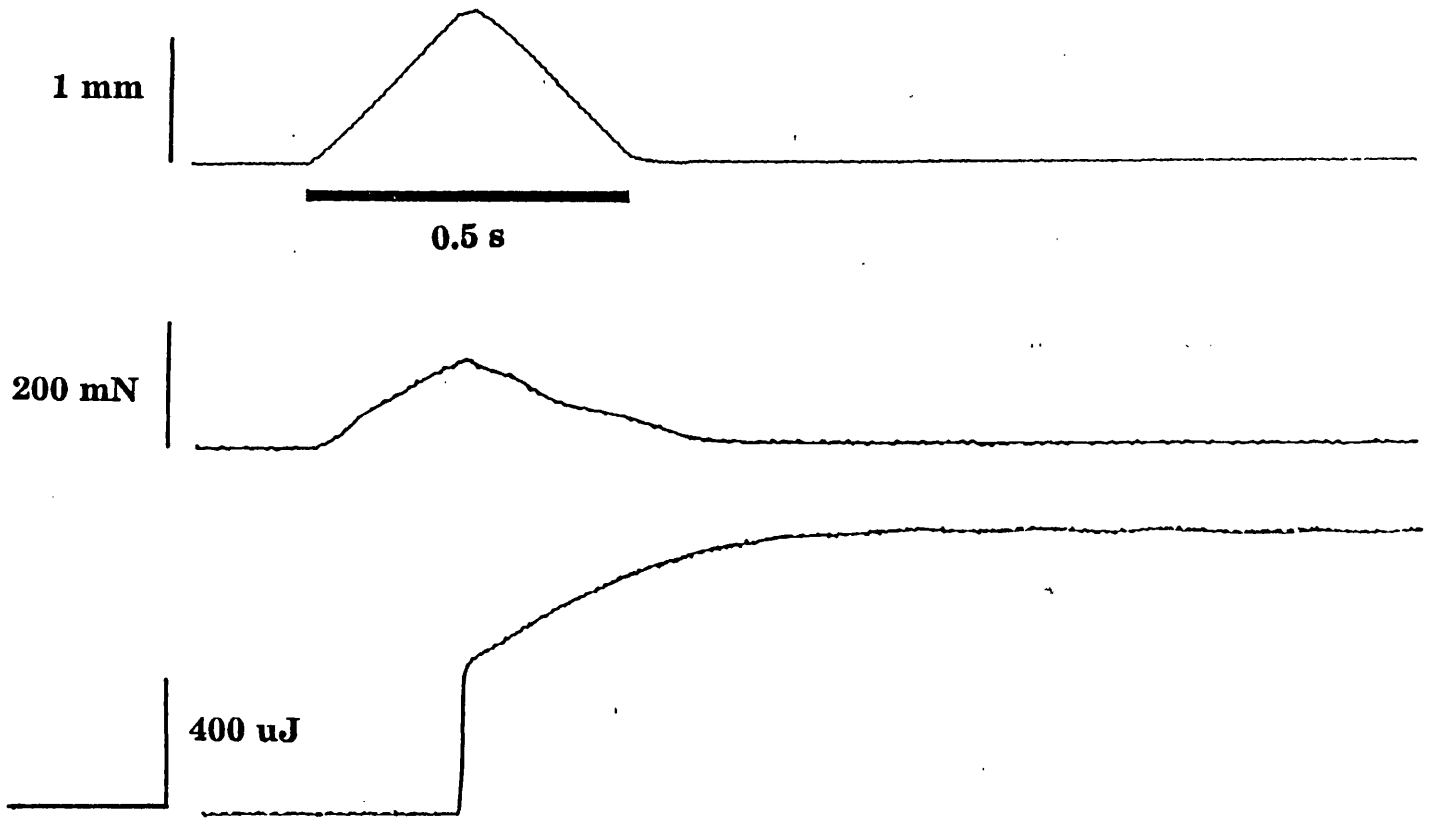


Fig. 7.1 Experimental records of a contraction in a stretching experiment (top: length; middle: force; bottom: heat record). Note that both stretch and release occur during the period of stimulation (shown by the bar), and that the time scale for the heat record is different. The tetanus duration is 0.5 s (shown by the bar). the stretch was started 0.01 s after the start of the stimulation. Both the stretch and the release last for about 0.24 s.

In stretching contractions

In the case of stretching, because the work is not produced by the muscle, instead, the work is done on the stretched muscle by the motor, the work to be added in order to obtain initial energy is a negative quantity, which is the sum of heat and work. The following are the procedures:

a) measure initial heat (h_i), total heat (h_t), external work (-W) done upon the muscle by the motor during stretching, and the external work (+W) produced by the muscle during shortening. Note that work has a negative value for the stretching period, and a positive value for the shortening period.

b) calculate recovery heat (h_r) by using $h_r = h_t - h_i$

c) calculate initial energy (E_i) by using $E_i = h_i + (TW)$

Note that TW (total work) is the sum of work done upon and by the muscle during stretching and subsequent shortening,

i.e. TW = (+W) + (-W)

d) work out the ratio of R_r to E_i. This ratio is called Recovery Ratio of Stretching (RRS)

7.3 Results

Four experiments were conducted and soleus muscles from different mice were used in each experiment. From these experiments, a total of 94 successful observations were made.

Efficiency

In the experiments, the active muscle was first stretched by the motor and then released. Both the stretch and the release occurred within the stimulation period. Therefore, the total work was consisted of two parts: a negative work done on the muscle and a positive work done by the muscle. For the purpose of calculating efficiency, the equation which should be used is

$$\varepsilon_i = \frac{(+W)}{(h_i + TW)}$$

$$\varepsilon_t = \frac{(+W)}{(h_t + TW)}$$

Note that the amount of negative work should be included in the initial energy.

In these experiments, because the recovery ratio was the main interest, efficiency was not systematically investigated. The highest efficiency values obtained by using the above equality was 0.16 for initial process and 0.09 for overall process. They are lower than those found in shortening experiments (0.23 and 0.11 respectively).

Recovery ratio

The values of recovery ratio is the main interest in these experiments. This value reflects the quantitative energetic relation between initial process and overall process. It is interesting to compare this ratio with that in shortening experiments.

Fig.7.2 shows the results from one experiment, comparing the recovery ratios both for isometric contractions and contractions containing stretching and releasing of active muscle. For simplicity, the latter will be referred to as "stretching" contractions. There is no difference in recovery ratios between the isometric and the "stretching" contractions. The two types of data fall around a single line corresponding to a recovery ratio of 1.2.

Fig. 7.2 A comparison of recovery ratio in a stretching experiment (mouse soleus, 25°C, each point for one observation). There is no difference in the ratio between working contraction and isometric contraction.

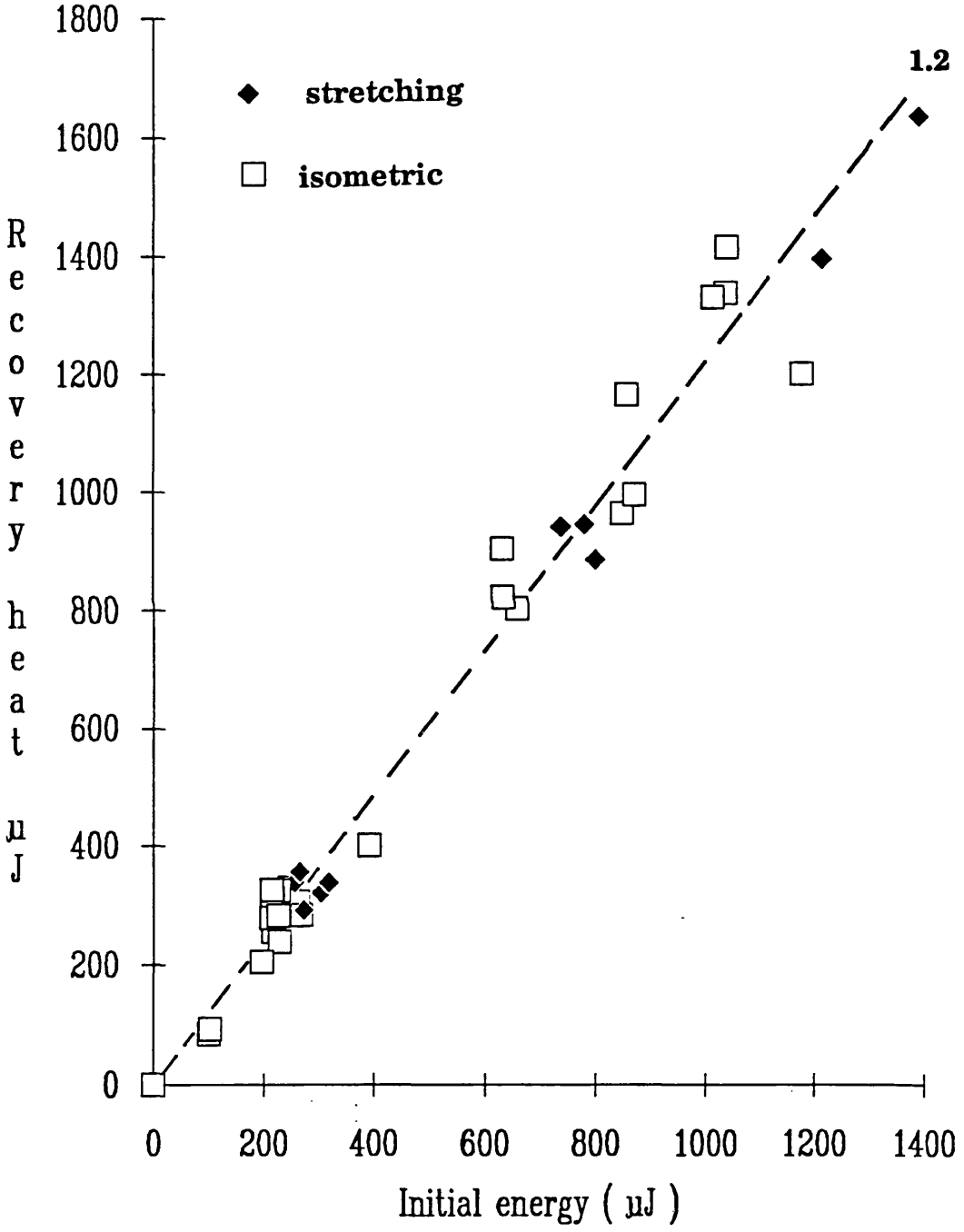


Table 7 is a summary of all four experiments of this type. From this table, it can be seen that although there is a tendency for the ratio to be less in the "stretching" experiments, there is no significant difference in the recovery ratios between isometric contractions in any one experiment. Although in one experiment, recovery ratios are rather lower, compared with the rest, the recovery ratios are the same for isometric contraction and for "stretching" contractions.

This result is the opposite to what has been found in shortening experiments in this project. Since in these experiments the muscle did undergo active shortening (by about half the distance used in the shortening experiments) the expectation would be that the recovery ratio would be greater than that for isometric contractions. This is not what was found. Therefore the stretch did have a consequence in altering the energetics of the recovery process. Either (1) the shortening that occurs immediately after a stretch does not produce the same effect on the recovery as normal shortening; or (2) a process occurs during stretching which is the opposite of that occurring during shortening and the two effects cancel out, to leave the recovery ratio unaltered. To distinguish these possibilities experiments with stretching alone, without shortening, would be required.

Tab. 7 Summary of recovery ratio in stretching experiments (mouse soleus, 25 C). There is no significant difference in recovery ratio between isometric and "stretching" contraction.

	isometric			stretching			comparison		
expt	mean	sem	n	mean	sem	n	pared t test		
1	0.69	0.04	7	0.6	0.04	7	n=7	t=1.084	P > 0.5
2	1.04	0.03	9	1.05	0.04	13	n=9	t=1.891	P > 0.1
3	1.27	0.04	18	1.19	0.03	11	n=11	t=1.046	P > 0.2
4	1.14	0.02	13	1.09	0.02	20	n=13	t=1.953	P > 0.05
4 expts.	*1.04*	*0.11*	*4*	*0.98*	*0.11*	*4*	*n=4*	*t=2.333*	*P>0.1*

CHAPTER 8 DISCUSSIONS

8.1 Efficiency in mouse soleus

In this study, it has been found that the maximum efficiency in mouse soleus muscles is low, compared with the efficiency values in other species. Table 8.1 compares the maximum efficiency in different animals, and compares these values with maximum shortening velocity for this muscle. The relevant references can be found from Chapter 1 (Table 1.1) and from Woledge et al (1985, Table 2.11).

As can be seen, although the maximum efficiency value in mouse soleus muscle in this study is considerably lower than those in tortoise or frog, they are quite comparable with those found in the soleus of rat and mouse by other workers. It is evident that the maximum efficiency does vary between species. Therefore, it is not altogether strange to obtain the low maximum efficiency in mouse soleus in this study.

Tab. 8.1 Comparison of efficiency with maximum speed of shortening in different species

* The references here are for efficiency values.

** All Vmax values here are from Woledge 1985(Tab.2.II).

Species	Vmax *	Efficiency		References*
	um/half-sarcomere	Initial	Overall	
Tortoise rectus femoris	0.28	0.72	0.36	(Woledge 1968)
Frog sartorius	1.29	0.45	0.18	(Hill 1964, 1939)
Rat soleus	9.1		0.15	(Heglund & Cavagna 1987)
Mouse soleus	11.5	0.23	0.11	(This study)

As can also be seen in this table, the maximum efficiency tends to be inversely related to the maximum velocity of shortening. The animals enjoying a high efficiency like tortoise tend to have a low speed of shortening.

From the evolutionary point of view, mice are sometimes considered "higher" than either tortoise or frog. Therefore, very naturally, questions are raised: why is it necessary for mice to have such a low efficiency; what kind of advantage have mice obtained in the course of evolution at the expense of such low efficiency?

With our available knowledge, these questions cannot be answered satisfactorily. Here, at least two reasons are suggested. First, as Wilkie (1960) argued, the high rate of energy transformation can only be achieved by setting a coupled system far away from its equilibrium. At such a position, the system possesses a high "driving force" and thus a high rate of energy transduction can be achieved. However, because the system is far away from its equilibrium, the transduction process is more irreversible. The more irreversible the process, the less efficient the process. This speculation seems to agree with the fact that maximum efficiency does seem to relate inversely with maximum speed of shortening in the animal kingdom.

Second, the results in this study strongly indicates that there is an extra process involved specifically in working contraction (more details about this in next part of this chapter) in mouse soleus muscle. This has not been reported in any other muscle studied so far. Although nothing is known about this extra process, it might well be involved, directly or indirectly, in chemomechanical transduction because only working contractions elicit this unknown process. It is hard to imagine, but it is possible, that this unknown process that occurs only during working contraction, has nothing to do with chemomechanical transduction. In this case, the low efficiency can be explained more easily, because the unknown process does not contribute to chemomechanical transduction, it is wasted in terms of work production. Therefore, the efficiency is low due to the existence of this extra unknown process which is not truly a "driving process".

Another possible reason for the low efficiency value in this study may be due to the temperature (25°C) used which is lower than the physiological temperature (about 37°C) of mammalian muscle. It is possible that the efficiency value at physiological temperature is higher. In Chapter 4, the efficiency value obtained at 15°C is compared with that obtained at 25°C. The efficiency values seem to be the same at these two temperatures. Nevertheless, neither of them is the physiological temperature and thus this may not be the highest value possible.

The reason for not making experiments at 37°C is that the oxygen supply to the isolated muscle at this temperature is not likely to be adequate. Because of the finding of an unknown process existing in working contraction in mouse soleus, it is impossible to assess the thermodynamic efficiency in the muscle. Nevertheless, if a reasonable assumption can be made according to available knowledge, an approximation can be made to indicate the thermodynamic efficiency. As will be seen later in Section 8.4, there may be a delayed ATP (PCr) splitting during the recovery period. This delayed ATP splitting is about 10% of that split during the initial period. Using the following values (for 0.5 s tetani) found in this study

h_i = 30 mJ/g, h_r = 38 mJ/g, W = 7 mJ/g,

the "thermodynamic efficiency" can be estimated on the basis that (1) ΔF_{PCr} = 45 kJ/mol (Kushmerick & Davies 1969), ΔH_{PCr} = 37 kJ/mol; (2) the delayed ATP splitting is 10% of that split during contraction; and (3) the unknown process is thermally neutral (see Section 8.4). The thermodynamic efficiency for the initial process (ε_i) can be calculated as below :

$$\varepsilon_i = \frac{\Delta H_{ATP}}{\Delta F_{ATP}} \times \frac{W}{1.1 \times (h_i + W)}$$

$$= \frac{37}{45} \times \frac{7}{1.1 \times (30 + 7)} = 14\%$$

This value is a reasonable estimate of the thermodynamic efficiency in mouse soleus. The true value of thermodynamic efficiency in mouse soleus cannot be assessed better until more information about the unknown process indicated by the results of this study is available.

8.2 Recovery ratio in mouse soleus muscle

It is totally unexpected to find any difference in recovery ratio between working and isometric contractions in mouse soleus muscle. This phenomenon was found to be absent in frog (Hartree 1928, Hill 1939 and this work) and in tortoise muscle (Woledge 1966). Therefore the possibility of the existence of such a phenomenon in a mammalian muscle had not previously been investigated. This raises the question whether this result is an artifact or a genuine physiological phenomenon. Some possible causes of such artefact will be reviewed and reasons given why they are not likely to have caused this phenomenon.

(a) Stimulation heat

In heat experiments, energy input due to the electric stimulation is usually carefully considered. If the stimulation heat is wrongly estimated or is not corrected for, an error will be caused in the initial heat measured and this would affect the recovery ratio. As the proportion of initial heat in the initial energy is different between isometric and working contraction such an error could conceivably cause a difference between RRW and RRI. However in this project, the stimulation heat is small because of the arrangement of stimulation electrodes used (as described in Chapter 3). Observations made on procaine treated muscle confirmed that stimulation energy was indeed negligible. In another study, Woledge and I have used nerve stimulation in experiments of this kind. This reduced stimulation heat even further; but the difference in recovery ratio between working and isometric contraction was seen as usual. Therefore, it is very unlikely that the difference between RRW and RRI is the consequence of stimulation heat error.

(b) Stability of the baseline of temperature recording

Good recovery heat measurement requires a stable heat baseline. Random heat baseline fluctuations cause errors in recovery ratio values, but there is no reason why these would affect RRW more

than RRI. As it was found that in every individual experiment, RRW was *always* bigger than RRI, it is unlikely that the difference between them is due to any heat baseline problem.

(c) Anoxic muscle core

An adequate oxygen supply is vital for muscle recovery metabolism. If the oxygen supply is insufficient, the recovery process will certainly be affected. As a result, recovery heat will be affected and this will, in turn, distort the recovery ratio. Therefore, in any experiment dealing with the recovery process, checking for an adequate oxygen supply is essential. In this project, this point was critically tested by a series of control experiments so that the possibility that the difference between RRW and RRI may be caused by an inadequate oxygen supply can be confidently eliminated for the following reasons.

The experiments were repeated at 15°C. If the different recovery ratio between shortening and isometric contraction was due to inadequate oxygen supply, this difference should disappear at the lower temperature, at which oxygen demand is less and oxygen supply is greater. However, the difference in recovery ratio was seen as usual in the low temperature experiments. Thus, it is very hard to imagine that the different recovery ratio can possibly be caused by shortage of oxygen.

Careful consideration was directed to the oxygen diffusion aspect. Even though there is no shortage of total oxygen supply, an anoxic core in the muscle may still develop due to a long diffusion distance. This distance may become bigger because one side of the muscle is in contact closely with the thermopile. This contact may prevent oxygen diffusion from that direction. Therefore, oxygen may have to travel a longer distance in order to reach the side of muscle which was "sealed" by the thermopile. In the case of using whole muscle preparation, this is a point needing to be checked carefully.

Based on this idea, the experiments on small muscle bundles were conducted. By using them, the distance of oxygen diffusion was greatly reduced. This, in turn, reduced any oxygen shortage related to diffusion limitation. Once more, the difference in recovery ratio was seen. It is thus not due to oxygen shortage related either to the shortage of total oxygen supply or to any diffusion problem.

Finally, if there was any oxygen shortage, it would be the working contraction that suffered more than the isometric contraction did, because the initial energy consumed would be greater and thus more oxygen would be needed for the recovery metabolism. Therefore, in the case of oxygen shortage, although both RRW and

RRI could be affected, the consequence should be that RRW would be smaller than RRI, not bigger as observed.

(d) Heat overestimation or work underestimation

The recovery ratio of isometric contraction is relatively easy to obtain correctly, compared with the recovery ratio for working contraction. In the later case, in order to calculate the recovery ratio, both heat and mechanical work measurements are needed, whereas in the case of isometric contraction, only heat measurements are needed. It is from this fact that some problems might arise.

In these experiments, heat was recorded from only part of the muscle while mechanical measurements (force, length change, and therefore, work) were recorded from the whole muscle. Then, according to the weight ratio (weight of the part of the muscle over the recording section of the thermopile to the total weight of the same muscle), the work produced by the part of the muscle which was over the recording section(s) of the thermopile was calculated. Obviously, this involves accurately cutting the muscle in order to weigh each part. If the cutting was not accurate, some error could result. The work might be underestimated and this, in turn, could give a higher recovery ratio for working contraction.

If the cutting were not accurate enough, one would expect this to introduce a random error. As a result of this random nature, one should sometimes see a lower recovery ratio in the working contractions, a situation never encountered in any of our experiments. Thus, the higher recovery ratio in shortening seems to have nothing to do with any random error in cutting.

Any systematic error in the cutting procedure would have affected the experiments on frog muscle as much as those on mouse muscle. Therefore, the fact that RRW = RRI in frog but not in mouse is an argument against the suggestion that the differences seen are due to a systematic error in this procedure.

There may be another possible reason for work underestimation or heat overestimation. If the muscle fibres are not uniform along the whole of their length, the work calculation could be wrong. Because the work calculation was based on the assumption that the muscle fibres shorten uniformly, so that the work done by any part of the muscle is proportional to its weight. In this study, this point has not been investigated systematically because of the lack of suitable equipment which could record heat from enough different parts of a muscle, particularly from the two ends of the muscle. Although in some experiments, heat was recorded from the

different sections of the thermopile, the part of the muscles used for heat recording was usually confined to the middle part of the muscles. For completeness a further study is needed to eliminate the possibility that the difference between RRW and RRI is due to non-uniform shortening along the fibres.

An example here illustrates how large this effect would have to be. In this study the observed values are: RRI = 1.0, RRW=1.2, initial efficiency = 0.2, weight ratio (the middle part to total muscle) = 1/3. Suppose that the true RRW = 1.0, the initial and recovery heat are correct, the observed initial energy (work + heat) is too small by 20%. If the apparent work was 20% of the initial energy (initial efficiency = 0.2), the true work should be 40% of the initial energy. Therefore the middle part of the muscle, which covers the recording sections of the thermopile and weighs 1/3 of the whole muscle actually produces 2/3 of the work, not 1/3. Therefore the effect would have to be very much larger to explain the observed effects. This seems very unlikely but is worthy of further investigation.

(e) Conditions change during experimental observation

During experimental observation, the condition of the muscle might change gradually. Did this change contribute to the higher RRW value? In our experiments, a paired observation order was strictly followed, so, if there were any changes in muscle condition, the change should be cancelled within this design. Moreover, under our experimental conditions, the muscle survived reasonably well. The tension and heat production were fairly constant probably because the tetanus was very brief and the stimulation voltage was not big. The deterioration of the muscle was therefore minimised.

(f) Initial and recovery processes overlap

In frog muscle at low temperature (0°C), the recovery process is very slow and there is a clear separation between initial and recovery processes. However, in mammalian muscle, the recovery process is quick, especially at higher temperatures. In this project, although mouse soleus muscle was used and the experiments were usually made at 25°C, the difference in recovery ratio was not caused by any overlap for the following reasons.

Firstly, because of the very brief tetanus (0.5 second) used, the recovery process did not significantly overlap with the initial process. This can be clearly seen from Fig. 4.5B, which shows the same heat production record as that in Fig.4.1, but expanded in order to see the detail. There is a level part immediately after the initial heat production in the curve. This illustrates that just after the initial phase, there is no substantial rate of recovery heat production in the contraction containing only one 0.5 second tetanus. In such a case, there is no recovery heat overlapping the initial heat.

Secondly, if there is a considerable overlap, the amount of "overlapped" recovery heat in working contractions should be larger than that in isometric contractions, because the larger the initial energy consumed, the faster the recovery metabolism is initiated. This should decrease RRW more than RRI. This is opposite to what has been found.

For contractions containing multiple 0.5s short tetani, there was indeed a substantial amount of "overlapped" recovery heat in the initial phase. However, this recovery heat had been corrected for as described in Section 4.1.2. This correction seems to work well because the difference between RRW and RRI is hardly dependent on the number of short tetani.

(g) Other technical aspects

In order to ensure that the difference between RRW and RRI is not due to any technical error, the frog muscle experiments were performed. The results were the same as those found by previous workers, i.e. these ratios are the same in frog muscles, in contrast to the result with mice. Thus, unless the technical error affects only one species it cannot be true that RRW is equal to RRI in both species.

In another series of experiments performed by Woledge and me, a new thermopile and different mechanical apparatus were used and all the equipment was recalibrated carefully. The difference in recovery ratio still existed in these experiments. So, it is unlikely that the difference between RRW and RRI is caused by any technical error, such as a calibration error.

From the above discussion, it is quite clear that the difference between RRW and RRI is unlikely to be due to any artifact. Therefore, apart from the incomplete treatment of possible variations along the length of muscle referred to under (d) above, it has to be concluded that the difference between RRW and RRI is not a consequence of artifacts, but a genuine physiological phenomenon, reflecting a change caused by shortening. And this

change can only be induced by a muscle during shortening, but not by the same muscle when contracting isometrically. And this phenomenon cannot be explained by our existing knowledge.

8.3 Recovery ratio in isometric contraction

The observed recovery ratio for isometric contraction in this study is 1.00 ± 0.04 (n=5, see Table 4.1). This value is in good agreement with the theoretical value predicted from mouse soleus. In mouse soleus at 25°C pH_i = 7.23 (Aickin & Thomas 1977), the expected RRI is about 0.95 (Woledge 1989a, also see Fig. 1.1). Because the observed RRI is the same as the theoretical value, the underlying process during initial or recovery process is PCr splitting or oxidative resynthesis of PCr. Therefore for isometric contraction in mouse soleus it seems that there is no mystery about the relation between initial and recovery process. This relation can be explained by our knowledge of muscle.

8.4 Possible reasons for the difference between RRW and RRI

Parvalbumin

It is well known that, in frog muscles, there exists a considerable

amount of parvalbumin (Pa), a calcium binding protein (Somlyo et al 1981). In frog muscle during an isometric contraction, part of heat production is due to the reaction

$$Ca^{++} + PaMg \text{ ------ } Mg^{++} + CaPa$$

This reaction is exothermic process producing about 25 kJ/mol calcium bound (Closset & Woledge, quoted in Curtin & Woledge 1978, Smith & Woledge 1982). There are increasing reports that this protein is responsible for the labile heat and for the "unexplained" enthalpy during isometric contraction in frog muscles.

It is widely accepted that, in mammalian muscles, there is no parvalbumin (Klug et al. 1983). This view is in line with the fact that there is no labile heat production in mouse soleus muscles (Fig. 8.1).

In this study, some observations were made in order to check this point. From Fig. 8.1, it can be seen that the initial heat production is quite linear. This linearity clearly shows that there is no labile heat production in our experimental mouse soleus muscles. There was no chemical analysis for parvalbumin in this study. However, based on the experiment observations, it is very unlikely that there is the existence of energetically significant amount of

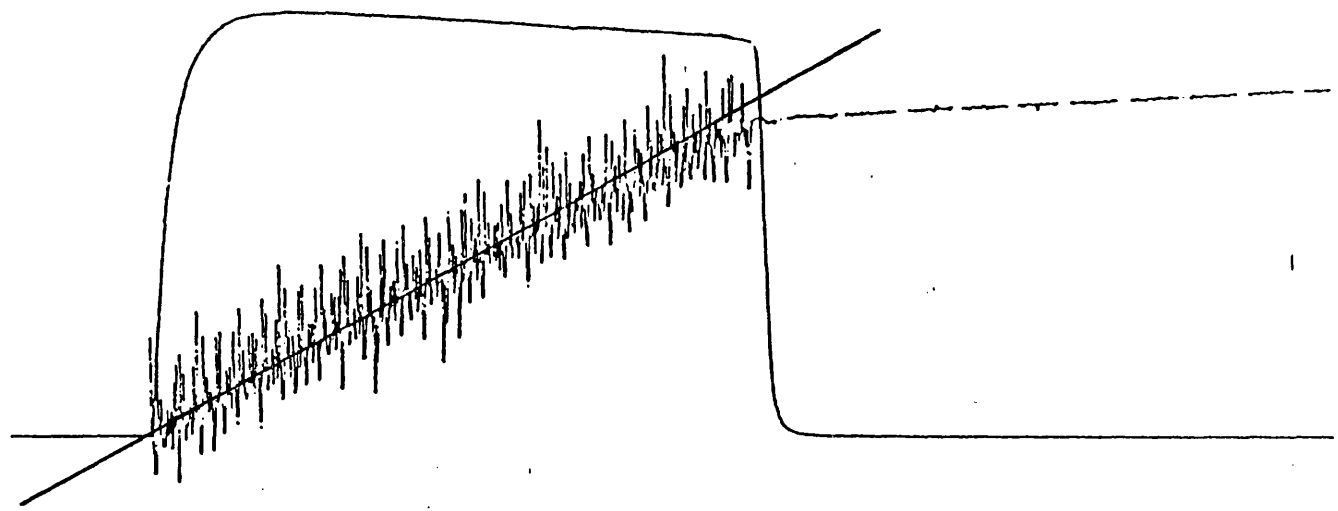


Fig. 8.1 **An experimental observation of initial heat production from a mouse soleus muscle at 25 C. The record shows that the heat rate is very linear (compare the superimposed straight line) and there is no labile heat production. The noise is a stimulus artifect.**

parvalbumin which can produce labile heat.

Because no labile heat production was found in any experiments in this study, and others have reported no presence of parvalbumin, it was not possible that the different recovery ratios were caused by parvalbumin.

A hypothesis

To explain the difference in recovery ratio between working and isometric contraction, a hypothesis is formed as below.
It is supposed that there is a crossbridge reaction occurring in the muscle during shortening, which does not occur during isometric contraction. This extra reaction is assumed to be thermally neutral ($\Delta H_x = 0$). Then during recovery, ATP splitting occurs to reverse this crossbridge process. Therefore, during recovery, extra heat is produced by two processes: (a) the reversal of the crossbridge process linked to ATP (PCr) splitting ($\Delta H = \Delta H_{PCr} - \Delta H_x \approx \Delta H_{PCr}$), and (b) the oxidative resynthesis of the ATP (PCr) split by process (a). Therefore, the energy production in the initial period for a working contraction is about the same as that expected from PCr splitting only, as in isometric contraction. However, during the subsequent recovery period, the recovery heat in a working

contraction is greater than that in the isometric contraction because the two extra processes (a) and (b) occur after the working contraction. This explains why RRW is higher than RRI.

Because the value of RRI (recovery ratio for isometric contraction) is close to the theoretical value, which is predicted from pH_i and ΔH_{PCr} in the living muscle, it is assumed in this hypothesis that PCr splitting is the only significant reaction occurring during isometric contraction. Therefore, RRI is treated as the true recovery ratio, reflecting the relation between PCr splitting (the initial process) and the subsequent oxidative resynthesis of PCr (the recovery process).

Based on the information obtained in this study we can see the quantitative consequences of this hypothesis. Assume RRI is the true recovery ratio; taking RRI = 1.0 and RRW = 1.2, the relative value for initial energy and recovery heat for a working contraction would be 1.0 and 1.2 respectively (the recovery heat in isometric contraction is 1.0). Therefore, in a working contraction, the extra recovery heat is 0.2, compared with isometric contraction. This extra recovery heat includes two parts: heat of process (a) and heat of process (b), these two are equal to each other because the recovery heat after PCr splitting is equal to the initial heat, as shown by the isometric results. So, this proportion of the heat from process (a) to the observed heat from the initial

process in the working contraction is 0.1. This means that 10% of the ATP splitting is delayed in the working contraction. Consider how many crossbridge cycles are involved in a working contraction of 0.5 second; take the initial energy as 30 mJ per gram wet weight muscle and ΔH_{PCr}=37 mJ/μmol, the total crossbridge cycles involved are about 0.8 μmol/g. The cycles which result in the delayed ATP split are 10% of these, i.e. about 0.08 μmol/g which is about 30% of the crossbridges in a muscle. Therefore, this is a reasonable value.

The assumption that during shortening a crossbridge reaction occurs that is reversed after shortening is not novel. Very strong evidence for such a process occurring during rapid shortening in frog muscle has been given by Homsher et al. (1981). The novelties in this hypothesis are to assume:

1. The reaction is approximately thermally neutral in mouse muscle whereas in frog muscle it is strongly exothermic. This hypothetical difference could explain why shortening heat is much less obvious or maybe absent in mouse muscle (Gibbs and Gibson 1972). In this study shortening heat production in mouse soleus has not been systematically investigated. However some results seem to indicate that there may be a little shortening heat in mouse soleus muscle (Fig. 8.2), although the phenomenon is much less obvious than in frog muscle.

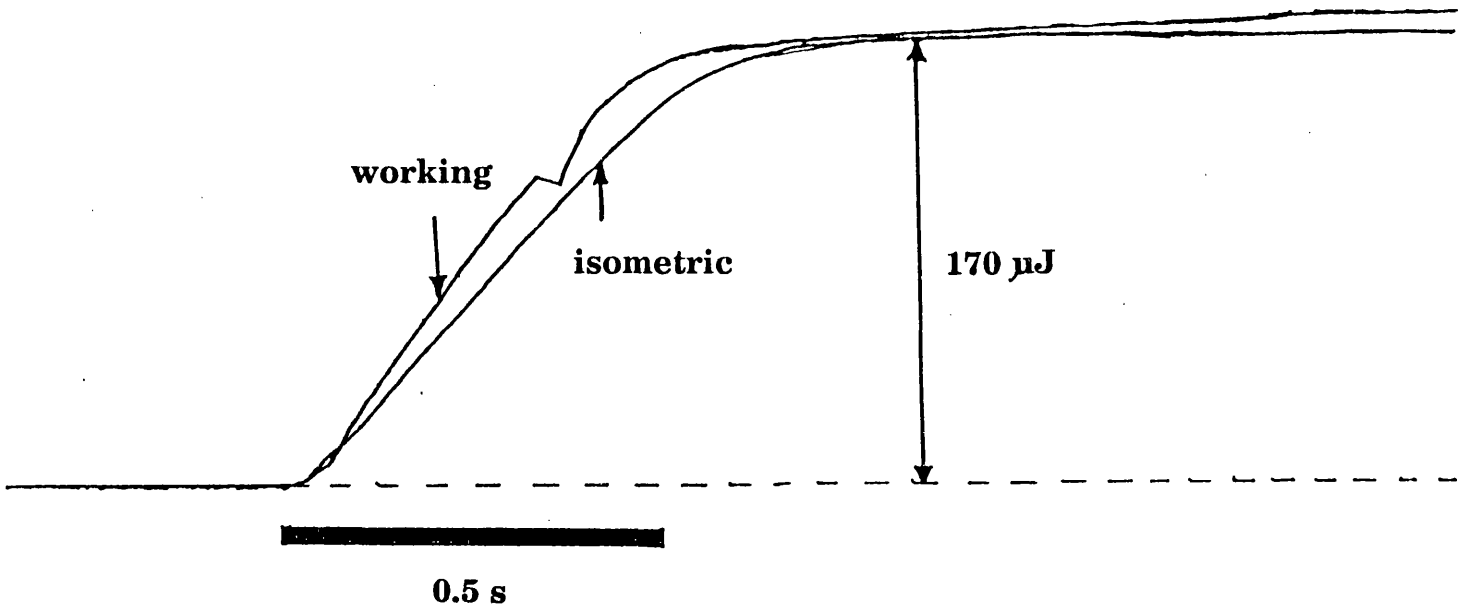


Fig. 8.2 A comparison of initial heat production from an isometric contraction with that obtained from a working contraction (mouse soleus muscle at 25 C). The bar shows the stimulation duration, during which a shortening occured. The interruption on the heat record for the working contraction is probably due to the movement of motor. The figure shows that the shortening heat in mouse soleus seems to be less obvious than that in frog muscle.

2. The process occurs during moderate speed shortening as well as during rapid shortening.

8.5 Comparison of this study with others'

Crow and Kushmerick (1982) predicted that there might be a thermal "energy balance" prevailing in mouse soleus muscle because they detected a chemical "energy balance" between initial and recovery processes. The recovery ratio found for isometric contraction in this study is 0.95 ± 0.03 (mean ± standard error of mean, 218 observations on 15 muscles), which is close to that predicted from the observed pH of about 7.2 and the values of ΔH_{PCr} reported by Woledge (Woledge & Reilly, 1987). Thus for isometric contractions, there may be a thermal "energy balance" in mouse soleus muscles. However, for working contraction, this is not likely to be the case.

Table 8.2 compares the results from Crow & Kushmerick and Gower & Kretzschmar with these in this study. There are some differences among these three works. Crow and Kushmerick's experiments were conducted at 20°C, on mouse soleus, with 66 Hz, 1.0 second tetanus duration. Gower and Kretzschmar's were at

****

Table 8.2 Comparison of results from 3 studies.

	Crow & Kushmerick	Gower & Kretzschmar	This study▲
Rates	n = 5	n = 10	n = 46
ΔPCr	2.33 ± 0.66	0.37 ± 0.03	---
ΔO_2	0.37 ± 0.10	---	---
h_i	(86 ± 24)	33 ± 2	72 ± 1
h_r	(88 ± 24)	---	88 ± 5
h_t	(175 ± 48)	---	159 ± 2
R	(1.02 ± 0.28)	---	1.22 ± 0.07

* ΔPCr & ΔO_2 in μmol/sec/g; h_i, h_r, h_t in mJ/sec/g

h_i: initial heat, h_r: recovery heat, h_t: total heat.

****

▲: The values used were from 46 observations of isometric contractions on 3 muscles which gave the same time-tension integral value as that in Crow & Kushmerick's experiments.

18°C, on rat soleus, with 20 Hz, 10.0 second tetanus duration.This study was at 25°C, on mouse soleus, with 50Hz, 0.5 second tetanus duration. The experiments chosen from this study for the purpose of this table have the same value (0.20 ± 0.01 N·m/g) as that (0.20 ± 0.11 N·m/g) in Crow and Kushmerick's experiments. In Table 8.2, values in brackets are calculated values (using Q_{10} = 3, pHi = 7.23, ΔH_{PCr} = 37 kJ/mol, P/O ratio = 6.3). The values not in brackets are experimental measurements. "n" is the number of observations.

Table 8.2 shows that the three study are basically compatible. Although the energy production in Gower and Kretzschmar's experiments was considerably lower than that in the other two studies, this is understandable. Because they used a) only 20 Hz, b) long tetanus (10 second), and c) rat soleus muscles. These three factors can explain the lower energy production in their experiments. It is known that larger animals generally have smaller metabolic rate per unit weight (Benedict 1938). So, taking the three factor into account, Gower and Kretzschmar's results are generally in line with the other two in Table 8.2 in terms of the size of the values, but the conclusion they draw is different from the other two studies. They concluded that there was an "energy imbalance" in isometric contractions.

Therefore, it is possible that in isometric contractions there is an "energy balance" prevailing in mouse soleus muscles. But, in the

case of working contraction, an unknown process may be involved. In this project, although a series of control experiments have been conducted and an independent set-up of apparatus also used, basically, only one method of investigation has been used, i.e. the thermal method.

8.6 Further experiments needed

Due to the unexpected phenomenon found in this project, namely, the difference between RRW and RRI, it is a sensible idea to reinvestigate the existence of this phenomenon by using other independent techniques. In order to clarify this situation, a chemical investigation of PCr splitting and oxygen consumption during and after working contraction is needed.

In this project, the phenomenon has only been found in mouse soleus muscles. In frog muscle, there is no such phenomenon, as was originally reported by Hartree. Hence, it is very interesting to know whether this is an exceptional case or not. In order to know this, more experiments should be conducted using a wide range of preparations obtained from different animal species.

ABBREVIATIONS

ATP: Adenosine triphosphate

PCr: Phosphocreatine

W: External work done by a shortening muscle

h_i: Initial heat, the amount of heat produced during contracting-relaxation process

E_i: Initial energy, energy produced during contractio-relaxation process, including heat and work

$$E_i = h_i + W$$

h_r: Recovery heat, the amount of heat produced during recovery process; recovery heat can be called as recovery energy.

h_t: Total heat, the amount of heat produced during contraction-relaxation-recovery process

$$h_t = h_i + h_r$$

E_t: Energy produced during contraction-relaxation-recovery process, including heat and work

$$E_t = h_t + W$$

ε_i: Initial efficiency, efficiency of contraction-relaxation process

$$\varepsilon_i = W / E_i$$

ε_t: Total efficiency, efficiency of contraction-relaxation-recovery process

$$\varepsilon_t = W / E_t$$

R: Recovery ratio, the ratio of recovery energy to initial energy

$$R = h_r / E_i$$

R_{PCr}: Recovery ratio due to PCr splitting only

RRW: Recovery ratio for working contraction

RRI: Recovery ratio for isometric contraction

ΔF_{PCr}: Molar free energy change due to PCr splitting

ΔH_{PCr}: Molar enthalpy change due to PCr splitting

ACKNOWLEDGEMENTS

I wish to thank Professor Roger Woledge for all his help and excellent supervision over last four years.

I also wish to thank all the people in this department for their various help during my study here in University College London, particularly, for all those in our laboratory.

REFERENCES

Aickin, C. C. & Thomas, R. C. (1976).

Intracellular pH of mouse soleus muscle.

J. Physiol. 260, 25-26.

Aickin, C. C. & Thomas, R. C. (1977).

Microelectrode measurement of the intracellular pH and buffering power of mouse soleus muscle fibres.

J. Physiol. 267, 791-810.

Benedict, F. G. (1938).

Vital energy: A study in comparative basal metabolism.

Washington, D. C.: Carnegie Institute of Washington. 215.

Benzinger, T. H., & Hems R. (1956).

Reversibilty and equilibrium of the glutaminase reaction, observed calorimetrically to find the free energy of adenosine triphosphate hydrolysis.

NMRI Research Report NM 000 018.17.01

Bozler, E. (1930).

The heat production of smooth muscle.

J. Physiol. (London) 69, 442-462.

Barclay, J. K. (1982).

Energetics of mammalian fast and slow twitch muscles.

Federation Proc. 41, 189-191.

Burk, D. (1929).

The free energy of glycogen-lactic acid break down in muscle.

Proc. Roy. Soc. B104, 153-170.

Cain, D. F. & Davies, R. E. (1962)

Breakdown of adenosine triphosphate during a single contraction of working muscle.

Biochem. Biophys. Res. Commun. 8, 361-366.

Crow, M. & Kushmerick, M. J. (1982).

Chemical energetics of slow- and fast-twitch muscles of the mouse.

J. Gen. Physiol. 79, 147-166.

Curtin, N. A. (1986).

Buffer power and intracellular pH of frog sartorius muscle.

Biophys. J. 50, 837-841p

Curtin, N. A. & Woledge, R. C. (1978)

Energy changes and muscular contraction.

Physiol. Rev. 58, 690-761.

Elzinga, G. & Leijendekker, W. J. (1988).

Recovery heat production of mouse extensor digitorum longus and soleus muscle in vitro.

J. Physiol. 400, 27p.

Feng, T. P. (1932).

The effect of length on the resting metabolism of muscle.

J. Physiol. (London) 74, 441-454.

Gibbs, C. L. & Gibson, W. R. (1970).

Energy production in cardiac isotonic contractions.

J. Gen. Physiol. 56, 732-750.

Gibbs, C. L. & Gibson, W. R. (1972).

Energy production of rat soleus muscle.

Am. J. Physiol. 223(4), 864-871p

Godfraind-de Becker, A. (1971)

The ratio of oxidative recovery heat to initial heat in toad sartorius.

J. Physiol. 217, 63p.

Godfraind-de Becker, A. (1973).

"La Restauration Post-tetanique du Muscle Strie Thermogenese et Fouorescence" Vander, Louvain.

Godfraind-de Becker, A. (1989)

Factors affecting aerobic recovery heat production and recovery reatio of frog sartorius.

J. Physiol. 419, 455-475.

Gower, D. & Kretzschmar, K. M. (1976).

Heat production and chemical change during isometric contraction of rat soleus muscle.

J. Physiol. 258, 659-671.

Hartree, W. & Liljestrand, G. (1926)

The recovery heat production in tortoise's muscle.

J. Physiol. 62, 94-97.

Hartree, W. (1928).

The identical source of work and heat in muscular contraction.

J. Physiol. 65, 385-388.

Heglund, N. C. & Cavagna, G. A. (1985).

Efficiency of vertebrate locomotory muscles.

J. Exp. Bio. 115, 283-292.

Heglund, N. C. & Cavagna, G. A. (1987).

Mechnical work, oxygen consumption, and efficiency in isolated frog and rat muscle.

Am. J. Physiol. 253(22), C22-C29.

Hill, A. V. (1939).

Recovery heat in muscle.

Pro. Roy. Soc. B, 127, 297-307p

Hill, A. V. (1964).

The efficiency of mechanical power development during muscular shortening and its relation to load.

Pro. Roy. Soc. London ser. B 159, 319-324p

Hill, A. V. (1965).

'Trails and Trails in Physiology'. Arnold, London.

Homsher, E., Irving, M. & Wallner, A. (1981).

High energy phosphate metabolism and energy libration associated with rapid shortening in frog skeletal muscle.

J. Physiol. 321, 423-436.

Huxley, A. F. (1957).

Muscle structure and theories of contraction.

Progress in Biophysics, 7, 255-318.

Infante, A. A. & Davies, R. E. (1965).

The effect of 2,4-dinitrofluorobenzene on the activity of striated muscle.

J. Biol. Chem. 240, 3996-4001.

Keden, O. & Caplan, S. R., (1965).

Degree of coupling and its relation to efficiency of energy conversion.

Trans. Faraday Soc. 61, 1897-1911.

Klug, G., Reichmann, H. & Rette, D. (1983)

FEBS Lett. 152, 180-182.

Kretzschmar, K. M. & Wilkie, D. K. (1972).

A new method for absolute heat measurement utilizing the Peltier effect.

J. Physiol. (London)224, 18-21.

Kretzschmar, K. M. & Wilkie, D. K. (1975).

The use of the Peltier effect for simple and accurate calibration of thermoelectric devices.

Pro. Roy. Soc. London Ser. B 190, 315-321.

Kushmerick, M.J. & Davies, R. E. (1969).

The chemical energetics of muscle contraction. II. The chemistry, efficiency and power of maximally working sartorius muscles.

Pro. Roy. Soc. London Ser. B 174, 315-353.

Mast, F., Elzinga, G. & Woledge, R. C. (1989).

Analysis of thermopile records from contracting isolated cardiac muscle.

Am. J. Physiol. (1990, in press)

Nwoye, L. O. & Goldspink, G. (1981).

Biochemical efficiency and intrinsic shortening speed in selected vertebrate fast and slow muscles.

Experientia 37, 856-857.

Ricchiuti, N. V. & Mommaerts, W.F.H.M. (1965).

Technique for myothermic measurements.

Physiologist 8, 259.

Smith, S. J. & Woledge, R. C. (1982).

Thermodynamic analysis of calcium binding to frog parvalbumin.

J. Muscle Res. Cell Motil. 3, 507

Somlyo, A. V. et al (1981).

Calcium realease and ionic changes in the sarcoplasmic reticulum of tetanized muscle: An Electron-Probe Study.

J. Cell Biol. 90, 577-594.

Wendt, I. R. & Gibbs, C. L. (1976)

Recovery heat production of mammalian fast- and slow-twitch muscles.

Am. J. Physiol. 230(6), 1637-1643.

Whipp, B. G. & Wasserman, K. (1969).

Efficiency of muscular work.

J. Appl. Physiol. 26, 644-648.

Wilkie, D. R. (1960).

Thermodynamics and interpretations of biological heat measurements.

Prog. Biophys. Biophys. Chem. 10, 259-298.

Wilkie, D. R. (1974).

The efficiency of muscular contraction.

J. Mechanochem. Cell Motility. 2, 257-267.

Wilkie, D. R. & Woledge, R. C. (1967).

The application of irreversible thermodynamics to muscular contraction. Comments on a recent theory by S. R. Caplan.

Proc. Roy. Soc. B. 169, 17-29.

Woledge, R. C. (1966).

The mechanical and thermal properties of skeletal muscles of the tortoise.

Ph.D thesis, The University of London.

Woledge, R. C. (1971).

Heat and chemical change in muscle.

Prog. Biophys. Mol. Biol. 22, 37-74.

Woledge, R. C., Curtin, N. A. & Homsher, E. (1985).

"Energetic aspects of muscle contraction".

Academic Press, London.

Woledge, R. C. & Reilly, P. J. (1987).

Molar enthalpy change for the hydrolysis of phosphorylcreatine under the condition in muscle cells.

Biophys. J. 54, 97-104.

Woledge, R. C. et al (1988)

The energetics of work and heat production by single muscle fibres from the frog.

"Molecular mechanism of muscle contraction" (in press)

Woledge, R. C. (1989a)

Energy transformation in living muscle.

"Energy transformations in cells and organisms"

(Proceedings of the 10th conference of the European Society for Comparative Physiology and Biochemistry)

Edited by Wolfgan Wieser & Erich Gnaiger, Geoger Thieme Verlag Stuttgart, New York.

Woledge, R. C. (1989b).

Calorimetric studies of initial and recovery processes in muscle.

"Energy transformations in cells and organisms"

(Proceedings of the 10th conference of the European Society for Comparative Physiology and Biochemistry)

Edited by Wolfgan Wieser & Erich Gnaiger, Geoger Thieme Verlag Stuttgart, New York.

Woledge, R. C. & Yin, C. (1989).

A comparison of recovery heat in mouse soleus muscle following shortening and isometric tatani.

J. Physiol. 415, 28p