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Abstract

The addition of propionate to the extracellular solution has been shown to cause decreases in intracellular pH in a large variety of tissues (Marrannes et al., 1979; De Hemptinne and Marrannes, 1979; Sharp and Thomas, 1981).

The substitution of chloride ions with 20 mM propionate in a 25 mM MOPS Ringer's solution at pH 6.80 was found in the present experiments to decrease the intracellular pH of resting frog sartorius muscles 0.2 pH units. Intracellular pH was determined using the distribution ratio of the weak acid DMO. Under similar conditions propionate was taken up by the muscle fibers resulting in an intracellular concentration of 10.5 ± 0.6 mM (SEM) after 60 minutes of equilibration.

In light of these results the following research questions were proposed; 1) What is the effect of externally administered propionate on lactate efflux from isolated frog sartorius muscles?, and 2) What model of lactate efflux is consistent with the effect of externally administered propionate on lactate efflux from isolated frog sartorius muscles?

Propionate added to the extracellular fluid bathing isolated frog sartorius muscles during recovery from electrical stimulation increased whole muscle lactate efflux. The magnitude of this increase was found to be buffer concentration dependent within the MOPS buffer concentrations 1 to 25 mM. The portion of the effect not attributed

to an extracellular pH change was determined by extrapolating the results to an infinite buffer concentration. At this buffer concentration propionate increased whole muscle lactate efflux 1.8 times control efflux values while estimated fiber efflux increased 2.1 times control values. The permeability coefficient for lactic acid, estimated from the estimated fiber efflux data was found to compare favorably with the value reported by Wolosin and Ginsburg (1975) for artificial lipid membranes.

The results from the post experiment tissue lactate analysis revealed that tissues exposed to extracellular solutions containing propionate had a significantly lower lactate level than did tissues exposed to control solutions. These results are consistent with the increase in lactate efflux observed after the substitution of chloride with propionate in the extracellular solution.

On the basis of these results and the work of Mainwood and Worsley-Brown (1975) it is suggested that lactate crosses the sarcolemma of frog sartorius muscles in association with hydrogen ions, most likely as lactic acid. The magnitude of this effect is transmembrane pH dependent.

Introduction

Lactic Acid: A Brief Introduction

Araki (1891a,b) was one of the first physiologists to conduct experiments into the mechanisms of lactate formation after Berzelius in 1841 reported finding lactic acid in the muscles of exhausted game (du Bois-Reymond, 1877). Araki found increases in lactic acid in blood and urine of many animals exposed to hypoxic conditions.

Fletcher and Hopkins (1907) defined in greater detail the effects of hypoxia and stimulation on lactate accumulation in muscle and demonstrated the removal of lactate from frog skeletal muscle when tissues were exposed to high oxygen tensions.

Hastings (1921) suggested that oxygen insufficiency may play a key role in lactate appearance in blood after brief bouts of intense exercise. Due to the fact that lactate levels in the blood were not elevated after moderately intense exercise, hypoxia due to circulation insufficiencies at higher work loads was thought to be the cause.

Groag and Schwarz (1927) further implicated the circulatory system in lactate accumulation when they showed that human subjects suffering from cardiac failure had higher blood lactate levels at a given submaximal work load than did healthy subjects.

Collazo and Lewicki (1925) presented data which failed to fit the theory of hypoxia being the cause of lactate formation in the body. It was found that normal subjects given 100 g of sucrose orally developed elevated blood lactate levels.

In addition high carbohydrate diets were found to have the same effect.

At about the same time, Meyerhof and Lohman (1926) working with fatigued amphibian muscle and Furasawa and Kerridge (1927) working with fatigued mammalian muscles, showed a reduction in pH of water extracts. This strongly suggests that it is lactic acid which is produced in active muscle.

The appearance of lactate ions in blood and surrounding fluids as reported, strongly suggests that the muscle fiber membrane is permeable either to the lactate ion or the lactic acid molecule. After more than 50 years the permeable species and its method of crossing the membrane is still debated in the literature.

Accumulation of Lactate in Muscle and Blood

Two conditions that lead to increases in intracellular lactate in muscle tissue are electrical stimulation and anoxia. These experimental techniques have enabled researchers to study the normal and elevated lactate levels in muscle and blood.

Sacks and Morton (1956) found that tetanic contraction induced by electrical stimulation of isolated cat gastrocnemius muscle increased lactic acid and pyruvic acid levels. Tissue lactate values went from 1.7 $\mu mol \cdot g^{-1}$ at rest to 24.9 $\mu mol \cdot g^{-1}$ after 30 seconds of tetanic stimulation. Repeated single twitches continued long enough to produce a steady state, resulted in tissue lactate levels rising to 17.6 $\mu mol\ g^{-1}$.

Corsi et al. (1969) using an isolated dog hindlimb preparation electrically stimulated with tetanic pulses, found that after 2 minutes of stimulation, muscle lactate reached a maximum of 20.1 $\mu mol \cdot g^{-1}$ and

then slowly declined. Resting control values of 1.5 $\mu mol \cdot g^{-1}$ were reported.

Karlsson et al. (1972b) used an isolated dog gracilis preparation and reported intracellular lactate levels in excess of 25 $\mu mol \cdot g^{-1}$ in some preparations. Resting values ranged from 0.7 to 3.9 $\mu mol\ g^{-1}$.

Hirche and his co-workers (1973) found high levels of lactate in isolated dog gastrocnemius muscles after the onset of electrical stimulation. Within 2 minutes of the onset of tetanic contractions the mean tissue lactate level rose from 3.5 $\mu mol \cdot g^{-1}$ at rest to 17.5 $\mu mol \cdot g^{-1}$. In accordance with the findings of Corsi et al. (1969) the intracellular lactate levels declined after 2 minutes of stimulation. Similar results were reported by Hirche et al. in 1975.

Similarly, high tissue lactate levels have been reported in experiments with human subjects. Diamant et al. (1968) have reported mean tissue lactate values of 19.1 $\mu mol \cdot g^{-1}$ (range 11.2 to 24.6 $\mu mol \cdot g^{-1}$) in biopsy samples taken from the M. quadriceps femoris after an exhaustive 3 minute work bout. Mean resting values were reported to be 3.9 $\mu mol \cdot g^{-1}$ (range 1.8 to 5.2).

Bergstrom and his research associates (1971) reported values of 12 $\mu mol \cdot g^{-1}$ fat free tissue in human biopsy samples obtained after 17 to 20 min of bicycle ergometer exercise at 1,000 $kpm \cdot min^{-1}$. Mean resting values of 0.8 $\mu mol \cdot g^{-1}$ fat free tissue were reported. In addition, a decline in tissue lactate was evident 17 to 20 min after the onset of exercise. This decline is in agreement with the results of Corsi et al. (1969) and Hirche et al. (1973, 1975), both of whom

found similar declines after the 2 minute exercise peak of muscle lactate in dogs.

Karlsson (1971a) and a research group led by Karlsson (1975), as well as Hermansen and Vaage (1977) have reported human skeletal muscle lactate values in excess of 25 $\mu mol \cdot g^{-1}$. Similarly, high values were also reported by Linnarsson et al. (1974). A large variation in muscle lactate values after maximal exercise has been reported however, even within the same research group. Between 1970 and 1975 Karlsson had reported peak human muscle lactate levels between 14.8 and 25 $\mu mol \cdot g^{-1}$. The variation is thought to be due to both subject variations and the mode and intensity of the exhaustive exercise employed (Karlsson and Saltin, 1970, 1971; Karlsson, 1971a,b,c; Karlsson et al., 1971, 1972a). Mean peak values appear, however, to be more of the order of 20 to 25 $\mu mol \cdot g^{-1}$ at a work load taxing 100% of maximum oxygen consumption or 100% maximal voluntary contraction (Karlsson et al., 1972a; Edwards et al., 1972).

Mean resting values are of the order of 1.0 to 2.0 $\mu mol \cdot g^{-1}$ with the increasing lactate levels being a nonlinear function of the work load (Karlsson, 1972a).

Similarly, high values have been reported in frog skeletal muscle as well. Mainwood et al. (1972, 1975) have reported peak levels between 20 and 25 $\mu mol \cdot g^{-1}$ in isolated, electrically stimulated sartorius muscles. Fitts and Holloszy (1976) found peak values of approximately 17 $\mu mol \cdot g^{-1}$ and resting values of 3.5 $\mu mol \cdot g^{-1}$. As with other species, it appears that the magnitude of the accumulation

of tissue lactate is a function of the stimulus parameters employed.

It would appear however, that the reported peak tissue lactate level in many species is very similar, with 20 to 25 $\mu mol \cdot g^{-1}$ being a reasonable estimate of the mean peak value. A value of between 1.0 and 4.0 $\mu mol \cdot g^{-1}$ is a reasonable estimate of resting tissue lactate levels.

Concomitant with this increase in intracellular lactate has been the observation of an increase in plasma lactate (Sacks and Morton, 1956; Diamant et al., 1968; Bergstrom et al., 1971; Karlsson et al., 1972a, 1972b, 1975; Hirche et al., 1973, 1975; Jorfeldt et al., 1978). On the basis of the change in plasma lactate concentration, many investigative groups have calculated muscle lactate efflux rates.

Lactate Efflux From Muscle Tissue

As efflux rates are a function of intracellular lactate levels, the stimulation rate of experimental tissues and the length of the stimulation interval play a key role in the rates of efflux observed in the literature. Due to the large variation in experimental models and protocols it is difficult to obtain agreement as to a maximum lactate efflux rate.

Hollanders (1968) reported efflux rates from electrically stimulated, perfused rat diaphragms of 1.4 $\mu mol \cdot g^{-1} \cdot min^{-1}$ (tetanic contractions). This is considerably higher than the mean value of 0.4 $\mu mol \cdot g^{-1} \cdot min^{-1}$ reported by di Prampero et al. (1970) for perfused dog gastrocnemius muscles (1 tetanus every 3 seconds). Graham et al.

(1976), using an isometric dog gastrocnemius preparation, reported a mean maximum efflux value of 0.48 $\mu mol \cdot g^{-1} \cdot min^{-1}$ when the nerve ending was stimulated at a frequency of 5 twitches per second. Hirche et al. (1975) have reported efflux values in excess of 3.0 $\mu mol \cdot g^{-1} \cdot min^{-1}$ in isotonically working, perfused dog gastrocnemius muscle stimulated at a rate of 86 tetani per minute. Mainwood and Worsley-Brown (1975) have reported some efflux rates from isolated frog sartorius muscles in excess of 0.75 $\mu mol \cdot g^{-1} \cdot min^{-1}$ when the tissues were exposed to alkaline solutions. Mean values are more in the range of 0.1 to 0.25 $\mu mol \cdot g^{-1} \cdot min^{-1}$ under physiological conditions of buffer concentration and pH.

Jorfeldt and his co-workers (1978) have reported a mean maximum efflux rate of lactate from the leg of exercising human subjects of 4.5 $mmol \cdot min^{-1}$. This is in agreement with the earlier work by Wahren et al. (1974) who found that most human subjects tested using a similar protocol had a maximum release rate of between 4 and 5 $mmol \cdot min^{-1}$. These values would be reduced considerably if normalized per gram of metabolising tissue.

In addition to the release rate of 4.5 $mmol \cdot min^{-1}$ reported by Jorfeldt et al., another important fact was evident. The release rate of lactate from the muscles of the leg rose approximately linearly with the muscle lactate concentration up to the value of 4.5 $mmol \cdot min^{-1}$, after which no further increase in efflux was evident despite a further increase in muscle lactate. The authors suggested that a translocation hindrance existed within the muscle as blood

flow and oxygen consumption in the leg continued to increase with subsequent workloads. The authors were unable to pinpoint the location of the hindrance. The hindrance may be in the cell membrane ie. a carrier process, or extracellular ie. inadequate diffusion or changes in the extracellular medium.

Karlsson et al. (1972b) used an isolated dog gracilis preparation and reported intracellular lactate levels in excess of 25 $\mu mol \cdot g^{-1}$ wet wt. Although a marked concentration gradient for lactate existed between muscle and blood, it was clear that venous lactate concentrations were not linearly related to muscle lactate when muscle levels exceeded 5 to 10 $\mu mol \cdot g^{-1}$ wet wt. This report suggests that the release of lactate from skeletal muscle was not entirely a function of the lactate gradient but may be dependent upon the interactive effects of other factors.

Karpatkin et al. (1964) suggested that a saturation type of efflux mechanism may be operating in the lactate efflux process from frog sartorius muscles. The authors reported a Vmax value of 0.8 $\mu mol \cdot ml^{-1}$ of intracellular $H_2O \cdot min^{-1}$. Kubler et al. (1966) have postulated that an active transport system might be involved in cardiac tissue.

Lactate efflux will be linearly related to the internal lactate ion concentration only if the ionic species is responsible for the measured efflux and the membrane potential and external lactate ion concentration are constant. With a pK of 3.7 (Lockwood et al.,1965), approximately 99.9% of the total intracellular lactate will be in the

ionic form. If, however, the small remaining fraction of lactate in the undissociated acid form is responsible for the measured efflux then the efflux will be linearly related to the total lactate gradient only if intracellular and extracellular pH are constant. This is clearly not the case during muscular contraction. This argument does not deny the existence of a carrier facilitated mechanism for lactate transport but suggests rather, that simultaneous drops in pH can produce "saturation-like" efflux kinetics if lactic acid can diffuse across the membrane rapidly.

It has also been suggested that the limited lactate efflux may be a function of changes in lactate diffusion through the extracellular space. Recall that lactate release from skeletal muscle is a two step process. Step one involves the passage through the membrane, while step two involves diffusion through the extracellular space. One of the earliest mechanisms proposed to explain the limited lactate efflux after fatigue in frog sartorius muscles was a swelling of the fibers. This was postulated to interfere with the diffusion of lactate through the extracellular space (Eggleton et al., 1928).

If however, step one of the lactate efflux process is rate limiting, then determination of the mechanism will rely heavily on knowledge of the species of lactate (ionic, nonionic) crossing the membrane.

The Movement of Lactate Across Cellular Membranes

The study of monocarboxylate movements across cellular membranes has been the focal point of investigation for many research groups since the early seventies. Two monocarboxylates of major interest have been lactate and pyruvate. The existence of a possible carrier system for the anionic transfer of these compounds has received much interest due to the successful identification and characterization of the transport protein mediating chloride-bicarbonate exchange in erythrocytes. The role played by this anion exchange system in lactate efflux has been determined by the use of specific inhibitory agents such as stilbenedisulfonate compounds (DIDS-4,4'-diisothiocyano-2,2'-stibenedisulfonic acid; DNDS-4,4'-dinitro-2,2' stilbenedisulfonic acid) (Lepke et al., 1976; Cabantchik et al., 1978). SH-group modifying mercurials (PCMBS-p-chloromercuriphenylsulphonic acid; PCMB-p-chloromercuribenzoate; Hg^{2+}) and dithiol compounds (DTNB-5, 5'-dithio-bis(2-nitrobenzoate); DTDP-4,4'-dithiodipyridine; several analogues) block that component of monocarboxylate transfer that is insensitive to stilbenedisulphonates while not affecting, with the exception of DTDP, the anion exchange system (Deuticke et al., 1982; Deuticke et al., 1978). This inhibitory pattern can be used to discriminate carrier pathways which mediate monocarboxylate and in our case lactate transfer.

Halestrap and Denton in 1974 were the first to reveal that pyruvate transport into rat liver cells and human erythrocytes could be substantially inhibited. Lactate uptake was also inhibited in

the erythrocyte experiments. Halestrap (1976) later partitioned out two carrier mechanisms for lactate and pyruvate in human erythrocytes using more specific inhibition. More recently, the work of Deuticke Beyer and Forst (1982) has resulted in a clearer understanding of the mechanisms active in lactate transport in human erythrocytes.

Deuticke et al. (1982) have shown that inhibition of the anion exchange system in erythrocytes can reduce lactate efflux. While the transfer of sulphate and oxalate was completely reduced in the presence of anion exchange inhibitors, the transfer of both lactate and glycolate was only partially inhibited. As well, the addition of mercurial compounds could further decrease lactate efflux. This further decrease in lactate efflux was found to be independent of chloride levels extracellularly. This provided more evidence for a second independent mechanism capable of transferring lactate. The clue to the nature of this specific monocarboxylate (versus anion) transfer system was to come from the work of Dubinsky and Racker (1978) and Spencer and Lehninger (1976). Dubinsky and Racker reported synchronous movements of H^+ (or OH^-) and lactate across the human erythrocyte membrane. Spencer and Lehninger working with Ehrlich ascites-tumour cells found similar results. In addition Deuticke and his co-workers (1982) studied the influence of extracellular pH modifications on the SH dependent efflux of lactate in erythrocytes and found that this transport mechanism reacted in a manner consistent with lactate/H^+ cotransport or lactate/OH^- exchange.

A small residual lactate efflux, ie. that remaining after both transport systems were inhibited was found to exist after maximum inhibitory agents were applied. Deuticke et al. (1982) suggested that a good candidate for the remaining inhibitor insensitive component of the lactate transfer may be nonionic diffusion of lactate via the lipid portion of the cellular membrane. This assumption was born out in that the pH dependency of the inhibitory insensitive lactate efflux was quite in agreement with nonionic diffusion of lactate through the lipid domain of the erythrocyte membrane. This was further born out by the increase in inhibitor insensitive lactate efflux in the presence of hexanol which has been shown to accelerate non-electrolyte diffusion in membranes while inhibiting protein-mediated transport (Deuticke, 1977). The possibility of nonionic diffusion of weak acids is not a new issue, however.

It has long been suspected that nonionic diffusion of the undissociated acid through the lipid phase of the membrane may account for lactate diffusion. Overton (1897) observed the influx of the weak acids, lactic and acetic acids into frog muscle. The author used osmotic swelling as an index of entry and found that the swelling was more pronounced in lactic acid and acetic acid solutions than in solutions of stronger acids. These results were explained by assuming membrane permeability to only the undissociated

molecule. Marrannes and De Hemptinne (1978) studied the effects of acid solutions on transient changes in conduction velocity in isolated cardiac fibers. The smaller the pK value of the anion, the smaller were the observed changes. The changes were absent when the fibers were placed in solutions of methanesulfonate or α-Ketoglutarate whose concentration of undissociated acid is neglibible. In addition, Marrannes and his co-worker found that between acids of approximately similar pK values, a relationship between the magnitude of the velocity changes and the lipid/water partition ratio of the organic acid existed. This suggests that the changes depend on the penetration of the undissociated form of the acid through the cell membrane. Work by De Hemptinne and Marrannes (1979) and Marrannes et al. (1979) has shown that rat soleus muscles and isolated heart fibers exhibit a drop in intracellular pH and a transient increase in extracellular surface pH when exposed to propionate. These findings are consistent with the concept of nonionic diffusion of propionic acid across the membrane.

It would appear then, that three methods of transporting or removing lactate from erythrocytes exists. They are nonionic diffusion, anion exchange (inhibited by stilbenedisulphonates) and a specific monocarboxylate transport (inhibited by SH-group modifying mercurials and dithiol compounds). The specific monocarboxylate transport system is coupled to intracellular acid base regulation and like monionic diffusion of lactate, is

transmembrane pH dependent. At present it is unclear as to whether specific monocarboxylate transport exists in skeletal muscle. Although an anion exchange system has been found in skeletal muscle (Aickin and Thomas, 1977), the role it may play in lactate transport is not known.

It is known that plasma lactate levels increase during intense exercise and that plasma bicarbonate levels decline. The question that arises is the degree of coupling of hydrogen ion release with lactate release from skeletal muscle. Many research groups have, however, studied the relative efflux rates of lactate and hydrogen ions from skeletal muscle in an effort to understand the species of lactate crossing skeletal muscle membranes and the method of release. As cited earlier, the work of Overton (1897), Marrannes and De Hemptinne (1978), Marrannes et al. (1979) and De Hemptinne and Marrannes (1979) suggests that weak organic acids such as lactic acid and propionic acid, cross the skeletal muscle membrane in the nonionized form or, in unison with net H^+ movements. It should be noted that differentiation of nonionic diffusion of compounds from carrier mediated OH^- exchange or H^+ cotransport is difficult. Both systems are transmembrane pH dependent, therefore pH dependency will not distinguish the transport systems from nonionic diffusion. Carrier inhibition is clearly the easiest method of separation.

Hill and his co-workers (1924) and Bock and his co-workers (1927) observed an extensive shift of the CO_2 dissociation curve of human blood in intense exercise. Hill concluded that lactate ions

reached the blood as lactic acid and Henderson, an original member of Bock's research group, concluded the same in 1928.

Much more recently, Roos (1975) concluded that it was predominantly the undissociated lactic acid molecule rather than the much more numerous lactate ions which permeated the fiber membrane of rat diaphragm. This was determined on the basis that the steady-state lactate distribution ratio was correlated with the transmembrane pH gradient rather than the membrane potential. Correlation with the membrane potential would suggest ionic movement of lactate. Roos used the distribution of the weak acid 5,5-dimethyl-2,4-oxazalidine dione (DMO) to compute the intracellular pH of the diaphragm fibers. Hinke and Menard (1976) have since reported comparison results of intracellular pH measured with DMO and microelectrodes. The error in intracellular pH estimates from DMO studies are such that the conclusions of Roos with respect to the role of pH in the steady state distribution of lactate may be incorrect.

Steinhagen et al. (1976) working with isolated dog gastrocnemius muscles showed an undetermined level of synchronous release of lactate and hydrogen ion in that reductions in interstitial hydrogen ion were mirrored by reductions in lactate release.

The work of Heisler (1972) on isolated rat diaphragm muscles stimulated in hypoxia revealed a somewhat different pattern. The efflux of hydrogen ions was much faster than that of lactate. The equilibration half time was less than 1 minute for hydrogen ions and approximately 6 minutes for lactate. Similar results were found by

Benade and Heisler in 1978, with isolated rat diaphragms and frog sartorius muscles. The results showed that the rate of efflux of hydrogen ions exceeded that of lactate ions by factors of about 14 and 50 in the case of diaphragm and sartorius muscle respectively. It is important to remember that the release of compounds from muscle tissue involves both the passage through the membrane and the diffusion through the interstitial space to the blood or bulk phase. The diffusion characteristics of lactate, hydrogen ion and the buffer species binding the proton may in fact be very different. This would result in different efflux kinetics through the interstitial space despite the fact that the proton may have crossed the membrane in conjunction with the lactate ion. In addition one must not neglect the possible mechanisms of hydrogen ion release which are independent of lactate release. Included in these systems are the Cl^-/HCO_3^- exchange and Na^+/H^+ exchange both of which have been found to exist in mammalian skeletal muscle (Aicken and Thomas, 1977).

The work of Mainwood and Worsley-Brown in 1975 clearly adds to the apparent controversy surrounding the release of hydrogen ion and lactate from skeletal muscle. In these experiments with isolated frog sartorius muscles, net hydrogen ion and net lactate release from electrically stimulated muscles was determined. At a low buffer concentration and an external pH of 8.0 appreciably more lactate entered the bathing medium than did hydrogen ion. While at a high buffer concentration and a similar external pH, larger amounts of hydrogen ions were released from the muscle, the ratio never became

one. The finding of a greater release of lactate than hydrogen ion is in direct opposition to the findings of Heisler (1972) and Benade and Heisler (1978)(cited earlier). These authors found that hydrogen ion release was greater than lactate release. More direct evidence for the independent movement of lactate ions comes from the effect of membrane depolarization on lactate efflux. Mainwood and Worsley-Brown (1975) found that frog sartorius membrane depolarization with potassium sulphate at a high buffer concentration (25 mM) and an external pH of 8.0 resulted in little if any decrease in lactate efflux. Similar experiments with the contralateral muscle bathed in a weakly buffered solution (1 mM) at the same external pH resulted in a 50% reduction in efflux. These results are consistent with 80-90% of the measured efflux in weakly buffered solutions being due to ionic movements of lactate. At high buffer concentrations, less than 10% of the total lactate efflux appears to be due to ionic movement of lactate as membrane potential has very little effect on the measured efflux

The Effect of Extracellular pH and Buffer Concentration on Lactate Efflux in Skeletal Muscle

MacLeod and Hoover (1917) have reported an increase in blood lactic acid following intravenous injections of sodium carbonate. MacLeod and Knapp (1918) found that alkaline venous injections of sodium carbonate resulted in considerable increases in urine lactic acid. Similar results with bicarbonate injections were obtained in artificial respiration experiments by Gesell et al. (1930).

Eggleton and Evans (1930) found that under-ventilation or hydrochloric acid injections lowered blood lactate whereas over-ventilation or sodium bicarbonate injections augmented blood lactate levels. These findings led the authors to state that "the important factor is not the bicarbonate concentration but the H^+ ion concentration or what amounts to the same thing; the acid-base ratio". The relationship does not appear to be consistent across all stimuli, however. Johnston and Wilson (1930) found an increase in blood lactic acid associated with increased acidity after hemorrhage in normal unanesthetized dogs. Gesell et al. (1932) found increases in blood lactic acid following sodium cyanide induced acidity. Bode et al. (1932) showed that blood lactic acid levels in man is related to more than simple hydrogen ion shifts. Alkalosis induced by ingestion of sodium bicarbonate and acidosis produced by ammonium chloride had no effect on the lactate concentration of blood.

Interpretation of this type of work is difficult. Although it appears that blood pH may play a role in the ability of lactate to be released from tissues, the effects of increased production of lactate on blood concentrations cannot be determined. An increase in intracellular pH due to bicarbonate movements can result in an acceleration of glycolysis due to the pH sensitivity of the rate limiting phosphofructokinase (PFK) reaction (Mansour, 1963; Danforth, 1965; Trivedi and Danforth, 1966). In addition, hypoxia due to cyanide or impaired blood flow is also a factor that may stimulate glycolysis. This glycolytic acceleration could result in increased intracellular

lactate production and ultimately increased blood lactate levels. Whether the changes in blood concentrations are a function of pH induced increases in production or release, or a combination of the two mechanisms, is unclear. Haldi (1933) has shown an increase in blood lactate following intravenous injections of sodium bicarbonate. Concomitant with this finding was the finding of parallel increases in tissue lactate.

These types of findings certainly confuse the interpretation of the role of extracellular pH in regulating lactate efflux (recall that efflux is computed on the basis of the rate of change of concentration).

Much more recently Mainwood et al. (1972) have found that isolated frog sartorius muscles fatigued and allowed to recover in a 25 mM bicarbonate solution showed a much greater loss of lactate than did muscles allowed to recover in a 1 mM solution. No differences in intracellular lactate were found immediately after the fatigue stimulation. This clearly showed that the influence of the bicarbonate concentration was not on production, but rather on release. This type of finding suggested to the authors that external bicarbonate limits the efflux of lactate from frog muscle.

Hirche et al. (1975) studied the efflux of lactate from the isolated working gastrocnemius of dogs during metabolic alkalosis and acidosis. Metabolic alkalosis was induced by sodium bicarbonate or tris hydroxymethyl aminomethane (THAM) infusion while acidosis was induced by hydrochloric acid infusion. It was reported that in

alkalosis (pH ≈ 7.5) the efflux rate of lactate was nearly three times higher than in the acidotic condition (pH ≈ 7.0 - 7.1) despite the fact that intracellular lactate concentrations were initially similar. The authors concluded that lactate efflux rates across muscle cell membranes is increased by high extracellular bicarbonate values in combination with low hydrogen ion activity and vice versa.

Steinhagen et al. (1976) working with an isolated working, blood perfused dog gastrocnemius muscle found that sodium bicarbonate induced alkalosis increased lactate efflux rates while L-arginine-hydrochloride induced acidosis suppressed efflux. Intracellular lactate levels were not reported, however. This makes the interpretation somewhat difficult as the changes in efflux rates of lactate may have been mediated directly through the blood pH change or via modifications in intracellular lactate due to the extracellular pH influence on intracellular metabolic processes as suggested previously.

Spriet et al. (1983) and Heigenhauser et al. (1983) have presented results in agreement with the work of Hirche and his co-workers (1975). Using a perfused rat hindquarter preparation, Spriet found that perfusate acidosis (decreased bicarbonate or increased PCO_2) reduced lactate efflux from the electrically stimulated muscle groups (stimulated for 20 min). Decreases in pH induced by bicarbonate decreases resulted in consistently lower effluxes over 20 minutes. Decreases in pH induced by increased PCO_2 suppressed effluxes only initially. Heigenhauser (same laboratory) found that after 5 minutes the intracellular lactate

concentrations of some muscle groups of the hindlimb preparation perfused with acidic solutions (decreased bicarbonates) were lower than control values. After 20 minutes, however, no significant difference in intracellular lactate were reported. It would appear that the reduction in lactate efflux after 20 minutes seen by Spriet under acidic conditions induced by bicarbonate decreases are not due to lower intracellular concentrations of lactate (ie. reduced gradient).

A major question which arises from the papers implicating bicarbonate in lactate efflux is determination of the origin of the effect. Is it a direct bicarbonate effect on lactate efflux or is it an extracellular or intracellular pH effect?

In 1975 Mainwood and Worsley-Brown examined the effects of extracellular pH and buffer concentration on the efflux of lactate from in vitro frog sartorius muscles. Effluxes were computed using a superfusion technique and the efflux curves were followed after raising the intracellular lactate levels by repetitive electrical stimulation for 200 seconds. Increasing the external pH from 7.0 to 8.0 resulted in a two- to three-fold increase in the peak efflux rate. The effect was found to be independent of the buffer system used. Similar results were found when buffer species with limited membrane permeability were used. This clearly suggests that the modulation of lactate efflux from frog skeletal muscle is not a specific bicarbonate effect nor an intracellular effect due to buffer diffusion altering intracellular pH. A reasonable alternative which

fits the data, is extracellular pH, be it directly or indirectly through buffer capacity (ie. low buffer capacity will result in pH shifts in the face of moderate acid loads). In agreement with this finding Sutton et al. (1981) on the basis of data obtained from human subjects suggested that the rate of lactate removal from muscle is impaired during acidosis. This is clearly in agreement with the data obtained from erythrocytes and supports the notion that lactate may be released by nonionic diffusion, hydrogen cotransport, or hydroxyl exchange (transmembrane pH dependent). This, however, is in disagreement with the finding that a membrane potential component of lactate efflux exists at low buffer concentrations (Mainwood and Worsley-Brown, 1975). One may easily suggest that lactate leaves skeletal muscle tissue in more than one form (ie. a membrane potential component and perhaps a pH dependent component) (Roos, 1975).

Models to Explain the Role of Extracellular pH in Lactate Efflux from Skeletal Muscle

On the basis of the effect of extracellular pH on lactate efflux, five models will be proposed to explain the role of extracellular pH. A rudimentary separation of the models into two groups may be made on the basis of whether hydrogen ion is removed from the intracellular space in conjunction with the lactate ion.

The Movement of Lactate Independent of Hydrogen Ion Movements

Model A - Anion Channel:

Hutter and Warner (1967a, b) and Woodbury and Miles (1973) have shown results which indicate that a relationship exists between

extracellular pH and anion conductance. Hutter and Warner found that alkaline external solutions (pH 9.8) increased Cl^- efflux from frog skeletal muscle while acidic solutions (pH 5.0) reduced the efflux. Boyle and Conway (1941) regarded the anionic diameter as a critical factor in diffusion through the membrane and favoured the view of a molecular pore or channel. It can be suggested that hydrogen ion binding to the inner membrane of the channel may be responsible for impeding anionic movements through the channel. Although it is possible that a channel may exist which allows lactate ions to pass through and that it may be interfered with by hydrogen ions, the work of Harris (1960) suggests that membrane permeability to anions larger than chloride is very low.

Ito et al. (1962) have suggested that the hydrated ion diameter of lactate is more than twice that of chloride. As reported earlier however, Mainwood and Worsley-Brown (1975) found a membrane potential component of efflux at low buffer concentrations suggesting uncoupled ionic movements of lactate.

Model B - Anion Exchange:

An alternative explanation for the extracellular pH influence on chloride fluxes arises out of the discovery of an anion exchange system as discussed earlier. The possibility of a large number of monocarboxylate compounds crossing the membrane via this mechanism has been fully discussed. It is not unreasonable to suggest that the protein moiety of the carrier may be sensitive to extracellular pH changes resulting in conformational changes due to neutralization

of fixed charges by bound hydrogen ion. The conformational changes may result in a loss of binding sites, thereby reducing lactate efflux.

Movement of Lactate in Conjunction with Net Hydrogen Ion Loss

Model C - Nonionic Diffusion:

If the undissociated lactic acid is the dominant species crossing the skeletal muscle membrane then extracellular pH would play an active role in regulating lactate efflux. For lactic acid to cross the membrane it is necessary to maintain the diffusion gradient for this species. An alkaline change in external pH would shift the lactate/lactic acid equilibrium towards lactate ion formation thereby reducing the concentration of lactic acid and increasing the transmembrane lactic acid gradient. A shift towards more acidic external solutions would create the opposite effect. In quantitative terms this is given by the Henderson-Hasselbalch equation:

$$pH = pK + \log \frac{1-\alpha}{\alpha} \quad (\text{where } \alpha = \text{undissociated fraction})$$

rearranging:

$$\alpha = 1/(1 + 10^{pH-pK})$$

It is important to note that a pH change of 0.3 pH units is all that is required to double or halve the concentration of the undissociated lactic acid species in solution.

Model D - Cotransport of Hydrogen Ion and Ionic Lactate:

Figure 1 outlines the basic characteristics of this carrier cotransport model for lactate. Extracellular alkalinization would favor the formation of the deprotonated carrier; $^{-}C^{+}$ ($^{-}C^{+}$, a carrier with a lactate and proton binding position) which is able to re-orient in the membrane. This would facilitate the return of the carrier to the inner surface.

Figure 1

Cotransport of Hydrogen Ion/Ionic Lactate

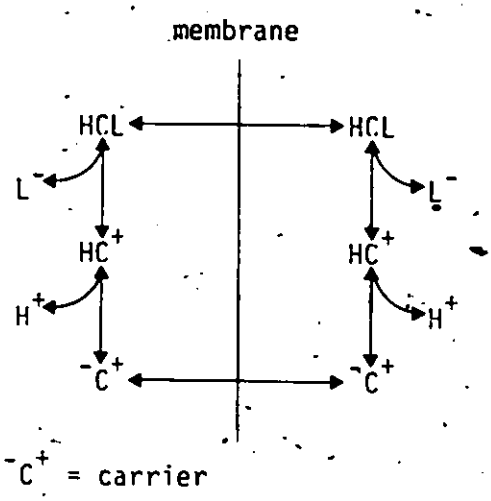
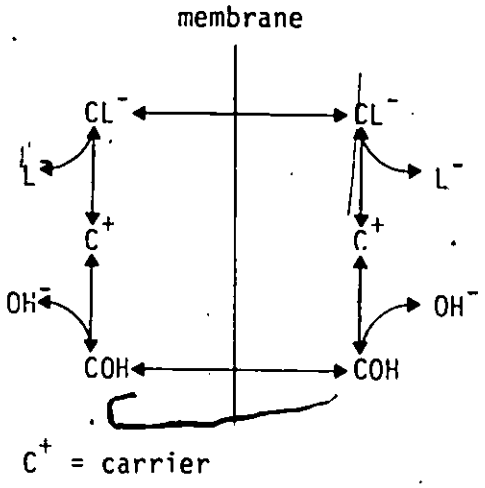


Figure 2

Lactate/Hydroxide Exchange



Figures 1 and 2 taken from Deuticke et al., 1982.

Model E - Lactate/Hydroxide Exchange:

Figure 2 outlines the basic characteristics of this carrier exchange model for lactate transport. The stimulating effect of extracellular alkalinization arises from the increasing concentration of an extracellular exchange partner for intracellular lactate. This would facilitate a carrier, mediating a lactate/hydroxide exchange to return to the inside of the fiber membrane at a higher rate.

Evidence for models C, D, or E, suggesting the movement of lactate in conjunction with a net hydrogen ion loss has been obtained in human erythrocytes and ascites-tumour cells as discussed previously. This was achieved by showing that lactate efflux was influenced by intracellular pH changes in a manner consistent with the models. In addition preliminary work by Mainwood and Worsley-Brown (1975) in frog sartorius muscles showed that intracellular acidification due to increased external PCO_2 at fairly constant extracellular pH resulted in increased efflux rates of lactate. This is consistent with a transmembrane pH dependency of lactate efflux as suggested by models C, D and E. More work is required to partition out the extracellular pH effect on lactate efflux from the intracellular effect, as the movement of an acid from the extracellular solution to the intracellular space will not only acidify the intracellular space but will cause extracellular alkalinization. Whether the increase in lactate efflux due to CO_2 movements has its origin in the intracellular or extracellular pH change remains unclear. In addition, a less volatile acidifying agent might be more appropriate, as CO_2 produces bicarbonate, a possible lactate exchange partner via the classic anion exchange

mechanism. Knowledge of the effect of intracellular pH changes at constant extracellular pH values will help us determine whether lactate efflux in skeletal muscle is transmembrane pH dependent (ie, is lactate released in conjunction with a hydrogen ion loss). The key to answering this question lies in finding a suitable intracellular acidifying agent.

The Effect of Externally Administered Compounds on Intracellular pH and Their Proposed Mode of Action

A large number of chemical compounds added to the external solution bathing cells of various types have been implicated in intracellular pH modifications.

In 1871 deVries reported that the only substance he found that readily penetrated the cells of red beets was ammonia. Entrance of the ammonia was verified by means of the color change that occurred in the plants cell pigments. The same change was observed when ammonia was added to a solution of the pigment material.

Overton, in 1897 demonstrated the entry of ammonia, primary, secondary and tertiary amines and various alkaloids into plant cells by observing the formation of intracellular precipitates caused by the interaction of these bases with intracellular tannin. In addition, it was observed that no precipitation was formed when the concentration of the uncharged species was reduced by acidification of the bathing solutions. Overton concluded that it is only the free, uncharged form of the base which can penetrate the membrane.

Warburg in 1910 dyed sea urchin eggs with a neutral red indicator and observed their intracellular alkalinization upon exposure to an ammonium chloride solution. It was also observed that no color change was evident when these cells were exposed to solutions containing strong bases.

Similar experiments were run by Harvey in 1911 using plant cells, animal cells and marine eggs. Exposure to ammonia and various amines once again resulted in intracellular alkalinity as measured by the neutral red dye color change.

In 1920, Jacobs demonstrated the permeability of the cell wall of the flower petal (Symphytum peregrinum) to molecular CO_2. The petals of this flower contain a natural indicator dye. The blue petal quickly turned red (ie. more acid) not only when immersed in water gassed with 100% CO_2, but even in a HCO_3-CO_2 mixture at pH = 7.4. In addition, it was found that the red petals of the rhododendron hybrid turned bright blue (ie. alkaline) not only when immersed in an alkaline NH_4OH solution, but even in an acid (pH 6.2) solution of $(NH_4)_2SO_4$ (Jacobs, 1922). This last result strongly suggests cell membrane permeability to NH_3 rather than NH_4^+.

The cell membrane preference for uncharged molecular species was found to hold for dye uptake as well. Harvey (1911) found that plant and animal cells take up basic dyes when dissolved in alkaline solutions but not in acidic solutions. The reverse was found to be the case for acid dyes.

It has also been shown that the rate of accumulation and final concentration of cresyl blue dye in the vacuole of (Nitella) is roughly proportional to the external concentration of the undissociated species of the dye (Irwin, 1926).

Osterhout and Dorcas (1925) working with marine algae, found that the steady-state vacuolar concentrations of CO_2 and H_2S are approximately the same as those of the undissociated form. Cooper and Osterhout (1930) directly measured the pH of the vacuolar fluid and found that it becomes alkaline when the cells are exposed to NH_4Cl.

By the early 1920's it was generally understood that CO_2 and NH_3 penetrated the cell on account of their lipid solubility, and changed intracellular pH by giving off protons in the case of CO_2 (after hydration) or by taking up protons in the case of NH_3.

Many years were to pass before it was shown that pH sensitive microelectrodes could be used to measure pH in muscle cells (Caldwell, 1954; Kostyuk & Sorokina, 1961). Caldwell (1958) used glass microelectrodes to study the intracellular pH of squid giant axons exposed to 100% CO_2. A prompt large sustained acidification was reported. No lower CO_2 concentrations or CO_2/HCO_3^- mixture were employed, however.

Much more recently, Thomas (1974) used microelectrodes of his own design to observe CO_2 and NH_3 induced intracellular pH changes in snail neurones. In addition to NH_3, the metabolic inhibitors anoxia, sodium azide and 2,4 dinitrophenol (DNP) all caused a slow fall in internal pH. As well the removal of external sodium had a slow

and somewhat variable influence on intracellular pH. Sodium was later found to play a major role in intracellular pH regulation in mouse soleus muscles (Aicken and Thomas, 1977). The influence of external CO_2 on intracellular pH is further supported by Thomas' 1976 work, in which CO_2 was used in part to measure the intracellular buffering power of snail neurones.

In addition, Boron (1977) using Hinke type microelectrodes found that intracellular pH increased dramatically (≈ 0.5 pH units) when giant barnacle muscle fibers were exposed to solutions containing NH_4Cl. The intracellular pH change was found to be related to the extracellular NH_3 concentration. In addition, increased CO_2 levels caused intracellular acidification. De Weer (1978) and Boron et al. (1979) found similar intracellular results using similar techniques in snail neurones and barnacle muscle fibers.

Hinke and Menard (1976) measured the intracellular pH of barnacle muscle fibers by the DMO and microelectrode methods during exposure to CO_2 or $(NH_4)_2SO_4$. Once more it was reported that CO_2 caused intracellular pH to drop while $(NH_4)_2SO_4$ caused intracellular pH to rise. In addition it was suggested that the weak acid distribution method of measuring intracellular pH compared favourably with the electrode values providing the DMO pH value was calculated from the values of the indicator compounds ^{14}C DMO and ^{3}H inulin obtained by extrapolating the slow uptake phase to time zero.

Roos and Boron (1978) working with rat diaphragm muscle found that transferring the muscle from CO_2 free to CO_2-containing solutions caused intracellular pH to fall. Subsequent return to the CO_2 free solution caused intracellular pH to rise. The distribution of the weak acid DMO was used to measure intracellular pH.

There appears to be little doubt as to the effect of cell exposure to CO_2 or NH_3 on intracellular pH. Another group of compounds which have been studied with respect to their effects on intracellular pH are weak organic acids. Marrannes, De Hemptinne and Leusen (1979) found that in sheep Purkinje fibers, rapid substitution of 20 mM Cl^- with 20 mM propionate at an extracellular pH of 6.8 resulted in intracellular acidosis and a transient alkalosis at the external surface of the cells. Similar intracellular pH changes were also observed with formate, butyrate, β-hydroxybutyrate, lactate, pyruvate and α-Cl-propionate. The degree and kinetics of the change was quite variable however. Intracellular pH was measured with a double-barrel pH sensitive electrode while surface pH was measured with an independent microelectrode. De Hemptinne and Marrannes (1979) observed a similar intracellular acidification and surface alkalosis in rat soleus fibers exposed to propionate.

These observations in conjunction with the previously cited finding by Marrannes and De Hemptinne (1978) that correlates the lipid solubility of weak acids to the transient changes in cardiac fiber conduction velocity, suggest that weak acids such as propionate penetrate the cell in the protonated form. The subsequent dissociation causes

intracellular acidosis. The transient surface alkalosis can be explained on the basis of the unprotonated form remaining in excess, acting as a proton sink causing the pH to rise.

In addition to its effects on interstitial and intracellular pH, propionate has been reported to hyperpolarize the membrane of many cell types. The magnitude of this change appears, however, to be quite variable (De Hemptinne and Marrannes, 1979; Marrannes et al., 1979)

A Role of Organic Acids and Intracellular Acidosis in the Suppression of Tension in Cardiac and Skeletal Muscle

The effect of many organic acids on skeletal muscle tension development has been studied by Foulks et al. (1977), while the effect of octanoate was studied by Caffier et al. (1982).

Octanoate (2-10 mM) was found by Caffier et al. (1982) to alter the characteristics of the isometric twitch in isolated bundles of frog skeletal muscle. The peak tension, the maximal rate of rise of tension, the time to peak tension, and the half relaxation time were all reduced. Tetanic tension was reduced by the same amount in percent (40%), as a single isometric twitch. As well the fusion frequency of tetanic contractions increased. It was also found that K^+ induced contractions were diminished. As a general statement 1-2 mM caffeine removed the octanoate effects described above. Caffeine is known to facilitate the release of Ca^{2+} from the sarcoplasmic reticulum (Weber and Herz, 1968). The authors suggest that these results are consistent with the hypothesis that the octanoate-induced changes in muscle contractions are due to a reduced Ca^{2+} release from tellular

stores. This is not to say that this inhibition of Ca^{2+} release is not mediated through intracellular pH decreases due to octanoic acid diffusion across the fiber membrane and subsequent dissociation. Whether inhibition of Ca^{2+} release is due to a direct octanoate inhibition or via suspected octanoate induced intracellular acidosis remains unclear at present.

Foulks and his co-workers (1977) found that the depressant effect of extracellular acidity on twitches and K^+ contractions in frog skeletal muscle was significantly accentuated in the presence of organic anions such as butyrate. Foulks proposed that these depressant effects may be produced by interference with the membrane stabilizing actions of divalent cations. This, it was suggested, would increase membrane fluidity. Once more intracellular pH was not measured. One must not neglect the effect the organic acids used by Caffier and Foulks may have had on intracellular pH. There is some evidence correlating decreases in intracellular pH to reductions in tension outputs in cardiac and skeletal muscle.

Vaughan Williams (1955) reported that intracellular pH decreases induced by changing pCO_2 resulted in reductions in contractile force of isolated rabbit auricles. McElroy and his co-workers (1958) found that CO_2 induced alterations in intracellular pH of guinea pig heart muscle resulted in a 70% reduction in contractility (pCO_2 changed from 5% to 20%). This effect was found to be reversible. Further evidence for the effect of intracellular acidosis on cardiac contractility was provided by the work of Lorkovic on frog heart strips. A rapid drop in twitch tension

resulted from exposure to solutions saturated with 80% CO_2 at a constant extracellular pH of 6.7. Hill (1955) has reported a 1 unit pH change in frog sartorius muscle equilibrated in the same solution. With the differences in cardiac and skeletal muscle buffer capacity, it is difficult to say exactly what the intracellular pH change would be. It is sufficient to say, however, that a substantial acidosis will occur. Results of a similar nature have been found for skeletal muscle as well.

Meyer et al. (1983) perfused isolated cat biceps brachii and soleus muscles with red blood cell suspension in Krebs-Henseleit equilibrated with 5 or 30 CO_2 (pH 7.2 and 6.6, respectively). Intracellular pH as measured by ^{31}p NMR spectra was found to decrease from 7.0 to 6.6 in both muscles. In the soleus muscle, this produced a 24 decrease in twitch tension, and a prolongation of both time to peak tension (5) and time to half relaxation (40). Tetanus rise and fall times were increased by 32 and 100 , respectively, but peak tetanic tension was unchanged. In the biceps muscle, however, a similar intracellular pH drop produced no significant changes in twitch or tetanus kinetics although peak tetanic tension was reduced by 10 . This reduction in tension is very much smaller than the reduction seen during physiological fatigue even though the magnitude of the intracellular pH change approximates that which is expected to occur during fatigue. It would appear that intracellular pH is not a direct source of fatigue. The question arises as to the means whereby intracellular pH decreases can reduce tension output in both

cardiac and skeletal muscle. Fabiato and Fabiato (1978) and Donaldson and Hermansen (1978) provided evidence to illustrate the role of intracellular pH in the suppression of tension. It was found in skinned frog semitendinosus muscle that if pH was reduced to 6.6 from 7.0, the maximum tension generated by the muscle would be 90% of that observed at pH 7.0. This effect was found to be due to a shift in the tension-pCa^{2+} curve due to the reduction in pH. In addition if the free Ca^{2+} concentration was not increased at pH 6.6 to a pCa^{2+} of 6.0 form 5.5 then tension generated by the muscle was only 80% of that which it could generate at pH 7.0. This type of work clearly implicates intracellular pH in the suppression of tension via modifications in the Ca^{2+} system. Allen and Orchard (1983a) have presented data supporting the hypothesis that the rapid drop in tension of mammalian papillary muscles exposed to acidic solutions follows a time course similar to the fall in intracellular pH. In addition the slow recovery of tension after the initial tension drop was accompanied by an increase in free Ca^{2+}. The increase in free Ca^{2+} could be stopped by metabolic inhibition suggesting that energy is required to increase the calcium levels. The suppression of the free Ca^{2+} levels also resulted in tension reductions (Allen and Orchard, 1983b). It appears that in cardiac muscle, decreases in tension occur as a direct effect of both a decline in intracellular pH and/or free Ca^{2+} levels. This is not to suggest that intracellular acidosis is the sole cause of fatigue, rather one possible mechanism. Recall that Caffier et al. (1982) have suggested that octanoate

suppression of twitch and tetanic tension was induced via inhibition of Ca^{2+} release from cellular stores. One can suggest that this inhibition may be due to the suspected intracellular pH change induced by octanoate exposure (octanoic acid diffusion and subsequent intracellular dissociation). A direct Ca^{2+} inhibition by octanoate cannot be ruled out.

Porzehl et al. (1969) have reported a marked decline in ATPase activity (indicative of power output capabilities) of extracted rabbit fibrils when the pH was lowered from 8.0 to 6.0. In addition the Ca^{2+} requirements for ATP splitting were increased during acidosis. Schadler (1967) found similar results for rabbit skeletal muscles which were glycerol extracted. Bozler (1968) also found an acidosis induced reduction in ATPase activity at constant pCa^{2+} values as measured by tension outputs of glycerol extracted fibers. Maximum ATPase activity was not changed. Kentish and Nayler (1977) found that acidosis depresses the Ca^{2+} sensitivity of the myofibrillar ATPase in both cardiac and white skeletal muscle thereby requiring higher Ca^{2+} levels to obtain similar ATPase activities. The maximum ATPase activities were depressed only slightly.

It would appear then that intracellular acidosis may suppress tension by two Ca^{2+} related mechanisms: (1) inhibition of Ca^{2+} release from sarcoplasmic reticulum, or (2) suppression of myofibrillar ATPase Ca^{2+} sensitivity, consequently shifting the tension pCa^{2+} curve to the right. Superimposed on these two potential fatigue mechanisms is the normal loss of Ca^{2+} during stimulation (Bianchi, 1982) which

may further suppress tension. It can be suggested that the drop in intracellular pH will influence tension only marginally (Meyer et al., 1983) but when coupled with the Ca^{2+} loss observed during muscular contractions, may reduce tension considerably by lowering free Ca^{2+} levels. However, this is not to deny other mechanisms of tension suppression (Wenger and Reed, 1976).

Regardless of the mechanism, a large body of evidence exists, some of which I have reported, implicating decreases in intracellular pH in the fatigue process in skeletal and cardiac tissue. In the light of these studies the work of Fitts and Holloszy (1976) and Tesch (1978) takes on a new importance. Fitts and Holloszy found a strong correlation between the levels of intracellular lactate and reductions in contractile force in frog sartorius muscles ($r = -0.99$, $p < 0.01$). Tesch found a close relationship ($r = -0.79$) between lactate concentration of muscle and performance time on a supramaximal bicycle ergometer test. As well, we must not forget the early work of Fletcher and Hopkins (1907) who found that a high lactate concentration occurred at the same time as muscle fatigue.

As lactic acid is the primary metabolic acid, the mechanisms of release of lactate from skeletal muscle tissue should be studied for the following reasons;

(1) to determine the role of lactate release in intracellular pH regulation during fatigue and recovery from fatigue.

(2) to determine the role of lactic acid induced pH changes in tension suppression during muscular activity.

Summary

The data to date suggests that extracellular pH influences lactate efflux from skeletal muscle. High extracellular pH values or high extracellular buffer capacities support high lactate efflux rates. On the basis of this effect five models are proposed to explain the role of extracellular pH in modulating lactate efflux. These models may be divided into two categories on the basis of whether hydrogen ion is released in conjunction with lactate release or whether lactate release is independent of hydrogen ion release. If hydrogen ion release is coupled to lactate release then the efflux rates of lactate from skeletal muscle tissue should be dependent upon the transmembrane pH gradient. This is to say that decreasing intracellular pH (increasing H^+ concentration) should facilitate increased lactate efflux as suggested by models C, D or E.

The literature suggests that the monocarboxylate propionic acid (CH_3CH_2COOH) may be a suitable intracellular acidifying agent. Data has been presented showing that the addition of propionate to the extracellular solution causes substantial drops in intracellular pH. Monitoring lactate efflux rates upon the addition of propionate to the external solutions bathing isolated muscle may help us to determine whether lactate efflux from skeletal muscle is dependent upon intracellular pH.

This is an important concept to muscle physiology in general, in that uncoupled hydrogen ion and lactate release from skeletal

muscle results in the cell disposing of the hydrogen ion load through alternate mechanisms. This is necessary for the maintenance of cellular integrity. As intracellular pH changes have been implicated in the impairment of many cellular functions, knowledge of the means of regulating lactic acidosis may come from experimental work dealing with the regulation of lactate release.

Research Questions

(1) What is the effect of externally administered propionate on lactate efflux from isolated frog sartorius muscles?

(2) What model of lactate efflux is consistent with the effect of externally administered propionate on lactate efflux from isolated frog sartorius muscles?

Materials and Methods

General

Animal Housing and Care

Frogs (Rana pipiens) were obtained from Anilab Enr'g of Quebec City. Upon their arrival, the animals were treated with antibiotics to destroy any infections they may have acquired during storage and transport.

The frogs were kept in a water filled Living Stream (Frigid Units Inc., Toledo, Ohio). The Living Stream was divided into three compartments so as to seperate newly arrived animals from those which were currently being used for experimental purposes. Each compartment contained a plexiglass platform which enabled the frogs to climb out of the water to feed.

The tap water in the compartments was maintained at approximately 17^0 C by heating the cold water in a large reservoir to the desired temperature prior to its addition to the Living Stream. Fresh water from the reservoir was continually added to the Living Stream so as to regulate the water temperature in the compartments. The temperature fluctuated approximately $\pm 2^0$ C due to the daily variations in tap water temperature (cold water outlet).

The animals were fed a diet of live mealworms three times a week. The mealworms were placed in a shallow dish on the plexiglass platform. The frogs ate at will and after a short period of time adjusted to this method of feeding. All animals were fed for a minimum of two weeks prior to being used in any experiment

Dissection Procedure Pre-Experiment Equilibration

Each animal was pithed after decerebration. The hind limb skin was then removed and the distal tendon of the sartorius isolated. A small

loop of 5'0' surgical silk was tied to the tendon. An estimate of proximal to distal tendon length was made and the distal tendon was then cut. The body of the sartorius was carefully dissected out and a small loop of 5'0' silk was tied to the proximal (pelvic) tendon and the tendon was then cut. The contralateral sartorius was removed in a similar manner. Muscles from the same animal were used as experimental and control pairs in all experiments. Throughout the dissection procedure, the muscles were kept moist with a Modified Ringer's solution. The solution used corresponded to the control Modified Ringer's solution being used in that days experiment.

Upon completion of the dissection procedure, the sartorius muscles were transferred to a large reservoir containing approximately 150 ml of the control Modified Ringer's solution being used in that days experiment. The solution was aerated with 100% oxygen. The oxygen was passed through a home-made CO_2 trap containing soda lime crystals and then through a custom built water equilibration unit containing 120 mM NaCl. These two processes ensured that CO_2 impurities were removed and that the dry gas from the cylinder reached the equilibration chamber saturated (Fig. 3). All experiments and equilibration procedures were run at room temperature and specific equilibration times will be given under the Methods section of each individual experimental series.

Experimental Solutions

Table 1 shows the composition in mM of the control Modified Ringer's solutions and the experimental Modified Ringer's solutions.

Figure 3

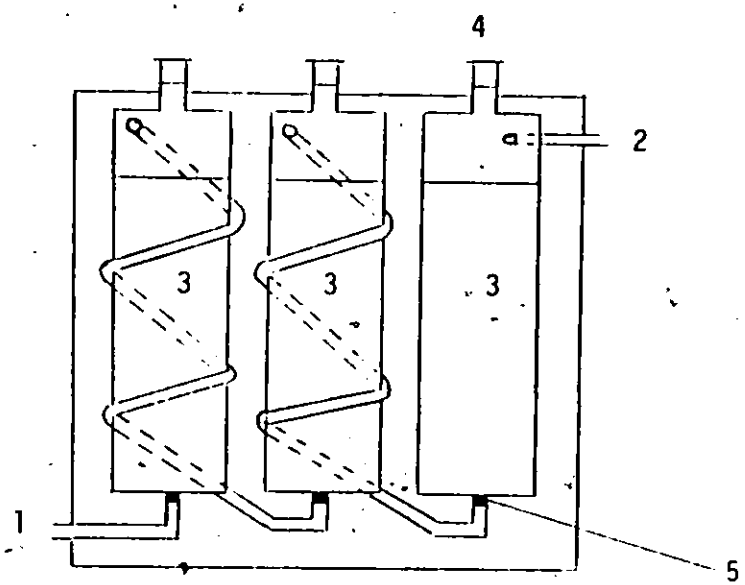
Water Equilibration Unit and Carbon Dioxide Trap

Water Equilibration Unit

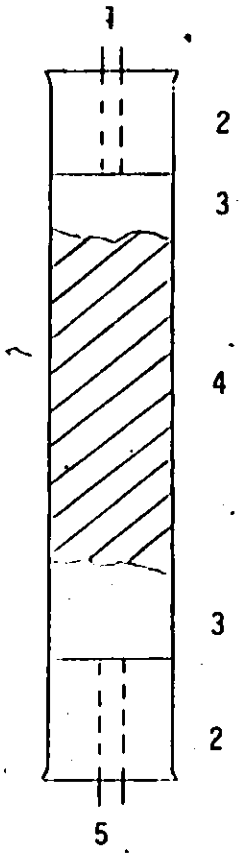
1. Dry oxygen input from carbon dioxide trap
2. Saturated oxygen output
3. 120 mM NaCl solution
4. Stopper
5. Sintered glass

Carbon Dioxide Trap

1. Oxygen output to water equilibration unit
2. Rubber stopper
3. Filter
4. CO_2 absorbent, soda lime
5. Oxygen input from tank



Water Equilibration Unit



Carbon Dioxide Trap

Table 1. Composition of solutions.

Solution #	Na^+	Cl^-	Ca^{2+}	K^+	Glucose	Total MOPS Buffer	Total Propionate
1	110.7	116.5	1.8	2.5	5.5	1.0	0
2	110.7	96.5	1.8	2.5	5.5	1.0	20.0
3	109.4	113.8	1.8	2.5	5.5	5.0	0
4	109.4	93.8	1.8	2.5	5.5	5.0	20.0
5	107.7	110.5	1.8	2.5	5.5	10.0	0
6	107.7	90.5	1.8	2.5	5.5	10.0	20.0
7	104.4	103.8	1.8	2.5	5.5	20.0	0
8	104.4	83.8	1.8	2.5	5.5	20.0	20.0
9	102.7	100.5	1.8	2.5	5.5	25.0	0
10	102.7	80.5	1.8	2.5	5.5	25.0	20.0
	Source NaCl NaOH Na Propionate (if applicable)	Source NaCl KCl $CaCl_2$	Source $CaCl_2$	Source KCl		Source MOPH	Source $NaCH_3CH_2COO$

pK values used in this thesis

MOPS	7.10
PROPIONATE	4.90
LACTATE	3.70
DMO	6.33

All solutions were made with certified reagents in distilled water and were titrated to pH 6.80. In addition all solutions were of equivalent osmolarity. This table encompassess all non-radioactive solutions used in this project. Note that the experimental solution varies from its corresponding control only in that 20 mM propionate has been substituted for 20 mM Cl^-. These solutions will be referred to as control and experimental solutions. All solutions were titrated to pH 6.80 with small amounts of NaOH or HCl and pH was measured with a Radiometer model PHM64 Research pH meter.

Specific Experimental Series

^{14}C Propionate Uptake in Resting Sartorius Muscles

Four sartorius muscles were dissected out and equilibrated for 3 hours as described previously, in control solution #9 which is buffered to pH 6.80 with 25 mM 2-N-morpholinopropanesulphonic acid (MOPS pK_a 7.10) (Table 1). After the 3 hour equilibration period, each muscle was suspended in an individually aerated tube (Fig. 4). The 100% oxygen was passed through the CO_2 trap and water equilibration unit was described previously (Fig. 3). The muscles were stretched to in vivo length and further equilibrated in 8 ml of the above solution for 15 min.

After 15 minutes the tubes were removed and replaced with fresh tubes containing experimental solution #10. In addition, trace amounts of ^{14}C sodium propionate and ^{3}H inulin (NEN) were added 1.2 X 10^6 dpm·ml^{-1} [^{14}C], 2.0 X 10^5 dpm·ml^{-1} [^{3}H], SA [^{14}C] 2.5 mCi·mmol^{-1}

[^{3}H] 287.5 mCi·g^{-1}.

After 60 minutes the muscles were removed from their individual tubes and the suture loops removed. The muscles were blotted and cut in half. One half was added to a pre-weighed crucible, reweighed and placed in a vacuum oven at 80^{o} C overnight for total tissue water determination (vacuum, 25 inches of Hg). The other half was placed in a pre-weighed scintillation vial and reweighed. To the vial, 0.5 ml of NCS tissue solubilizing agent (Amersham) was added and the vial placed in a 40^{o} C water bath for 16 to 20 hours. After digestion the vial was cooled and 0.017 ml of glacial acetic acid was added. To this neutralized mixture, 15 ml of OCS scintillation cocktail (Amersham) was added and the mixture was vortexed and then cold and dark adapted for 24 hours to further reduce chemiluminesence. The sample was then double label counted using internal standardization. Blanks were prepared as above for determination of background counts.

The radioactive bathing mediums were vortexed and 0.100 ml aliquots were added to each of 4 vials containing 15 ml of PCS scintillation cocktail (NEN). Each bathing medium was prepared in this manner. The samples were vortexed and cold and dark adapted for 24 hr. The samples were then double label counted using internal standardization. Aliquots of cold solution #10 were taken and prepared as above for determination of background counts.

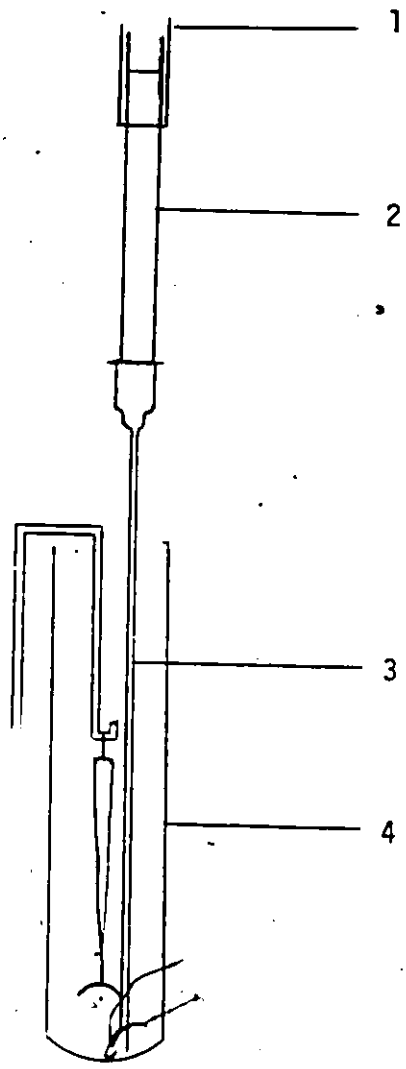
The pH of the remaining bath medium was determined using the Radiometer Model PHM64 Research pH meter with a Semimicro Radiometer Electrode Model GK2321C.

Using ^{3}H inulin as an extracellular water marker, it was possible to calculate the intracellular concentration of ^{14}C propionate.

Figure 4

Individually Aerated Tube

1. Oxygen input
2. 1 ml syringe
3. 75 mm, 22 gauge needle
4. 10 ml tube


1
2
3
4

The Effect of Externally Administered Propionate on Intracellular pH of Resting Frog Sartorius Muscles as Measured by the Distribution Ratio of 5,5-dimethyl-2,4-oxazolidine dione (DMO)

Four pairs of sartorius muscles were dissected out and equilibrated for 3 hours in control solution #9 as described previously. After the 3 hour equilibration period each muscle was suspended in an individually aerated tube (Fig. 4). The muscle was stretched to in vivo length and further equilibrated in 8 ml of the above solution for 15 minutes.

After 15 minutes the tubes were removed and replaced with fresh solutions. One of each pair of muscles was returned to control solution #9 to which had been added trace amounts of ^{14}C DMO 1.3×10^6 dpm·ml^{-1} [^{14}C], S.A. 55.4 mCi/mmol and ^{3}H inulin 3.7×10^4 dpm·ml^{-1} [^{3}H], S.A. 287.5 mCi/g. The other muscle of each pair was returned to experimental solution #10 to which had been added identical amounts of ^{14}C DMO and ^{3}H inulin.

After 60 minutes the muscles were removed from the individual tubes and prepared for total tissue water and double label counting in an identical manner as has been described in the previous section for ^{14}C propionate uptake determination.

The distribution ratio of the weak acid DMO was used to estimate intracellular pH according to the method of Waddell and Butler (1959) and the modified algorithm of Hinke and Menard (1978) (Appendix 1).

The Effect of Externally Administered Propionate on Whole Muscle Lactate Efflux from Frog Sartorius Muscles Bathed in 25 mM MOPS Ringer's Solution pH 6.80.

Paired sartorius muscles were dissected out and equilibrated for 90 minutes in control solution #9. One muscle was then transferred to a small plexiglass efflux chamber 54 mm x 17 mm x 5 mm (Fig. 5). The suture loop attached to the distal tendon was secured to the bottom of the chamber by way of a stainless steel rod which slid into parallel notches in the sides of the chamber. The proximal suture loop was attached to a stainless steel pin which passed out of the top of the chamber through a teflon ring. The pin was attached to a light silver chain which linked the pin to a Grass Model FT03C force transducer. The transducer was mounted in the arm of a standard inch worm apparatus. By manually adjusting the height of the transducer above the efflux chamber, it was possible to set the muscle length to in vivo values. The 3.4 ml of control solution #9 which bathed the muscle in this chamber was aerated with 100% oxygen by flowing the oxygen into the chamber through a sintered glass insert in the bottom of the chamber. The flow of approximately 17 $ml \cdot min^{-1}$ was regulated with a Gilmont flowmeter. As per the methods described previously, this gas was passed through a home made CO_2 trap containing soda lime and a custom made water equilibration unit. The front plate of the chamber was held

in place with 4 small wing nuts and was sealed with water insoluble Dow Corning high vacuum silicone grease. When the front plate of the chamber was in place, the muscle was suspended between two 34 mm X 6 mm platinum electrodes.

A known amount of solution was added to the chamber by way of a 5 ml Gilson Autopipette inserted into a hole high on the front face of the chamber. Solutions were evacuated by way of a 10 ml disposable syringe fixed to a needle epoxied into the front face of the chamber.

In order to raise intracellular lactate levels, the sartorius muscle was electrically stimulated. A Grass SD8 stimulator delivered 1 ms pulses at a frequency of 70 Hz. This output was modulated with a Grass SD9 stimulator to give 175 ms trains of pulses.

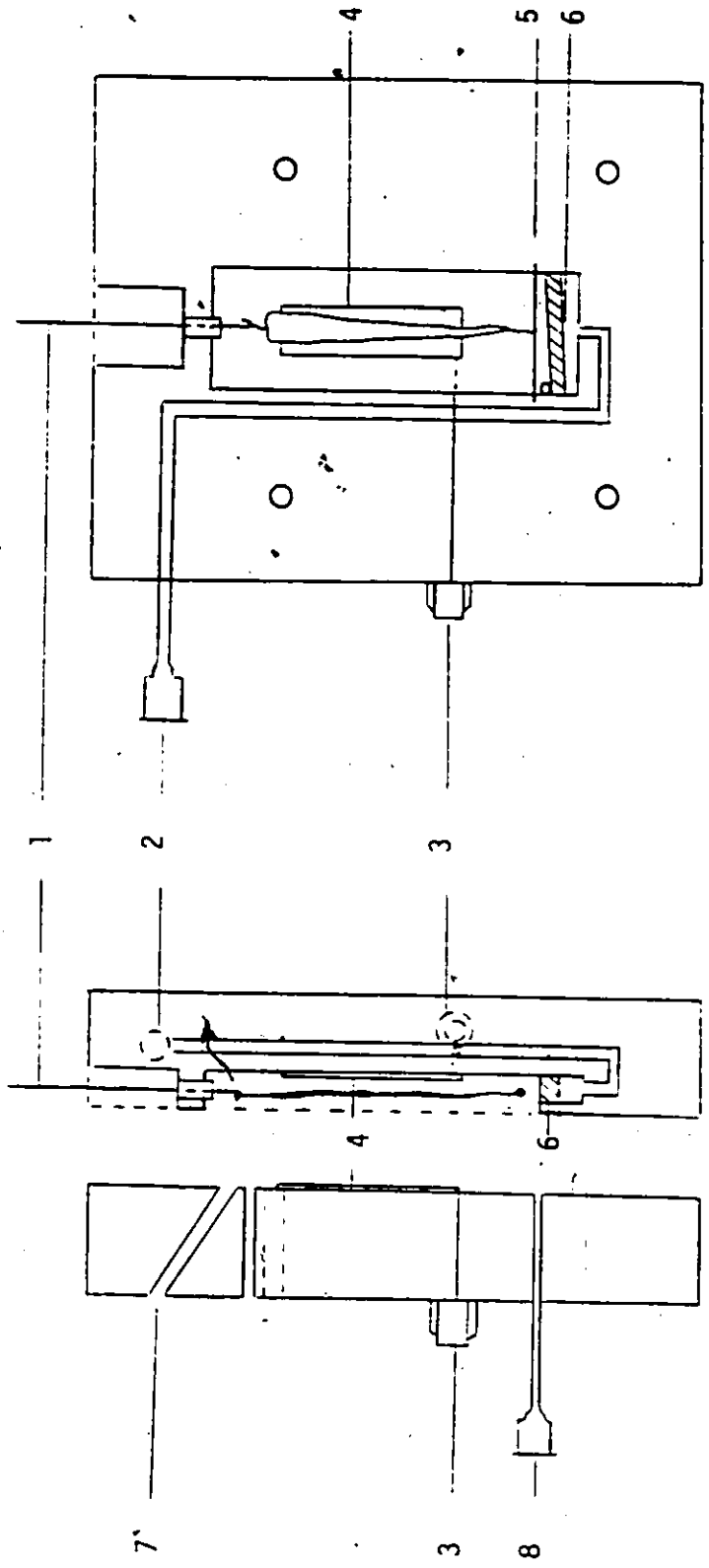
Determination of the stimulus voltage necessary to elicit maximum tetanic tension was achieved by stepwise increases in voltage output until no further increase in tetanic tension occurred. This voltage value was then doubled to assure that supramaximal trains of pulses were delivered. A homemade current booster was used to increase the current across the electrodes. The relationship between the Grass SD8 voltage output and the output voltage after passing through the current booster is represented in Appendix 2.

The output from the Grass transducer was balanced with a simple balancing circuit and was amplified with a Philbrick Researchers Model MP Operational Amplifier. The output was simultaneously monitored

Figure 5

Efflux Chamber

1. Stainless steel pin for tension measurements
2. Oxygen input
3. Electrode input
4. Platinum electrode
5. Stainless steel bar
6. Sintered glass
7. Injection port
8. Withdrawl port



Front View

Side View

on a Tektronics Model T912 storage oscilloscope and a Model 5D Grass chart recorder.

After the muscle length and stimulus parameters were set as described above, the preparation was equilibrated in control solution #9 for a further 60 minutes. The solution in the chamber was changed at 10, 20, 30, 45 and 60 minutes of accumulated time.

The muscle was then fatigued by administering one supramaximal train of pulses every 3 seconds for 4 minutes. The solution was then changed and changed every 10 minutes for 50 minutes resulting in 5 post fatigue collection intervals.

The experimental muscle varies from control in that the experimental muscle was exposed to experimental solution #10 from time 20 minutes to 50 minutes post fatigue.

All effluent samples were frozen and subsequently analyzed for lactate. A mean interval whole muscle efflux was computed using the blotted tissue weight including suture loops and the time the solution was exposed to the muscle (interval time). The order of presentation of experimental and control conditions within paired muscles was randomly chosen as only one efflux chamber was available.

No correction was made for residual volumes of solution remaining in the chamber after evacuation although theoretically the fluid remaining in the chamber after the sample was withdrawn would contribute a lactate load to the next interval. The actual residual volume of chambers such as the one used in the present study is approximately 75 µl as determined by using ^{14}C sucrose (Lubek, M.Sc. Thesis).

This residual volume is less than 2.5% of the total bath volume.

The values calculated as the efflux rates can be corrected for this residual volume. Letting V represent the amount of fluid withdrawn from the muscle bath; C_1, the concentration of the substance from the last sample; C_2, the concentration of the substance in the present sample; E_2, the amount of substance which will efflux from the muscle tissue into the present sample; and X, the residual volume, then:

$$C_2 = \frac{E_2 + C_1 X}{V + X}$$

Using this calculation it can be shown that the difference between the measured amount of the substance and the actual amount due to efflux from the muscle into the present sample is represented by:

$$X\ (C_2 - C_1)$$

The use of this formula reveals that the maximal error introduced by this residual volume is extremely small, and thus no correction factor was applied.

The Effects of Externally Administered Propionates on Whole Muscle Lactate Efflux from Frog Sartorius Muscles Bathed in 4 Buffer Concentrations of MOPS Ringer's Solution pH 6.80

The materials and methods are the same as those outlined in the previous section with the exception that solutions #1, 3, 5 and 7 were used as control solutions and solutions #2, 4, 6 and 8 as the

corresponding experimental solutions. These correspond to buffer concentrations of 1, 5, 10 and 20 mM MOPS respectively.

In addition, lactate efflux was monitored through 3 post fatigue intervals rather than 5. After 30 minutes the muscle was removed from the chamber and blotted. The suture loops were removed and the muscle was quickly frozen between stainless steel blocks pre-cooled in liquid nitrogen. The frozen tissue was weighed and stored at -70^0 C for future intracellular lactate analysis. The time required to remove the muscle from the chamber, blot the muscle, trim the sutures and freeze the muscle was approximately 45 seconds.

In this series of experiments trimmed frozen muscle weights were used in the computation of mean whole muscle lactate efflux rates.

Tissue Extraction of Lactate and Preparation of Effluent Samples for Biochemical Assay

Tissues were extracted for lactate analysis as follows: a known weight of frozen muscle was added to a pre-cooled glass homogenizing tube containing a known volume of ice cold 6% perchloric acid (80 mg tissue$\cdot ml^{-1}$ PCA) and homogenized by hand on ice. The homogenate was left to stand 10 minutes on ice. The pestle was not removed and after 10 minutes was used to thoroughly mix the homogenate. A measured volume of homogenate was poured into a centrifuge tube (Eppendorf) and was spun at 8,800 G at 4^0 C for 10 minutes (Eppendorf Centrifuge 5413). A known volume of the supernatant was decanted and neutralized (pH > 7.4) with a known volume of 2.5 M potassium carbonate so as to precipitate

the perchlorate. The pH was checked with litmus paper and the mixture was recentrifuged for 10 min at 8,800 G. The supernatant was decanted and frozen at -70^{0} C for biochemical assay. Refer to Appendix 3 for more details as to the appropriate dilution factors used in the tissue lactate calculations.

Due to the low bath concentration of lactate at the end of the collection intervals it was necessary to concentrate the samples. This was achieved by evaporating off the water in 3.25 ml of the sample in a vacuum oven at 80^{0} C (vacuum, 25 inches of Hg). The precipitate was then reconstituted to 0.65 ml thereby concentrating the lactate in the sample by a factor of five. This enabled one to measure the lactate concentration in a reliable range of the assay. This procedure resulted in no loss of lactate (Appendix 4).

Biochemical Assay

Lactate was assayed in both the tissue extract and effluent samples by measuring the reduction of NAD (Boehringer-Mannheim) in the presence of lactate dehydrogenase (LDH) (Boehringer-Mannheim). This was done by monitoring the increase in optical density at 340 nm (37^{0} C) with a Beckman DU7 spectrophotometer. The pyruvate formed in the reaction was trapped with semicarbazide as described by Olson (1962).

20 µl NAD (40 mg·ml^{-1}) and 10 µl LDH (20 mg·ml^{-1}) were pipetted into a quartz cuvette together with 0.9 ml freshly made buffer containing semicarbazide (1.5 g glycine, 2.23 g semicarbazide HCl dissolved in 8.0 ml DH_2O and adjusted to pH 9.8 with 3 M NaOH).

The optical density was read until steady and then 0.2 ml of the sample was added. The optical density increased and readings were taken every 1 minute for 55 minutes. The same volume of Ringer's solution (control or experimental) in the case of effluent samples or the same volume of neutralized perchloric acid, in the case of tissue samples was used as a correction factor for dilution or contamination. The volume of sample was often reduced to 0.1 ml in the case of concentrated samples.

Net whole muscle lactate efflux in the case of effluent samples was computed on the basis of the sample concentration and was normalized per gram of tissue per minute (Appendix 3).

A Two Compartment Diffusion Model for Lactate Diffusion from Frog Sartorius Muscles

In order to compute the fiber lactate efflux from whole muscle efflux values, an estimate of the amount of lactate contained within the interstitial space of the sartorius muscle must be made.

Since the frog sartorius muscle is considered a flat sheet, the system is symmetrical with diffusion occurring through both surfaces of the sheet. This system can adequately be described by considering diffusion from one surface (X = 0) to the center of the sheet (X = 1). The required equations are very similar to those used for steady-state oxygen and glucose uptake and diffusion (Hill, 1965; Mainwood and Cechetto, 1977). In Hill's model, the diffusion space corresponds to the total tissue space and diffusion is assumed to occur equally through the cells and the extracellular medium. For lactate

however, diffusion is confined to the extracellular space and lactate efflux is defined as the release of lactate from the whole muscle. Diffusion within the cells is not considered.

The relationships between substrate concentration (Y) and the depth in the tissue sheet (X) when diffusion is not confined to the extracellular space are given by Hill (1965, pp. 216-217) as follows:

1) $$\frac{d^2Y}{dX^2} = \frac{a}{K}$$

where a is the efflux rate per unit tissue volume and K is the diffusion constant. Solving this equation to obtain Y at different depths gives:

2) $$Y = Yo + \frac{alX}{K} - \frac{aX^2}{2K}$$

where Yo is the external concentration. To find the total amount of substrate (Q) within a tissue ½ sheet of area 1 cm^2, the integral of Y is required.

3) $$Q = \int_0^1 Y \cdot dX = Yol + \frac{al^3}{3K}$$

These equations are appropriate for lactate diffusion providing the differences arising from the restriction of diffusion to the extracellular space is accounted for. This restriction implies that: (A) the area for diffusion through any plane parrallel to the surface is only a fraction (α) of the total area of the plane and (B) the path length for diffusion at any depth (X) from $0 \rightarrow 1$ is increased by a factor (λ) because of tortuosity.

Modifying equations 1-3 to take these factors into account results in the following equations:

4) $$\frac{d^2Y}{dX^2} = \frac{a}{K\alpha}$$

5) $$Y = Yo + \frac{al\chi\lambda^2}{K\alpha} - \frac{a(X\lambda)^2}{2K\alpha}$$

To obtain Q, the integral of Y over the whole path length ($l\lambda$) must be found using the appropriate cross sectional area (α):

6) $$Q = \alpha \int_0^l Y dX = \alpha \left[Yol\lambda + \frac{a(l\lambda)^3}{3K\alpha} \right]$$

But, the extracellular space within this tissue ½ section (Vel) must be equal to the total volume of the diffusion pathways ($l\lambda\alpha$) so that equation (6) becomes:

7) $$Q = VelYo + \frac{a(l\lambda)^3}{3K}$$

Q amount of lactate in extracellular space ($\mu mol \cdot cm^{2-1}$ tissue area)

Ve 0.20 $ml \cdot ml^{-1}$ tissue

l maximum diffusion distance (cm)

λ 1.55

K 6.6×10^{-4} $cm^2 \cdot min^{-1}$

Yo bath concentration of lactate at end of collection interval ($\mu mol \cdot ml^{-1}$)

a steady-state lactate efflux ($\mu mol \cdot ml^{-1}$ tissue $\cdot min^{-1}$)

The text explanation of these derivations is taken directly from Mainwood and Cechetto (1977).

The efflux of lactate during the first 60 minutes following fatigue is not steady-state. A mean whole muscle efflux over a four or ten minute interval may be considered a steady-state estimate of the mean whole muscle efflux during that interval. The use of a steady-state diffusion model is then appropriate, although only an approximation.

A number of assumptions have been made to facilitate the use of the model;

(1) The extracellular volume of the tissue (V_E) is assumed to be 0.20 ml·ml tissue^{-1}.

(2) The tortuosity factor (λ) is assumed to be 1.55 (McLennan, 1957).

(3) The diffusion coefficient for lactate is assumed to be 6.6×10^{-4} cm^2·min^{-1} (Hill, 1965).

(4) The maximum diffusion distance (l) is obtained from a linear regression analysis done by Dr. J.M. Renaud (Ph.D. Thesis, 1982). A strong correlation was found between the maximum diffusion distance ($\frac{1}{2}$ muscle thickness) and muscle weight. This analysis was produced from data collected from toad sartorius muscles (Appendix 5).

(5) The steady-state lactate efflux (a), in μmol·ml tissue^{-1}·min^{-1} was derived from knowledge of the efflux in μmol·g^{-1}·min^{-1} and an assumed tissue density of 1.05 g·ml^{-1}.

(6) The amount of lactate contained in the extracellular space (Q) obtained from equation 7 is expressed as μmol per 1 cm^2 of tissue surface. The true estimate of the amount of lactate

contained in the extracellular space of the tissue is computed from an estimate of the surface area of a flat sheet of tissue and may be estimated from knowledge of the tissue volume and the tissue thickness, 2(l). Tissue volume is computed using the assumed tissue density of 1.05 g·ml tissue^{-1}. The tissue area is then simply the tissue volume divided by 2(l). Mean fiber efflux for any interval can then be estimated by adding to the bath lactate level (μmol) the amount of lactate contained in the extracellular space at the end of the interval (Q_E). In addition the amount of lactate contained in the extracellular space at the beginning of the same interval (Q_E) must be subtracted from the bath level. The result of this calculation is an estimate of the amount of lactate which crossed the sarcolemma membranes of the muscle during the collection interval in question. It is important to recall that the amount of lactate which is present in the extracellular space at the end of an interval is used as the amount present at the beginning of the next interval. Preliminary studies have shown that resting whole muscle efflux from frog sartorius muscles is extremely low (≈ 20 nmol·g^{-1}·min^{-1}). Based on this efflux rate the amount of lactate present at the beginning of the stimulation interval is insignificant. Therefore no correction for this amount of lactate is made in the computation of mean fiber efflux rates during the fatigue interval. Equation 8 is used to calculate an estimate of the mean fiber efflux for

any interval.

8) $MFE = (BL + Q_E - Q_B) \times \frac{1}{TW} \times \frac{1}{CT}$

MFE mean fiber efflux (μmol).

BL bath lactate level (μmol).

Q_E amount of lactate in extracellular space at the end of the collection interval (μmol).

Q_B amount of lactate in extracellular space at the beginning of the collection interval (μmol).

TW tissue weight (g).

CT time of collection (min).

Results

^{14}C Propionate Uptake in Resting Sartorius Muscles

Four sartorius muscles were incubated for 60 minutes in solution #10, a 25 mM MOPS Ringer containing 20 mM propionate. To this solution trace amounts of ^{14}C propionate and ^{3}H inulin had been added as described under Materials and Methods. After the 60 minute equilibration period the tissue was analyzed for total water, [^{14}C] and [^{3}H] as described previously. If inulin is assumed to be confined to the extracellular space, then the counts of ^{14}C propionate may be expressed per unit of intracellular water. The results from the four tissues are presented in Table 2. The mean intracellular [^{14}C] concentration was found to be $1.04 \times 10^{5} \pm 6.14 \times 10^{3}$ dpm per millilitre of intracellular water (SEM). If one assumes that 60 minutes is sufficient time to ensure adequate mixing of ^{14}C and unlabelled propionate between the extracellular and intracellular compartments, then it can be assumed that the specific activity (S.A.) of the two compartments is the same. Based on this assumption the concentration of propionate in the intracellular water can be computed. A mean value of 10.5 ± 0.6 mM intracellular propionate was estimated to be present at the end of the 60 minute equilibration period (Table 2).

The Effect of Externally Administered Propionate on Intracellular pH of Resting Frog Sartorius Muscles Determined by the ^{14}C DMO Distribution Ratio

One sartorius muscle (control) was equilibrated for 60 minutes in a 25 mM MOPS Ringer's solution (Solution #9). Trace amounts of

the weak acid ^{14}C DMO and the extracellular volume marker ^{3}H inulin were added to the bath solution as described under Materials and Methods. After 60 minutes the tissue was analyzed for total water, [^{14}C] and [^{3}H] as described under Materials and Methods. The intracellular pH was then computed using the distribution of the weak acid DMO according to the assumptions of Waddel and Butler (1959) and the algorithm of Hinke and Menard (1978), Appendix 1. The contralateral muscle (experimental) was treated in an identical manner with the exception that 20 mM propionate was substituted for chloride ions in the bath solution (Solution #10). The results of 4 pairs of muscles are presented in Table 3. The intracellular pH values have been converted to hydrogen ion concentrations in Table 4. A mean control intracellular hydrogen ion concentration of $1.19 \times 10^{-7} \pm 1.6 \times 10^{-8}$ M (SEM) was found to be significantly different from the mean experimental value of $1.89 \times 10^{-7} \pm 9.8 \times 10^{-9}$ M (ANOVA $P < .05$, Summary Table 1, Appendix 6). The intracellular hydrogen ion concentrations correspond to a mean control tissue pH of 6.92 and a mean experimental tissue pH of 6.72.

<u>The Effect of Externally Administered Propionate on Whole Muscle Lactate Efflux from Frog Sartorius Muscles Bathed in 25 mM MOPS Ringer's Solution pH 6.80 (Solution #9 and #10)</u>

One sartorius muscle (control) was mounted in the efflux chamber and stimulated for 4 minutes in 25 mM MOPS Ringer (Solution #9). The chamber was emptied and the medium subsequently analyzed for lactate as described under Materials and Methods. The quantity of lactate accumulated in the chamber during each of five subsequent 10 minute intervals was determined in the same manner. The contralateral sartorius muscle (experimental) was treated in an identical manner with the exception that 20 mM propionate was

substituted for chloride ions during the third, fourth and fifth 10 minute intervals (Solution #10). The results of the lactate determinations from 6 pairs of muscles treated in this manner are presented in Figure 6. No significant differences were found to exist between experimental and control whole muscle efflux rates during the stimulation interval, (299.8 ± 17.7 $nmol \cdot g^{-1} \cdot min^{-1}$ (SEM) Experimental, 260.0 ± 19.8 $nmol \cdot g^{-1} \cdot min^{-1}$ Control). In the following 10 minute interval whole muscle efflux rates reached a measured maximum (382.7 ± 17.4 $nmol \cdot g^{-1} \cdot min^{-1}$ Experimental, 352.2 ± 17.0 $nmol \cdot g^{-1} \cdot min^{-1}$ Control) and then declined steadily in the case of the control tissues to reach a value of 141.4 ± 10.1 $nmol \cdot g^{-1} \cdot min^{-1}$ during the fifth 10 minute collection interval. Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in experimental whole muscle lactate efflux rates to a mean value of 411.7 ± 36.6 $nmol \cdot g^{-1} \cdot min^{-1}$ or 174% ± 14 (SEM) of control efflux rates (ANOVA, $p < .05$, Summary Table 2, Appendix 6). The experimental whole muscle efflux rate declined at a faster rate after propionate substitution, than did control whole muscle efflux rates, but was still 133% of control values during the fifth collection interval.

The Effects of Externally Administered Propionate on Whole Muscle Lactate Efflux From Frog Sartorius Muscles Bathed in Four Buffer Concentrations of MOPS Ringer's Solution pH 6.80 (Solutions #1 through #8)

One sartorius muscle (control) was mounted in the efflux chamber and stimulated for 4 minutes as per the previous experiment.

The chamber was emptied and the bath medium was analyzed for lactate as described under Materials and Methods. The quantity of lactate accumulated in the chamber during each of three subsequent 10 minute intervals was determined in the same manner and was used to calculate a mean whole muscle efflux rate. The contralateral sartorius muscle (experimental) was treated in an identical manner with the exception that 20 mM propionate was substituted for chloride ions during the third and final 10 minute interval. The results of 18 pairs of muscles exposed to 1, 5, 10 or 20 mM MOPS Ringer's solutions are presented below.

1 mM MOPS Ringer's Solution (Solutions #1 and #3). The results of the lactate determinations from 4 pairs of muscles exposed to 1 mM MOPS Ringer's solution and treated as described above are presented in Figure 7. No significant differences were found to exist between experimental and control whole muscle efflux rates during the stimulation interval (109.7 ± 6.0 $nmol \cdot g^{-1} \cdot min^{-1}$ (SEM) Experimental, 144.7 ± 22.9 $nmol \cdot g^{-1} \cdot min^{-1}$ Control). In the following 10 minute interval efflux rates reached a measured maximum (146.4 ± 4.5 $nmol \cdot g^{-1} \cdot min^{-1}$ Experimental, 174.3 ± 25.8 $nmol \cdot g^{-1} \cdot min^{-1}$ Control) and then declined gradually in the case of the control tissues to reach a value of 114.3 $nmol \cdot g^{-1} \cdot min^{-1}$ ± 9.5 during the third 10 minute collection interval. Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in experimental whole muscle lactate efflux rates to a mean value of 414.3 ± 18.8 $nmol \cdot g^{-1} \cdot min^{-1}$ or 375% ± 53 (SEM)

control whole muscle efflux rates (ANOVA, $p < .05$, Summary Table 5, Appendix 6).

5 mM MOPS Ringer's Solution (Solutions #3 and #4). The results of the lactate determinations from 4 pairs of muscles exposed to 5 mM MOPS Ringer's solution and treated as described previously are presented in Figure 8. No significant differences were found to exist between experimental and control whole muscle efflux rates during the stimulation interval (173.4 ± 4 25.6 $nmol \cdot g^{-1} \cdot min^{-1}$ (SEM) Experimental, 189.4 ± 29.1 $nmol \cdot g^{-1} \cdot min^{-1}$ Control). In the following 10 minute interval efflux rates increased, reaching a peak value of 235.8 ± 25.3 $nmol \cdot g^{-1} \cdot min^{-1}$ in the case of the experimental tissues. The control graph reached a mean whole muscle efflux rate of 231.5 ± 3.0 $nmol \cdot g^{-1} \cdot min^{-1}$ and remained somewhat constant during the second 10 minute interval (232.8 ± 24.3 $nmol \cdot g^{-1} \cdot min^{-1}$) before it declined abruptly to a value of 177.5 ± 16.7 $nmol \cdot g^{-1} \cdot min^{-1}$ during the third 10 minute interval. The experimental tissues showed a decline in mean efflux rates during the second 10 minute interval (210.0 ± 20.2 $nmol \cdot g^{-1} \cdot min^{-1}$). Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in experimental whole muscle lactate efflux rates to a mean value of 433.6 ± 27.8 $nmol \cdot g^{-1} \cdot min^{-1}$ or 252% ± 31 (SEM) of control whole muscle efflux rates (ANOVA, $p < .05$, Summary Table 6, Appendix 6).

10 mM MOPS Ringer's Solution (Solutions #5 and #6). The results of the lactate determinations from 6 pairs of muscles exposed to 10 mM MOPS Ringer's Solution and treated as previously described are presented in Figure 9. No significant differences were found to exist between experimental and control whole muscle efflux rates during

the stimulation interval (155.0 ± 22.9 nmol·g^{-1}·min^{-1} (SEM) Experimental, 196.0 ± 30.6 nmol·g^{-1}·min^{-1} Control). In the following 10 minute interval efflux rates reached a measured maximum (258.3 ± 28.7 nmol·g^{-1}·min^{-1} Experimental, 281.8 nmol·g^{-1}·min^{-1} Control) and then declined steadily in the case of control tissues to a value of 189.5 nmol·g^{-1}·min^{-1} during the third 10 minute interval. Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in whole muscle lactate efflux rates to a mean value of 412.7 ± 49.8 nmol·g^{-1}·min^{-1} or 226% ± 28 (SEM) of control whole muscle efflux rates (ANOVA, $p < .05$, Summary Table 7, Appendix 6).

20 mM MOPS Ringer's Solution (Solutions #7 and #8). The results of the lactate determinations from 4 pairs of muscles exposed to 20 mM MOPS Ringer's solution and treated as described previously are presented in Figure 10. No significant differences were found to exist between experimental and control whole muscle efflux rates during the stimulation interval (248.1 ± 20.3 nmol·g^{-1}·min^{-1} (SEM) Experimental, 251.2 ± 29.2 nmol·g^{-1}·min^{-1} Control). In the following 10 minute interval efflux rates reached a measured maximum (369.9 nmol·g^{-1}·min^{-1} Experimental, 367.5 nmol·g^{-1}·min^{-1} Control) and then declined steadily in the case of control tissues to a value of 212.3 ± 24.0 nmol·g^{-1}·min^{-1} during the third and final 10 minute interval. Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in whole muscle lactate efflux rates to a mean value of 385.6 ± 29.2 nmol·g^{-1}.

min^{-1} or 190% ± 30 (SEM) of control whole muscle efflux rates (ANOVA, $p < .05$, Summary Table 8, Appendix 6).

The Effects of External MOPS Buffer Concentration on Whole Muscle Lactate Efflux

A comparison of the whole muscle lactate efflux rates during the first 10 minute interval suggests that high external buffer concentrations may increase lactate efflux rates. As no significant differences were found to exist between control and experimental whole muscle efflux rates during the first 10 minute interval, pooled mean values for each buffer concentration were compared (Table 5). The pooled mean whole muscle efflux rate for tissues bathed in 10 mM MOPS Ringer's solution (270.1 ± 18.5 $nmol \cdot g^{-1} \cdot min^{-1}$ (SEM)) was found to be significantly higher than the pooled mean whole muscle efflux rate for tissues bathed in 1 mM MOPS Ringer's solution (160.4 ± 13.2 $nmol \cdot g^{-1} \cdot min^{-1}$). In addition the pooled mean whole muscle efflux rate for tissues bathed in 20 mM MOPS Ringer's solution (368.7 ± 16.6 $nmol \cdot g^{-1} \cdot min^{-1}$) was found to be significantly higher than the pooled mean whole muscle efflux rate for tissues bathed in 1 mM (160.4 ± 13.2 $nmol \cdot g^{-1} \cdot min^{-1}$), 5 mM (233.7 ± 13.6 $nmol \cdot g^{-1} \cdot min^{-1}$) or 10 mM MOPS Ringer's solution (270.1 ± 18.5 $nmol \cdot g^{-1} \cdot min^{-1}$) (ANOVA, $p < .05$, Summary Table 4, Appendix 6).

The Influence of External MOPS Buffer Concentration on Experimental Whole Muscle Efflux Rates

Expressed as a percent of control, the mean whole muscle experimental lactate efflux during the 30 minute interval was found

to be 375% ± 53 (SEM), 252% ± 31, 226% ± 28, 190% ± 30, and 174% ± 14 for tissues bathed in 1, 5, 10, 20 and 25 mM MOPS Ringer's solution, respectively. The data on which the percent of control efflux values are computed are presented in Appendix 7. A decreasing non linear function was found to define the relationship between the experimental whole muscle efflux expressed as a percent of control, and buffer concentration of the Ringer's solution for the five buffer concentrations studied in the efflux experiments (Figure 11). A first order exponential with an asymptote was fitted to the data. The parameters of the function were determined by least square estimates as obtained from BMDP Statistical Software Package P3R, a non-linear regression. The curve drawn through the experimental data points is defined by the equation;

$$Y = 234.6\ e^{-0.216\ X} + 183.2$$

where Y is experimental whole muscle efflux rate expressed as a percent of control,

X is MOPS buffer concentration in mM.

The mean deviation of the data points above and below the theoretical curve is less than 9 units. The maximum deviation of any data point from the theoretical curve is 7%. This suggests that the theoretical curve adequately describes the data. It is evident from the equation of the curve that if the buffer concentration is extrapolated to infinity, the experimental whole muscle efflux as a percent of control efflux values is 183.2%.

Tissue Lactate Levels at the End of the Efflux Experiments

At the end of the efflux experiments, control and experimental tissues were frozen for lactate analysis as outlined under Materials and Methods. Experimental tissues showed mean tissue lactate levels of 10.6 ± 1.0 $\mu mol \cdot g^{-1} \cdot min^{-1}$ (SEM), 8.8 ± 1.2 $\mu mol \cdot g^{-1}$, 8.7 ± 1.0 $\mu mol \cdot g^{-1}$ and 6.8 ± 0.6 $\mu mol \cdot g^{-1}$ for tissues bathed in 1, 5, 10 and 20 mM MOPS Ringer's solutions (Table 6). Control tissues showed mean tissue lactate levels of 11.1 ± 0.5 $\mu mol \cdot g^{-1}$, 11.5 ± 1.0 $\mu mol \cdot g^{-1}$, 11.5 ± 1.0 $\mu mol \cdot g^{-1}$ and 10.1 ± 1.6 $\mu mol \cdot g^{-1}$ for tissues bathed in 1, 5, 10 and 20 mM MOPS Ringer's solutions (Table 5). Preliminary analysis showed no significant differences between experimental tissue lactate levels at the four buffer concentrations (ANOVA, Summary Table 9, Appendix 6). A similar result was found for control tissues at the four buffer concentrations (ANOVA, Summary Table 10, Appendix 6). The mean of all experimental tissue lactate values (8.7 ± 0.6 SEM, n = 18) was found to be significantly lower than the mean of all control tissue lactate values (11.1 ± 0.5, n = 18) (ANOVA 2, $p < .05$, Summary Table 11, Appendix 6).

Estimates of Tissue Lactate Levels Immediately After Stimulation

Estimates of tissue lactate levels immediately after stimulation can be made by adding to the individual post experiment tissue lactate values, the amount of lactate lost due to the corresponding tissue efflux. This method assumes, however, that all lactate present at the end of the stimulation interval is lost from the tissue solely through efflux. This ignores any fraction of lactate lost after stimulation through oxidation.

Experimental tissues showed mean tissue lactate estimates of 17.5 ± 0.9 $\mu mol \cdot g^{-1}$ (SEM), 17.6 ± 1.1 $\mu mol \cdot g^{-1}$, 17.6 ± 1.8 $\mu mol \cdot g^{-1}$ and 17.2 ± 0.9 $\mu mol \cdot g^{-1}$ for tissues bathed in 1, 5, 10 and 20 mM MOPS Ringer's solutions (Table 7). Control tissues showed mean tissue lactate estimates of 15.6 ± 0.6 $\mu mol \cdot g^{-1}$, 18.2 ± 0.9 $\mu mol \cdot g^{-1}$, 18.6 ± 1.3 $\mu mol \cdot g^{-1}$ and 18.9 ± 2.5 $\mu mol \cdot g^{-1}$ for tissues bathed in 1, 5, 10 and 20 mM MOPS Ringer's solutions (Table 7). Preliminary analysis showed no significant differences between experimental tissue lactate estimates at the four buffer concentrations (ANOVA, Summary Table 12, Appendix 6). A similar result was found for control tissues at the four buffer concentrations (ANOVA, Summary Table 13, Appendix 6). The mean of all experimental tissue lactate estimates (17.5 ± 0.7 $\mu mol \cdot g^{-1}$; n = 17) was not significantly lower than the mean of all control tissue lactate estimates (17.8 ± 0.8 $\mu mol \cdot g^{-1}$, n = 18) (ANOVA 2, $p > .05$, Summary Table 14, Appendix 6).

Lactate Efflux from Muscle Fibers as Computed on the Basis of a Two Compartment Diffusion Model

If the extracellular compartment of the isolated preparation is not assumed to be in equilibrium with the bulk phase, then an estimate of the amount of lactate in the extracellular space is dependent upon the diffusion characteristics of lactate, the whole muscle efflux of lactate and the maximum diffusion distance (½ muscle thickness). As the sartorius is considered to be a flat sheet, the steady-state oxygen diffusion model of Hill (1965) with the modifications and assumptions as outlined under Materials and Methods, is appropriate for the estimation of extracellular lactate. Knowledge of the bath lactate level at the end of a collection interval and knowledge of the extracellular lactate at the beginning and end of the collection

interval enables one to estimate the mean rate of lactate efflux across the sarcolemma membranes of the isolated sartorius muscle. The fiber efflux rates for tissues exposed to 1, 5, 10 and 20 mM MOPS Ringer's solutions were computed from the corresponding whole muscle efflux rates as presented previously in this section of the thesis. All raw data required for the fiber efflux computations are presented in Appendix 7.

Fiber Efflux Rates of Lactate for Tissues Exposed to 1 mM MOPS Ringer's Solution (Solutions #1 and #2). The results of the diffusion model computations from 4 pairs of muscle exposed to 1 mM MOPS Ringer's solution are presented in Figure 12. No significant differences were found to exist between experimental and control fiber efflux rates during the stimulation period (169.4 ± 4.9 $nmol \cdot g^{-1} \cdot min^{-1}$ (SEM) Experimental, 218.2 ± 25.3 $nmol \cdot g^{-1} \cdot min^{-1}$ Control). Fiber efflux rates steadily declined from this measured maximum value during stimulation to reach a value of 104.7 ± 8.7 $nmol \cdot g^{-1} \cdot min^{-1}$ during the third 10 minute collection interval in the case of the control tissues. Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically signficant increase in fiber lactate efflux rates to a mean value of 487.7 ± 24.2 $nmol \cdot g^{-1} \cdot min^{-1}$ or 482% ± 67 of control fiber efflux rates (ANOVA, $p < .05$, Summary Table 16, Appendix 6).

Fiber Efflux Rates of Lactate for Tissues Exposed to 5 mM MOPS Ringer's Solution (Solutions #3 and #4). The results of the diffusion model computations from 4 pairs of muscles exposed to 5 mM MOPS Ringer's solution are presented in Figure 13. No significant

differences were found to exist between experimental and control fiber efflux rates during the stimulation interval (261.9 ± 26.4 nmol·g^{-1}·min^{-1} (SEM). Experimental, 263.9 ± 28.3 nmol·g^{-1}·min^{-1} Control). Fiber Efflux rates steadily declined from this measured maximum value during stimulation to reach a value of 151.2 ± 21.8 nmol·g^{-1}·min^{-1} during the third 10 minute collection interval in the case of the control tissues. Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in fiber lactate efflux rates to a mean value of 486.7 ± 32.1 nmol·g^{-1}·min^{-1} or 345% ± 54 of control fiber efflux rates (ANOVA, $p < .05$, Summary Table 17, Appendix 6).

Fiber Efflux Rates of Lactate for Tissue Exposed to 10 mM MOPS Ringer's Solution (Solutions #5 and #6). The results of the diffusion model computations from 6 pairs of muscles exposed to 10 mM MOPS Ringer's solution are presented in Figure 14. No significant differences were found to exist between experimental and control fiber efflux rates during the stimulation interval (250.0 ± 32.0 nmol·g^{-1}·min^{-1} (SEM) Experimental, 318.6 ± 48.1 nmol·g^{-1}·min^{-1} Control). The fiber efflux increased during the first 10 minute interval after stimulation in the experimental tissues to reach a measured maximum value of 290.6 ± 46.6 nmol·g^{-1}·min^{-1}. The fiber efflux then declined to a value of 209.3 ± 23.1 nmol·g^{-1}·min^{-1} during the next interval. The fiber efflux for the control tissues steeply declined from its measured maximum value during stimulation to a value of 177.8 ± 18.4 nmol·g^{-1}·min^{-1} during the third 10 minute collection interval.

Following the addition of propiohate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in fiber lactate efflux rate to a mean value of 459.5 ± 54.7 $nmol \cdot g^{-1} \cdot min^{-1}$ or 272% ± 38 of control fiber efflux rates (ANOVA, $p < .05$, Summary Table 18, Appendix 6).

Fiber Efflux Rates of Lactate for Tissues Exposed to 20 mM MOPS Ringer's Solution (Solutions #7 and #8). The results of the diffusion model computations from 4 pairs of muscles exposed to 20 mM MOPS Ringer's solution are presented in Figure 15. No significant differences were found to exist between experimental and control fiber efflux rates during the stimulation interval (405.5 ± 11.3 $nmol \cdot g^{-1} \cdot min^{-1}$ (SEM) Experimental, 415.8 ± 49.3 $nmol \cdot g^{-1} \cdot min^{-1}$ Control). Fiber efflux rates declined very little during the first ten minute collection interval in both experimental and control tissues (402.0 ± 19.0 $nmol \cdot g^{-1} \cdot min^{-1}$ Experimental, 400.0 ± 29.8 $nmol \cdot g^{-1} \cdot min^{-1}$ Control). The fiber efflux dropped sharply after the first 10 minute interval to reach a value of 189.6 ± 22.6 $nmol \cdot g^{-1} \cdot min^{-1}$ in the case of the control tissues. Following the addition of propionate to the experimental tissues at 20 minutes, there was an immediate and statistically significant increase in fiber lactate efflux rates to a mean value of 410.9 ± 31.6 $nmol \cdot g^{-1} \cdot min^{-1}$ or 226% ± 35 of control fiber efflux rates (ANOVA, $p < .05$, Summary Table 19, Appendix 6).

The Effects of External MOPS Buffer Concentration on Lactate Efflux from Muscle Fibers

A comparison of the fiber efflux rates of lactate during the stimulation interval reveals a trend towards increasing fiber efflux values with increasing buffer concentrations. As no significant differences were found to exist between control and experimental fiber efflux rates during this interval, pooled mean values for each buffer concentration were compared (Table 8). The pooled mean fiber efflux rate for tissues bathed in 20 mM MOPS Ringer's solution (410.6 ± 23.7 $nmol \cdot g^{-1} \cdot min^{-1}$ (SEM)) was found to be significantly higher than the pooled mean fiber efflux for tissues bathed in 1 mM (198.8 ± 15.9 $nmol \cdot g^{-1} \cdot min^{-1}$), 5 mM (262.8 ± 17.7 $nmol \cdot g^{-1} \cdot min^{-1}$) or 10 mM MOPS Ringer's solution (284.3 ± 29.7 $nmol \cdot g^{-1} \cdot min^{-1}$) (ANOVA, $p < .05$, Summary Table 15, Appendix 6).

The Influence of External MOPS Buffer Concentration on Experimental Fiber Efflux Rates

Expressed as a percent of control, the mean experimental fiber lactate efflux rate during the 30 minute interval was found to be 482% ± 67, 345% ± 54, 272% ± 38 and 226% ± 35 for tissues bathed in 1, 5, 10 and 20 mM MOPS Ringer's solutions, respectively. In addition, the experimental fiber efflux for tissues exposed to 25 mM MOPS Ringer's solution was found to be 210% ± 18. Individual fiber efflux data for the 25 mM experiments were not presented, as tissue weights in these experiments were determined with the suture loops attached (see Materials and Methods). As the maximum diffusion distance is computed from tissue weight, a gross over-estimation of diffusion distance and ultimately fiber efflux will

occur. The effect of this error is neutralized when experimental fiber efflux is computed as a percent of control values, where control values are computed in an identical manner. The data from which the percent of control efflux values are computed are presented in Appendix 7.

A decreasing non-linear function was found to define the relationship between the experimental fiber efflux expressed as a percent of control, and buffer concentration of the Ringer's solution for the five buffer concentrations studied (Figure 16). A first order exponential with asymptote was fitted to the data. The parameters of the function were determined by least square estimates as obtained from BMDP Statistical Software Package P3R, a non-linear regression. The curve drawn through the experimental data points is defined by the equation;

$$Y = 320.5\ e^{-0.169\ X} + 210.3$$

where Y is experimental fiber efflux expressed as a percent of control,

X is MOPS buffer concentration in mM.

The mean deviation of the data points above and below the theoretical curve is less than 3.5 units. The maximum deviation of any data point from the theoretical curve is less than 2.5%. This suggests that the theoretical curve adequately describes the data. It is evident from the equation of the curve that if the buffer concentration is extrapolated to infinity, the experimental fiber efflux as a percent of control efflux values will be 210.3%.

Table 2. The uptake of ^{14}C propionate in resting frog sartorius muscles.

Muscle I.D.	Weight of Counted Tissue (g)	Water gH_2O / 100 g Tissue	V_t Water [illegible] Tissue (ml)	D_c Tissue ^{14}C (DPM)	D_H Tissue ^3H (DPM)	$[B_c]$ Bath $[^{14}C]$ ($DPM \cdot ml^{-1}$)	$[B_H]$ Bath $[^3H]$ ($DPM \cdot ml^{-1}$)	Extra-cellular Water (ml)	Intra-cellular Water (ml)	^{14}C in Extra-cellular Water (DPM)	Intra-cellular ^{14}C (DPM)	Intra-cellular $[^{14}C]$ Contentration ($DPM \cdot ml\ H_2O_i$)	Intra-cellular Propionate Concentration (mM)
1	0.0320	80.5	0.0258	3019.2	7723.6	197830.0	11972415.1	0.0065	0.0193	1285.9	1733.3	89808.3	9.08
2	0.0396	79.2	0.0314	3961.5	10194.8	193266.7	1198173.3	0.0085	0.0229	1685.3	2276.2	99397.3	10.03
3	0.0438	80.1	0.0351	4743.4	11907.9	200772.5	1202956.3	0.0099	0.0252	1987.6	2755.8	109357.1	10.89
4	0.0314	80.2	0.0252	3387.8	6314.1	196921.8	1209223.4	0.0052	0.0200	1024.0	2363.8	118190.0	12.00
$\bar{X}$ S.E.M.						1.98×10^5 $\pm 8.23 \times 10^2$						1.04×10^5 $\pm 6.14 \times 10^3$	10.5 $\pm$ 0.6

Table 3. The effect of externally administered propionate on intracellular pH as determined by the ^{14}C DMO distribution ratio.

Muscle I.D.	Weight of Counted Tissue (g)	% Water $\frac{gH_2O}{100\ g\ Tissue}$	V_t Water Content of Tissue (ml)	D_C Tissue ^{14}C (DPM)	D_H Tissue ^{3}H (DPM)	$[B_C]$ Bath $[^{14}C]$ $(DPM \cdot ml^{-1})$	$[B_H]$ Bath $[^{3}H]$ $(DPM \cdot ml^{-1})$	pHe Extra-cellular pH	pHi Intra-cellular pH estimate
Control 1	0.0293	81.0	0.0237	1077.2	10778.7	38360.9	1276178.7	6.80	6.94
Experimental 1	0.0318	81.7	0.0260	874.8	13776.1	36764.3	1312601.7	6.81	6.72
Control 2	0.0454	76.5	0.0347	1735.1	13190.3	37084.0	1259427.0	6.80	7.02
Experimental 2	0.0431	76.9	0.0331	1081.9	10276.1	36938.2	1292733.8	6.82	6.72
Control 3	0.0301	80.5	0.0242	858.1	10301.5	36434.4	1340343.0	6.80	6.78
Experimental 3	0.0249	81.2	0.0202	638.3	8473.4	36789.4	1274246.5	6.81	6.67
Control 4	0.0265	77.3	0.0205	981.4	8731.4	36915.7	1331926.8	6.80	7.00
Experimental 4	0.0316	78.6	0.0248	867.3	10527.0	35985.2	1310851.4	6.81	6.78

Table 4. The effect of externally administered propionate on intracellular $[H^+]$ as determined by the ^{14}C DMO distribution ratio.

	Control pH	Control $[H_i^+]$ M	$[H_i]/[H_o]$	Experimental pH	Experimental $[H_i^+]$ M	$[H_i]/[H_o]$
Experiment 1	6.94	1.15×10^{-7}	0.72	6.72	1.90×10^{-7}	1.20
Experiment 2	7.02	9.57×10^{-8}	0.61	6.72	1.90×10^{-7}	1.20
Experiment 3	6.78	1.66×10^{-7}	1.05	6.67	2.12×10^{-7}	1.34
Experiment 4	7.00	1.01×10^{-7}	0.64	6.78	1.64×10^{-7}	1.04
MEAN of $[H^+]$	$1.19 \times 10^{-7} \pm 1.6 \times 10^{-8}$ SEM pH 6.92**			$1.89 \times 10^{-7} \pm 9.8 \times 10^{-9}$* pH 6.72**		

*Significantly different from control. ANOVA $P < 0.05$.

**Computed from mean hydrogen ion concentration.

$pH_o = 6.8$, $[H_o^+ = 1.58 \times 10^{-7}$ M]

Table 5. Pooled whole muscle lactate efflux during the first 10 minute interval of recovery.

	Buffer Concentration (mM)			
	1	5	10	20
Whole Muscle Efflux	160.3±13.2	233.7±13.6	270.1±18.5*	368.7±16.6**
$nmol \cdot g^{-1} \cdot min^{-1} \pm SEM$ (n)	(8)	(7)	(12)	(8)

*Significantly different from 1 mM values $P < 0.05$ ANOVA.

**Significantly different from 1 mM, 5 mM and 10 mM values $P < 0.05$ ANOVA.

Table 6. Tissue lactate levels after 30 minutes of recovery.

Tissue	Buffer Concentration (mM)				
	1	5	10	20	$\bar{X}$
Control $\mu mol \cdot g^{-1} \pm SEM$ (n)	11.1±0.5 (4)	11.5±1.0 (4)	11.5±1.0 (6)	10.1±1.6 (6)	11.1±0.5 (18)
Experimental $\mu mol \cdot g^{-1} \pm SEM$ (n)	10.6±1.0 (4)	8.8±1.2 (4)	8.7±1.0 (6)	6.8±0.6 (4)	8.7±0.6* (18)

*Experimental lactate levels are significantly different from controls. ANOVA $P < .05$.

Table 7. Estimate of tissue lactate levels immediately after stimulation.

Tissue	Buffer Concentration (mM)				
	1	5	10	20	$\bar{X}$
Control	15.6±0.6	18.2±0.9	18.6±1.3	18.9±2.5	17.8±0.8
$\mu mol \cdot g^{-1} \pm SEM$ (n)	(4)	(3)	(6)	(4)	(17)
Experimental	17.5±0.9	17.6±1.1	17.6±1.8	17.2±0.9	17.5±0.7
$\mu mol \cdot g^{-1} \pm SEM$ (n)	(4)	(4)	(6)	(4)	(18)

Table 8. Pooled fiber lactate efflux during the stimulation interval.

	Buffer Concentration (mM)			
	1	5	10	20
Fiber Efflux $nmol \cdot g^{-1} \cdot min^{-1} \pm SEM$ (n)	193.8±15.9 (6)	262.8±17.7 (7)	284.3±29.7 (8)	410.6±23.7* (8)

*Significantly different from 1 mM, 5 mM and 10 mM values $P < 0.05$ ANOVA.

Figure 6

Whole muscle lactate efflux from paired isolated muscles bathed in 25 mM MOPS Ringer ± SEM, n = 6.

● Control solution #9 .

■ Experimental solution #10

* Significantly different from control efflux $p < .05$

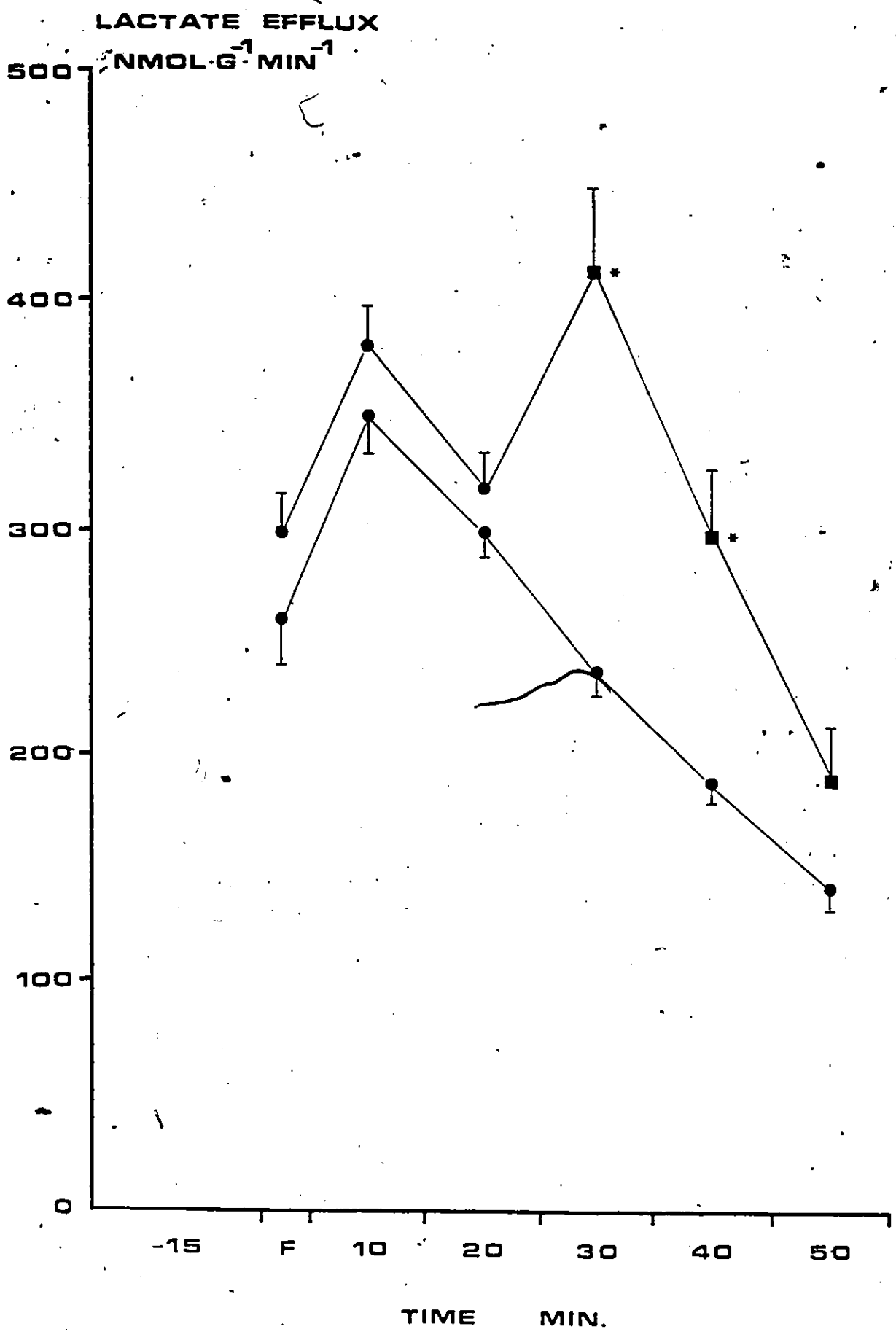

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
-15
F
10
20
30
40
50
TIME MIN.

Figure 7

Whole muscle lactate efflux from paired isolated muscles bathed in 1 mM MOPS Ringer ± SEM, n = 4 unless otherwise marked.

● Control solution #1

■ Experimental solution #2

* Significantly different from control efflux $p < .05$

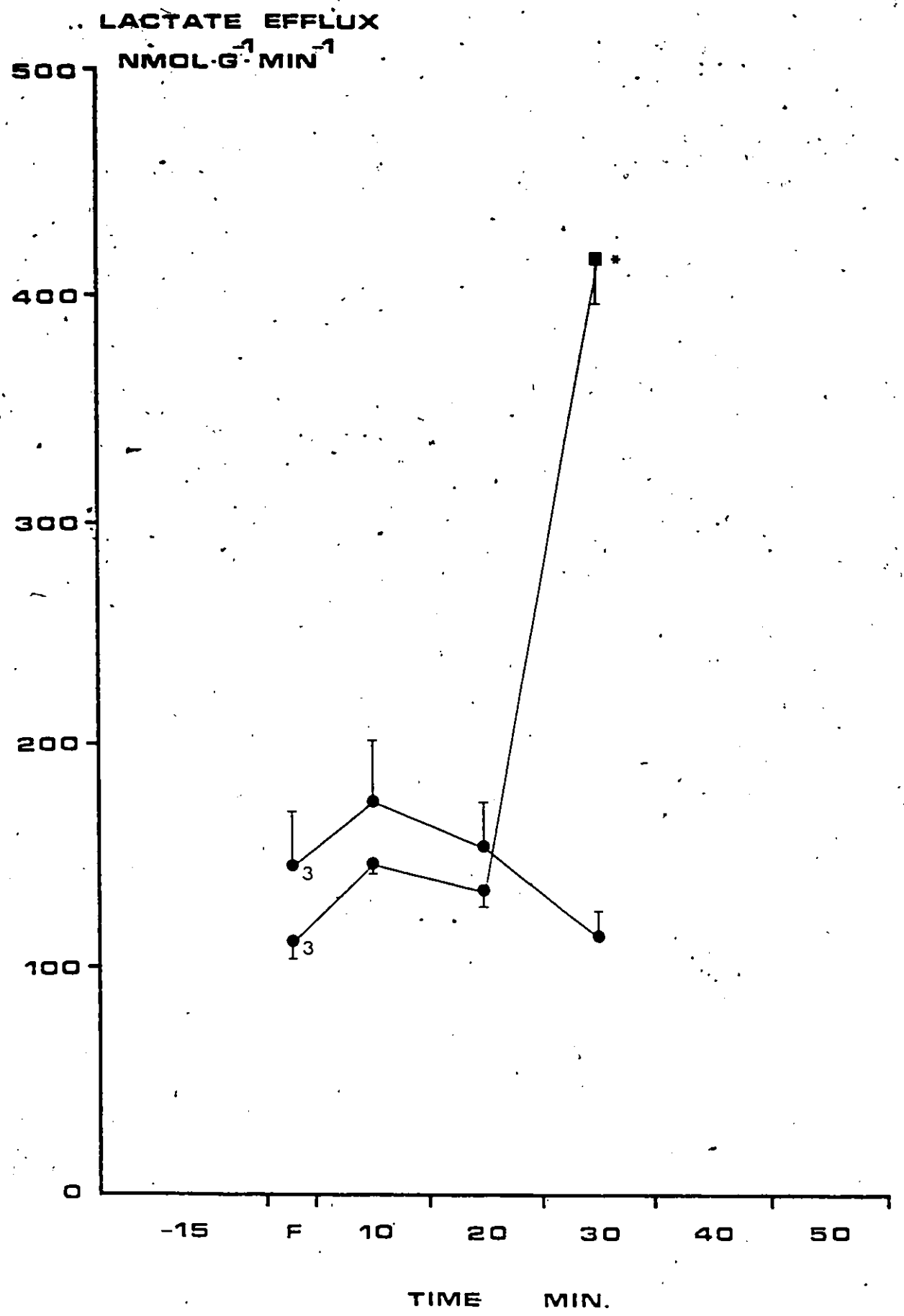

LACTATE EFFLUX
NMOL·G⁻¹ MIN⁻¹
500
400
300
200
100
0
3
3
-15
F
10
20
30
40
50
TIME MIN.

Figure 8

Whole muscle lactate efflux from paired isolated muscles bathed in 5 mM MOPS Ringer ± SEM, n = 4 unless otherwise marked.

● Control solution #3

■ Experimental solution #4

* Significantly different from control efflux $p < .05$

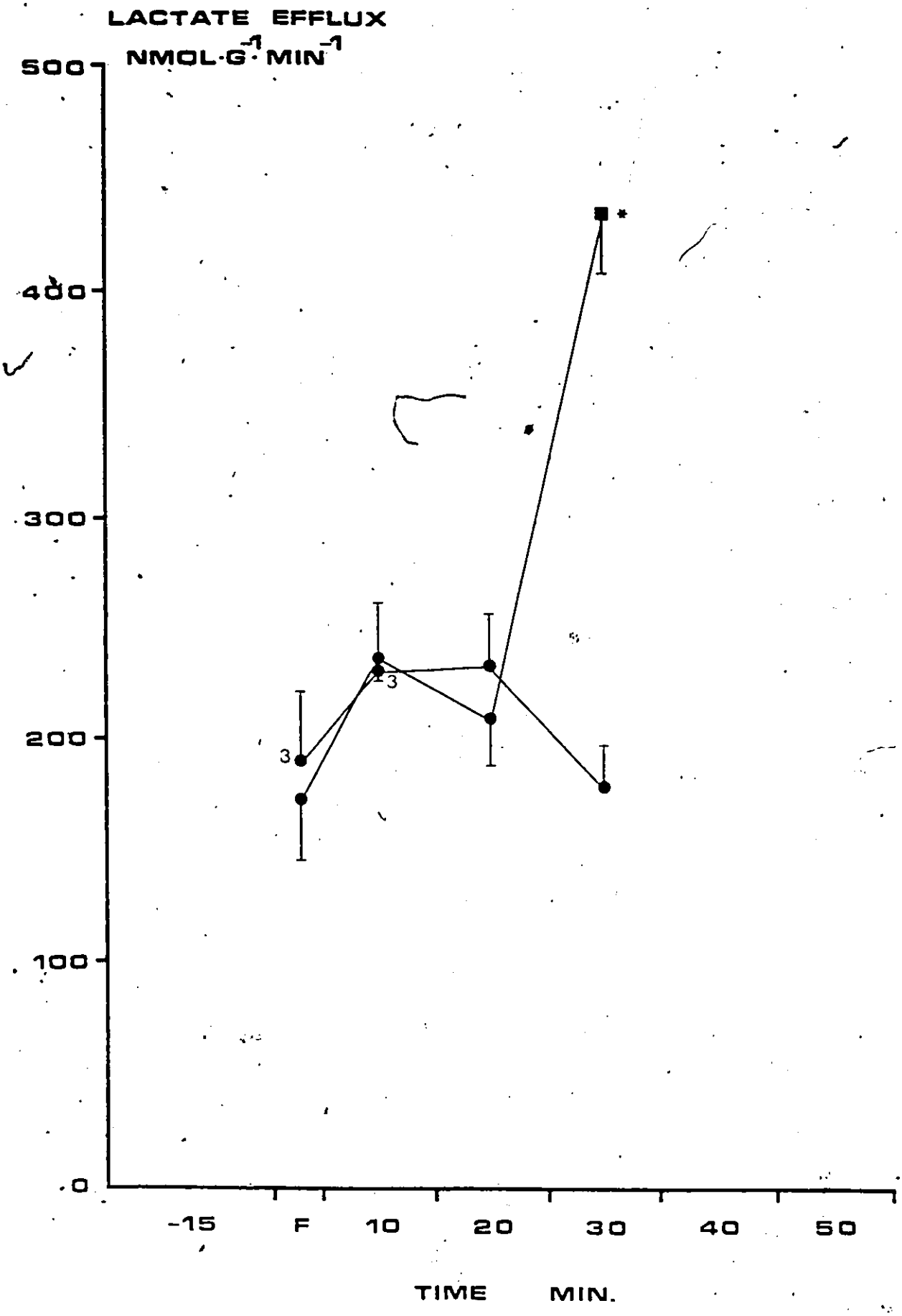

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
3
3
*
-15
F
10
20
30
40
50
TIME MIN.

Figure 9

Whole muscle lactate efflux from paired isolated muscles bathed in 10 mM MOPS Ringer ± SEM, n = 6 unless otherwise marked.

● Control solution #5

■ Experimental solution #6

* Significantly different from control efflux $p < .05$

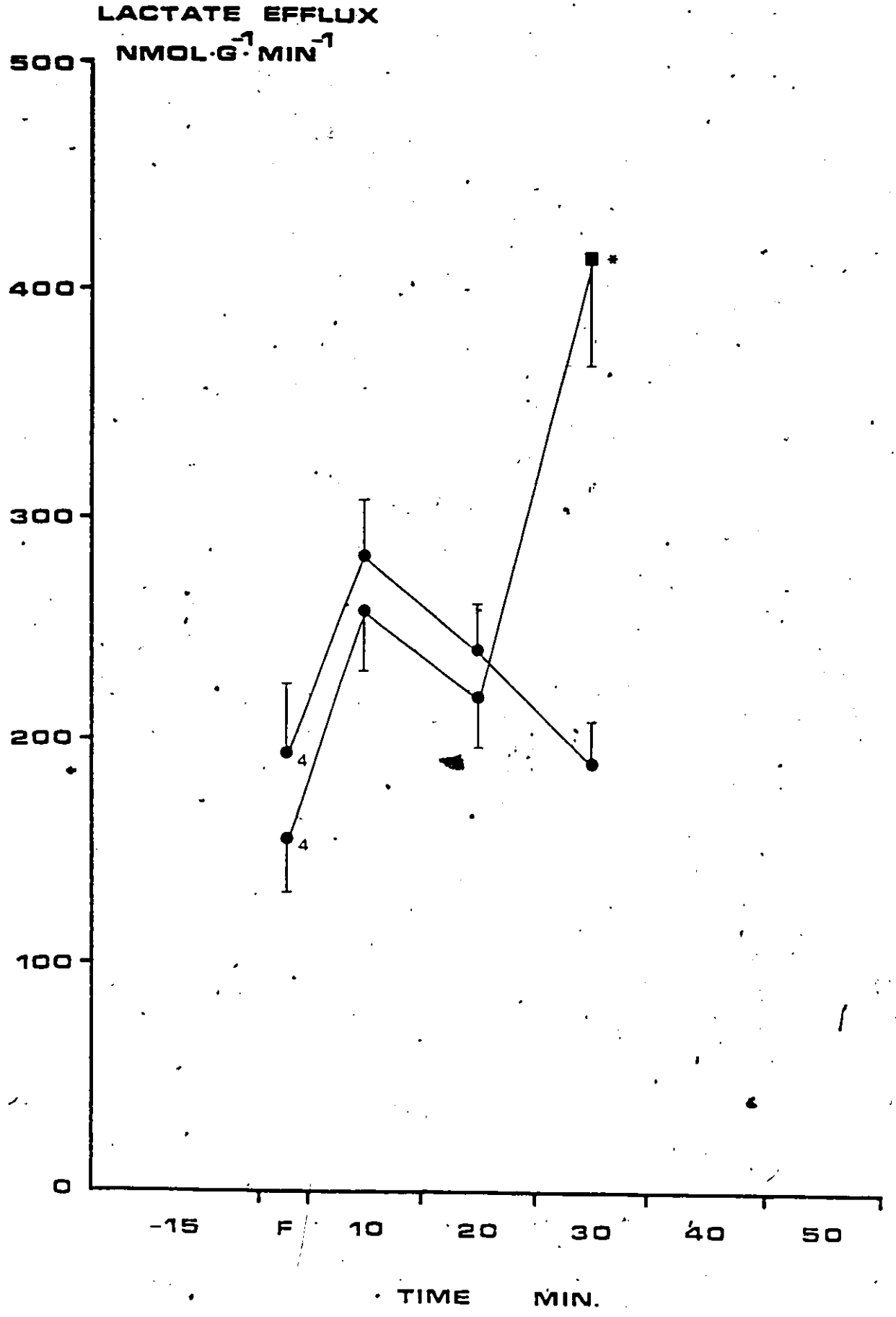

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
4
4
*
-15
F
10
20
30
40
50
TIME MIN.

Figure 10

Whole muscle lactate efflux from paired isolated muscles bathed in 20 mM MOPS Ringer ± SEM, n = 4 unless otherwise marked.

● Control solution #7

■ Experimental solution #8

* Significantly different from control efflux $p < .05$

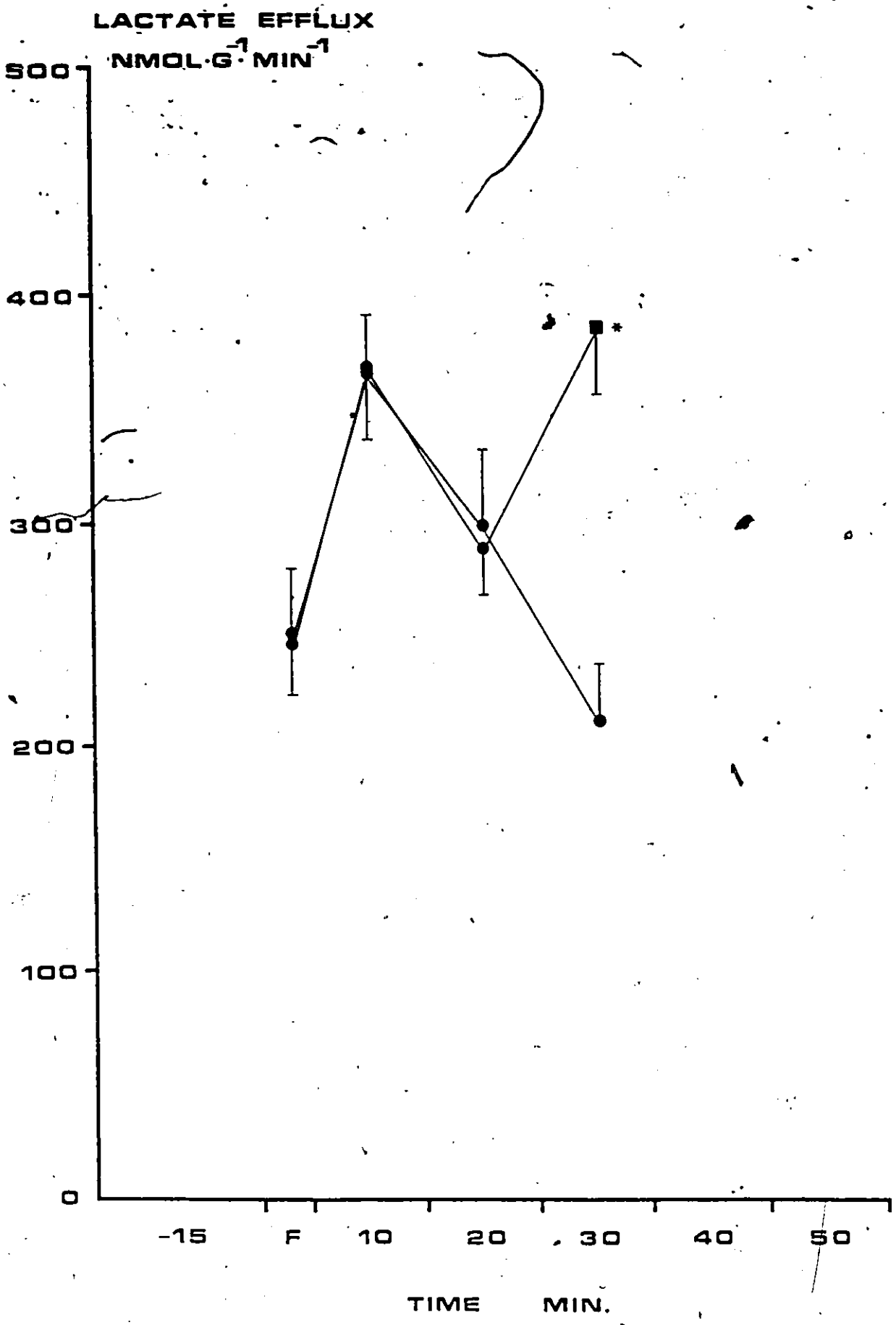

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
-15
F
10
20
30
40
50
TIME MIN.

Figure 11

Experimental whole muscle lactate efflux during the final 10 minute interval of recovery (20-30 minutes) as a percent of control efflux. Data is presented for five buffer concentrations. n = 4 for 1, 5 and 20 mM buffer concentration, n = 6 for 10 and 25 mM buffer concentrations. The curve is defined by the equation;

$$y = 234.6\, e^{-0.216\, X} + 183.2$$

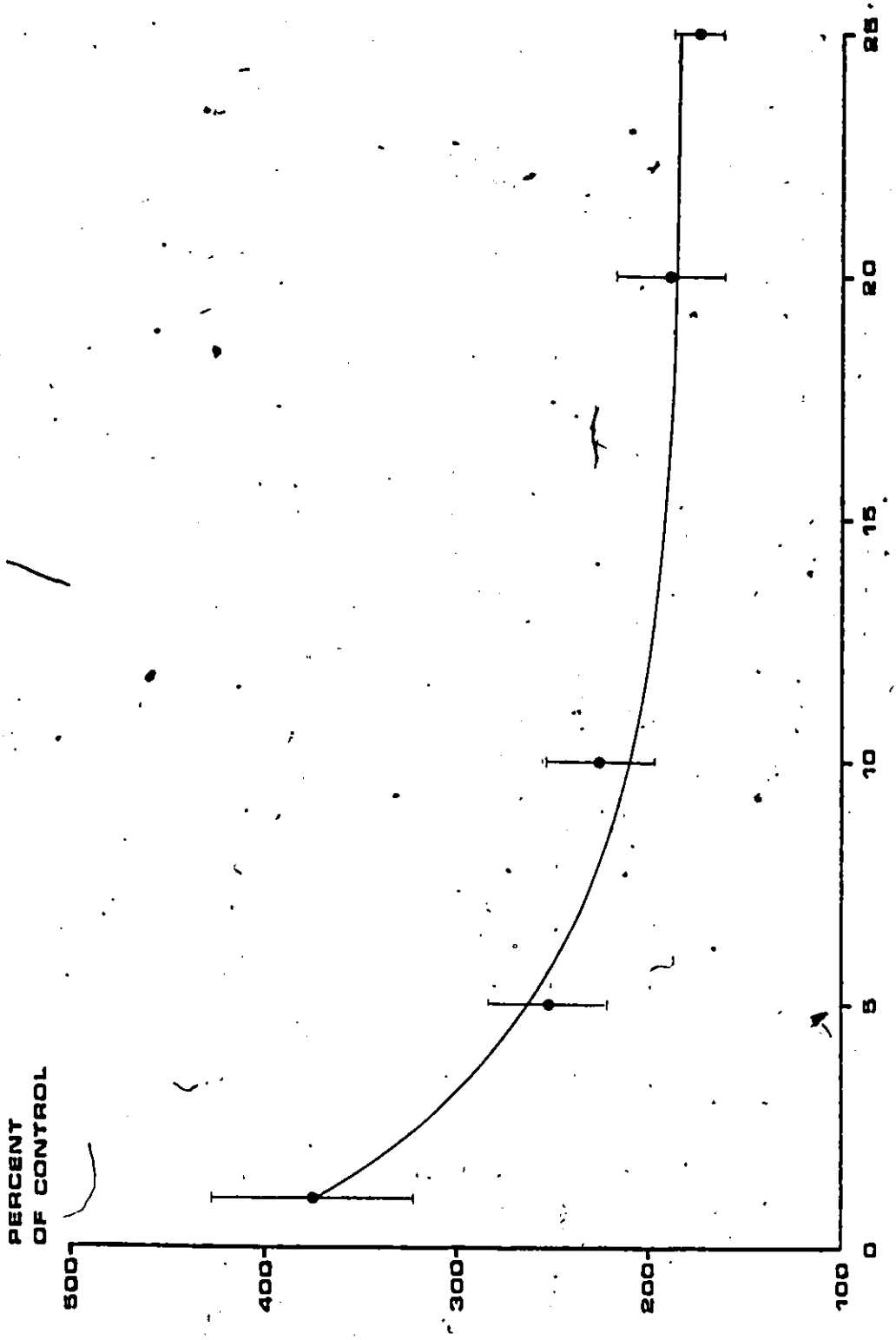

PERCENT
OF CONTROL
500
400
300
200
100
0
5
10
15
20
25
BUFFER CONC.
mM

Figure 12

Lactate efflux from muscle fibers of paired isolated muscles bathed in 1 mM MOPS Ringer ± SEM, $n_{\sim}$ = 4 unless otherwise marked.

● Control solution #1

■ Experimental solution #2

* Significantly different from control efflux $p < .05$

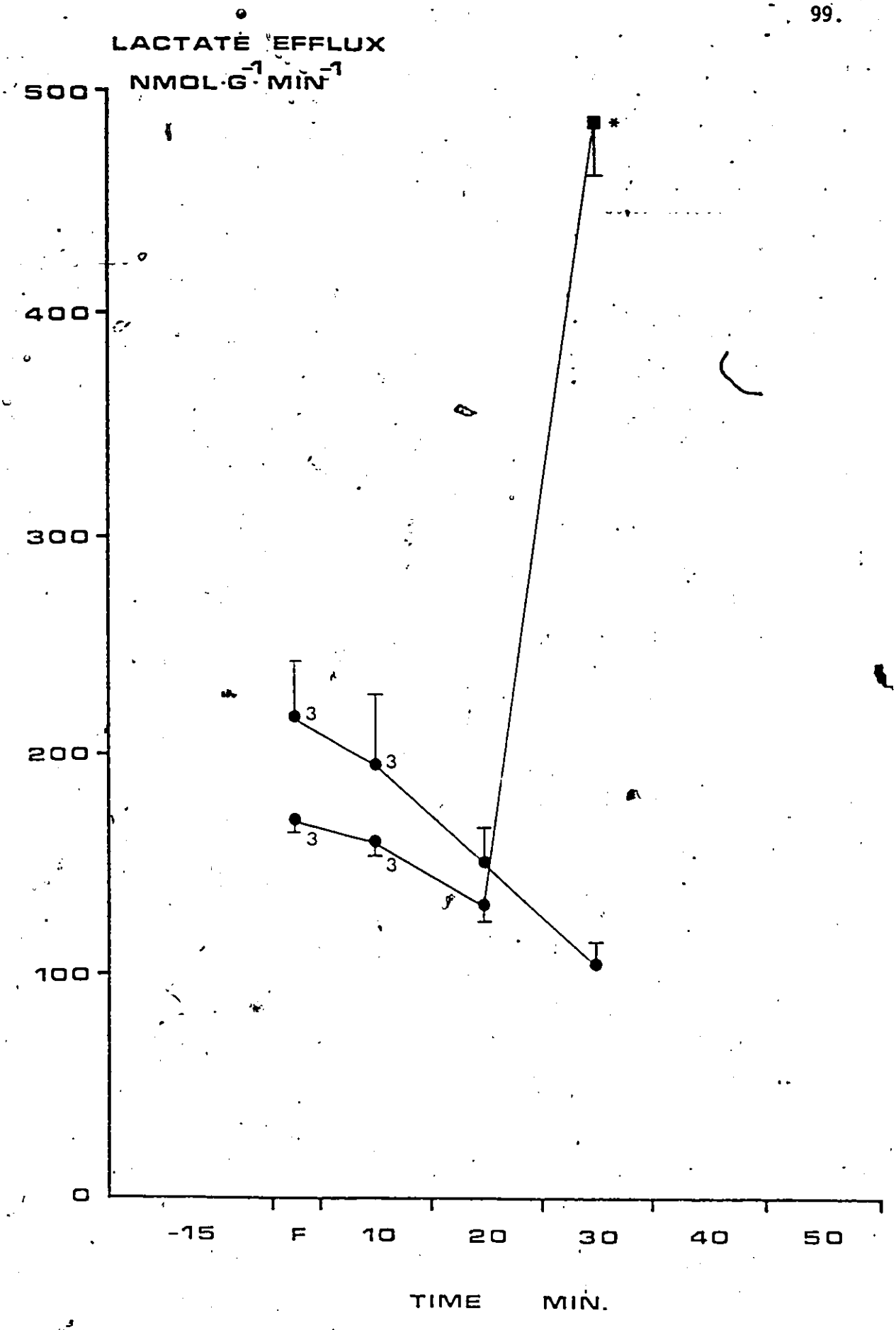

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
3
3
3
3
*
-15
F
10
20
30
40
50
TIME MIN.

Figure 13

Lactate efflux from muscle fibers of paired isolated muscles bathed in 5 mM MOPS Ringer ± SEM, n = 4 unless otherwise marked.

● Control solution #3

■ Experimental solution #4

* Significantly different from control efflux $p < .05$

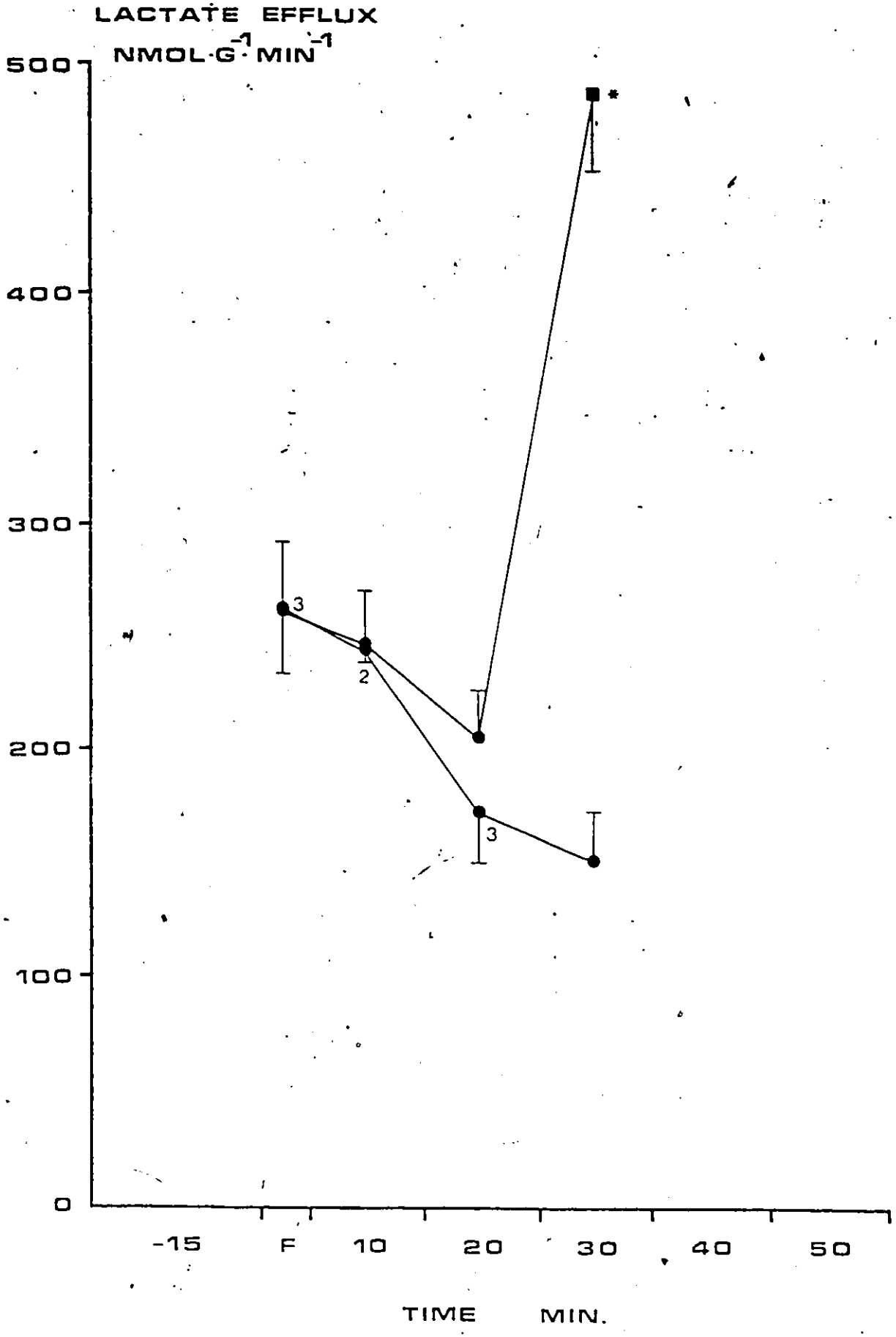

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
3
2
3
*
-15
F
10
20
30
40
50
TIME MIN.

Figure 14

Lactate efflux from muscle fibers of paired isolated muscles bathed in 10 mM MOPS Ringer ± SEM, n = 6 unless otherwise marked.

● Control solution #5

■ Experimental solution #6

* Significantly different from control efflux $p < .05$

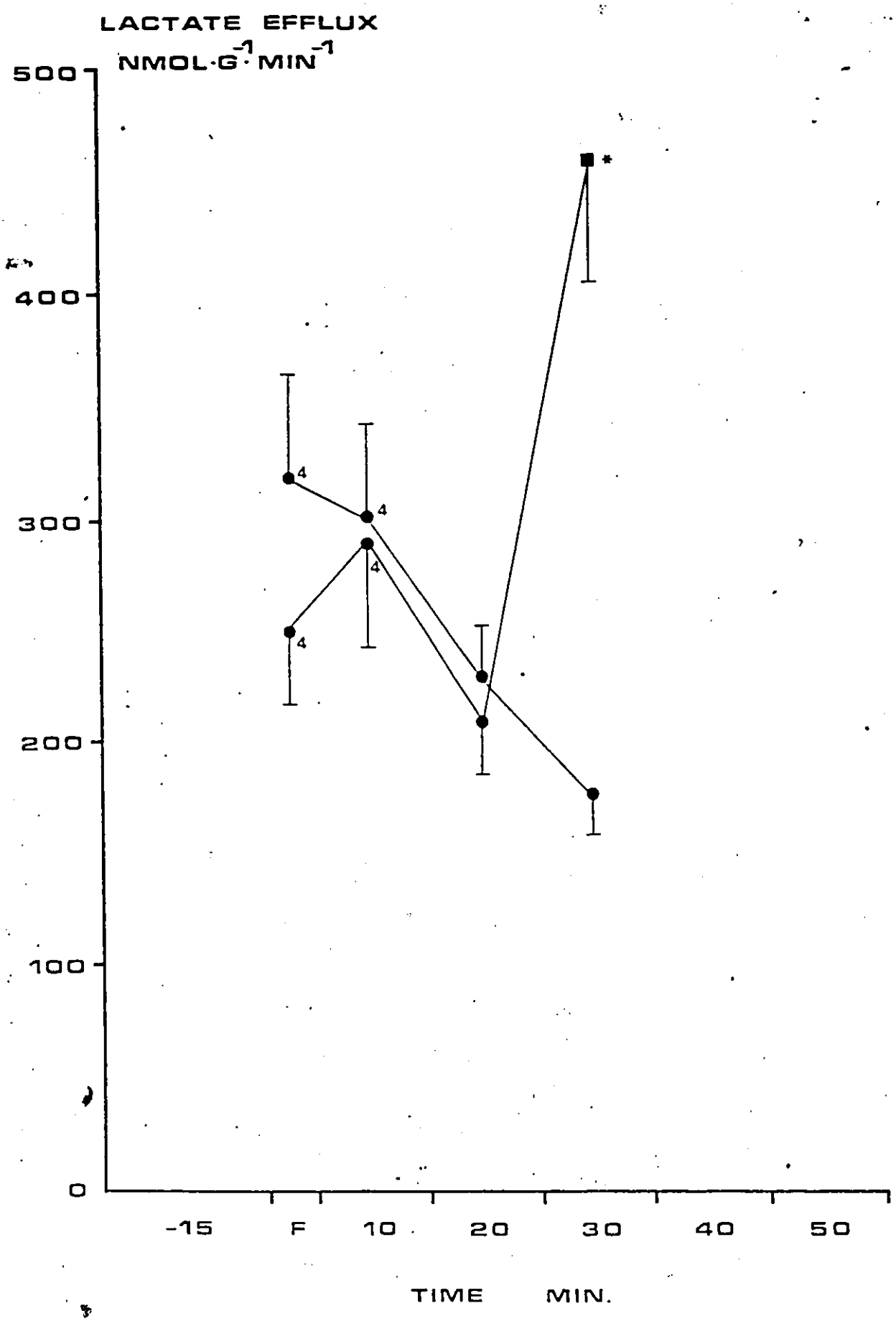

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
4
4
4
4
*
-15
F
10
20
30
40
50
TIME MIN.

Figure 15

Lactate efflux from muscle fibers of paired isolated muscles bathed in 20 mM MOPS Ringer ± SEM, n = 4.

● Control solution #7

■ Experimental solution #8

* Significantly different from control efflux $p < .05$

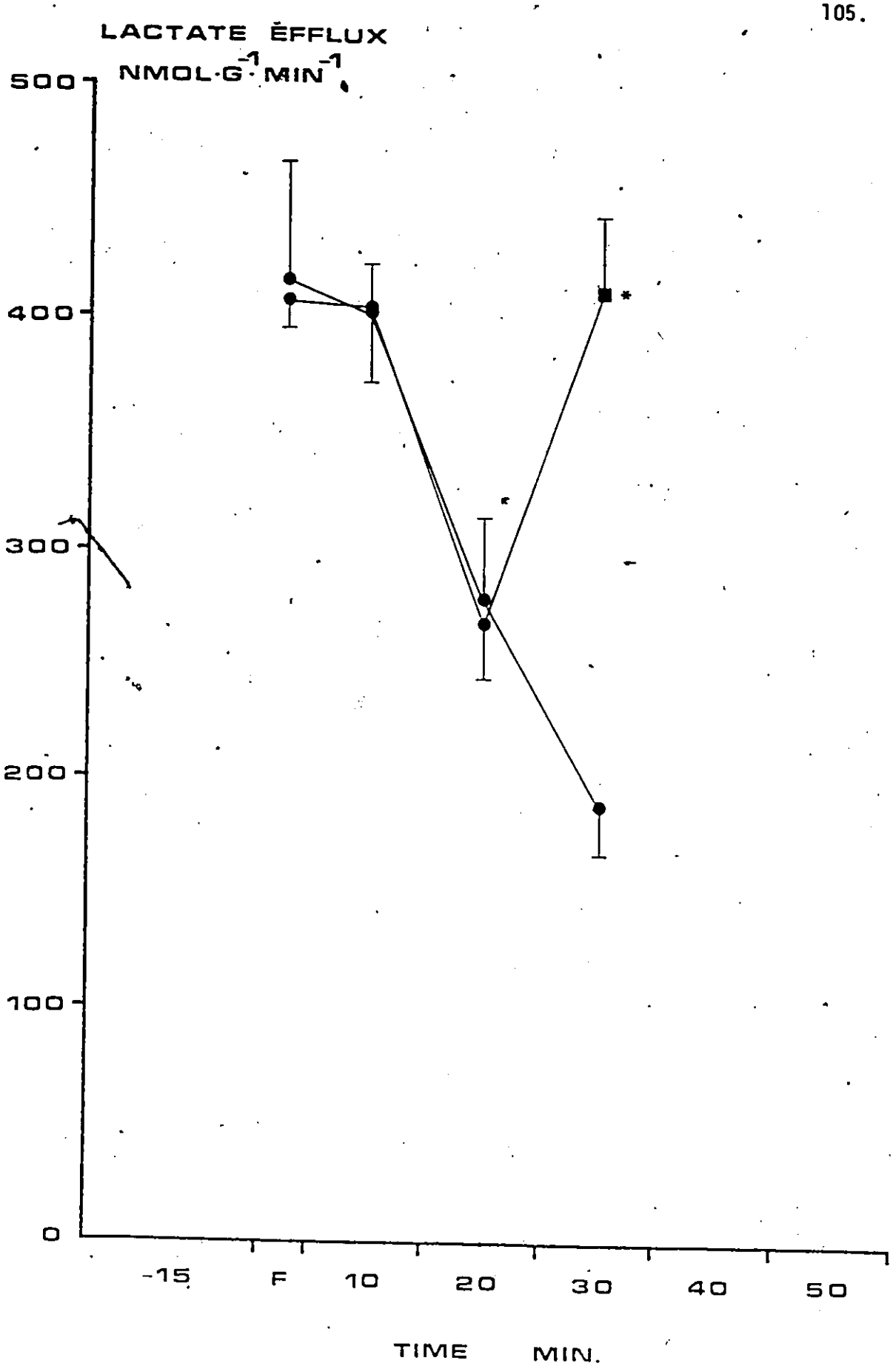
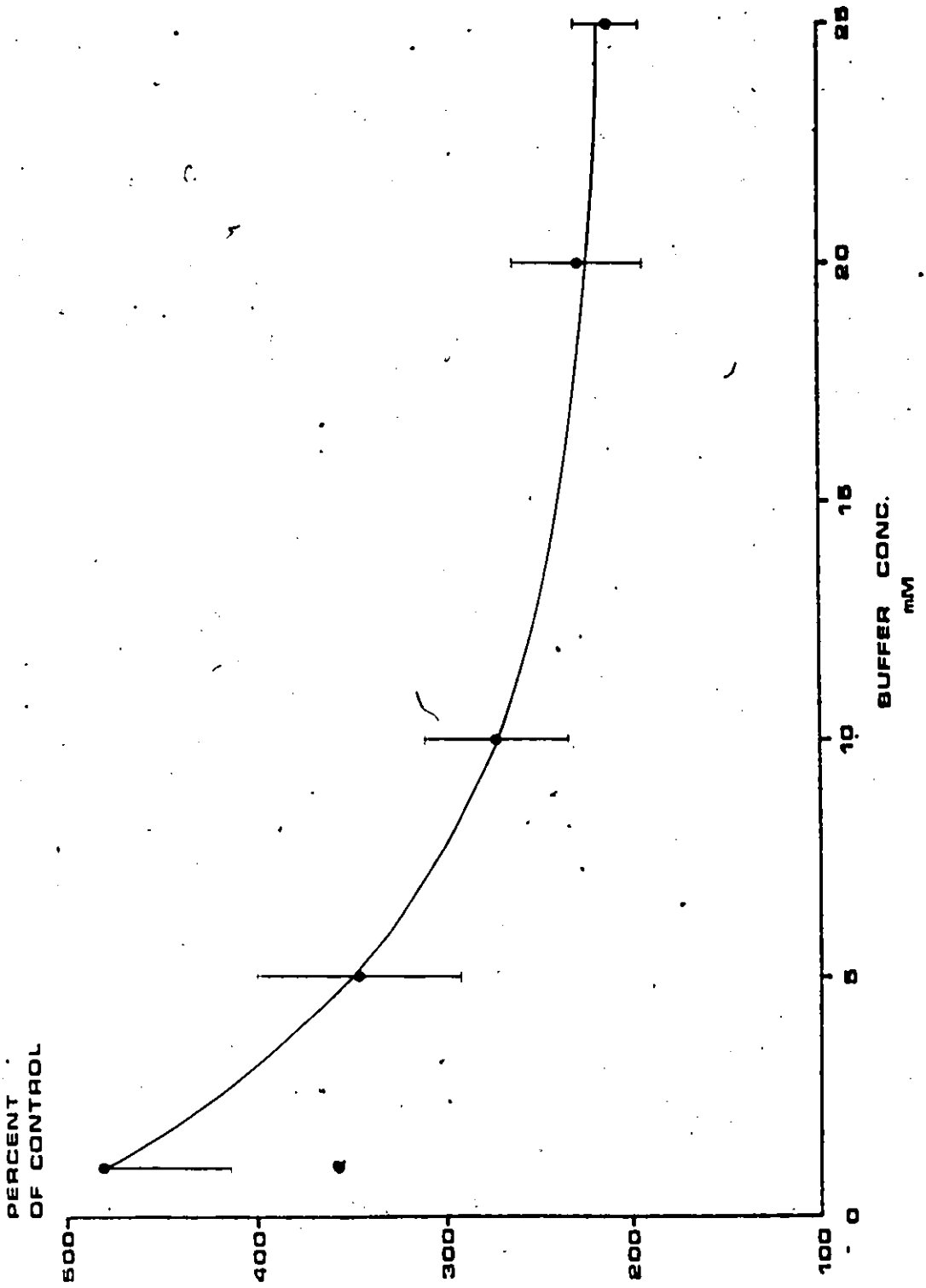

LACTATE EFFLUX
NMOL·G⁻¹·MIN⁻¹
500
400
300
200
100
0
-15
F
10
20
30
40
50
TIME MIN.

Figure 16

Experimental fiber lactate efflux during the final 10 minute interval of recovery (20-30 minutes) as a percent of control efflux. Data is presented for five buffer concentrations, n = 4 for 1, 5 and 20 mM buffer concentrations, n = 6 for 10 and 25 mM buffer concentrations. The curve is defined by the equation;

$$Y = 320.5\ e^{-0.169\ X} + 210.3$$


PERCENT
OF CONTROL
500
400
300
200
100
0
5
10
15
20
25
BUFFER CONC.
mM

Discussion

The discussion is divided into three main sections. The first is a discussion of the effect of propionate addition to the external solution on the intracellular pH of resting frog sartorius muscles. As propionate has been reported to acidify the intracellular space of a large number of tissues, it is important that its effect on frog sartorius muscles be discussed.

In light of the findings of the first section, the effect of propionate addition on lactate efflux from frog sartorius muscles will be discussed.

Finally, this section of the thesis will conclude with a discussion of the experimental data, in light of the five models of lactate efflux outlined in the Introduction.

Propionate Uptake and Intracellular pH

It is apparent from the results presented in Table 2 that a significant amount of ^{14}C propionate is taken up by the muscle fibers at rest. The mean value of $1.04 \times 10^5 \pm 6.14 \times 10^3$ $dpm \cdot ml^{-1}$ intracellular water is computed on the assumption that inulin is a satisfactory extracellular water marker in frog sartorius muscles. Sperelakis et al. (1978) have suggested on the basis of ^3H sucrose uptake and washout experiments, that the extracellular compartment may be made up of three compartments. The smallest of these three components may be inaccessible to the larger inulin molecule. This does not appear to be the case, however, as the measurement of extracellular space in frog sartorius muscles with inulin (24.8 ± 0.8%) and albumin (21.9 ± 0.5%)

are not much smaller than the sucrose space (26.5 ± 1.0%) (Tasker et al., 1959). Reliable measures of intracellular propionate require a sensitive measure of extracellular fluid, and it can be suggested that inulin may underestimate the extracellular space due to its inability or impeded ability to penetrate the smallest compartment of the Sperelakis model. On the basis of the result of Tasker et al. (1959) this error would be extremely small and would underestimate the extracellular space of the muscle by approximately 1.7%. The experiments of Neville (1979) also indicate that inulin gives an adequate estimate of extracellular space.

Therefore an estimate of intracellular propionate after 60 minutes of equilibration can be made using the known specific activity of the external solution. On the basis of this specific activity a mean intracellular propionate concentration of 10.5 ± 0.6 mM was computed to be present at this time.

If one assumes that all the propionate present in the intracellular space was obtained from extracellular sources by way of nonionic diffusion through the lipid portion of the membrane, then a significant reduction in intracellular pH would be expected upon dissociation. The pK of propionate (4.9) would favor this dissociation reaction. If an intracellular buffer capacity of 35 mM H^+ per pH unit change is assumed to be a reasonable estimate of frog sartorius muscles, then a pH drop of approximately 0.3 pH units would occur (Izutsu, 1972; buffer capacity of bull frog skeletal muscle). This is in agreement with the findings of Marrannes et al. (1979) who found a similar pH drop in sheep Purkinje fibers exposed to an external propionate concentration of 20 mM at an extracellular pH of 6.80, as in this experiment. De Hemptinné and Marrannes (1979) found that identical

conditions in rat soleus muscles resulted in a drop of 0.2 pH units. The discrepancy in the magnitude of the propionate effect may reflect a difference in membrane permeability or intracellular buffer capacity. Sharp and Thomas (1981) have reported a 0.52 ± 0.14 pH unit drop in the intracellular pH of crab muscle fibers exposed to 100 mM propionate at an external pH of 7.5. All three investigative groups used microelectrode techniques to measure intracellular pH.

If, however, the membrane is weakly permeable to the anion species of propionate, then the membrane potential would result in a slow loss of intracellular propionate. Propionate would then behave as a shuttle with a net transfer of protons that is greater than the measured net propionate movement. Under these conditions, the present method employed to calculate the intracellular pH change (buffer capacity and propionate concentration) would underestimate the pH effect. If, however, an endogenous supply of propionate were present before the addition of external propionate, then this method would over-estimate the pH effect.

The effect of the external administration of 20 mM propionate on intracellular pH at rest was determined much more directly by the DMO technique. The results presented in Table 4 show a 0.20 pH unit decline in intracellular pH. An uncertainty of the accuracy of this result is introduced as a result of the need to estimate extracellular volume as discussed previously. Despite the problems with the DMO technique for estimating intracellular pH, Hinke and Menard (1978) have reported reasonable agreement with electrode determinations of pH (the reader is encouraged to read Hinke and Menard, 1978, for a thorough

discussion of the sources of errors in the DMO technique). At a DMO estimated pH value of 6.7, the electrode reading showed a value closer to 6.5. This discrepancy decreases towards zero as the electrode value approaches 7.0. The underestimation of pH in this range by the DMO technique has been explained on the basis of an artifact resulting from the use of the continually increasing plateau phase isotope values. The uptake of the isotopes was found by Hinke and Menard to contain a rapid uptake phase followed by a slow plateau phase uptake. On the basis of this discussion of the DMO technique, the propionate induced pH shift reported in this paper may be an underestimation of the peak shift.

The discrepancy between the theoretical pH change computed on the basis of an assumed buffer capacity and intracellular propionate concentration, and the estimate of the intracellular pH change computed on the basis of the DMO distribution ratio, may also be explained on the basis of suspected proton pump activity. Both Marrannes et al. (1979) and De Hemptinne and Marrannes (1979) have reported a slow intracellular alkalinization following the rapid propionate acidification. Boron (1977), Roos and Boron (1978) and Boron et al. (1979) have all reported a similar phenomenon in barnacle and rat diaphragm muscle. This result has been attributed to a proton extrusion mechanism involving the exchange of intracellular Cl^- for extracellular HCO_3^-. This exchange mechanism has been found to be sensitive to 4-acetomido-4'-isothiocyanostilbene-2,2' disulphonic acid (SITS) in snail neurones (Thomas, 1976) and squid axons (Russell, 1976). Aicken and Thomas (1977) have also reported a Na^+/H^+ exchange mechanism in mouse soleus muscle which is thought to work in conjunction with the

anion exchange mechanism. The Na^+/H^+ exchange was found to be amiloride sensitive. If these proton extrusion systems are operating in amphibian skeletal muscle, then a similar slow intracellular alkaline phase would occur after the initial intracellular acidification upon propionate exposure. The slow alkaline phase will increase the uptake of propionate as the undissociated acid due to the shift in the intracellular propionate equilibration towards dissociated propionate with the increasing pH. The influx of more undissociated acid may not be followed by a decrease in intracellular pH due to the acid extrusion mechanisms. The use then, of the total intracellular propionate level and the assumed buffer capicity, will over-estimate the pH shift as the acid extrusion mechanisms would increase the muscles "apparent" buffer capacity.

The proposed acid extrusion systems will also influence the estimate of the intracellular pH by the DMO distribution ratio. The weak acid distribution technique is an estimate of mean steady-state muscle pH. The suspected slow alkalinization following rapid propionate induced acidification would result in the underestimation of the peak pH shift, due to DMO equilibration with respect to this phase.

On the basis of the discussed sources of errors in the methods of pH estimation employed in this thesis (1) DMO, 2) Buffer capacity estimate in conjunction with propionate uptake), it would appear that the magnitude of the difference between the methods may not nearly be as great as reported. It is clear, however, that the addition of propionate to the external solution bathing <u>resting</u> sartorius muscles causes intracellular acidification. The magnitude of the effect is between 0.2 and 0.3 pH units.

The Effects of External Buffer Concentration on Whole Muscle and Estimated Fiber Lactate Efflux.

The rate of lactate efflux from whole muscle and the estimated fiber efflux was found to be buffer concentration dependent (Table 5 and 8). An increase in efflux is observed with increases in buffer concentration between 1 and 20 mM. This is in agreement with the work of Mainwood and Worsley-Brown (1975) who found similar results in frog sartorius muscles exposed to an imidazole buffer. In addition, the authors found that increasing the pH of the external solution resulted in increases in peak efflux rates. This effect was found to occur with all buffer systems used and occurred fairly rapidly when the pH of the external solution was changed, suggesting that the effect may be extracellular.

The buffer concentration dependent efflux observed in the present experiments may be explained on the basis of the inability of the lower buffer concentration to maintain a stable external pH in the face of the initial large acid efflux from the muscle. This would result in a decrease in pH of the external solution and a subsequent decrease in efflux.

As the buffer concentration increases, the peak lactate efflux continues to increase. This increase in efflux would be expected to occur until a buffer concentration was reached which was capable of maintaining a stable external pH despite the addition of metabolic acids to the bath solution. An infinitely large buffer concentration would achieve this but for practical purposes, a much lower buffer concentration would suffice. Clearly the buffer concentration required

is a function of the relationship between the pk of the buffer and the pH of the external solution.

The Effects of External Buffer Concentration on Propionate Induced Increases in Whole Muscle and Estimated Fiber Lactate Efflux

The magnitude of the propionate effect on lactate efflux is dependent upon buffer concentration. These results are presented in Figures 11 and 16. In these figures the efflux observed after the substitution of chloride with propionate are presented as a percent of the paired control efflux value. The data was normalized to its own control so as to remove the effect of different tissue lactate levels on efflux. Although no significant differences were observed in tissue lactate levels immediately after stimulation between tissues incubated and stimulated in different buffer concentrations (Table 7), a difference would be expected after 20 minutes of recovery due to the different efflux rates observed between tissues bathed in different buffer concentrations.

One can see that the magnitude of the effect of propionate on lactate efflux is greater at lower buffer concentrations for both whole muscle and estimated fiber efflux values. At a 1 mM buffer concentration the whole muscle efflux is 372% of control efflux values, while the estimated fiber efflux is 481% of control efflux values. At a 25 mM buffer concentration the whole muscle efflux is 184% of control efflux values, while the estimated fiber efflux is 215% of control efflux values. The differences between whole muscle and estimated fiber efflux is interpreted as being due to the time required for lactate to diffuse through the interstitial space. The effect of propionate on lactate efflux is clearly minimized at

high buffer concentrations. What is the mechanism whereby buffer concentration influences the propionate induced lactate efflux?

The observed decrease in intracellular pH discussed previously, strongly suggests that propionate crosses the sarcolemma in the undissociated form and subsequently dissociates. In addition to the decrease in intracellular pH presented in Table 4, Marrannes et al. (1979) and De Hemptinne and Marrannes (1979) also reported a transient increase in surface pH on the external side of the membrane. The movement of an undissociated acid from the extracellular space to the intracellular space will result in intracellular acidification and extracellular alkalinization. The chemical reactions in the aqueous unstirred layers of solution adjacent to a membrane can have a major influence on the diffusion of solutes across biological membranes. The chemical reactions of primary importance in this study are those that depend on the maintenance of adequate buffer capacity in the unstirred layer bordering the external side of the membrane (Gutnecht and Tosteson, 1973). The magnitude of the external alkalosis is dependent upon the external buffer capacity which is in part a function of buffer concentration.

At a low buffer concentration the magnitude of this extracellular alkalosis will be significant. Recall that increases in external pH favor increases in lactate efflux. The propionate effect on lactate efflux observed at low buffer concentrations may be the summation of both intracellular and extracellular pH changes due to propionate movement.

This, however, is somewhat of an oversimplification. The control tissues bathed in low buffer concentrations display a suppressed efflux rate. It has been previously suggested that this is due to a drop in extracellular pH due to the acid efflux. The movement of propionate will help to neutralize this effect in the experimental tissues. The net effect is that the experimental tissues may have a more stable extracellular pH than the control tissues, and may not necessarily exhibit an increase in pH. The end result is the same, however, in that experimental tissues would have a higher extracellular pH than their controls. Only at an infinitely large buffer concentration will the pH of the extracellular solution be stable at pH 6.80. At this buffer concentration, any effect of extracellular pH changes on lactate efflux will be removed and the effect of the propionate induced intracellular pH decrease isolated. At this infinitely large buffer concentration, the effect of propionate on lactate efflux will be minimized. For this reason, an asymptote was included in the equation of the line (Figures 11 and 16). Extrapolating the equation of the line to this buffer concentration reveals that the effect of propionate on whole muscle lactate efflux is to increase the efflux to 183% of control values (1.83 X control). The effect on estimated fiber efflux is to increase the efflux to 210% of control values (2.10 X control).

Before one can attribute the non-extracellular pH component of propionate induced lactate efflux to the intracellular pH decrease observed at rest, it is important to discuss two alternate explanations. The post experimental tissue lactate data clearly does not support the hypothesis that propionate may increase lactate efflux by stimulating lactate production. The pooled experimental

tissue lactate levels were significantly lower than control values (Table 6). A lower experimental tissue lactate level is expected due to the significant increase in experimental efflux observed. In addition the sum of (a) the post experimental tissue lactate levels and (b) the total amount of lactate lost from the tissue via efflux reveals similar results for experimental and control tissues (Table 7). This clearly supports the suggestion that increased lactate production is not responsible for the increase in efflux.

A second explanation for the increase in efflux arises from the finding that in erythrocytes a small fraction of the lactate crossing the membrane is transported by the classical anion exchange mechanism as discussed in the Introduction. It can be argued that if lactate is a transportable substrate for this mechanism then perhaps propionate is as well. The addition of propionate to the external solution may favor increased efflux due to the availability of an anion exchange partner for lactate. Spencer and Lehninger (1976) have found that various cyano-cinnamate compounds and thiol group reagents inhibit lactate transport in ascites-tumour cells, yet had no effect on the simple unsubstituted monocarboxylate acetate and propionate. Thiol group reagents block specific monocarboxylate transport across many membranes while cyano-cinnamates block both anion exchange and specific monocarboxylate transport (Deuticke, 1982, unpublished results; Halestrap, 1976).
De Hemptinne et al. (1983) have reported that the rate of intracellular acidification induced by extracellular L-lactate and pyruvate was greatly depressed in the presence of α-cyano-4-hydroxycinnamate in sheep Purkinje fibers. This inhibition however, had no effect on the acidification induced by propionate. It would appear then, that propionate and acetate cross the membrane by a different mechanism and that the addition of propionate to the

external solution would not favor increased lactate efflux via lactate/propionate exchange. Spencer and Lehninger (1976) have suggested that acetate and propionate probably cross membranes by passive diffusion, presumably, as the nonionized species as suggested by the pH changes observed. In addition, the observed intracellular pH decrease can not be explained on the basis of lactate/propionate anion exchange.

A Consistent Model to Explain the Lactate Efflux

We are left then with the hypothesis that nonionic movements of propionate across the sarcolemma membrane and the subsequent intracellular acidosis as reported, is responsible for the increase in efflux. Of the five models proposed in the Introduction to explain the influence of extracellular pH changes on lactate efflux;

A) anion channel

B) anion exchange

C) nonionic diffusion

D) hydrogen ion/ionic lactate cotransport

E) lactate/hydroxide exchange

the first two are inconsistent with the effects of decreased intracellular pH on lactate efflux.

Model A suggests that proton binding to an anion channel impedes lactate ion movements through the channel thereby reducing the net lactate efflux. For this model to be consistent with the observed results of increased lactate efflux with a decrease in intracellular pH reported in this paper, and the effects reported by Mainwood and Worsley-Brown (1975) with external pH manipulations, the binding

of protons entering the channel from the extracellular side and intracellular side would have to have opposite effects. Protons entering the channel from the extracellular side of the channel would inhibit efflux while those entering from the intracellular side would facilitate efflux. This appears highly unlikely.

Model B requires a somewhat analogous mechanism in that increases in extracellular hydrogen ions would favour a reduction in the carrier binding sites by conformational changes, yet increases in intracellular hydrogen ions would produce an increase in binding sites. This is highly unlikely in an exchange carrier protein.

Models C, D and E are consistent with the increase in lactate efflux observed with a decrease in intracellular pH. With respect to model C, decreases in intracellular pH will shift the lactate/lactic acid equilibrium towards the formation of lactic acid, thereby increasing the intracellular concentration of lactic acid. This would increase the transmembrane lactic acid gradient favoring an increased efflux. A 0.3 pH unit drop is all that is necessary to double the intracellular concentration of lactic acid without changing the total intracellular lactate concentration. Increases in external buffer concentration will favor holding external pH constant in light of the strong acid flux out of the muscle. This maintenance of external pH will keep external lactic acid concentrations low, thereby maintaining the transmembrane lactic acid gradient. An increase in extracellular pH will have a similar result as extracellular lactic acid concentrations will be reduced.

Models D and E are somewhat analogous and are based on the inability to distinguish proton movements from hydroxide movements in the opposite direction. Intracellular acidification in the case of Model D (Figure 1) would favor the formation of the protonated carrier and would facilitate the reorientation of the carrier to the outer surface of the membrane. Intracellular acidification in the case of Model E (Figure 2) would favor the release of hydroxide from the carrier at the inner surface of the membrane and free the binding site for lactate binding and subsequent exchange.

A large body of evidence derived from non-skeletal muscle sources has suggested that lactate is transported across membranes in part, by carrier proteins. This evidence was reviewed in the Introduction and is the rational for including models D and E. Much more recently, De Hemptinne et al. (1983) have reported that the rate of intracellular acidification induced by extracellular L-lactate and pyruvate was greatly depressed in the presence of α-cyano-4-hydroxy-cinnamate in sheep cardiac Purkinje fibers. Hydroxycinnamates, as discussed previously in this section, are non-specific inhibitors of anion exchange and monocarboxylate transport in other non-muscle tissues. This is more evidence for the possible existence of carrier mediated cotransport of lactate and protons as suggested by models D and E. To date however, these systems have not been shown to exist in skeletal muscle.

It is not possible to determine on the basis of the reported data, which of the three models is responsible for the movement of lactate across the sartorius membrane. Wolosin and Ginsburg (1975)

reported a lactic acid permeability coefficient through artificial lipid films of 5 X 10^{-5} $cm \cdot s^{-1}$ at 25° C. Assuming a diffusion area of 2.3 X 10^3 $cm^2 \cdot g^{-1}$ of tissue (Mobley and Eisenberg, 1975) and using the estimated fiber efflux rates of lactate for tissues exposed to 20 mM MOPS Ringer's solution, it is possible to compute a permeability coefficient. The only remaining variables are knowledge of the mean intracellular lactic acid concentration and the mean interstitial lactic acid concentration. The mean interstitial lactic acid concentration is computed with the diffusion model while the intracellular lactic acid concentration is derived from knowledge of intracellular lactate levels and an estimate of the intracellular pH; assuming, 1) a buffer capacity of 35 mM H^+ per pH unit change, 2) equimolar amounts of H^+ and lactate are present, 3) resting pH is 6.93 as determined by DMO distribution. The computed permeability coefficients of 4.1 X 10^{-5} ± 1.2 X 10^{-5} $cm \cdot s^{-1}$ SEM for control tissues and 6.8 X 10^{-5} ± 8.4 X 10^{-6} $cm \cdot s^{-1}$ for experimental tissues compare favourably with the value of Wolosin and Ginsburg (1975). It should be noted that the permeability coefficient calculated for tissues exposed to propionate, 6.8 X 10^{-5} $cm \cdot s^{-1}$ is based on an assumed intracellular pH effect due to propionate of 0.2 pH units. If the effect is of the order of 0.3 pH units as reported by Marrannes et al. (1979), then the coefficient value becomes 5.2 X 10^{-5}.

These calculations do not rule out the existence of lactate carrier systems, rather they suggest that the propionate induced intracellular pH decrease is sufficient to account for the significant increase in lactate efflux without a change in the permeability of the membrane to lactic acid.

Despite the inability to conclusively determine the mode of lactate transport, it is suggested from the results of these experiments and the work of Mainwood and Worsley-Brown (1975), that a large fraction of lactate crosses the membrane in combination with proton movements. This is based on the finding that decreases in intracellular pH increase lactate efflux while decreases in extracellular pH decrease lactate efflux. The results of this paper verify the hypothesis put forward by Mainwood and Worsley-Brown (1975) that the efflux of lactate across frog sartorius muscle membranes is dependent upon the maintenance of a steep transmembrane pH gradient.

What are the implications of this finding? This may be important in the regulation of skeletal muscle metabolism. The role of increased blood flow to metabolizing tissues is to deliver oxygen and other substrates. In addition to this role, blood plays the important role of removing metabolites, including CO_2, hydrogen ions and lactate. As long as the capacity of the blood to buffer the acid load entering it at the capillary bed is sufficient, no significant drop in pH will be observed. This will favor the release of more lactate in conjunction with hydrogen ions. As the buffering capabilities of the blood are exceeded, a precipitous drop in blood pH will result and lactate efflux from metabolizing tissues will be reduced. If the blood pH drop is of sufficient magnitude then significant reductions in efflux will occur. This will result in an accumulation of intracellular hydrogen ions if lactate is released in combination with hydrogen ions. This could result in a slowing of glycolysis due to the sensitivity of the rate

limiting phosphofructokinase reaction to the hydrogen ion concentration (Mansour, 1963; Danforth, 1965; Trivedi et al., 1966). The net result is that the production of lactic acid is inhibited via a simple cascade mechanism.

The pH mediated suppression of tension in skeletal muscle, as discussed in the Introduction may be regulated by the same mechanism. The accumulation of lactic acid due to impaired release may impair or inhibit the tension generating mechanism.

Although for this discussion we have dealt with the pH gradient between intracellular space and blood, in actual fact the gradient responsible for in vivo movements of lactate is the gradient between intracellular space and interstitial space. Steinhagen et al. (1976) have shown in working dog gastrocnemius muscle that interstitial pH is lower than venous blood pH but follows venous pH changes. The magnitude of the pH gradient between interstitial space and venous blood will be a function of the diffusion distance and the capillary blood flow rate.

The findings of this paper can be summarized by addressing the research aims of this thesis.

Primary Research Questions

1) What is the effect of externally administered propionate on lactate efflux from isolated frog sartorius muscles?

   Externally administered propionate increases the rate of lactate efflux. The magnitude of the effect is dependent upon the buffer concentration.

2) What model of lactate efflux is consistent with the effect of externally administered propionate on lactate efflux from isolated frog sartorius muscles?

Models involving cotransport of hydrogen ions with lactate are consistent with the effect of externally administered propionate on lactate efflux (ie. models requiring the maintenance of a steep transmembrane pH gradient). The estimated permeability coefficient for lactic acid movement across the sacolemma, calculated from the estimated fiber efflux values are in agreement with the value reported for lactic acid movements across artificial lipid bilayers (Wolosin and Ginsburg, 1975). This suggests that lactate is released from frog sartorius muscles as lactic acid.

Secondary Findings

1) The substitution of 20 mM propionate for chloride ions in MOPS Ringer's solution results in a decline in intracellular pH in resting frog sartorius muscles exposed to the solution.

2) Lactate efflux rates from frog sartorius muscles are dependent upon the external MOPS buffer concentration.

Appendix 1

DMO Algorithm for Intracellular pH Estimate

(Hinke and Menard, 1978)

$$pH_{DMO} = pK' + Log_{10}\left[\left[\frac{[B_H]V_t}{D_H} - 1\right]^{-1}\left[\frac{[B_H]D_c}{[B_c]D_H} - 1\right]\left[10^{pHe-pK'} + 1\right] - 1\right]$$

Where

B_H = [^{3}H] inulin in dpm·unit bath volume^{-1}

B_c = [^{14}C] DMO in dpm·unit bath volume^{-1}

D_H = total ^{3}H (dpm) in a blotted portion of tissue

D_c = total ^{14}C (dpm) in a blotted portion of tissue

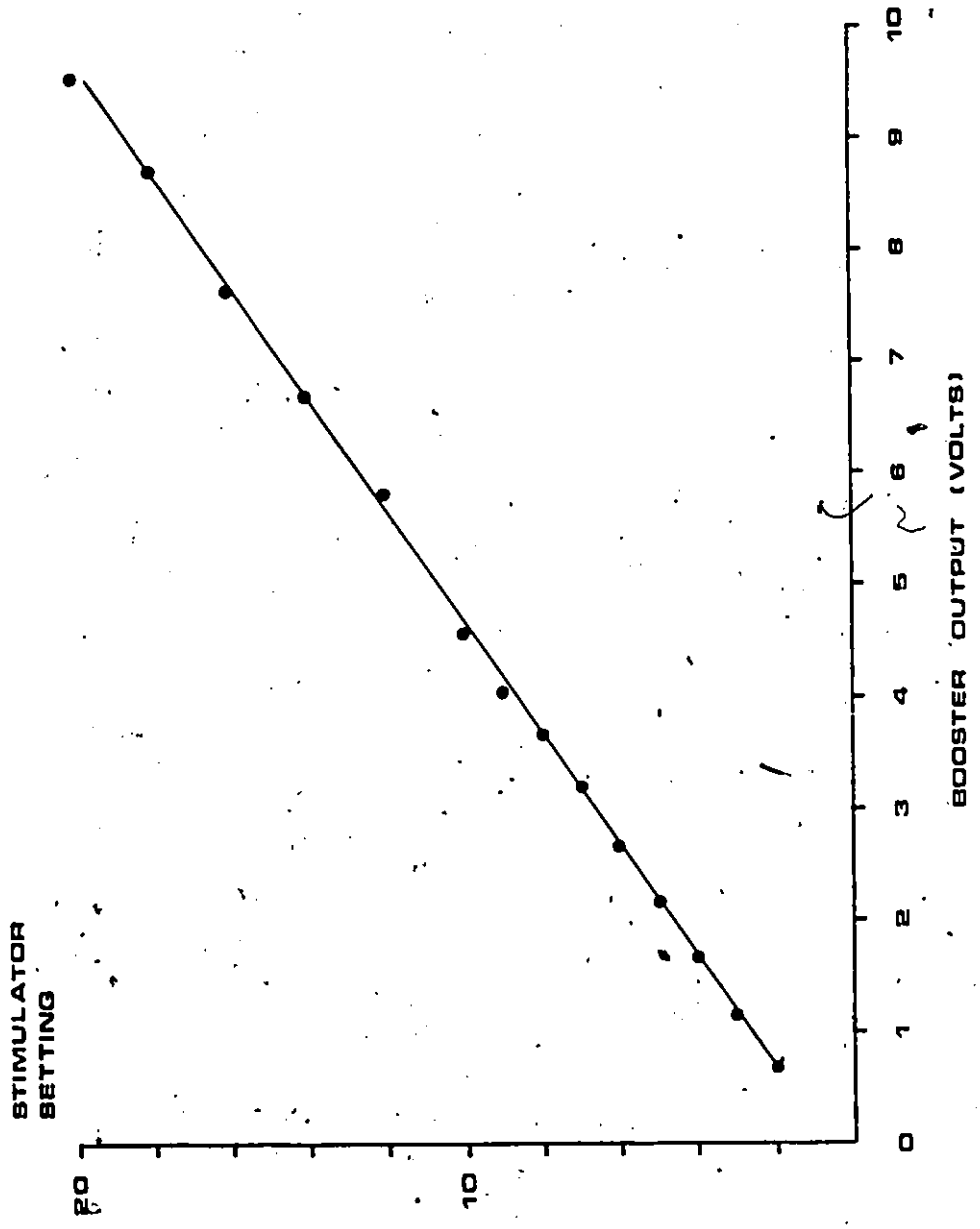
V_t = total tissue water volume in ml.

pK' = pKa of DMO (6.33)

pHe = extracellular pH

Appendix 2

Current booster voltage output as a function of Grass SD8 Stimulator Setting.


STIMULATOR SETTING
20
10
0
1
2
3
4
5
6
7
8
9
10
BOOSTER OUTPUT (VOLTS)

Appendix 3

Tissue Lactate Calculations

$$\text{Tissue Lactate Concentration } (\mu mol \cdot g^{-1}) = \frac{\Delta \text{Optical Density}}{6.22} \times \frac{A}{B} \times C \times \frac{1}{D} \times E \times \frac{1}{F}$$

A = total cuvette volume (ml)

B = sample volume (ml)

C = total neutralized volume (ml)

D = volume homogenate neutralized (ml)

E = total homogenate volume (ml)

F = weight of tissue homogenized (g)

6.22 = extinction coefficient

Mean Whole Muscle Efflux Calculations

$$\text{Whole Muscle Efflux } (\mu mol \cdot g^{-1} \cdot min^{-1}) = \frac{\Delta \text{Optical Density}}{6.22} \times \frac{A}{B} \times C \times \frac{1}{5} \times \frac{1}{E} \times \frac{1}{F}$$

A = total cuvette volume (ml)

B = sample volume (ml)

C = bath volume (ml)

5 = factor by which original samples were concentrated

E = tissue weight (g)

F = length of collection interval

6.22 = extinction coefficient

Appendix 4

The Effects of Evaporation on Lactate Concentration In Effluent Samples

5 X 2.5 ml samples of a 250 µM Lithium Lactate standard in distilled water were placed in a vacuum oven at 80° C for four hours (vacuum, 10 to 15 inches of Hg). After complete evaporation the contents of the vial were reconstituted back to 2.5 ml. 0.2 ml Aliquots of each vial's mixed contents were then used for the enzymatic determination of lactate (Refer to Biochemical Assay Section; Materials and Methods). 0.2 ml Aliquots from five vials containing 250 µM Lithium Lactate standards were also assayed for lactate by the same method. Five means were computed on the basis of repeated samples. A one-way analysis of variance was run for differences between groups. No significant difference was found at the $p = .05$ level ($F = 0.013$ df 1,8).

Data (mm pen deflection on Unicam Spectrophotometer) Evaporated Samples

62.50, 65.50, 65.75, 60.50, 65.50 $\bar{X}$ 63.95

Non-Evaporated Samples

65.40, 64.25, 57.75, 65.5, 66.75 $\bar{X}$ 63.75

Due to the peculiarly low value of 57.75 in the non-evaporated group, a mean value was substituted and the analysis repeated. No significant difference was found at the $p = .05$ level ($F = 1.30$ df 1,8). No degrees of freedom were removed by the substitution of the mean value of 65.25 for the value of 57.75 in the analysis.

On the basis of this data, it is concluded that evaporation at 80^{o} C does not add or substract to the lactate of the sample.

Appendix 5

Estimate of Maximum Diffusion Distance (l) From Toad Sartorius Weights

(Renaud, 1982)

A linear regression was run on data taken from 21 toad sartorius muscles (Buro americanus). The equation of the line of best fit is as follows:

$$Y = 0.158 + 0.00472\ X \qquad r = .795$$

Where X is sartorius weight in milligrams and

Y is maximum diffusion distance (l) in millimeters.

Total muscle thickness is then 2(l).

Appendix 6

Summary of the Analyses of Variance.

Table 1. Summary of ANOVA for data on intracellular hydrogen ion concentration under experimental and control conditions.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Eobs	Fc
Between Groups	9.68×10^{-15}	1	9.68×10^{-15}	13.67	5.99
Within Groups	4.25×10^{-15}	6	7.08×10^{-16}		
Total	1.39×10^{-14}				

Table 2. Summary of ANOVA for data on whole muscle lactate efflux under experimental or control conditions; 25 mM buffer concentration, 30 minute interval.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	93033.6	1	93033.6	21.4	4.96
Within Groups	43561.4	10	4356.1		
Total	136595.0	11			

Table 3. Summary of ANOVA for data on whole muscle lactate efflux under experimental or control conditions; 25 mM buffer concentration, 40 minute interval.

Summary for the Analysis of Variance					
Sources of Varation	ss	df	MS	Fobs	Fc
Between Groups	38363.5	1	38363.5	13.84	4.96
Within Groups	27727.0	10	2772.7		
Total	66090.5	11			

Table 4. Summary of ANOVA for data on whole muscle lactate efflux (pooled data) during the first 10 min interval of recovery across four buffer concentrations.

	Summary for the analysis of variance				
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	180016.9	3	60005.6	23.85	2.91
Within Groups	78007.6	31	2516.4		
Total	258024.5	34			

Scheffé Multiple Comparisons reveal that;

Whole muscle efflux from tissue exposed to 10 mM buffer is greater than the efflux from tissue exposed to 1 mM buffer.

Whole muscle efflux from tissue exposed to 20 mM buffer is greater than the efflux from tissue exposed to 1 mM buffer, 5 mM buffer and 10 mM buffer.

Table 5. Summary of ANOVA for data on whole muscle lactate efflux under experimental or control conditions; 1 mM buffer concentration.

Summary of the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	180060.0	1	180060.0	202.3	5.99
Within Groups	5341.3	6	890.2		
Total	185401.3	7			

Table 6. Summary of ANOVA for data on whole muscle lactate efflux under experimental or control conditions; 5 mM buffer concentration.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	131225.6	1	131225.6	62.30	5.99
With Groups	12638.4	6	2106.4		
Total	143864.0	7			

Table 7. Summary of ANOVA for data on whole muscle lactate efflux under experimental or control conditions; 10 mM buffer concentration.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	149544.0	1	149544.0	17.61	4.96
Within Groups	84919.2	10	8491.92		
Total	234463.2	11			

Table 8. Summary of ANOVA for data on whole muscle lactate efflux under experimental or control conditions; 20 mM buffer concentration.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	60083.1	1	60083.1	21.08	5.99
Within Groups	17105.4	6	2850.9		
Total	77188.5	7			

Table 9. Summary of ANOVA for data on post experiment, experimental tissue lactate levels across four buffer concentrations.

Summary for the Analysis of Variance					
Sourses of Variation	ss	df	MS	Fobs	Fc
Between Groups	28.7	3	9.6	2.04	3.34
Within Groups	65.8	14	4.7		
Total	94.5	17			

Table 10. Summary of ANOVA for data on post experiment control tissue lactate levels across four buffer concentrations.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	Ms	Fobs	Fc
Between Groups	5.2	3	1.7	0.34	3.34
Within Groups	71.4	4	5.1		
Total	76.6	17			

Table 11. Summary of ANOVA 2 for data on post experiment tissue lactate levels under experimental or control conditions and across four buffer concentrations.

	Summary for the Analysis of Variance				
Sources of Variation	ss	df	MS	Fobs	Fc
Between Factor A Group	48.4	1	48.4	9.59	4.20
Between Factor B Buffer concentration	27.4	3	9.14	1.81	2.95
Between AXB	9.7	3	3.2	0.64	2.95
Within Error	141.3	28	5.0		
Total	226.8	35			

Table 12. Summary of ANOVA for data on post stimulation, experimental tissue lactate levels across four buffer concentrations.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	0.4	3	0.1	0.02	3.34
Within Groups	127.6	14	9.1		
Total	128.0	17			

Table 13. Summary of ANOVA for data on post stimulation, control tissue lactate levels across four buffer concentrations.

	Summary for the Analysis of Variance				
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	29.4	3	9.8	0.96	3.41
Within Groups	132.2	13	10.2		
Total	161.6	16			

Table 14. Summary of ANOVA 2 for data on post stimulation tissue lactate estimates under experimental or control conditions and across four buffer concentrations.

	Summary of the Analysis of Variance				
Sources of Variation	ss	df	MS	Fobs	Fc
Between Factor A Group	1.0	1	1.01	0.10	4.21
Between Factor B Buffer concentration	14.3	3	4.8	0.49	2.96
Between AXB	15.7	3	5.2	0.54	2.96
Within Error	260.5	27	9.6		
Total	291.5	34			

Table 15. Summary of ANOVA for data on fiber efflux (pooled data) during the stimulation interval across four buffer concentrations.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	176640.6	3	58880.2	14.50	2.99
Within Groups	101487.3	25	4059.5		
Total	278127.9	28			

Scheffé Multiple Comparisons reveal that; fiber efflux from tissues exposed to 20 mM buffer is significantly different from efflux from tissues exposed to 1, 5 or 10 mM buffer concentrations.

Table 16. Summary of ANOVA for data on lactate efflux from muscle fibers under experimental or control conditions; 1 mM buffer concentration.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	293492.9	1	293492.9	222.54	5.99
Within Groups	7912.9	6	1318.8		
Total	301405.8	7			

Table 17. Summary of ANOVA for data on lactate efflux from muscle fibers under experimental or control conditions; 5 mM buffer concentration.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	225120.5	1	225120.5	74.68	5.99
Within Groups	18085.2	6	3014.2		
Total	243205.7	7			

Table 18. Summary of ANOVA for data on lactate efflux from muscle fibers under experimental or control conditions; 10 mM buffer concentration.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	238064.7	1	238064.7	23.80	4.96
Within Groups	100016.4	10	10001.6		
Total	338081.1	11			

Table 19. Summary of ANOVA for data on lactate efflux from muscle fibers under experimental or control conditions; 20 mM buffer concentration.

Summary for the Analysis of Variance					
Sources of Variation	ss	df	MS	Fobs	Fc
Between Groups	97925.3	1	97925.3	32.37	5.99
Within Groups	18148.7	6			
Total	116074.0	7			

Appendix 7

Raw Data for Estimation of Lactate Efflux From Muscle Fibers

1 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance wt. (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
1 Control	F	4	55.9	.042	.1878	.1972	.012	.042	.01762	-	.2666
	10	10			.2418	.2539	.040	.135	.02278	.01762	.2501
	20	10			.1894	.1989	.031	.106	.01784	.02278	.1808
	30	10			.1256	.1319	.021	.070	.01183	.01784	.1145
8 Control	F	4	98.7	.062	-	-	-	-	-	-	-
	10	10			.1345	.1412	.039	.133	.04886	-	-
	20	10			.1495	.1570	.043	.148	.05431	.04886	.1555
	30	10			.1151	.1209	.033	.114	.04181	.05431	.1028
15 Control	F	4	66.9	.047	.1362	.1430	.011	.036	.01929	-	.2066
	10	10			.1874	.1968	.037	.125	.02664	.01929	.1978
	20	10			.1680	.1764	.033	.112	.02388	.02664	.1633
	30	10			.1293	.1358	.025	.086	.01838	.02388	.1203
17 Control	F	4	77.2	.052	.1100	.1155	.010	.034	.02198	-	.1813
	10	10			.1336	.1403	.030	.103	.02703	.02196	.1400
	20	10			.1096	.1151	.025	.085	.02185	.02703	.1034
	30	10			.0872	.0916	.020	.067	.01739	.02185	.0810
Mean	F	4			.1447 ± .0229 (SEM)						.2182 ± .0253 (SEM)
	10	10			.1743 ± .0258						.1960 ± .0318
	20	10			.1541 ± .0169						.1508 ± .0166
	30	10			.1143 ± .0095						.1047 ± .0087

1 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance Wt. (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
1 Experimental	F	4	62.5	.045	.1211	.1272	.009	.030	.01465	-	.1786
	10	10			.1462	.1535	.027	.091	.01775	.01465	.1506
	20	10			.1285	.1349	.024	.080	.01561	.01775	.1246
	30	10			.3775	.3964	.069	.236	.04584	.01561	.4260
8 Experimental	F	4	99.3	.063	-	-	-	-	-	-	-
	10	10			.1344	.1411	.039	.133	.04957	-	.1157
	20	10			.1206	.1266	.035	.120	.04448	.04957	.4949
	30	10			.3937	.4134	.115	.391	.14520	.04448	
15 Experimental	F	4	70.4	.049	.1075	.1129	.009	.030	.01715	-	.1674
	10	10			.1562	.1640	.032	.110	.07502	.01715	.1674
	20	10			.1465	.1538	.030	.103	.02346	.02502	.1441
	30	10			.4229	.4441	.088	.298	.06774	.02346	.4862
17 Experimental	F	4	75.2	.051	.1006	.1056	.009	.030	.01877	-	.1621
	10	10			.1487	.1561	.033	.112	.02784	.01877	.1610
	20	10			.1380	.1449	.031	.104	.02584	.02784	.1356
	30	10			.4633	.4865	.102	.348	.08675	.02584	.5438
Mean	F	4			.1097 ± .0060 (SEM)						.1694 ± .0049(SEM)
	10	10			.1464 ± .0045						.1597 ± .0049
	20	10			.1334 ± .0056						.1300 ± .0062
	30	10			.4144 ± .0188						.4877 ± .0242

5 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance wt. (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate -BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
4 Control	F	4	65.2	.047	.1634	.1716	.013	.043	.02179	-	.2484
	10	10			.2255	.2368	.043	.147	.03019	.02179	.2383
	20	10			.2255	.2368	.043	.147	.03019	.03019	.1329
	30	10			.1810	.1901	.035	.118	.02423	.03019	.1120
5 Control	F	4	97.8	.062	-	-	-	-	-	-	-
	10	10			.2343	.2460	.067	.229	.08320	-	-
	20	10			.1926	.2022	.055	.188	.06839	.08320	.1771
	30	10			.1478	.1552	.043	.145	.05249	.06839	.1320
13 Control	F	4	57.9	.043	.1574	.1653	.011	.036	.01599	-	.2245
	10	10			.2347	.2465	.040	.136	.02394	.01599	.2486
	20	10			.2102	.2207	.036	.122	.02144	.02394	.2064
	30	10			.1579	.1658	.027	.091	.01610	.02144	.1479
14 Control	F	4	41.8	0.36	.2475	.2599	.012	.041	.01232	-	.3189
	10	10			-	-	-	-	-	-	-
	20	10			.3029	.3181	.037	.127	.01514	-	-
	30	10			.2231	.2343	.027	.093	.01115	.01514	.2129
Mean	F	4			.1894 ± .0291 (SEM)						.2639 ± .0283 (SEM)
	10	10			.2315 ± .0030						.2435 ± .0051
	20	10			.2328 ± .0243						.1721 ± .0214
	30	10			.1775 ± .0167						.1512 ± .0218

5 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance Wt. (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
4 Experimental	F	4	69.1	.048	.2078	.2182	.017	.057	.03173	-	.3210
	10	10			.2369	.2488	.048	.164	.03632	.03173	.2440
	20	10			.2029	.2131	.041	.140	.03111	.03632	.1951
	30	10			.4452	.4675	.090	.308	.06825	.03111	.4995
5 Experimental	F	4	96.7	.061	.1038	.1090	.012	.040	.03572	-	.1958
	10	10			.1680	.1764	.048	.162	.05800	.03572	.1906
	20	10			.1558	.1636	.044	.150	.05379	.05800	.1508
	30	10			.3947	.4145	.112	.382	.13627	.05379	.4803
13 Experimental	F	4	65.0	.046	.1663	.1746	.013	.043	.02202	-	.2501
	10	10			.2461	.2584	.047	.160	.03272	.02202	.2626
	20	10			.2433	.2555	.047	.158	.03235	.03272	.2425
	30	10			.5075	.5329	.097	.330	.06723	.03235	.5614
14 Experimental	F	4	41.5	0.35	.2158	.2266	.011	.036	.01058	-	.2806
	10	10			.2902	.3047	.035	.120	.01429	.01058	.2981
	20	10			.2381	.2500	.029	.099	.01172	.01429	.2324
	30	10			.3870	.4064	.047	.161	.01905	.01172	.4056
Mean	F	4			.1734 ± .0256 (SEM)						.2619 ± .0264 (SEM)
	10	10			.2353 ± .0253						.2488 ± .0224
	20	10			.2100 ± .0202						.2052 ± .0208
	30	10			.4336 ± .0278						.4867 ± .0321

10 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance wt. (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
3 Control	F	4	79.1	.053	.2109	.2215	.020	.067	.04440	-	.3521
	10	10			.3014	.3165	.070	.238	.06369	.0440	.3258
	20	10			.2663	.2796	.062	.211	.05628	.06369	.2574
	30	10			.2147	.2255	.050	.170	.04537	.05628	.2011
7 Control	F	4	88.3	.057	.1854	.1947	.019	.065	.05098	-	.3284
	10	10			.2344	.2461	.061	.207	.06469	.05096	.2500
	20	10			.1735	.1822	.045	.153	.04788	.06469	.1542
	30	10			.1301	.1366	.034	.115	.03590	.04788	.1167
9 Control	F	4	66.4	.047	.2675	.2809	.021	.071	.03787	-	.4099
	10	10			.3824	.4016	.075	.254	.05342	.03787	.4059
	20	10			.3126	.3283	.061	.208	.04367	.05342	.2986
	30	10			.2400	.2520	.047	.159	.03353	.04367	.2242
16 Control	F	4	65.6	.047	.1201	.1261	.009	.032	.01624	-	.1838
	10	10			.2062	.2165	.040	.135	.02800	.01624	.2237
	20	10			.1780	.1869	.034	.117	.02417	.02800	.1725
	30	10			.1346	.1413	.026	.088	.01828	.02417	.1252
18 Control	F	4	58.2	.043	-	-	-	-	-	-	-
	10	10			.2929	.3076	.050	.170	.03022	-	-
	20	10			.2537	.2664	.043	.148	.02618	.03022	.2474
	30	10			.2091	.2196	.036	.122	.02158	.02618	.2017
19 Control	F	4	68.8	.048	-	-	-	-	-	-	-
	10	10			.2738	.2875	.055	.188	.04155	-	-
	20	10			.2532	.2659	.051	.174	.03843	.04155	.2484
	30	10			.2083	.2187	.042	.143	.03161	.03843	.1979
Mean	F	4			.1960 ± .0306 (SEM)						.3186 ± .0481 (SEM)
	10	10			.2818 ± .0249						.3014 ± .0410
	20	10			.2395 ± .0221						.2297 ± .0225
	30	10			.1895 ± .0187						.1778 ± .0184

10 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance ℓ Wt. (mg)	ℓ (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
3 Experimental	F	4	85.3	.056	.1147	.1204	.011	.038	.02898	-	.1963
	10	10			.1946	.2043	.048	.162	.04935	.02898	.2138
	20	10			.1583	.1662	.039	.132	.04015	.04935	.1440
	30	10			.2308	.2424	.057	.193	.05853	.04015	.2478
7 Experimental	F	4	89.8	.058	.1582	.1661	.017	.057	.04534	-	.2849
	10	10			.2318	.2434	.061	.208	.06666	.04534	.2554
	20	10			.2029	.2131	.054	.182	.05835	.06666	.1934
	30	10			.3784	.3974	.106	.359	.10886	.05835	.4560
9 Experimental	F	4	61.6	.045	.2181	.2290	.016	.054	.02551	-	.3227
	10	10			.3921	.4117	.071	.242	.04605	.02551	.4262
	20	10			.3248	.3411	.059	.200	.03815	.04605	.3119
	30	10			.5575	.5854	.101	.343	.06548	.03815	.6012
16 Experimental	F	4	65.8	.047	.1291	.1356	.010	.034	.01759	-	.1960
	10	10			.2431	.2553	.047	.160	.03325	.01759	.2670
	20	10			.1906	.2001	.037	.125	.02607	.03325	.1791
	30	10			.3706	.3892	.072	.244	.05069	.02607	.4082
18 Experimental	F	4	62.6	.045	-	-	-	-	-	-	-
	10	10			.2171	.2280	.040	.136	.02646	-	-
	20	10			.2151	.2259	.040	.135	.02622	.02646	.2153
	30	10			.5437	.5709	.100	.340	.06626	.02622	.6071
19 Experimental	F	4	73.3	.050	-	-	-	-	-	-	-
	10	10			.2713	.2849	.058	.199	.04780	-	-
	20	10			.2242	.2354	.048	.164	.03950	.04780	.2124
	30	10			.3952	.4150	.085	.290	.06963	.03950	.4367
Mean	F	4			.1550 ± .0229 (SEM)						.2500 ± .0320 (SEM)
	10	10			.2583 ± .0287						.2906 ± .0466
	20	10			.2193 ± .0231						.2093 ± .0231
	30	10			.4127 ± .0498						.4596 ± .0547

20 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance wt. (mg)	l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_E (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
2 Control	F	4	105.8	.066	.2528	.2655	.031	.105	.10893	-	.5055
	10	10			.3831	.4023	.119	.405	.16561	.10893	.4364
	20	10			.2948	.3096	.092	.312	.12744	.16561	.2588
	30	10			.2213	.2324	.069	.234	.09567	.12744	.1911
6 Control	F	4	75.1	.051	.1681	.1765	.015	.051	.03135	-	.2741
	10	10			.2936	.3083	.065	.221	.05480	.03135	.3255
	20	10			.2196	.2306	.048	.165	.04099	.05480	.2013
	30	10			.1620	.1701	.036	.121	.03024	.04099	.1468
11 Control	F	4	66.9	.047	.2839	.2981	.022	.076	.04019	-	.4342
	10	10			.4275	.4489	.084	.286	.06077	.04019	.4583
	20	10			.3767	.3956	.074	.252	.05355	.06077	.3659
	30	10			.2742	.2879	.054	.183	.03898	.05355	.2518
12 Control	F	4	64.5	.046	.2993	.3143	.023	.077	.03893	-	.4493
	10	10			.3658	.3841	.069	.236	.04777	.03893	.3796
	20	10			.3074	.3228	.098	.198	.04014	.04777	.2951
	30	10			.1915	.2011	.036	.124	.02501	.04014	.1688
Mean	F	4			.2512 ± .0292 (SEM)						.4158 ± .0497 (SEM)
	10	10			.3675 ± .0279						.4000 ± .0298
	20	10			.2996 ± .0322						.2803 ± .0345
	30	10			.2123 ± .0240						.1896 ± .0226

20 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance Wt (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
2 Experimental	F	4	105.4	.067	.2003	.2103	.025	.085	.08549	-	.4044
	10	10			.3247	.3410	.101	.342	.13904	.08549	.3753
	20	10			.2340	.2457	.072	.247	.10019	.13904	.1975
	30	10			.3173	.3332	.098	.334	.13586	.10019	.3507
6 Experimental	F	4	75.4	.051	.2314	.2430	.021	.070	.04344	-	.3761
	10	10			.3375	.3544	.075	.254	.06360	.04344	.3636
	20	10			.2957	.3105	.066	.223	.05572	.06360	.2853
	30	10			.4432	.4654	.098	.334	.08351	.05572	.4798
11 Experimental	F	4	67.3	.048	.2685	.2819	.021	.072	.03855	-	.4107
	10	10			.4075	.4279	.081	.274	.05874	.03855	.4371
	20	10			.3047	.3200	.060	.205	.04392	.05874	.2826
	30	10			.4240	.4452	.084	.285	.06111	.04392	.4400
12 Experimental	F	4	61.8	.045	.2924	.3070	.021	.072	.03446	-	.4307
	10	10			.4098	.4303	.074	.253	.04849	.03446	.4321
	20	10			.3238	.3400	.059	.200	.03831	.04849	.3072
	30	10			.3578	.3757	.065	.221	.04234	.03831	.3641
Mean	F	4			.2481 ± .0203 (SEM)						.4055 ± .0133(SEM)
	10	10			.3699 ± .0226						.4020 ± .0190
	20	10			.2896 ± .0194						.2682 ± .0242
	30	10			.3856 ± .0292						.4109 ± .0316

25 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance Wt. (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
1A Control	F	4	105.5	.066	.2722	.2858	.034	.115	.11645	-	.5485
	10	10			.3220	.3381	.100	.340	.13821	.11645	.3429
	20	10			.2623	.2754	.081	.277	.11258	.13821	.2383
	30	10			.1944	.2041	.060	.205	.08344	.11258	.1667
3A Control	F	4	109.2	.067	.3021	.3172	.039	.132	.14099	-	.6250
	10	10			.3681	.3865	.118	.403	.17234	.14099	.3978
	20	10			.3055	.3208	.098	.334	.14303	.17234	.2790
	30	10			.2445	.2567	.079	.267	.11448	.14303	.2184
4A Control	F	4	122.9	.074	.1684	.1768	.024	.083	.10624	-	.3849
	10	10			.2813	.2954	.102	.346	.17800	.10624	.3399
	20	10			.2630	.2762	.095	.323	.16642	.17800	.2534
	30	10			.2263	.2376	.082	.278	.14320	.16642	.2073
5A Control	F	4	106.9	.066	.2686	.2820	.034	.115	.11879	-	.5467
	10	10			.3761	.3949	.118	.402	.16688	.11879	.4210
	20	10			.3265	.3428	.097	.329	.14483	.16688	.2871
	30	10			.2739	.2876	.086	.293	.12153	.14483	.2523
6A Control	F	4	85.8	.056	.2526	.2652	.026	.087	.06475	-	.4422
	10	10			.3793	.3983	.096	.325	.09758	.06475	.4171
	20	10			.3110	.3266	.078	.266	.08001	.09758	.2895
	30	10			.2349	.2466	.059	.202	.06043	.08001	.2126
7A Control	F	4	110.4	.068	.2958	.3106	.038	.131	.14192	-	.6180
	10	10			.3866	.4059	.126	.427	.18608	.14192	.4268
	20	10			.3274	.3437	.106	.361	.15758	.18608	.3012
	30	10			.2397	.2517	.078	.265	.11538	.15758	.2018
Mean	F	4			.2600 ± .0198 (SEM)						.5275 ± .0392 (SEM)
	10	10			.3522 ± .0170						.3909 ± .0162
	20	10			.2993 ± .0121						.2747 ± .0918
	30	10			.2356 ± .0106						.2098 ± .0113

25 mM Buffer Concentration

Muscle ID	Interval ID	Length of Collection Interval (min)	Maximum Diffusion Distance Wt (mg)	Maximum Diffusion Distance l (cm)	Whole Muscle Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Whole Muscle Efflux a ($\mu mol \cdot ml^{-1} \cdot min^{-1}$)	Bath Concentration Yo ($\mu mol \cdot ml^{-1}$)	Total Bath Lactate BL (μmol)	End of Interval Extr. Lact. Q_E (μmol)	Beginning of Interval Extr. Lact. Q_B (μmol)	Fiber Efflux ($\mu mol \cdot g^{-1} \cdot min^{-1}$)
1A Experimental	F	4	100.4	.063	.2953	.3101	.035	.119	.11157	-	.5741
	10	10			.3347	.3514	.099	.336	.12688	.11157	.3499
	20	10			.2498	.2623	.074	.251	.09470	.12688	.2179
	30	10			.2621	.2752	.077	.263	.09936	.09470	.2666
3A Experimental	F	4	110.0	.068	.3636	.3818	.047	.160	.17285	-	.7565
	10	10			.3891	.4086	.125	.428	.18556	.17285	.4006
	20	10			.3375	.3544	.109	.371	.16096	.18556	.3149
	30	10			.4273	.4487	.138	.470	.20379	.16096	.4662
4A Experimental	F	4	116.9	.071	.2529	.2655	.035	.118	.14035	-	.5525
	10	10			.3352	.3520	.115	.392	.18659	.14035	.3749
	20	10			.3082	.3236	.106	.360	.17159	.18659	.2951
	30	10			.4392	.4612	.151	.513	.24449	.17156	.5021
5A Experimental	F	4	100.4	.063	.3000	.3150	.035	.120	.11334	-	.5810
	10	10			.4329	.4545	.128	.435	.16411	.11334	.4838
	20	10			.3410	.3581	.101	.342	.12927	.16411	.3059
	30	10			.4273	.4487	.119	.404	.16194	.12927	.4349
6A Experimental	F	4	79.6	.053	.3324	.3490	.021	.072	.07099	-	.4491
	10	10			.4272	.4486	.100	.340	.09166	.07099	.4531
	20	10			.3593	.3773	.084	.286	.07709	.09166	.3410
	30	10			.5361	.5629	.126	.427	.11502	.07709	.5841
7A Experimental	F	4	114.0	.070	.2543	.2670	.034	.116	.13237	-	.5447
	10	10			.3773	.3962	.127	.430	.19701	.13237	.4339
	20	10			.3183	.3342	.107	.363	.16620	.19701	.2914
	30	10			.3783	.3972	.127	.431	.19753	.16620	.4056
Mean	F	4			.2998 ± .0177 (SEM)						.5763 ± .0409 (SEM)
	10	10			.3827 ± .0174						.4160 ± .0209
	20	10			.3190 ± .0.57						.2944 ± .0169
	30	10			.4117 ± .0366						.4431 ± .0434

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