Journal of Physiology (1988), **397**, *pp.* 643–655
With 5 text-figures
Printed in Great Britain

EFFECT OF CARBON DIOXIDE ON HEAT PRODUCTION OF FROG SKELETAL MUSCLES

BY T. KITANO

From the Department of Physiology, Medical College of Oita, Oita 879-56 Japan

(*Received 4 March 1987*)

SUMMARY

1. Maintenance heat produced in tetani of frogs' sartorius muscles (*Rana japonica*) was measured under various values of intracellular pH (pH_i) brought about by increasing the CO_2 concentration in Ringer solution. The pH_i values were measured using ^{31}P nuclear magnetic resonance from the chemical shifts of the inorganic phosphate resonance. The pH_i was $7{\cdot}10 \pm 0{\cdot}009$ (mean ± S.E. of the mean, $n = 10$) in the gas mixture of 5% CO_2/95% O_2 at 4 °C and it was reduced to $6{\cdot}44 \pm 0{\cdot}001$ ($n = 23$) in 45% CO_2.

2. As CO_2 was increased, the maximum force was decreased and relaxation was prolonged. This is in accordance with the results of Edman & Mattiazzi (1981) and Curtin (1986).

3. An increase in CO_2 induced a reduction of the maintenance heat production, which can be divided into stable and labile heats (Aubert, 1956). The stable heat, which is produced with a steady rate during contraction, was decreased as CO_2 was increased. The labile heat, which is produced with an exponentially declining rate, was not significantly altered by increasing CO_2 within the range studied.

4. The effect of previous contractile activity on the labile heat production, i.e. the time course of repriming of the labile heat, could be described by an equation with two exponential terms in 5% CO_2 in accordance with the result of Peckham & Woledge (1986). The time course of repriming of the labile heat was not affected by increasing CO_2 to 20%.

INTRODUCTION

It is well known that extracellularly added CO_2 can easily penetrate into the intracellular milieu, and is combined with water as carbonic acid which dissociates into bicarbonate and hydrogen ions and decreases the intracellular pH (pH_i) (see review by Roos & Boron, 1981). It is well established that the concentration of intracellular H^+ has influence on many biological systems (Roos & Boron, 1981). In muscle contraction, effects of pH on contractile properties of skinned muscle fibre have been studied (Fabiato & Fabiato, 1978). Edman & Mattiazzi (1981) and Curtin (1986) studied the effects of increasing extracellular CO_2 on contractile properties of intact skeletal muscles and have reported that maximal force was decreased and the relaxation of force was slowed with increasing CO_2. These results are similar to those of fatiguing muscle (Dawson, Gadian & Wilkie, 1978, 1980; Edman & Mattiazzi,

1981). In fact, it is considered that a decrease in pH_i is one of the causes of muscular fatigue (Dawson *et al.* 1978, 1980).

Heat production during tetanic contraction, i.e. maintenance heat production, could be divided into two components; one is labile heat and the other is stable heat (Aubert, 1956). The stable heat is probably derived from actomyosin ATPase and the sarcoplasmic reticulum Ca^{2+}-ATPase activities (Homsher, Mommaerts, Ricchiuti & Wallner, 1972). The amount of labile heat is very similar to the amount of unexplained energy, which does not come from ATP splitting associated with contraction (Woledge, Curtin & Homsher, 1985). One hypothesis for the origin of unexplained energy is that it comes from the binding of Ca^{2+} to parvalbumin (Woledge *et al.* 1985).

In this paper, effects of increasing CO_2 on tetanic force and the labile and stable heats were studied. Moreover, the effect of previous activity of muscle on the extent of labile heat, i.e. the repriming of labile heat, was observed at normal and increased CO_2 concentration. The values of pH_i at each CO_2 concentration used were determined by using ^{31}P nuclear magnetic resonance (^{31}P NMR).

Preliminary accounts of this work have already appeared (Kitano, Kometani, Tanokura & Yamada, 1986).

METHODS

^{31}P nuclear magnetic resonance

In order to determine the pH_i at various CO_2 concentrations in Ringer solution, ^{31}P NMR studies were performed on thirty-two sartorius muscles from sixteen frogs (*Rana japonica*) at 4 °C. The total weight of the muscles amounted to 1·47 g. The muscles were dissected and mounted in a chamber, diameter 20 mm, in which they could adequately be oxygenated by superfusing the Ringer solution at a rapid rate of 60 ml min^{-1} (Yamada & Tanokura, 1983). The ^{31}P NMR spectra were obtained on a Bruker WM200WB wide-bore spectrometer operating at 81 MHz. Free induction decays were obtained by 60 deg radio-frequency pulses repeated at intervals of 4 s. The pH_i was estimated from the chemical shifts of inorganic phosphate (P_i) resonance relative to that of the phosphocreatine (PCr) resonance according to Dawson, Gadian & Wilkie (1977). Frog Ringer solution contained (mM): NaCl, 97; KCl, 2·5; $CaCl_2$, 1·8; $NaHCO_3$, 18. The Ringer solution was bubbled with O_2/CO_2 gas mixture. The percentage of CO_2 in the gas mixture used was 5, 10, 20 and 45%. The concentration of $NaHCO_3$ (18 mM) in the Ringer solution was kept constant at each CO_2 concentration. The extracellular pH (pH_o) at each CO_2 concentration was measured using a pH electrode (HM5B, TOA).

Heat measurements

Maintenance heat. Two thermopiles of electroplated type were used for heat measurement (Ricchiuti & Mommaerts, 1965). Their sensitivities were 5·14 and 7·26 mV K^{-1}. The equivalent half-thickness (Hill, 1965) of each of them was 14 μm as estimated from heat capacities of the materials constituting them. The output of the thermopile was amplified by an Ancom 15c-3a chopper amplifier with low-pass filter (cut-off frequency, 200 Hz), and recorded on a Nicolet 2090 digital oscilloscope. Pairs of sartorius muscles of *Rana japonica* were mounted vertically on both sides of the thermopile and the tibial ends of the muscles were connected to the force transducer of the type described by Jewell, Kretzschmar & Woledge (1967), while at the other end, the pelvic bone was firmly held in a clamp. The length of the muscles was adjusted to their resting length *in situ* (measured with the frog legs in the standard position; Hill, 1965). The muscles were stimulated directly via platinum electrodes located near the top and the bottom ends of the muscles, using square pulses of 5 ms duration at about 15 Hz at 4 °C. The thermopile was placed in a glass vessel containing the Ringer solution so that the muscles could be immersed in the aerated solution between periods of heat production measurement. The entire system was immersed in a bath

thermostated at 4 °C by using a circulator (HAAKE, F3-C). The correction for heat loss was carried out in the usual way (Hill, 1965). The mean value of exponential rate constants for heat loss was $0{\cdot}051 \pm 0{\cdot}005$ s^{-1} (mean ± S.E. of the mean). The amount of stimulus heat, which was measured in the presence of 1·5 μM-tetrodotoxin, was about 0·5 mJ g^{-1} s^{-1} at the stimulus strength and frequency used. No correction for thermopile lag and stimulus heat was needed in this study. The Ringer solution and the way of changing CO_2 concentration were the same as described in ^{31}P NMR measurements.

Aubert (1956) has shown that heat production during tetanic contraction, i.e. maintenance heat production, consists of two parts, the labile and the stable heats. This is expressed by the equation below:

$$h = h_a/a\,(1-e^{-at}) + h_b t, \quad (1)$$

where t is time in seconds, h the maintenance heat production, h_a the labile heat rate, a the rate constant for labile heat, and h_b the stable heat rate. The observations of the heat at 0·4 s intervals were fitted to eqn (1) by adjusting the three parameters h_a, h_b and a. The modified Gauss–Newton method (Hartley, 1961) was used for this non-linear curve fitting. There were no systemic errors inherent in the analysis because plots of the residuals against time showed random scatter.

Repriming of the labile heat. Aubert (1967) has shown that the labile heat is reduced in a second tetanus repeated at short intervals, the extent of reduction depending on the intervals between the two contractions (the repriming of the labile heat). The two 5 s tetani at 4 °C were repeated at different intervals of 5, 10, 25, 60, 180, 300, 480, 600 and 900 s at 5 and 20 % CO_2. Muscles were allowed to recover for at least 20 min between sets of two tetani. The labile heats in the first and the second tetani were obtained by fitting the maintenance heat production with eqn (1), and the fraction of the labile heat in the second to that in the first tetanus was calculated at each interval studied.

Statistical analysis

One-factor analysis of variance (ANOVA) was used to assess the statistical significance of the effect of increasing CO_2. The null probability, P, of less than 0·05 was considered significant.

RESULTS

The relation between CO_2 concentration and pH_i

To ascertain how pH_i was changed as CO_2 was increased, resting sartorius muscles were studied with ^{31}P NMR. Figure 1 shows a ^{31}P NMR spectrum of muscles equilibrated with 5% CO_2, and shows the well-known characteristics of ^{31}P NMR spectrum of frog muscles (Dawson *et al.* 1977; Yamada & Tanokura, 1983). An accumulation of 256 scans (17 min) was needed to attain a sufficient signal to noise ratio. The intensity of the P_i resonance is small indicating that oxygenation was sufficient. On top of P_i peak, resonances in the vicinity of P_i peak corresponding to various CO_2 concentrations are also shown, magnified 4-fold. As CO_2 was increased, the chemical shift of the P_i peak was reduced (shifted upfield). The intensity of P_i peak was increased when CO_2 was increased in the range from 5 to 45%. The concentration of P_i at 45% CO_2 was estimated to be $6{\cdot}3 \pm 0{\cdot}3$ mM (mean ± S.E. of the mean), assuming that the resting muscles at 5% CO_2 contained PCr of 27 mmol kg^{-1} (Dawson *et al.* 1977). The peak height of PCr fell proportionally and reached the level as shown by the horizontal bars near the peak of PCr.

Table 1 summarizes the values of pH_i, as well as pH_o, attained at each CO_2 concentration.

Effect of CO_2 on force and maintenance heat

Since the values of pH_i at each CO_2 concentration were determined by using ^{31}P NMR, results will be described in the following by using the values of pH_i instead of

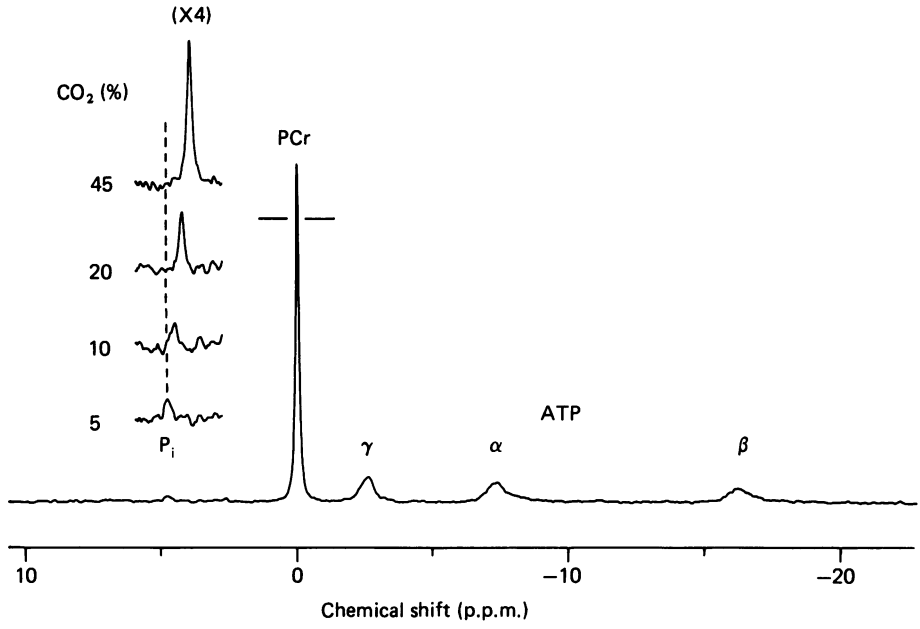

Fig. 1. ^{31}P NMR spectrum obtained from thirty-two resting sartorius muscles of *Rana japonica* aerated by 5% CO_2/95% O_2. Chemical shifts are described downfield from PCr. Inset shows the spectrum in the vicinity of P_i peak, magnified 4-fold, of muscles equilibrated with 5, 10, 20 and 45% CO_2. The horizontal bar near the peak of PCr represents the level to which the peak of PCr changed at 45% CO_2.

TABLE 1. Extracellular pH (pH_o) and intracellular pH (pH_i) values at various CO_2 concentrations (see text)

CO_2 (%)	5	10	20	45
pH_o	7·0	6·7	6·4	6·1
δ_{obs} (p.p.m.)	4·76±0·011	4·50±0·004	4·23±0·002	3·95±0·001
pH_i	7·10±0·009	6·90±0·003	6·69±0·002	6·44±0·001

The pH_o was measured with a pH electrode and the pH_i was obtained with ^{31}P NMR by measuring the chemical shifts of P_i peak (δ_{obs}) relative to that of the PCr resonance (Dawson *et al.* 1977). The δ_{obs} and pH_i are shown as the mean±1 S.E. of the mean from ten to twenty-three measurements in thirty-two sartorius muscles (*Rana japonica*). The bicarbonate concentration in the Ringer solution was fixed at 18 mM throughout.

CO_2 concentration. Figure 2 shows typical records of force developed and heat produced by tetanically stimulating a pair of sartorius muscles that were equilibrated with various CO_2 concentrations. As pH_i was decreased, the maximal force was reduced and at the same time relaxation was remarkably prolonged as has already been observed by Edman & Mattiazzi (1981) and Curtin (1986). In some cases active force was not maintained but tended to decline slightly at 45% CO_2 (pH_i 6·44) as seen in Fig. 2. For this and for the increase of P_i level in ^{31}P NMR (Fig. 1) the results with 45% CO_2 should be considered with some caution. Figure 3*A* shows that the mean values of maximal force in 5 s tetani were reduced with pH_i.

As seen in Fig. 2 the maintenance heat was also decreased with force as pH_i was decreased. Table 2 summarizes the amount of labile heat (h_a/a), labile heat rate

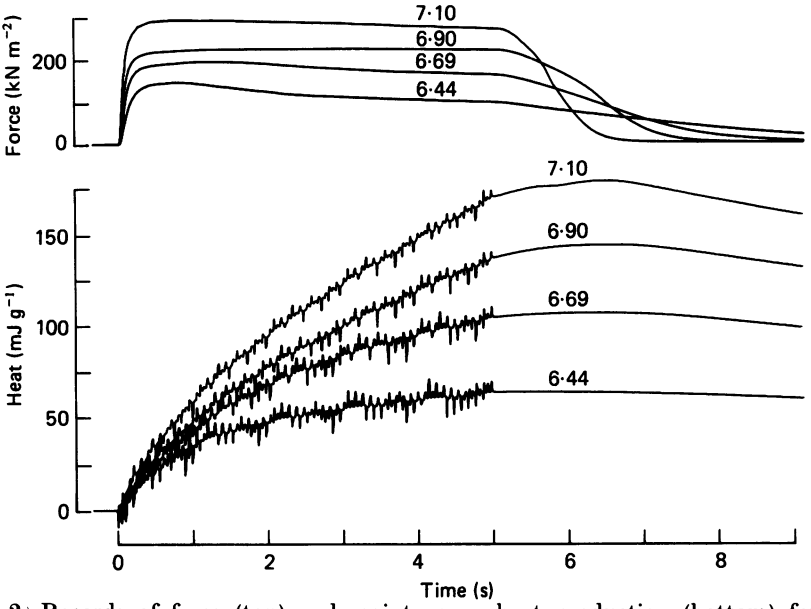

Fig. 2. Records of force (top) and maintenance heat production (bottom) from the sartorius muscles of *Rana japonica* at 5, 10, 20 and 45% CO_2. The pH_i values which were obtained by ^{31}P NMR are indicated on each curve. Heat records are not corrected for heat loss.

TABLE 2. Labile heat (h_a/a), labile heat rate (h_a), rate constant for labile heat (a) and stable heat (h_b) at each pH_i (CO_2), which are expressed as mean ± 1 S.E. of the mean from seventeen to nineteen observations in six frog muscles

pH_i (CO_2)	7·10 (5%)	6·90 (10%)	6·69 (20%)	6·44 (45%)
h_a/a (mJ g^{-1})	26·8 ± 1·81	25·0 ± 2·24	26·4 ± 2·36	24·0 ± 3·46
h_a* (mJ g^{-1} s^{-1})	40·9 ± 2·62	38·4 ± 1·84	32·8 ± 1·25	29·8 ± 2·67
a (s^{-1})	1·61 ± 0·01	1·76 ± 0·21	1·39 ± 0·12	1·53 ± 0·17
h_b** (mJ g^{-1} s^{-1})	26·7 ± 1·15	22·6 ± 1·16	18·4 ± 1·21	9·21 ± 1·12

The asterisks indicate that the mean values are significantly affected by increasing CO_2 (* $P < 0.05$, ** $P < 0.01$, ANOVA).

(h_a), rate constant of labile heat (a) and stable heat rate (h_b) obtained by the non-linear curve fitting of the maintenance heat at each CO_2 concentration. The residuals associated with the curve-fitting procedure were not significantly different between analyses at each CO_2. The labile heat (h_a/a) and stable heat rate (h_b) at each pH_i are plotted in Fig. 3*B*. The labile heat did not change significantly, while the stable heat rate was decreased with pH_i. Thus the decrease in total maintenance heat was mainly due to the decrease in the stable heat rate.

As shown in Fig. 2, relaxation of force was prolonged as pH_i was decreased. Figure 4 shows the mean values of the time from the last stimulus to 50% mechanical relaxation (t_{50}) and shows that t_{50} was increased significantly with decrease of pH_i.

Force and maintenance heat recovered well when CO_2 was decreased from 45 to 5%, but it took about 6 h to get full recovery.

Effect of CO_2 on repriming of the labile heat

Figure 5*A* shows the force and heat records associated with two 5 s isometric tetanic contractions repeated at an interval of 60 s. The force produced in the second tetanus was slightly smaller than that in the first one. The total maintenance heat in the second tetanus was markedly smaller than that in the first. Components of the

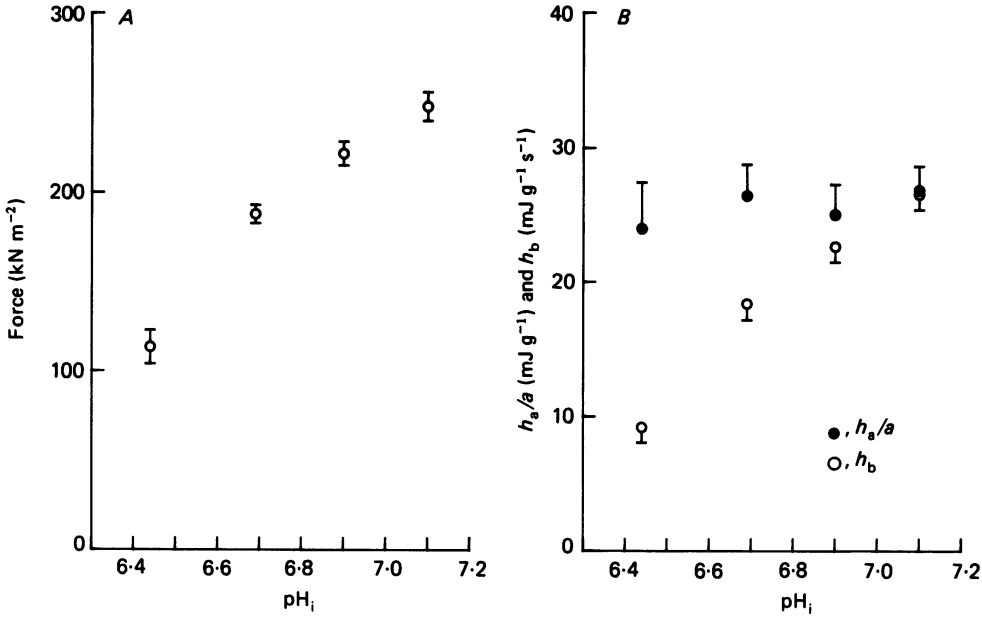

Fig. 3. Effect of pH_i on maximal force and maintenance heat production. *A*, maximal force normalized by cross-sectional area. Vertical lines represent ±1 S.E. of the mean (seventeen to nineteen observations in six mucles). *B*, amount of labile heat (h_a/a) and stable heat rate (h_b) normalized by blotted muscle weight. ●, the mean values of labile heat, and ○, stable heat rate, both of which were obtained from seventeen to nineteen observations in six muscles. Vertical lines represent ±1 S.E. of the mean. Both maximal force and stable heat rate were significantly decreased with pH_i ($P < 0{\cdot}01$, ANOVA), but the labile heat was not decreased with pH_i significantly.

maintenance heat, the stable and the labile, were evaluated using eqn (1). The results showed that the stable heat was almost unaffected in the second tetanus (the ratio of that in the second to that in the first tetanus was 0·96), whereas the labile heat was considerably decreased in the second tetanus (the ratio, 0·67). The reduction of the labile heat in the second contraction shortly after the first one and the subsequent recovery, the repriming of the labile heat, have been studied by Aubert (1967), Curtin & Woledge (1977) and Peckham & Woledge (1986). Figure 5*B* shows the time course of repriming of the labile heat at pH_i 7·10 and 6·69. The time course cannot adequately be described by a single exponential but can be described by the following equation below with two exponential terms, in accordance with Peckham & Woledge (1986) and E. Homsher (personal communication):

$$E = 1-(a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2}), \quad (2)$$

where t is time in seconds, E the labile heat in the second tetanus relative to the first one, a_1 and a_2 are constants, and τ_1 and τ_2 the two time constants. Figure 5*B* also shows the curves obtained by using a non-linear curve-fitting procedure (continuous lines) and shows that there are two components, rapid and slow, in the time course of repriming. The time courses of repriming of the labile heat at pH_i 7·10 and 6·69

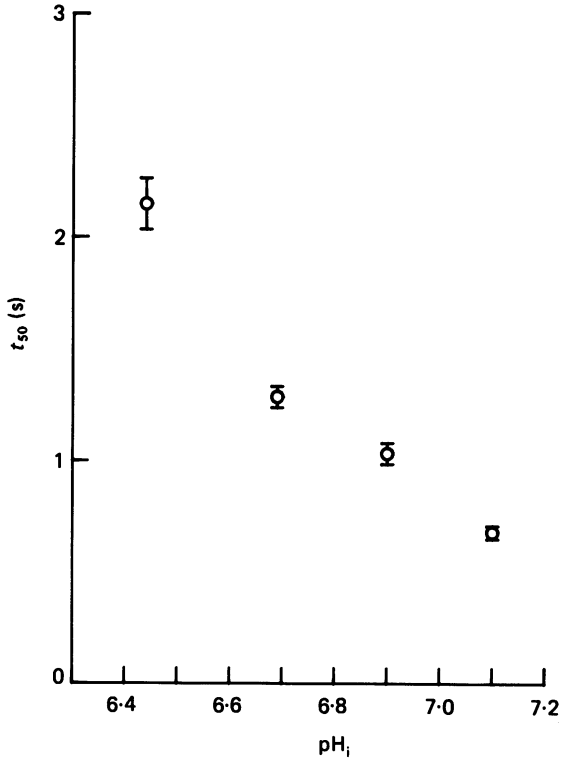

Fig. 4. Effect of pH_i on the time from the last stimulus to 50% mechanical relaxation (t_{50}). ○ represent the mean values from seventeen to nineteen observations in six muscles. Vertical lines show ±1 S.E. of the mean. t_{50} is significantly increased with lowering pH_i ($P < 0·01$, ANOVA).

were almost identical. Table 3 summarizes the values for a_1, a_2, τ_1 and τ_2 for pH_i 7·10 and 6·69. It should be noted that, muscles were allowed to recover for 20 min between the sets of two tetani. If repriming is not complete during this period, the amplitude and the time constant of the slow component of repriming would be underestimated.

DISCUSSION

CO_2 concentration and pH_i

The pH_i determined by ^{31}P NMR on resting muscles equilibrated with 5% CO_2 was slightly alkaline (7·10) and higher than the pH_o (7·0) at 4 °C. In the results obtained by using pH-sensitive microelectrodes on frog skeletal muscles (15 mM-$NaHCO_3$ and 5% CO_2, Bolton & Vaughan-Jones, 1977), however, the pH_i determined at 25 °C is

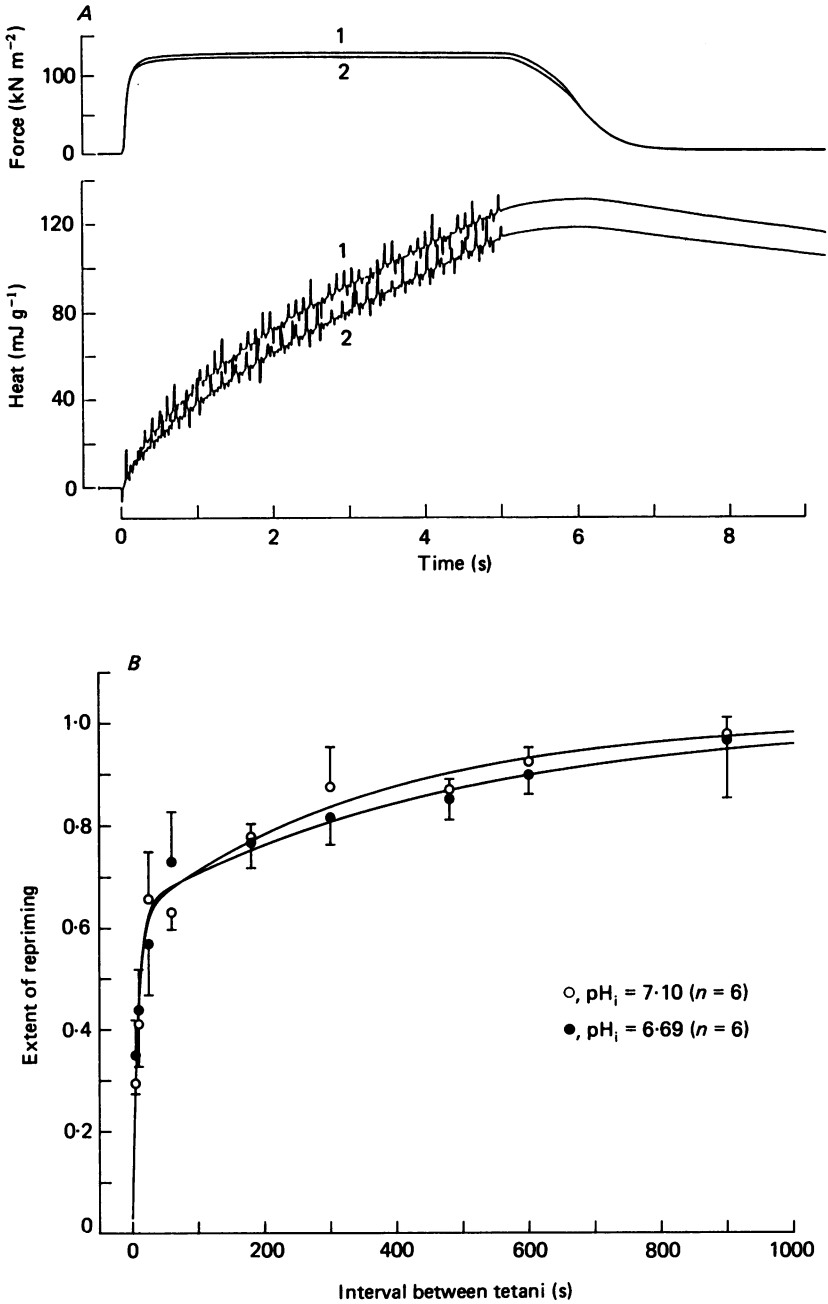

Fig. 5. *A*, force and heat production in two tetani separated by an interval of 60 s. The traces 1 are for the first tetanus and traces 2 for the second one. *B*, effect of pH_i on the repriming of the labile maintenance heat. Labile maintenance heat in the second tetanus expressed as a fraction of that in the first tetanus is plotted against the interval between two successive tetani. Each point represents the mean of six observations in six muscles. ○, pH_i 7·10; ●, pH_i 6·69. Vertical lines represent ±1 S.E. of the mean. The continuous lines are best-fit curves obtained by using eqn (2) and the values described in Table 3 at pH_i 7·10 and 6·69.

slightly acidic (6·9) and is lower than the pH_o (7·2). Abercrombie, Putnam & Roos (1983) have also shown the similar result (pH_i 6·8 at pH_o 7·35) at 22 °C (24 mM-$NaHCO_3$ and 5% CO_2). The pH_i determined by increasing CO_2 at 4 °C was also slightly higher than that in other observations at higher temperature (18 °C, Stella, 1929; 25 °C, Izutsu, 1972). The discrepancies between the results obtained by ^{31}P NMR and by microelectrode study may be explained by considering the temperature

TABLE 3. The values of the constants in eqn (2) that describes the time course of repriming of the labile heat

pH_i (CO_2)	a_1	a_2	τ_1 (s)	τ_2 (s)
7·10 (5%)	0·62	0·38	8·46	360·9
6·69 (20%)	0·62	0·36	8·60	486·6

dependence of both pH_i and pH_o (see Roos & Boron, 1981, pp. 332, 355; Hill, 1965, p. 245).

Effect of CO_2 on force produced in tetani

As CO_2 was increased the maximum force produced by tetanic stimulation was decreased and relaxation from contraction was prolonged. The effect of increasing CO_2 was similar to that of fatigue. Dawson *et al.* (1978, 1980) have shown using ^{31}P NMR that in fatigued muscle not only pH_i but also the concentrations of other metabolites such as P_i, ADP and PCr are altered, and that the concentration of such metabolites other than H^+ also affects both the force and the rate of relaxation. Among these metabolites the diprotonated form of P_i ($H_2PO_4^-$) has been shown to be responsible for the decrease in force (Dawson, Smith & Wilkie, 1986; Nosek, Fender & Godt, 1987). The total concentration of P_i at 45% CO_2 (pH_i 6·44) in resting muscles was 6·3 mM (Fig. 1), so that the concentration of $H_2PO_4^-$ was calculated to be 4·7 mM at this pH_i using pK of 6·88. According to Nosek *et al.* (1987) 5 mM-$H_2PO_4^-$ depresses force by about 15%. Since force was depressed by more than 50% at 45% CO_2 (Fig. 3*A*), the larger part of the depression in force by increasing CO_2 is caused by lowering pH_i.

In the present study the results of ^{31}P NMR showed that in resting muscles the concentration of PCr was reduced and that of P_i was increased when CO_2 was increased (Fig. 1). This may arise from the glycolysis and respiratory chain in resting muscles being suppressed by acidification (Roos & Boron, 1981). In the heat measurements, 5 s tetani were repeated at 20 min intervals. If the metabolic recovery from contraction is retarded by increasing CO_2, the change in the concentration of metabolites should increase as contractions are repeated and the effect of them on force might be more significant.

Effect of CO_2 on stable heat

As CO_2 was increased the stable heat was decreased as well as the force produced. It is thought that the stable maintenance heat is derived from the enthalpy change of PCr splitting, which follows ATP splitting by actomyosin ATPase as well as Ca^{2+}-ATPase of the sarcoplasmic reticulum (Homsher *et al.* 1972). The enthalpy change for PCr splitting is decreased as pH is lowered; the value at pH 6·44 is

reduced to about 84% of that at pH 7·1 (Alberty, 1968, 1969; Woledge, 1972). Thus in order to ascertain whether the decrease in the stable heat by increasing CO_2 was explained only by the pH dependence of the enthalpy change for PCr splitting, the amounts of PCr split to account for the stable heat at each pH_i were calculated using the values of enthalpy changes appropriate to each pH_i. The amount of PCr splitting calculated is still significantly decreased with pH_i ($P < 0{\cdot}01$, ANOVA, data not shown).

The following possibilities might account for the decrease. (1) Release of Ca^{2+} from the sarcoplasmic reticulum may be suppressed, (2) the contractile activation involving troponin–tropomyosin system may be suppressed, and/or (3) the activity of both actomyosin and Ca^{2+}-ATPases may be suppressed by increasing CO_2.

The twitch activation heat measured at 5, 20 and 45% CO_2 was not significantly different among these CO_2 values (Kitano *et al.* 1986). It is believed that the twitch activation heat is proportional to the amount of Ca^{2+} released (Woledge *et al.* 1985). Thus it is likely that the amount of Ca^{2+} released during contractile activation is not affected as pH_i is lowered. In the process of contractile activation, the competition between H^+ and Ca^{2+} for the Ca^{2+} binding sites of troponin must be considered as well. The capacity as well as affinity for Ca^{2+} binding of skeletal muscle troponin is not affected by the decrease in pH from 7·0 to 6·2 (Stull & Buss, 1978) and from 7·2 to 6·5 (Ogawa, 1985).

Thus, it can be concluded that the effect of increasing CO_2 on contractile activation is probably small, and that the decrease in the stable heat with pH_i is mainly due to the reduced rate of both actomyosin- and Ca^{2+}-ATPases. Edman & Mattiazzi (1981) have studied the effects of fatigue and altered CO_2 concentration on isometric force and velocity of shortening at zero load. From the results they have suggested that the effects of both fatigue and pH_i may be partly on the contractile activation, but mainly on the actomyosin-ATPase activity.

Effect of CO_2 on labile heat

As pH_i was decreased, the labile heat obtained was not altered, nor was the time course of repriming of the labile heat. The origin of the labile heat has not yet been unequivocally shown, but it has been suggested to be related to the enthalpy change associated with Ca^{2+} binding to parvalbumin (Woledge *et al.* 1985). According to this hypothesis, the result that the labile heat is not affected by the increase in pH_i agrees well with that little pH change has been observed for Mg^{2+}–Ca^{2+} exchange of parvalbumin (Moeschler, Schaer & Cox, 1980; Tanokura & Yamada, 1985).

The above hypothesis also suggests that the increase in calcium bound to parvalbumin after a conditioning tetanus causes a decrease in the labile heat in the second tetanus, the extent of the decrease depending on the time elapsed after the conditioning tetanus, i.e. the repriming of the labile heat. At the physiological pH, the rate of release of Ca^{2+} bound to parvalbumin (rate constant at 20 °C, 1·5 and 1·16 s^{-1} for the two isozymes of bull-frog parvalbumin, Ogawa & Tanokura, 1986*b*) is slower than the rate of Ca^{2+} uptake by the sarcoplasmic reticulum (rate constant at 15 °C, 40–60 s^{-1} for the rapid component and 2–3 s^{-1} for the slow one of the sarcoplasmic reticulum from bull-frog, Ogawa, Kurebayashi, Irimajiri & Hanai, 1981) and thus may determine the rate of repriming of the labile heat. The result that

the time course of repriming of the labile heat was not affected by the decrease in pH_i could be explained on the basis that the rate of Ca^{2+} release from parvalbumin was not affected by increasing CO_2.

On the other hand, according to calorimetric studies of Ca^{2+} binding to parvalbumin the expected amount of heat produced by the Mg^{2+}–Ca^{2+} exchange of parvalbumin can account for only half to two-thirds of the labile maintenance heat (Tanokura & Yamada, 1985; Smith & Woledge, 1986). Moreover, Ogawa & Tanokura (1986*a*) have estimated that as many as half or more of the binding sites of parvalbumin are occupied by Ca^{2+} even in resting muscles. Thus the component of the labile heat derived from binding of Ca^{2+} to parvalbumin may be lower than two-thirds and an additional contribution to the labile heat other than Ca^{2+} binding to parvalbumin must be sought.

The time course of repriming of the labile heat can be fitted with two exponential terms; a component with a time constant of about 8·5 s, and another one with a time constant of about 400 s at 4 °C. About 60% of the labile heat corresponds to the rapid component of repriming, and the other 40% to the slow one. Since the rate of the fast component is rather similar to the rate of Ca^{2+} release from parvalbumin (time constant estimated to be 5·8 s by taking the dissociation rate constant from parvalbumin to be about 1 s^{-1} at 20 °C, Ogawa & Tanokura, 1986*b*, and by assuming a Q_{10} of 3), this component might be associated with the process leading to availability of Ca^{2+} sites on parvalbumin after conditioning contraction.

A post-contractile utilization of PCr has recently been demonstrated (Yamada & Tanokura, 1983; Kawano, Tanokura & Yamada, 1986). The amount is about equal to that of myosin heads in muscle (0·3 mmol kg^{-1} muscle) and the time constant of the post-contractile PCr utilization is more than about 100 s at 4 °C. It might be possible that the slow component seen in the course of repriming of the labile heat (time constant 400 s, see above) corresponds to this process.

I am grateful to Professor K. Yamada, Drs K. Kometani, M. Tanokura and Y. Kawano for continual help and advice throughout this work. I would like to thank Professor R. C. Woledge and Dr N. A. Curtin for critical comments and helpful discussions on the manuscript.

REFERENCES

ABERCROMBIE, R. F., PUTNAM, R. W. & ROOS, A. (1983). The intracellular pH of frog skeletal muscle: its regulation in isotonic solutions. *Journal of Physiology* **345**, 175–187.

ALBERTY, R. A. (1968). Effect of pH and metal ion concentration on the equilibrium hydrolysis of adenosine triphosphate to adenosine diphosphate. *Journal of Biological Chemistry* **243**, 1337–1343.

ALBERTY, R. A. (1969). Standard Gibbs free energy, enthalpy, and entropy changes as a function of pH and pMg for several reactions involving adenosine phosphates. *Journal of Biological Chemistry* **244**, 3290–3302.

AUBERT, X. (1956). *Le Couplage Energetique de la Contraction Musculaire*. Brussels: Editions Arscia.

AUBERT, X. (1967). In *Symposium on Muscle, Symposia Biologica Hungarica*, 8, ed. ERNST, E. & STRAUB, F. B., pp. 187–190. Budapest: Akademiai Kiado.

BOLTON, T. & VAUGHAN-JONES, R. D. (1977). Continuous direct measurement of intracellular chloride and pH in frog skeletal muscle. *Journal of Physiology* **270**, 801–833.

CURTIN, N. A. (1986). Effects of carbon dioxide and tetanus duration on relaxation of frog skeletal muscle. *Journal of Muscle Research and Cell Motility* **7**, 269–275.

CURTIN, N. A. & WOLEDGE, R. C. (1977). A comparison of the energy balance in two successive isometric tetani of frog muscle. *Journal of Physiology* **270**, 455–471.

DAWSON, M. J., GADIAN, D. G. & WILKIE, D. R. (1977). Contraction and recovery of living muscles studied by ^{31}P nuclear magnetic resonance. *Journal of Physiology* **267**, 703–735.

DAWSON, M. J., GADIAN, D. G. & WILKIE, D. R. (1978). Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature* **274**, 861–866.

DAWSON, M. J., GADIAN, D. G. & WILKIE, D. R. (1980). Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorus nuclear magnetic resonance. *Journal of Physiology* **299**, 465–484.

DAWSON, M. J., SMITH, S. & WILKIE, D. R. (1986). The [$H_2PO_4^-$] may determine cross-bridge cycling rate and force production in living fatiguing muscle. *Biophysical Journal* **49**, 268a.

EDMAN, K. A. P. & MATTIAZZI, A. R. (1981). Effects of fatigue and altered pH on isometric force and velocity of shortening at zero load in frog muscle fibres. *Journal of Muscle Research and Cell Motility* **2**, 321–334.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology* **276**, 233–255.

HARTLEY, H. O. (1961). The modified Gauss–Newton method for the fitting of non-linear regression functions by least squares. *Technometrics*, vol. 3, No. 2.

HILL, A. V. (1965). *Trails and Trials in Physiology*. London: Edward Arnold.

HOMSHER, E., MOMMAERTS, W. F. H. M., RICCHIUTI, N. V. & WALLNER, A. (1972). Activation heat, activation metabolism and tension-related heat in frog semitendinosus muscles. *Journal of Physiology* **220**, 601–625.

IZUTSU, K. T. (1972). Intracellular pH, H ion flux and H ion permeability coefficient in bullfrog toe muscle. *Journal of Physiology* **221**, 15–27.

JEWELL, B. R., KRETZSCHMAR, M. & WOLEDGE, R. C. (1967). Length and tension transducers. *Journal of Physiology* **191**, 10–12*P*.

KAWANO, Y., TANOKURA, M. & YAMADA, K. (1986). Time-resolved ^{31}P-NMR studies on isolated skeletal muscles of the bullfrog (*R. catesbeiana*). *Journal of Physiology* **377**, 99*P*.

KITANO, T., KOMETANI, K., TANOKURA, M. & YAMADA, K. (1986). Effect of lowering intracellular pH on the maintenance heat of isolated frog skeletal muscle. *Journal of Physiology* **377**, 98*P*.

MOESCHLER, H. J., SCHAER, J. J. & COX, J. A. (1980). A thermodynamic analysis of the binding of calcium and magnesium ions to parvalbumin. *European Journal of Biochemistry* **111**, 73–78.

NOSEK, T. M., FENDER, K. Y. & GODT, R. E. (1987). It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibers. *Science* **236**, 191–193.

OGAWA, Y. (1985). Calcium binding to troponin C and troponin: effects of Mg^{2+}, ionic strength and pH. *Journal of Biochemistry* **97**, 1011–1023.

OGAWA, Y., KUREBAYASHI, N., IRIMAJIRI, A. & HANAI, T. (1981). Transient kinetics for Ca uptake by fragmented sarcoplasmic reticulum from bullfrog skeletal muscle with reference to the rate of relaxation of living muscle. *Advances in Physiological Sciences* **5**, 417–435.

OGAWA, Y. & TANOKURA, M. (1986*a*). Steady-state properties of calcium binding to parvalbumins from bullfrog skeletal muscle: effects of Mg^{2+}, pH, ionic strength, and temperature. *Journal of Biochemistry* **99**, 73–80.

OGAWA, Y. & TANOKURA, M. (1986*b*). Kinetic studies of calcium binding to parvalbumins from bullfrog skeletal muscle. *Journal of Biochemistry* **99**, 81–89.

PECKHAM, M. & WOLEDGE, R. C. (1986). Labile heat and changes in rate of relaxation of frog muscles. *Journal of Physiology* **374**, 123–135.

RICCHIUTI, N. V. & MOMMAERTS, W. F. H. M. (1965). Technique for myothermic measurements. *Physiologist* **8**, 259.

ROOS, A. & BORON, W. F. (1981). Intracellular pH. *Physiological Reviews* **61**, 296–434.

SMITH, S. J. & WOLEDGE, R. C. (1985). Thermodynamic analysis of calcium binding to frog parvalbumin. *Journal of Muscle Research and Cell Motility* **6**, 757–768.

STELLA, G. (1929). The combination of carbon dioxide with muscle: its heat of neutralization and its dissociation curve. *Journal of Physiology* **68**, 49–66.

STULL, T. J. & BUSS, J. E. (1978). Calcium binding properties of beef cardiac troponin. *Journal of Biological Chemistry* **253**, 5932–5938.

TANOKURA, M. & YAMADA, K. (1985). A calorimetric study of Ca^{2+} binding to two major isotypes of bullfrog parvalbumin. *FEBS letters* **185**, 165–169.

WOLEDGE, R. C. (1972). *In vitro* calorimetric studies relating to the interpretation of muscle heat experiments. *Cold Spring Harbor Symposia on Quantitative Biology* **37**, 629–634.

WOLEDGE, R. C., CURTIN, N. A. & HOMSHER, E. (1985). *Energetic Aspects of Muscle Contraction*. London: Academic Press.

YAMADA, K. & TANOKURA, M. (1983). Post-contractile phosphocreatine splitting in muscle as revealed by time-resolved ^{31}P nuclear magnetic resonance. *Japanese Journal of Physiology* **33**, 909–919.