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Hydrogen ion changes and contractile behavior in the perfused rat heart

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The effect of acid–base alterations was analyzed using isolated rat hearts perfused at constant coronary perfusion pressure, and stimulated to contract at constant rate.

The amount of shortening in the major axis and its derivative were measured to assess myocardial contractility. Both the 'respiratory' and 'metabolic' alterations affected the contractile behavior to the same extent. In the physiological range studied by us, acidosis depresses and alkalosis increases myocardial contraction. However, acidosis seems to depress contractility more than the enhancement produced by the same change in pH towards the alkalotic side. When either amount of shortening or max dl/dt was plotted as a function of hydrogen ion activity ($^aH^+$) a linear correlation was obtained, either with pure 'metabolic' or 'respiratory' acid–base induced alterations (correlation coefficients higher than $-.95$; $P < .01$). Our findings suggest that in the range studied by us, contraction of the perfused rat heart following acid–base alterations, is a linear function of hydrogen ion activity.

pH and the heart; myocardial contractility; pCO_2 and the heart; bicarbonate and the heart

Introduction

Since the work of Gremels and Starling [11] it has been well established that changes in pH elicited by altering the pCO_2 can affect myocardial contractility.

There is, however, no consensus regarding the effect of changes in pH at constant pCO_2 upon myocardial contractility [4–7, 11, 26]. Whereas some investigators have reported that changes in pH at constant pCO_2 do not affect contractile behavior [11], other reports indicate that myocardial contractility is affected by metabolic acid–base alterations [18, 25, 27, 28]. These conflicting results may be explained partially by different preparations, different species, and differences in the extent of changes in acid–base status.

The present experiments were designed to evaluate the quantitative relation between acid–base alterations and contractile behavior in the perfused rat heart.

There are two main objectives in this communication. The first is to present data indicating that in the perfused rat heart a change in pH affects myocardial contractility independent of the way in which the change is produced ('respiratory' or 'metabolic' alterations). The second is to examine the quantitative relation between acidosis and alkalosis, and cardiac performance in this preparation.

Methods

Isolated hearts from white rats weighing 250 g were perfused at 37 °C using the Langendorff technique [21]. Details about the preparation have

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been reported previously [22]. The hearts were perfused through the coronary arteries and were beating empty. The small amount of solution draining into the left ventricle through the Thebesian vessels was drained by a small polyvinyl catheter inserted into the left ventricle at the apex. The hearts were stimulated at a rate of 240 min^{-1} with two ring-shaped platinum electrodes stitched to the epicardial surface of the right ventricle. To prevent interference from atrial beats, total heart block was performed by cutting the bundle of His. The displacement of the heart in the major axis was recorded by a DC-100 Sanborn displacement transducer (weight : 1 g). The signal was electronically differentiated in order to obtain the instantaneous velocity of shortening (dl/dt). The ECG was usually obtained from electrodes attached to the lateral part of the left ventricle. Mechanical and electrical activity were amplified with Sanborn preamplifiers and recorded on magnetic tape and a Sanborn oscillographic recorder at a paper speed of 100 mm/sec. Figure 1 shows the mechanical records of a typical experiment.

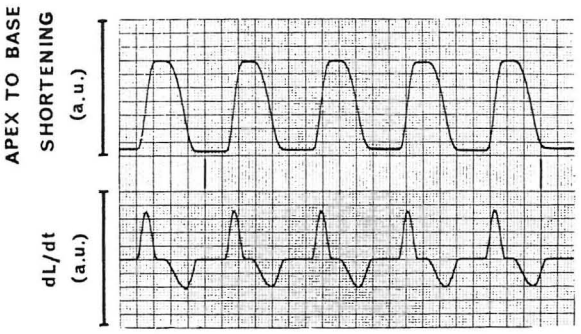

Fig. 1. Record from a typical experiment showing amount of shortening (arbitrary units) and its derivative (1 mm = .01 sec).

The experimental set up allowed us to switch rapidly from one Ringer's solution with a given sodium bicarbonate concentration and a given pCO_2, to another solution with the same pCO_2 but a different bicarbonate concentration, or with the same bicarbonate concentration but a different pCO_2. In this way, either pure 'respiratory' or 'metabolic' acid–base alterations were produced at constant coronary perfusion pressure and heart rate. The pH was constantly monitored by means of Radiometer flow electrodes and a Radiometer pM 27 pH meter. Samples of the solution were obtained after equilibration periods of approximately 10 minutes for pH and pCO_2 measurements.

In the first series of experiments, performed in 10 hearts, changes in pH at constant pCO_2 ('metabolic' changes) were produced by altering the sodium bicarbonate concentration and keeping sodium and osmolarity constant. The mechanical response to five different pH values was analyzed and the experiment was finished in approximately one hour after the stabilization period. Preliminary experiments showed no statistically significant decay of the mechanical performance of the preparation over a 60-minute period after the equilibration period.

A different random order to exposure was used for each heart and bicarbonate concentrations of 5; 10; 20; 40 and 60 mM/l were used to change the pH at a constant pCO_2 of approximately 35 mmHg. The different pH values obtained were 6.86 ± 0.16, $7.12 \pm .024$, $7.41 \pm .076$, $7.65 \pm .024$ and $7.80 \pm .018$. Coronary flow was measured by collecting the effluent draining from the heart.

In a second series of experiments performed in 9 hearts, the changes in pH were produced by changing pCO_2 in the gas mixture at a constant sodium bicarbonate concentration of 20 mM/l. Again the sequence of the changes was randomized. The different pCO_2 used were: 17, 36, 55 and 68 mm Hg. The pH values obtained with these 'respiratory' changes were: $7.67 \pm .022$, $7.37 \pm .020$, $7.19 \pm .018$ and $7.07 \pm .014$. The composition of the Ringer's solution in mM was: $Na^+ = 149$, $K^+ = 4.7$, $Ca^{++} = 2.6$, $Mg^{++} = 2.1$, $Cl^- = 138$, $CO_3H^- = 20$, $PO_4H^- = .4$, and glucose = 11. In both series of experiments, the condition in which pH was approximately normal (pCO_2 = 35 mm Hg, $NaHCO_3$ = 20 mM/l) was considered the control condition. The results were expressed as percentage of change from control either in amount of shortening or maximal dl/dt. Regression lines by the least square method and correlation coefficients were performed using a Hewlett–Packard desk computer model 9100 B. Student's t-test was used to determine significance of these data with a P value of .05 or less considered significant.

Results

1. Changes in pH at constant pCO_2

Figure 2 shows the heart's mechanical response to changes in pH at constant pCO_2. Bicarbonate concentration was altered in a range of 5 to 60 mM/l. Both the extent of shortening in the major axis and maximal dl/dt increased when pH was increased from 7.40 to 7.80. However, when our results were compared quantitatively, contractility* decreased by 47 % when pH was changed from 7.4 to 7.0 whereas the change in pH from 7.4 to 7.8 enhanced myocardial contractility by only 17 %.

* Contractility was assessed by the measurement of the amount of shortening in the major axis and its derivative.

2. Changes in pH at constant sodium bicarbonate concentration

Figure 3 shows the mechanical effects produced by altering pCO_2 at a constant bicarbonate concentration of 20 mM/l.

The changes in pCO_2 in the range from 17 to 68 mm Hg produced essentially the same changes which were found in experiments in which pH was

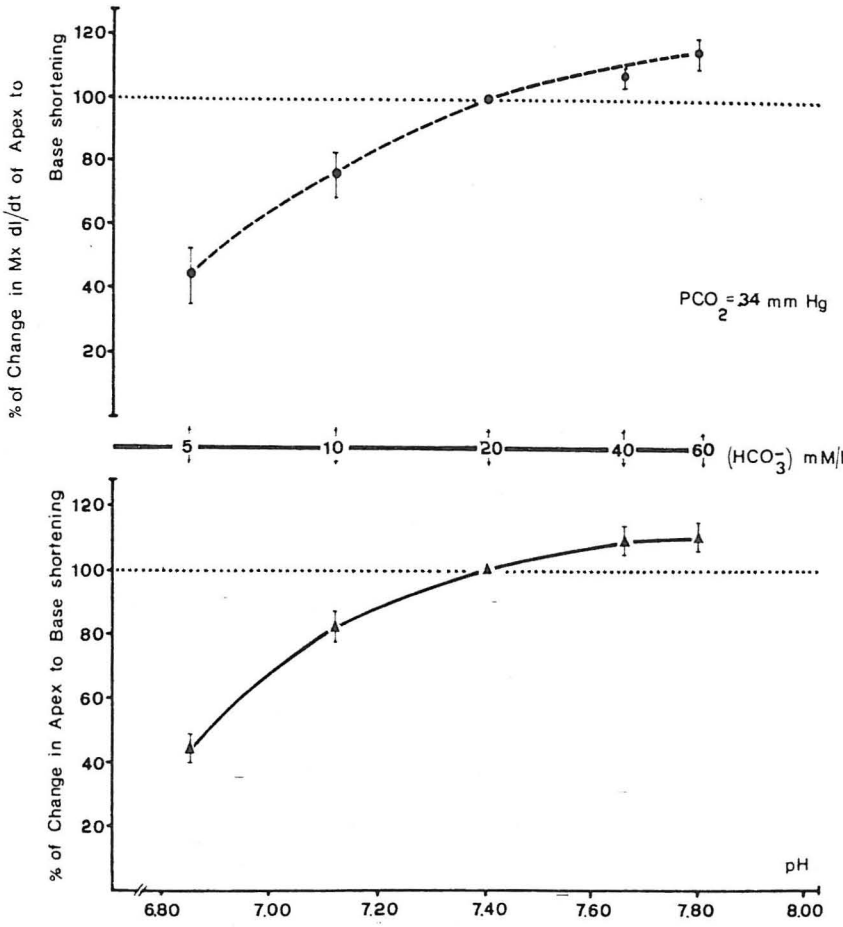

Fig. 2. Relationship between pH changes at constant pCO_2 (metabolic changes) and maximal dl/dt (upper pannel) an amount of shortening (lower panel). Note the inverse and nonlinear relationship between pH and the mechanical parameters use to assess myocardial contractility. Both the acidotic and alkalotic points are statistically significant from the acidotic to alkalot side.

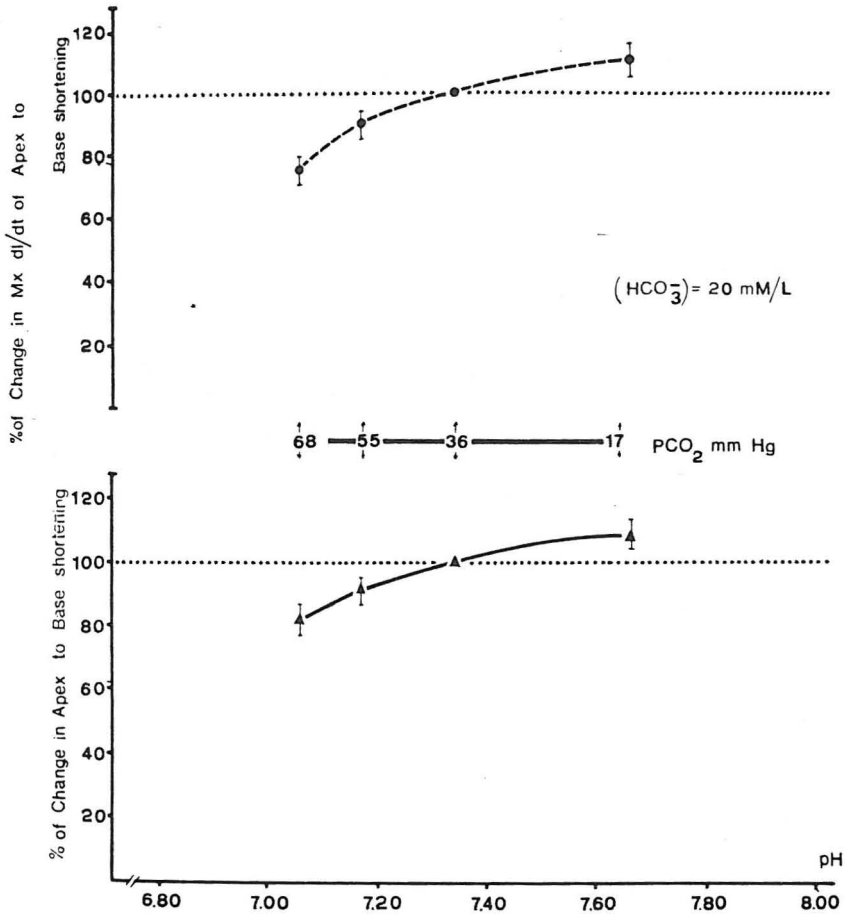

Fig. 3. Relationship between pH changes at constant bicarbonate concentration (respiratory changes) and maximal dl/dt (upper pannel) and amount of shortening (lower pannel). Both the acidotic and alkalotic points are statistically significant from control. Slopes are statistically different from the acidotic to the alkalotic side.

altered by changing $NaHCO_3$ concentration at constant pCO_2. With 'respiratory' alterations, contractility was again more affected by acidosis than by alkalosis, as it is reflected by the slopes of the relationship between pH versus amount of shortening or max dl/dt. When compared, the slopes of the plots produced by 'respiratory' (Fig. 3) and 'metabolic' (Fig. 2) alterations were not significantly different. However, both plots have statistically different slopes for the 'acidotic' and 'alkalotic' alterations ($P < .01$) suggesting that alkalosis influences cardiac performance to a lesser extent than acidosis does.

Figures 4 and 5 show how mechanical activity is affected in the perfused rat heart when the data present above were replotted as a function of hydrogen ion activity after 'metabolic' (Fig. 4) or 'respiratory' (Fig. 5) acid–base alterations. There was a highly significant correlation between $^aH^+$ and myocardial contractility as assessed by max dl/dt or amount of shortening. With changes in $^aH^+$ at constant pCO_2 max dl/dt (%) was = $(-.60 \pm .03) \times (^aH^+) + 125 \pm 2.6$ and amount of shortening (%) = $(-.58 \pm .04) \times (^aH^+) + 121 \pm 2.9$. The correlation coefficients (r) were $-.95$ and $-.96$, respectively ($P < .01$). When the changes in $^aH^+$ were produced at constant $NaHCO_3$ concentration, max dl/dt (%) = $(-.49 \pm 0.9) \times (^aH^+ + 120 \pm 5.1$ and amount of shortening (%) = $(-.37 \pm .06) \times (^aH^+) + 113 \pm 3.4$). The correlation coefficients were $-.96$ and

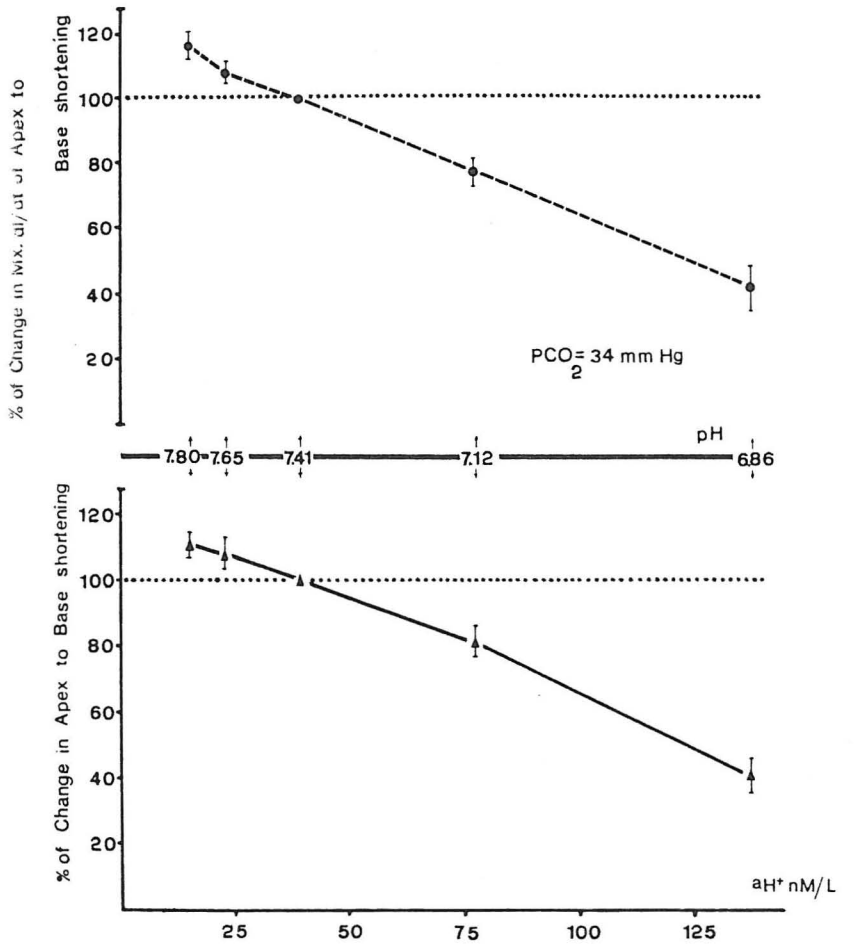

Fig. 4. Plot showing the linear relationship between hydrogen ion activity ($^aH^+$) and maximal dl/dt (upper panel) with metabolic acid–base alterations and amount of shortening (lower panel). The correlation coefficients (r) were −.95 and −.96, respectively.

−.97. respectively, It is evident now that for a given change in hydrogen ion activity (either below or above normal values) contractility is affected to the same extent. No significant differences were found in the slopes of the relationship between cardiac performances and hydrogen ion activity for the 'metabolic' versus the respiratory acid–base alterations.

No significant changes in coronary flow were found during these acid–base changes although this variable usually increased slightly following a decrease in contractility.

Discussion

In the perfused rat heart at constant coronary perfusion pressure and heart rate, contractility assessed either by the amount of shortening in the major axis or by maximal velocity of shortening, is affected by 'metabolic' or 'respiratory' acid–base alterations. In the range studied by us, acidosis depressed contractility and conversely, alkalosis enhanced myocardial performance. It is unlikely that small changes in coronary flow could account for the changes in contractility, since this variable did not change significantly during the experiments. It is also unlikely that a variable response to circulating catecholamines could have influenced our results in this in vitro preparation, although the possibility of pH changes producing an effect upon the nerve endings or beta receptors was not explored

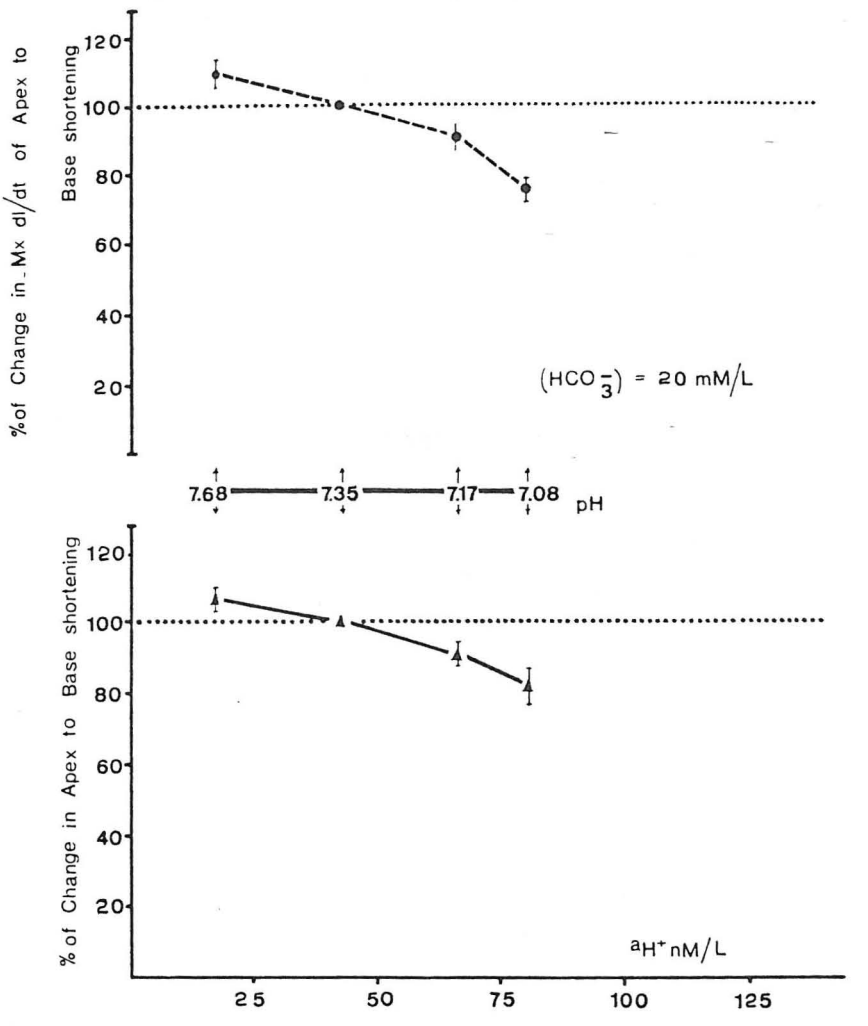

Fig. 5. Plot showing the linear relationship between $^aH^+$ and the mechanical parameters used to assess contractility in preparation. The correlation coefficients were −.96 and −.97, respectively.

When compared quantitatively, the changes in pH at constant pCO_2 or at constant bicarbonate concentration affect myocardial performance to the same extent. This was reflected in the fact that the slopes of the plots of pH versus the amount of shortening or pH versus max dl/dt were not significantly different either with respiratory or metabolic acid–base alterations.

One of the most interesting findings of these experiments, however, was the quantitative relationship between pH and contractility either with metabolic or respiratory alterations. Contractility changed less for a given change in pH to the alkalotic side than with alterations obtained to acidotic side. However, when the mechanical pa meters used to assess contractility were plotted a function of hydrogen ion activity, a linear co lation was obtained (Figs. 4 and 5) with either ' piratory' or 'metabolic' changes. In view of th findings, it appears that if the definition of alkal or acidosis were a change in $^aH^+$, heart contract would not be affected to a different extent by dosis or alkalosis, at least in the range of $^aH^+$ in study. The explanation for these observations be due to the fact that pH has a logarithmic lationship with hydrogen ion activity. Figu

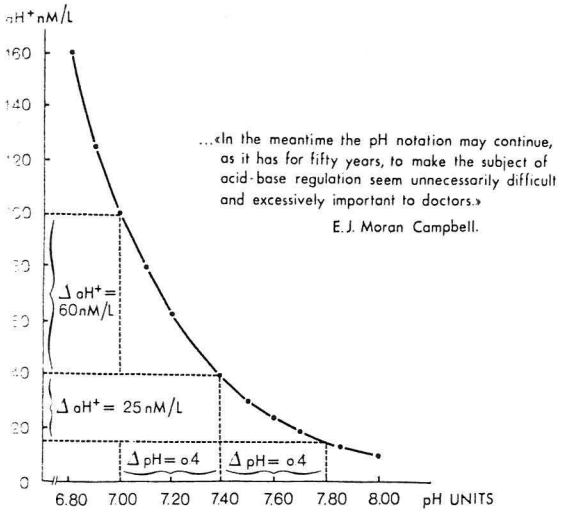

Fig. 6. This figure shows how a change in pH from 7.4 to 7.8 will decrease $^aH^+$ by approximately 25 nM/l, whereas the same change in pH to the acidotic side will produce an increase of 60 nM/l. The logarithmic relationship between pH and $^aH^+$ perhaps can explain the nonlinear relationship between contractility and pH in the rat heart.

illustrates how a given change in pH to the alkalotic side is associated with a smaller change in hydrogen ion activity than the same change in pH to the acidotic side does. The sentence of E. J. Moran Campbell quoted in the same figure is relevant to our findings [11]. In connection with this controversial aspect, a healthy skepticism has developed towards the pH notation in accordance with the Huckabee [16] suggestion that "nature cannot recognize or extract a logarithm". At least the mechanical performance of the perfused rat heart responds in a linear fashion to hydrogen ion activity and not to the minus log of $^aH^+$.

The present experiments do not establish the mechanism by which $^aH^+$ affects myocardial contractility in the rat heart. Several possibilities should be discussed in connection with our findings. Kata and Hecht [17] have suggested an interesting theory based on a possible competition between Ca^{++} and $^aH^+$. They stated that the level of contractility in the myocardium will be determined by the number of troponin binding sites which are bound to calcium, if the quantities of calcium ion normally released are insufficient to provide enough calcium for each troponin molecule. In the acidotic heart, however, the increase in hydrogen ion activity would displace the troponin-bound calcium and thereby decrease the number of active interactions. The increased (H^+) would also increase the affinity of the sarcoplasmic reticulum for calcium and decrease the release of bound calcium [23]. An increase in hydrogen ion activity could affect the contractile proteins directly or through their sensitivity to Ca^{++} [3]. In connection with this hypothesis the finding that in low sodium solutions, an increase in pCO_2 was followed by a decrease in contractility that was smaller than the one observed when the muscle was immersed in a solution with normal sodium concentration [12]. Low sodium medium enhances calcium uptake [19]. In this way, low sodium or high calcium concentrations could share a common mechanism.

The results presented here are not in agreement with those reported in some previous investigations (4–8, 11, 12, 20, 26) in which we are unable to show significant changes in contractility with pH changes at constant pCO_2. Some of these discrepancies may be due to species differences. Previous work by Opie [24] in the rat heart supports the fact that metabolic acidosis depressed contractility. In experiments performed by us in cat papillary muscle or the dog heart lung prepration [4–6, 20] only changes in pH brought by pCO_2 are able to affect contractility due perhaps to the fact that CO permeates the cell rapidly and could change intracellular pH rapidly. The greater penetrating power of CO_2 for the cell membrane was suggested by Gremels and Sarling in 1923 [11] and Hartree and Hill [13] have concluded that CO_2 penetrates the cell walls of skeletal muscle causing a lowering of pH more quickly than other acids do. The rat heart muscle is very resistant to digitalis [1, 9. 10], has negative staircase [15] and can be tetanized more easily than other species [14]. Perhaps bicarbonate is able to leak into the cell of the rat heart muscle and change intracellular pH to the same extent as pCO_2 and this could be another physiological difference of the rat heart muscle. In addition we cannot discard completely the possibility of other factors such as heart rate, temperature, or buffer capacities playing additional roles in exploring the differences between the rat's response as opposed to other species.

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