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Intracellular Acid-Base Regulation. I. The Response of Muscle Cells to Changes in CO_2 Tension or Extracellular Bicarbonate Concentration *

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The relationship between the acidity of cells and that of the extracellular fluid is not well defined. Except for the special case of the red blood cell, there is little sytematic information about the pH of normal cells exposed to varying external acid-base conditions. There is evidence that changes in CO_2 tension readily influence internal acidity, whereas, with the exception of red cells, it has been claimed that changes in extracellular bicarbonate concentration have relatively little effect (2–10). The current general opinion is, therefore, that most cell membranes, although freely permeable to carbon dioxide, are much less permeable to bicarbonate (8, 11–13). On the other hand, it is known that exogenous loads of fixed acid or alkali seem to be distributed in intracellular as well as extracellular buffers (14–17). Although this implies that acute changes in extracellular bicarbonate induce shifts of hydrogen, hydroxyl, or bicarbonate ions between cells and extracellular fluid, the resultant changes in cellular pH have never been clearly described.

Such information would seem to be essential for a clear understanding of the physiology of acid-base disorders. The limiting problem has been that of measuring cell pH. The theoretical and practical difficulties in this field have been fully discussed by Caldwell (11). We have recently modified the DMO [1] method of Waddell and Butler (7) by using 2-C^{14}-labeled DMO, and have demonstrated that it can be satisfactorily used to estimate mean cell pH in the intact rat diaphragm preparation (18). Certain theoretical problems relating to the use of DMO are considered in the Appendix of the present paper, and it is shown that despite possible internal heterogeneity of cells, a weak acid such as DMO can still yield physiologically useful information about cellular acidity.

The purpose of the present work was to employ the C^{14}-DMO method in a systematic examination of the behavior of rat skeletal muscle exposed *in vitro* to different steady conditions of external acidity. Changes in cell electrolyte and water content were also studied. In one series of experiments external $[HCO_3]$ was varied while CO_2 tension was held constant; in another group of experiments $[HCO_3]$ was fixed and CO_2 tension was varied. In this manner it was possible to study the separate effects of respiratory and metabolic acid-base disturbances on cell pH. We have found, contrary to prevailing opinion, that the acidity of muscle cells is readily influenced by external $[HCO_3]$ as well as by CO_2 tension. Striking differences were noted between the effects of external acidosis and alkalosis on cell pH.

Methods

Intact diaphragms from 60- to 90-g Sprague-Dawley rats were incubated in 1.5 L of modified Krebs-Ringer bicarbonate solution as previously described (18, 19). In each experiment the diaphragms were equilibrated at 37° C for 4 to 6 hours under constant external conditions to assure attainment of a steady state.

[1] 5,5-Dimethyl-2,4-oxazolidinedione.

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In one group of experiments, CO_2 tension was held constant at 34 to 41 mm Hg, and the bicarbonate concentration was set at different levels between 2 and 80 mEq per L to achieve the desired degree of external acidosis or alkalosis. Osmolality was kept constant by reciprocal changes in chloride. pH and CO_2 content of the medium, checked at appropriate intervals during each experiment, were steady; the pH and calculated P_{CO_2} never varied by more than 0.03 U and 3 mm Hg, respectively.

In a second group of experiments, different CO_2 tensions were produced in the medium by varying the percentage of CO_2 in the gas mixture between 1.5 and 30%, while keeping bicarbonate concentration constant at 22 to 23 mEq per L. In any single experiment P_{CO_2} did not change by more than 2 mm Hg, and bath pH did not vary by more than 0.03 pH U.

At the beginning of the final hour of incubation, inulin (8 g per L) and C^{14}-DMO [2] (50 μc per L) were added to the bath. Except as otherwise noted, diaphragms were removed for analysis after 4 or 6 hours of incubation. Determinations of total water, radioactivity, inulin, potassium, sodium, and chloride were carried out as previously described, and intracellular pH was calculated as before (18), with the inulin space as a measure of the extracellular fluid. Bath pH was measured with a Radiometer pH meter at 37° C; CO_2 content of the medium was determined manometrically and CO_2 tension calculated from pH and CO_2 content. All analyses of tissues were carried out on pools of three diaphragms.

Effect of carrier DMO. It has been shown that tracer quantities of C^{14}-DMO have the same distribution in skeletal muscle under normal conditions as does carrier DMO (18). To determine whether this is also true under widely varying acid-base conditions, three additional experiments were carried out at bath pH values of 6.76, 6.96, and 8.02, in which intracellular pH was calculated from the distribution of C^{14}-tracer DMO before and after the addition of 300 mg per L of chemical DMO. In each experiment there was no significant difference between the mean of four values of intracellular pH measured before, and four values measured after, the addition of the extra DMO.

Theoretical problems in the use of DMO posed by the possible heterogeneity of cells. Waddell and Butler (7) and Irvine, Saunders, Milne, and Crawford (20) have dealt adequately with the general theory of weak acids as applied to the use of DMO for the measurement of cell pH. Neither group, however, has considered in any detail the theoretical problem arising from the probably unequal distribution of hydrogen ion concentration within various subcellular structures. Caldwell (11) has shown that in such a heterogeneous system the apparent mean hydrogen ion concentration calculated from the distribution of a weak acid is not equal to the true mean hydrogen ion concentration of the system. He demonstrates that weak acids can be employed to calculate the true mean hydroxyl ion concentration of heterogeneous systems, but weak bases must be used to determine the mean hydrogen ion concentration.

This criticism of weak acid indicators does not, however, vitiate the usefulness of the DMO method. In the Appendix of this paper, it is demonstrated that $\overline{pH}_{HA} = pK_w - \overline{pOH}$ (Appendix, Equation 4), where $\overline{pH}_{HA}$ is the apparent mean pH of a heterogeneous system derived from the distribution of HDMO or any weak acid, $\overline{pOH}$ is the true mean pOH of the system, and pK_w has its usual meaning. It is apparent, therefore, that the "cell pH" value obtained by the DMO method bears a constant relationship to the true mean pOH of the cell. The "pH" determined by DMO thus provides a meaningful measure of the acid-base condition of the cell as a whole, even though it does not give a precise indication of either the true mean pH ($\overline{pH}$) or the pH at any specific site in the cell.

If the cell is indeed heterogeneous with respect to its internal pH, not only will methods based on weak acid indicators be affected, but also those employing microelectrodes. The latter can measure hydrogen ion activity only in the microsegment of the cell with which the hydrogen-permeable portion of the electrode has direct contact; they cannot measure any collective property of the intact cell. Such considerations serve to emphasize that no available method for cell pH is without theoretical as well as practical problems (11). The DMO technique, however, probably provides a reasonably satisfactory approximation of the over-all acid-base state of the cell.

Results

The demonstration of a steady state. Under normal conditions, the $[H^+]$ concentration and electrolyte content of the rat diaphragm preparation used in these studies remain steady for at least 8 hours. To determine whether a comparably steady state exists when diaphragms are exposed to different acid-base conditions, we carried out several preliminary experiments in which muscle pH was determined after varying periods of exposure to an abnormal external bicarbonate concentration or P_{CO_2}. The results of four such experiments are shown in Figure 1, which illustrates cellular $[H^+]$ plotted against duration of incubation.

The Figure shows that muscle $[H^+]$ changes relatively little after the initial hour of incubation and is essentially constant during the final 4 hours. No substantial number of observations was made before 1 hour, but in a few experiments we observed that a virtually steady state had been established by 30 or 45 minutes. In all of the sub-

[2] 2-C^{14}-DMO, 3.8 to 5.0 mc per mmole, obtained from New England Nuclear Corp., Boston, Mass.

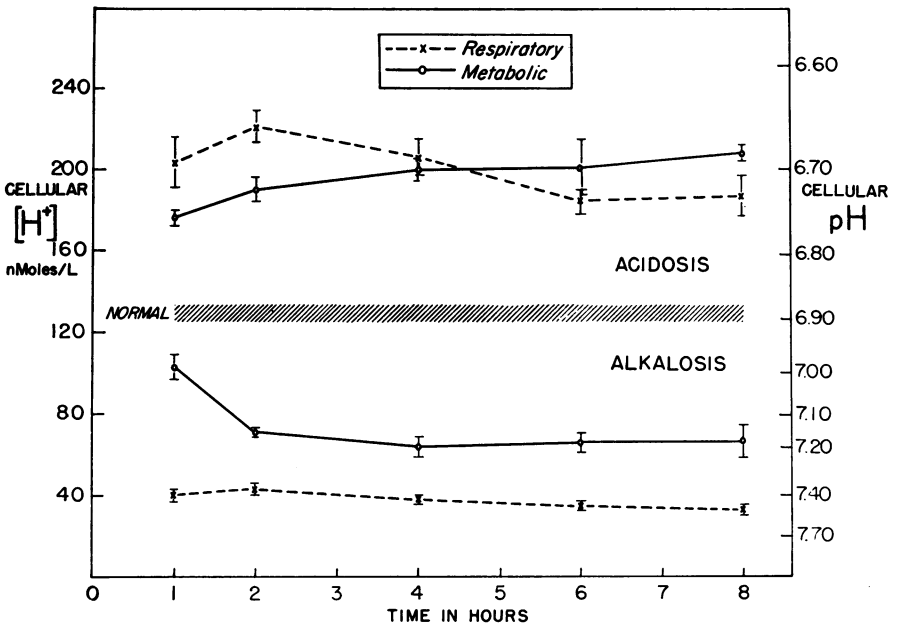

FIG. 1. DEMONSTRATION OF AN APPROXIMATELY STEADY STATE OF pH IN MUSCLE. Changes in cellular acidity are plotted against time of incubation for four separate experiments at different external acid-base conditions. Each point represents the mean ± standard error of the mean of at least four analyses. The shaded horizontal line indicates the normal level of muscle acidity.

sequent experiments described below, half the diaphragms were removed after 4 hours of incubation and half after 6 hours. There were no consistent or statistically significant differences [3] between the data obtained under given conditions at these two time intervals, and therefore results were pooled. All subsequent data are thus the means of observations made at 4 and 6 hours during an essentially steady state.

Effect of external $[HCO_3^-]$ *on intracellular acidity.* Tables I and II show the effects of lowering and raising extracellular bicarbonate, re-

[3] Differences described as "significant" have a p value of < 0.01 by the Student t test. When the difference between means is described as "not significant," the p value is > 0.05.

TABLE I

Effects of progressive extracellular metabolic acidosis on muscle composition †*

No. of analyses	Medium			Muscle						
	$[HCO_3^-]$	$[H^+]$	pH	Total H_2O	ECW	$[H^+]$	pH	$[K^+]$		$[Na^+]$
	mEq/L	*nmoles/L*		%	%	*nmoles/L ICW*		*mEq/100 g DW*	*mEq/L ICW*	*mEq/L ICW*
89	22.8	40.7	7.39	76.6 ± 0.6	23.4 ± 2.6	129 ± 39	6.89	34.0 ± 0.9	150 ± 5	8.0 ± 4.4
8	13.5	70.8	7.15	77.1 ± 0.7‡	22.4 ± 1.9‡	118 ± 12	6.93			12.7 ± 5.2§
8	10.7	91.2	7.04	75.9 ± 0.4	22.3 ± 1.2	134 ± 13	6.87	31.4 ± 1.3	141 ± 6	16.1 ± 6.7
8	7.69	106	6.98	76.8 ± 0.7	19.8 ± 1.0	134 ± 9	6.87	32.2 ± 1.4	133 ± 8	29.4 ± 10.5
8	7.71	110	6.96	76.3 ± 0.2	20.5 ± 1.3‡	139 ± 6	6.86	29.1 ± 1.6	124 ± 8	29.0 ± 5.3
8	8.18	120	6.92	76.4 ± 0.5	23.1 ± 2.9	137 ± 10	6.86	32.9 ± 0.9	145 ± 4	14.6 ± 6.3
8	6.08	137	6.86	76.2 ± 0.3	20.2 ± 1.2	159 ± 7	6.80	31.1 ± 1.4	132 ± 5	21.7 ± 5.8
8	5.80	146	6.84	76.5 ± 0.6	20.4 ± 2.2	162 ± 11	6.79	27.6 ± 1.1	116 ± 8	61.5 ± 15.6
8	5.66	145	6.84	76.7 ± 0.5	21.9 ± 2.4	151 ± 22	6.82	30.6 ± 2.3	128 ± 11	18.2 ± 4.2‖
8	4.86	175	6.76	75.3 ± 0.3	21.4 ± 1.3	173 ± 5	6.76	30.1 ± 1.2	138 ± 5	23.1 ± 5.6
16	4.82	200	6.70	77.0¶	23.0¶	203 ± 25	6.69	31.6 ± 2.0	137 ± 9	
8	4.27	209	6.68	76.0 ± 0.6‡	20.6 ± 1.1‡	224 ± 16	6.65	32.2 ± 2.3‡	139 ± 9‡	25.5 ± 4.1‡

* All experiments carried out at a constant P_{CO_2} of 34 to 41 mm Hg.
† ECW = extracellular water, ICW = intracellular water, DW = dry weight, and nmoles = nanomoles (10^{-9} moles). In Tables I to IV all data on muscle composition are given as means ± standard deviation. The number of determinations of muscle $[H^+]$ is the same as the number of analyses; the number of determinations of other muscle constituents is the same except where otherwise noted.
‡ Mean of seven determinations.
§ Mean of eight determinations.
‖ Mean of six determinations.
¶ Assumed space.

TABLE II

Effects of progressive extracellular metabolic alkalosis on muscle composition †*

No. of analyses	Medium			Muscle						
	[HCO_3^-]	[H^+]	pH	Total H_2O	ECW	[H^+]	pH	[K^+]		[Na^+]
	mEq/L	*nmoles/L*		%	%	*nmoles/L ICW*		*mEq/100 g DW*	*mEq/L ICW*	*mEq/L ICW*
7	29.8	30.0	7.52	78.0 ± 1.3‡	24.6 ± 2.4‡	115 ± 24	6.94	39.0 ± 3.1‡	161 ± 17‡	8.3 ± 8.8
8	29.5	27.7	7.56	76.8 ± 0.5	20.6 ± 1.9§	75.5 ± 12	7.12	34.8 ± 1.1	144 ± 5	18.6 ± 5.8
8	33.9	26.0	7.58	78.0 ± 1.0	25.2 ± 2.8§	116 ± 36	6.93	36.8 ± 1.1	154 ± 11	15.3 ± 8.6
8	34.8	25.6	7.59	76.5 ± 0.4	21.5‖	98.4 ± 19.7	7.01	37.7 ± 1.9	161 ± 9	13.3 ± 6.4
8	45.4	20.4	7.69	77.0 ± 0.7	17.6 ± 1.6	67.4 ± 13.5	7.17	36.8 ± 2.0	143 ± 4	24.9 ± 7.1
8	45.3	19.8	7.70	76.5 ± 0.4	18.0 ± 3.2	68.7 ± 13.9	7.16	35.4 ± 1.2	142 ± 8	35.0 ± 11.0
8	64.4	13.3	7.88	78.0 ± 0.6§	21.0 ± 1.7§	76.2 ± 17.5	7.12	35.3 ± 0.7	144 ± 6	
7	67.3	12.9	7.89	77.7 ± 0.6‡	23.2 ± 1.0	73.9 ± 11.6	7.13	33.7 ± 2.8	138 ± 10	25.3 ± 7.7¶

* All experiments carried out at a constant P_{CO_2} of 34 to 41 mm Hg.
† See footnote to Table I for abbreviations.
‡ Mean of eight determinations.
§ Mean of seven determinations.
‖ Assumed space.
¶ Mean of five determinations.

spectively, while P_{CO_2} is held constant at an approximately normal level. The external concentrations of bicarbonate and hydrogen ion and the pH in the medium are given in the second, third, and fourth columns of each Table, and the hydrogen ion concentration and pH of the muscle are listed in the seventh and eighth columns. The results of these experiments are also given graphically in Figure 2, which shows the relationship between the external and internal acidity of the muscle over the entire range of external [HCO_3^-] chosen for study. With the exception of the nor-

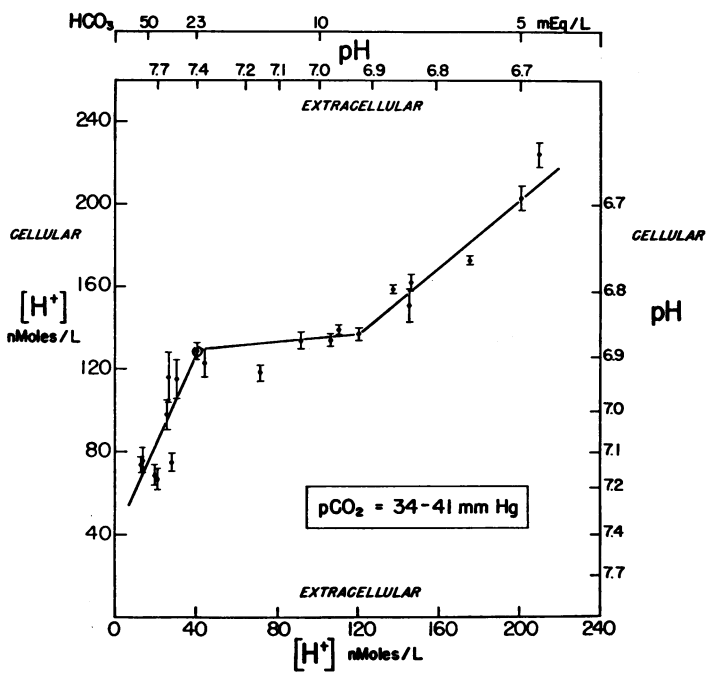

FIG. 2. THE RELATIONSHIP BETWEEN EXTRACELLULAR AND INTRACELLULAR ACIDITY AS THE FORMER IS CHANGED BY VARYING THE EXTERNAL [HCO_3^-]. P_{CO_2} was held constant in each experiment, at levels between 34 and 41 mm Hg. The normal value at an external [H^+] of 40 nmoles per L (pH 7.4) is shown as an encircled point and represents the mean ± standard error of the mean of 89 analyses. All other points represent the mean ± standard error of the mean of seven to 16 analyses. The data are in Tables I and II. The curve was drawn through the points by inspection.

TABLE III

*Effects of progressive respiratory acidosis on muscle composition**

No. of analyses	Medium			Muscle						
	P_{CO_2}	[H+]	pH	Total H_2O	ECW	[H+]	pH	[K+]		[Na+]
	mm Hg	*nmoles/L*		%	%	*nmoles/L ICW*		*mEq/100 g DW*	*mEq/L ICW*	*mEq/L ICW*
89	39	40.7	7.39	76.6 ± 0.6	23.4 ± 2.6	129 ± 39	6.89	34.0 ± 0.9	150 ± 5	8.0 ± 4.4
7	47	50.1	7.30	76.5 ± 0.8†	23.8 ± 1.0†	128 ± 16	6.89	33.8 ± 1.9†	150 ± 9†	16.6 ± 20.2‡
8	49	52.5	7.28	76.2 ± 1.0§	22.6 ± 1.3§	127 ± 16	6.90	32.7 ± 1.3§	145 ± 6§	14.2 ± 5.5§
8	65	69.2	7.16	76.8 ± 1.2	21.0 ± 2.1	134 ± 15	6.87	33.4 ± 1.6†	141 ± 3	13.3 ± 4.7‡
8	68	72.4	7.14	77.0 ± 0.4	20.7 ± 1.6	121 ± 7	6.92	33.5 ± 1.1	137 ± 6	17.5 ± 5.3
8	70	78.2	7.11	77.1 ± 0.4	23.8 ± 2.1	122 ± 6	6.91	33.3 ± 1.5	144 ± 8	16.6 ± 7.6
8	83	91.2	7.04	76.7 ± 0.5	23.4 ± 2.2	149 ± 15	6.83	32.8 ± 1.4§	144 ± 6§	5.2 ± 5.5‖
8	88	95.5	7.02	76.5 ± 0.9	25.0 ± 2.4	149 ± 14	6.83	31.5 ± 1.3§	143 ± 4§	11.8 ± 9.7
8	106	112	6.95	76.4 ± 0.4	22.5 ± 2.3	170 ± 11	6.77	32.7 ± 2.0	144 ± 8	12.3 ± 7.3
8	109	118	6.93	76.3 ± 0.8§	23.7 ± 2.6§	182 ± 14	6.74	30.3 ± 1.8§	136 ± 9§	21.9 ± 6.8§
8	121	134	6.87	77.2 ± 0.9	23.0 ± 3.0	179 ± 10	6.75	31.8 ± 2.9	134 ± 14	26.4 ± 12.4
8	122	135	6.87	76.7 ± 0.5	20.2 ± 2.7	188 ± 16	6.72	32.1 ± 1.6	133 ± 8	29.3 ± 12.5
9	136	148	6.83	76.5¶	23.5¶	197 ± 20	6.71			
8	147	158	6.80	77.4 ± 0.9	24.3 ± 1.9	195 ± 21	6.71	31.4 ± 1.9‡	136 ± 10‡	29.0 ± 7.9‡
8	201	209	6.68	74.9 ± 0.8	22.2 ± 3.3	240 ± 8	6.62	30.9 ± 1.4	148 ± 10	15.3 ± 4.3‖

* All experiments carried out at a constant extracellular $[HCO_3^-]$ of 22 to 23 mEq per L.
† Mean of eight determinations.
‡ Mean of six determinations.
§ Mean of seven determinations.
‖ Mean of five determinations.
¶ Assumed space.

mal value, each datum plotted is the mean ± standard error of the mean of seven to 16 observations. The normal value, at an external [H+] of 40 nmoles per L (pH 7.4), is the mean of 89 analyses and is indicated by the encircled point.

It is apparent from Table I and Figure 2 that intracellular [H+] was not affected by a considerable initial increase in the acidity of the medium. External [H+] could be increased approximately threefold, from 40 to 120 nmoles per L (pH 7.39 to 6.92), without significant increase in internal [H+]. When external [H+] was increased beyond this point, however, there was a definite and proportional increase in cellular acidity.

In contrast to the effect of external acidosis, any degree of alkalinization of the medium caused a proportional alkalinization of intracellular fluid. As shown by the data in Table II and illustrated in Figure 2, there was a striking and direct relationship between internal and external [H+] whenever the latter was reduced below normal.

Effect of CO_2 tension on intracellular acidity. Tables III and IV show the data obtained when the diaphragms were incubated at different CO_2 tensions, while the external bicarbonate concentration was held constant betwen 22 and 23 mEq per L. Figure 3 shows the relationship between external and internal acidity in these experiments, plotted in the same manner as Figure 2. Except for the normal value, each point in Figure 3 represents the mean ± standard error of the mean of seven to 12 observations.

As clearly shown in Table III and in Figure 3, the steady-state acidity of the muscles was found to be relatively unaffected by initial increases in P_{CO_2}. At a P_{CO_2} of 68 to 70 mm Hg, with an increase in external [H+] of nearly twofold ([H+] 72 to 78 nmoles per L, pH 7.11 to 7.14), cellular acidity

TABLE IV

*Effects of progressive respiratory alkalosis on muscle composition**

No. of analyses	Medium			Muscle						
	P_{CO_2}	[H+]	pH	Total H_2O	ECW	[H+]	pH	[K+]		[Na+]
	mm Hg	*nmoles/L*		%	%	*nmoles/L ICW*		*mEq/100 g DW*	*mEq/L ICW*	*mEq/L ICW*
8	28	30.9	7.51	77.9 ± 0.5†	23.3 ± 1.4†	88.0 ± 21.1	7.06	34.9 ± 0.9†	141 ± 6†	9.9 ± 9.1
8	28	31.1	7.51	77.7 ± 0.7	22.2 ± 2.1	95.4 ± 12.6	7.02	36.4 ± 2.0	146 ± 8	6.4 ± 4.6
8	19	21.6	7.67	77.8 ± 0.6	23.6 ± 1.4	68.2 ± 6.0	7.17	33.4 ± 1.8	137 ± 7	15.2 ± 6.2
8	18	20.7	7.68	78.0 ± 1.1	18.8 ± 2.7	63.9 ± 8.2	7.19	35.2 ± 1.1‡	131 ± 3‡	27.0 ± 5.9
8	10	11.2	7.95	77.4 ± 0.6	21.4 ± 2.2	43.0 ± 8.1	7.37	34.4 ± 2.2‡	141 ± 11‡	23.7 ± 11.8‡
12	9.2	10.7	7.97	76.5§	22.0§	35.7 ± 5.6	7.45			

* All experiments carried out at a constant extracellular $[HCO_3^-]$ of 22 to 23 mEq per L.
† Mean of seven determinations.
‡ Mean of six determinations.
§ Assumed space.

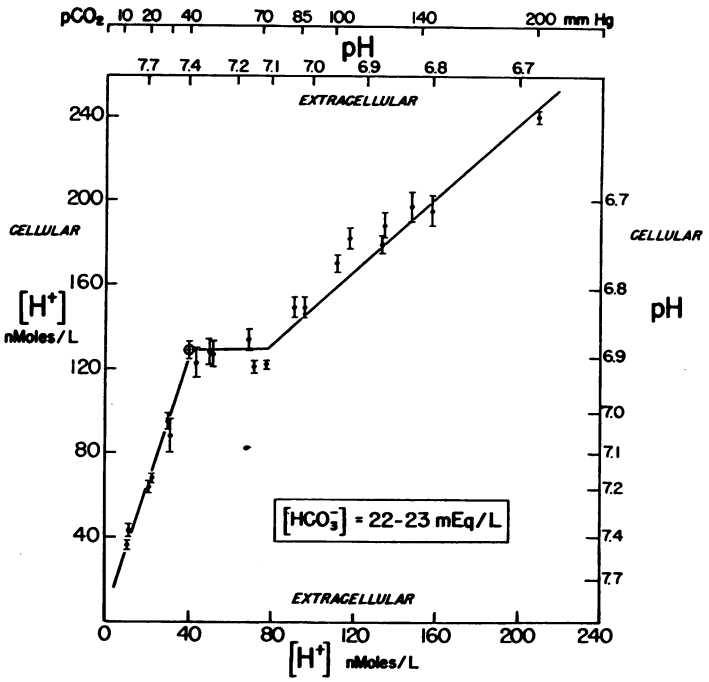

FIG. 3. THE RELATIONSHIP BETWEEN EXTRACELLULAR AND INTRACELLULAR ACIDITY AS THE P_{CO_2} OF THE MEDIUM IS VARIED. External $[HCO_3^-]$ was held constant in each experiment at levels of 22 to 23 mEq per L. Except for the encircled normal value each point represents the mean ± standard error of the mean of seven to 12 analyses. The data are in Tables III and IV. The curve was drawn through the points by inspection.

was still unchanged. Beyond this point, however, further increases in CO_2 tension caused proportional increases in the muscle acidity.

When, on the other hand, CO_2 tension was lowered (Table IV), there was a definite and proportional alkalinization of the cell. The striking difference in cellular reaction to raising and lowering P_{CO_2} is best seen by inspection of Figure 3. Although the muscle $[H^+]$ was unaffected when P_{CO_2} and external $[H^+]$ nearly doubled, a reduction in P_{CO_2} of only 10 mm Hg and a fall in external $[H^+]$ of only 10 nmoles per L sufficed to produce a significant reduction in muscle acidity.

Thus, the diaphragm muscle was found to be resistant to acidosis up to a point, but quite sensitive to alkalosis, regardless of whether these acid-base disturbances were produced by changing the P_{CO_2} or the concentration of $[HCO_3^-]$ in the medium. Beyond a certain degree of external acidosis, changes in either P_{CO_2} or $[HCO_3^-]$ were capable of producing a proportional degree of intracellular acidosis.

A comparison of the effects of P_{CO_2} *and* $[HCO_3^-]$. A more direct comparison of the effects of P_{CO_2} and $[HCO_3^-]$ is facilitated by Figure 4, which shows the two curves superimposed. The curve referred to as "respiratory" describes the effect of P_{CO_2}; that referred to as "metabolic" describes the effect of external $[HCO_3^-]$. The lines were calculated from the data by the method of least squares, and the derived equations are also indicated.

Obviously the general shape of the two curves is similar, but the flat portion of the respiratory curve is shorter, and the cellular $[H^+]$ values in the extreme acidotic range are higher. At an external $[H^+]$ of 120 nmoles per L produced by lowering external $[HCO_3^-]$, internal $[H^+]$ is 137 ± 10 nmoles per L, which is not significantly different from the normal. At essentially the same external $[H^+]$ produced by raising P_{CO_2}, the internal $[H^+]$ is 182 ± 14 nmoles per L, which is significantly higher than either the normal diaphragms or those exposed to the same degree of

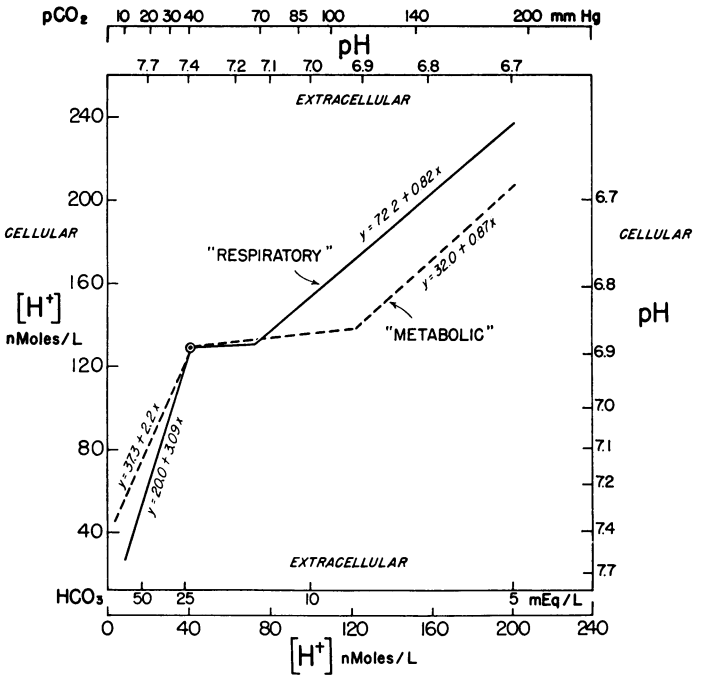

FIG. 4. A COMPARISON OF THE EFFECTS OF CHANGING P_{CO_2} ("RESPIRATORY") AND EXTERNAL $[HCO_3^-]$ ("METABOLIC") ON CELLULAR ACIDITY. The P_{CO_2} scale at the top applies only to the respiratory curve, and the lower $[HCO_3^-]$ scale only to the metabolic curve. The slopes were calculated by the method of least squares from the data shown in Figures 2 and 3.

metabolic acidosis. Furthermore, at all degrees of external acidosis greater than this the muscles exposed to respiratory acidosis are significantly more acidic than those exposed to metabolic acidosis. Figure 4 shows that the slopes of this portion of both curves are virtually the same. In the alkaline region, the slopes of both curves are significantly steeper, indicating that a given absolute change in external $[H^+]$ in the alkaline range has a greater effect on cell $[H^+]$ than does the same change in the very acid range. Although the Figure shows the respiratory slope in the alkaline region to be slightly more steep than the metabolic slope, this difference is not significant.

Effects of external acidity on muscle water and electrolyte content. Columns 5, 6, and 9 to 11 in Tables I to IV present the data on water and electrolyte content. In Table V these data are summarized as mean values and grouped according to the nature of the acid-base disturbance.

TABLE V

*Effect of acidosis and alkalosis on muscle water and electrolyte content**

Condition†	Total water	Cell water	K		Na	Cl
	g/100 g DW	*g/100 g DW*	*mEq/100 g DW*	*mEq/L ICW*	*mEq/L ICW*	*mEq/L ICW*
Normal	327 ± 12 (89)	227 ± 5 (89)	34.0 ± 0.9 (89)	150 ± 5 (89)	8.0 ± 4.4 (89)	
Acidosis	326 ± 10 (180)	231 ± 10 (179)	32.1 ± 1.6 (169)	138 ± 7 (161)	19.3 ± 7.6 (160)	
Respiratory	327 ± 11 (102)	230 ± 10 (102)	32.4 ± 1.6 (98)	140 ± 7 (98)	17.8 ± 8.7 (92)	
Metabolic	323 ± 9 (78)	233 ± 9 (77)	31.7 ± 1.6 (71)	135 ± 7 (63)	21.3 ± 6.0 (68)	14.7 ± 5.7 (59)
Alkalosis	346 ± 8 (102)	250 ± 9 (92)	35.7 ± 1.7 (98)	145 ± 7 (97)	18.3 ± 7.4 (87)	
Respiratory	349 ± 5 (39)	251 ± 10 (39)	34.9 ± 1.6 (35)	140 ± 7 (35)	15.6 ± 7.2 (35)	
Metabolic	344 ± 13 (63)	248 ± 8 (53)	36.2 ± 1.8 (63)	149 ± 7 (62)	20.0 ± 7.5 (52)	9.8 ± 4.2 (36)

* Data are given as mean values ± standard deviation. Numbers in parentheses are the number of analyses.
† Increase or decrease of CO_2 tension in the medium is "respiratory," and changes in external $[HCO_3^-]$ are called "metabolic."

For this purpose, all experiments in which CO_2 tension was changed are called "respiratory acidosis" or "respiratory alkalosis," and the term "metabolic" is similarly used to describe those experiments in which external $[HCO_3^-]$ was changed. Table V also gives the derived figures for cell water, calculated from the data on total water and extracellular water shown in Tables I to IV.

Table V shows that total tissue water was significantly increased in both types of alkalosis but was unaffected by acidosis. There was no significant or consistent change in the inulin space with any type of acid-base disturbance, and therefore the increased total water in alkalosis was due to an expansion of the calculated cell water. This is shown in Table V by the almost identical rise in mean cell water and total water when expressed per unit dry solids.

Tissue potassium content, expressed per unit dry weight, was slightly but significantly increased in alkalosis as shown in Table V. It was also slightly but significantly reduced below normal in acidosis. Owing to the concomitant rise in cell water, the calculated intracellular concentration of potassium did not rise in alkalosis, but it did fall significantly in acidosis. In Figure 5, the potassium content of each group of diaphragms shown in Tables I to IV is plotted against the extracellular pH. There was no consistent difference in the effects of P_{CO_2} and $[HCO_3^-]$ at a given external pH. The rise in tissue potassium seemed to be greatest with mild degrees of extracellular alkalosis, and there was a suggestion that potassium content dropped off with further increase in alkalosis. The distribution of the points in the acidotic range suggested that loss of tissue potassium was roughly proportional to the severity of the extracellular acidosis over the entire range studied.

Intracellular sodium concentration was not different in acidosis and alkalosis but was significantly increased above normal in both types of disturbance.[4] In Figure 6, a relation between

[4] Since percentage changes in cell water were relatively small (Table V), it is obvious that the expres-

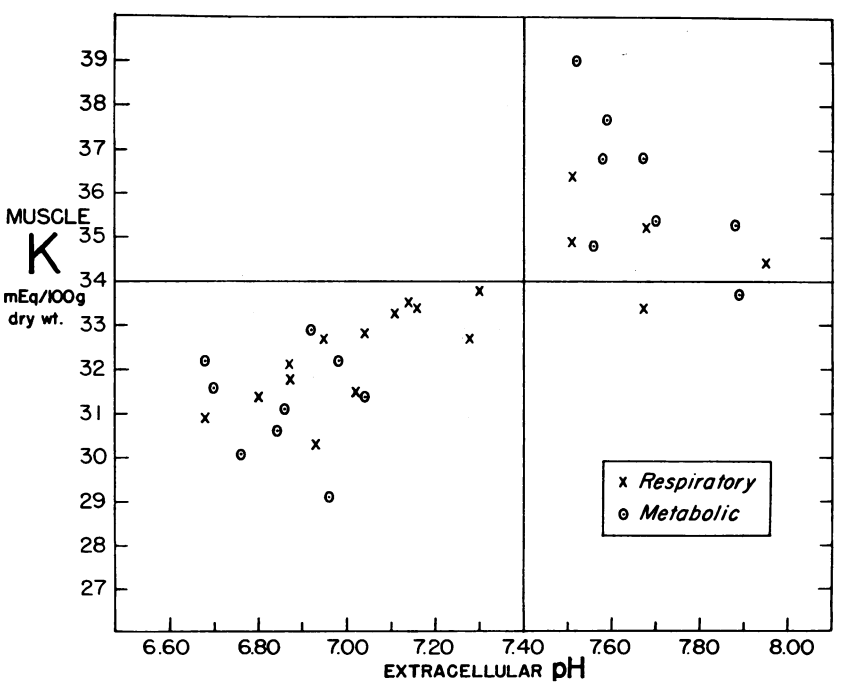

FIG. 5. THE RELATIONSHIP BETWEEN EXTRACELLULAR pH AND THE POTASSIUM CONTENT OF MUSCLE. Each point represents the mean of six to 16 analyses. Those labeled "metabolic" are found in Tables I and II, those labeled "respiratory" in Tables III and IV. The intersecting straight lines indicate the normal mean values for each parameter.

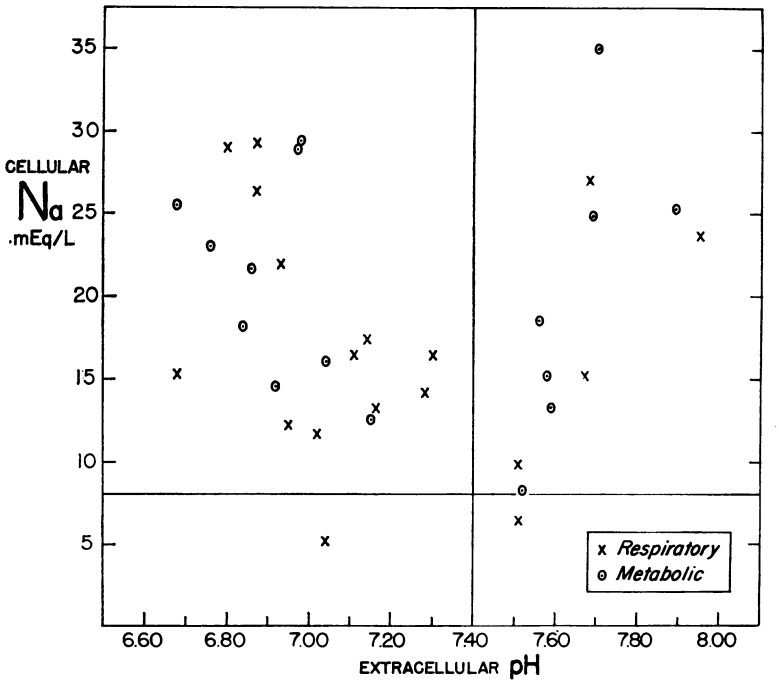

FIG. 6. THE RELATIONSHIP BETWEEN EXTRACELLULAR pH AND THE CONCENTRATION OF SODIUM IN CELLULAR WATER. Each point represents the mean of five to eight analyses. Those labeled "metabolic" are found in Tables I and II, those labeled "respiratory" in Tables III and IV. The intersecting straight lines indicate the normal values for each parameter.

the intracellular sodium concentration and the degree of extracellular acidosis or alkalosis is apparent. The lowest intracellular sodium concentration occurred under normal acid-base conditions; progressively severe acidosis or alkalosis, of either variety, appeared to cause increasing accumulation of intracellular sodium.

Table V also presents data on intracellular chloride, which were obtained only in the metabolic experiments. Unfortunately no normal control data were obtained in these experiments, but from data on chloride obtained in previous experiments (19) the values found in both acidosis and alkalosis seem very likely to represent a significant increase above normal. There was slightly but significantly more intracellular chloride in acidosis than in alkalosis; this difference is probably accounted for by differences in intracellular [HCO_3^-], since cellular sodium concentrations were the same.

sion of these data either in terms of sodium content per unit dry weight or as concentration per liter of cell water would give similar results. To facilitate direct comparison with intracellular [HCO_3^-], we chose to give [Na^+] and [Cl^-] data in terms of concentration.

Discussion

These observations demonstrate that changes in extracellular pH produced by variation in bicarbonate concentration affect the internal acidity of intact skeletal muscle almost as readily as do changes produced by variations in CO_2 tension. This conclusion is contrary to the prevailing opinion that muscle cells, although freely permeable to CO_2, are virtually indifferent to the extracellular [HCO_3] concentration (8, 11–13).

The current view rests primarily on the studies of Wallace and Hastings (3) and Wallace and Lowry (4), in which muscle pH was calculated from total tissue CO_2 content. In one series of experiments on anesthetized cats, muscle pH was found to be essentially unaffected by variations in serum pH from 7.00 to 7.55 produced by injections of $NaHCO_3$ or HCl (3). These observations, however, are not inconsistent with the data reported in the present work, for we have

shown that metabolic acidosis does not significantly increase internal acidity until the extracellular pH is well below 7.00, and that external metabolic alkalosis does not have a definite effect until the pH is above 7.55. Furthermore, in the alkalosis experiments of Wallace and Hastings, elevation of extracellular [HCO_3^-] was followed by a compensatory rise in Pco_2 to 50 to 60 mm Hg. As we show in the next paper (21), such levels of Pco_2 can moderate the intracellular effect of an elevated external [HCO_3^-] concentration.

In a separate series of *in vitro* experiments (4), Wallace and Lowry found that the internal pH of rat skeletal muscle was unaffected when the external pH was varied widely by changing the [HCO_3^-] concentration over the range of 0 to 70 mEq per L. However, these equilibrations were carried out anaerobically and, in one-half the experiments, at a temperature of 6° C. We have found that the normal relationship between internal acidity is apparently dependent upon the metabolic activity of the muscle cell and is drastically changed when temperature is lowered (22). Wallace and Lowry's results, therefore, do not represent the normal behavior of muscle and are not in conflict with our observations.

We are aware of only one other comparison of the effects of external Pco_2 and [HCO_3^-] on the internal pH of mammalian skeletal muscle. Using DMO in intact dogs, Waddell and Butler (7) recently reported that muscle cell pH was significantly reduced when plasma pH was lowered to 6.92 by an increased CO_2 tension (126 mm Hg) but was only slightly reduced (from 7.03 to 6.90) when plasma pH was lowered to 6.99 by intravenous infusion of HCl. In light of the data summarized in Figure 4 of the present work, this apparent lack of effect of HCl acidosis on muscle pH was very likely due simply to failure to lower external pH below the critical level of 6.9. It is interesting, however, that Waddell and Butler found that elevation of blood pH to 7.54 and 7.56, with infusion of $NaHCO_3$ or hyperventilation, respectively, had virtually the same very slight, alkalinizing effect on muscle pH. Our own data show that an external pH of about 7.55 seems to be the threshold level for intracellular alkalosis, and that there is no significant difference between the cellular alkalinizing effects of respiratory and metabolic changes.

Thus there is no evidence that mammalian skeletal muscle is normally unaffected by changes in external [HCO_3^-]. The present data demonstrate, to the contrary, that the relationship between internal and external acidity has similar general characteristics whether pH is changed with Pco_2 or HCO_3^-.

There are, however, significant quantitative differences between the effects of Pco_2 and HCO_3^- when the external medium is markedly acidified. As shown in Figure 4, at all external pH levels below about 7.1 a rise in Pco_2 has a greater acidifying effect on the cell than does a fall in [HCO_3^-]. It is apparent, therefore, that it is not the external [H^+] alone that determines internal [H^+], and the question must be raised as to the roles that Pco_2 and external [HCO_3^-] may of themselves play in regulating cell pH. The present experiments were not designed to study the interaction between these two physiologic parameters, nor were they intended to show how much variation in internal pH could occur at any given external pH. These matters are the subject of the following paper (21).

From recent observations of the effect of increasing Pco_2 on the interstitial fluid [HCO_3^-] in isolated rat diaphragm, Stegemann (23) has concluded that HCO_3^- ions generated intracellularly during the buffering of H_2CO_3 must be able to move readily out of cells and that muscle cell membranes must be readily permeable to bicarbonate. It should be appreciated, however, that the apparent ability of the HCO_3^- ion to effect changes in [H^+] and [HCO_3^-] on the opposite side of the muscle cell membrane need not be the direct result of movements of HCO_3^- ions. Neither Stegemann's experiments nor our own results with changing external [HCO_3^-] can distinguish between the possible flux of HCO_3^- or OH^- ions in one direction and the countermovement of H^+ ions in the other. Our own data, as noted below, do not even require the conclusion that there has been net transfer of acid or base across the cell membrane.

The distinctive shape of the two curves shown in Figure 4 has a superficial resemblance to the titration curve of a buffer, and it may reasonably be asked whether the plateau portion of these curves could not be explained simply by the titration of intracellular buffers with pK's in the re-

gion of 6.9. The metabolic curve is, however, not easily described in such terms because there is no information about how changes in external [HCO_3^-] affect the net flux of acid or base across the muscle cell membrane. As far as the present observations are concerned, the effects of external [HCO_3^-] on internal pH could be the result either of net movement of undetermined quantities of acid or base, or of changes in the metabolic production or consumption of acidic or basic compounds within the cell. The plateau could thus be explained either by the existence of a limited barrier to the net flux of H^+, OH^-, or HCO_3^- ions or by compensatory changes in the internal metabolism of acids or bases.

The respiratory curve, on the other hand, does in fact represent the CO_2 titration of diaphragm muscle, for CO_2 is known to freely diffuse across cell membranes. As such, this curve can be compared with "CO_2 dissociation curves" previously described for skeletal muscle by earlier investigators (24–26). Such curves, whether for frog muscle *in vitro* at 18 or 22° (24, 25) or for dog muscle *in vivo* at 38° (26), have been described as a single continuous function, quite different from the triphasic shape of the respiratory curve in Figure 3.[5] The discrepancy between our own results and the experiments on frog muscle is, very probably, explained by the relatively low temperatures at which the latter were conducted, for we have found that the CO_2 titration curve is temperature dependent (22). The dog muscle experiments cannot be explained by temperature differences, but close examination of the data from this work (26) suggests that the total CO_2 content of muscle may in fact have risen more rapidly between P_{CO_2} 40 and 70 mm Hg than at higher P_{CO_2} levels. If this interpretation is valid, then there would be relatively less change in muscle pH over the P_{CO_2} range in question, and the results of the dog experiments would be consistent with the present data.

[5] In most CO_2 dissociation curves, the total CO_2 content of the tissue, rather than cell [H^+], is plotted against P_{CO_2}. Our respiratory curve in Figure 3 is therefore not strictly comparable. However, it is easy to calculate tissue CO_2 content or [HCO_3^-] from cell [H^+] and P_{CO_2}, and it can readily be demonstrated that the triphasic plot of cell [H^+] versus P_{CO_2} gives rise to a comparably triphasic plot of cell [HCO_3^-] versus P_{CO_2}.

Although these experiments provide no clues to the mechanisms responsible for the shape of the curves describing the cellular response to changing P_{CO_2} or external [HCO_3^-], the physiological implications of these curves are clear. Resting skeletal muscle seems to be well adapted to resist acidosis, but is much more vulnerable to alkalosis. It is tempting to speculate that the position of the normal cell on these curves may reflect the development of cellular mechanisms for dealing with the constant threat of severe metabolic acidosis during exercise or anoxia. By contrast, reductions in [H^+] concentration are much less frequently encountered and are not usually so severe. Muscle cells would therefore have less need for resistance to excessive alkalinity and for this reason may not have developed appropriate mechanisms.

Much has been written about the response of sodium and potassium in mammalian skeletal muscle to changes in acid-base balance. Most of the data derive from *in vivo* experiments in which interpretation of the changes in muscle electrolyte is complicated by concomitant changes in the extracellular concentration of potassium. In the present work, extracellular sodium and potassium concentrations were kept constant and normal, and the effects of acidosis and alkalosis were therefore unmodified by other systemic factors.

The relationship between muscle potassium and extracellular pH (Figure 5) is consistent with the concept of a net H^+–K^+ exchange, but does not establish whether the net movement of potassium was directly coupled with H^+ or was accomplished by other means. Similar observations on the potassium content of rat diaphragm *in vitro* have been reported by Rogers (27). He further observes that with more severe degrees of alkalosis, muscle potassium tends to decrease again. In our experiments, as shown in Figure 5, there is a suggestion of a similar trend, the significance of which is uncertain.

The behavior of muscle sodium content was clearly different from that of potassium. In contrast to potassium, sodium appeared to accumulate in muscle in acidosis as well as alkalosis. These data cannot be compared with those of Rogers because his data on sodium content were not corrected for extracellular space (27). An explanation for the relationship shown in Figure 6 is not

apparent. Sodium transport in frog skin has been reported to fall progressively as external pH is reduced from 8.4 to 6.5 (28), but this could not explain the increased sodium content in alkalosis. In metabolic acidosis muscle chloride rose as much as did sodium (Table V), which would be consistent with a reduction in the active transport of sodium. In metabolic alkalosis, on the other hand, the rise of chloride was significantly less than that of sodium; presumably the discrepancy is accounted for by increased cellular [HCO_3^-]. Calculation of intracellular [HCO_3^-] from the pH and P_{CO_2} gives values that check very closely with the difference between intracellular sodium and chloride in acidosis as well as alkalosis.

Intracellular water increased only in alkalosis, despite equal increases in intracellular sodium in acidosis and alkalosis. This increase in cell hydration was very likely due to the increased potassium content in alkalosis. Loss of potassium in acidosis was osmotically balanced by a gain in sodium and chloride, and hence there was no significant change in tissue water.

Summary

C^{14}-labeled DMO (5,5-dimethyl-2,4-oxazolidinedione) was used to measure the steady-state intracellular pH in rat diaphragm muscle while the latter was exposed *in vitro* either to varying CO_2 tensions and a normal external bicarbonate concentration or to varying bicarbonate concentrations and a normal CO_2 tension. Changes in water and electrolyte content were also studied.

Under the conditions of these experiments the relationship between internal and external hydrogen ion concentration could be described by two complex curves which, although generally similar in shape, had slightly different loci in the region of extreme extracellular acidity.

Alkalinization of the extracellular medium, whether by reduction of P_{CO_2} or by increase of bicarbonate, caused a similar and proportionate degree of alkalinization of muscle cells, together with a rise in potassium, sodium, and water content. On the other hand, acidification of the medium had no discernible effect on cell pH until either the P_{CO_2} was raised above 70 mm Hg (pH 7.1) or the external bicarbonate concentration was reduced to less than 7 mEq per L (pH 6.9). Extracellular acidosis lowered the muscle potassium content, but muscle sodium was increased, and the water content of the cells was unchanged.

These data show that, contrary to prevailing opinion, the pH of muscle cells is readily influenced by the external bicarbonate concentration as well as by CO_2 tension. These studies also demonstrate that resting skeletal muscle resists external acidosis much more efficiently than it does external alkalosis.

Appendix

Limitations of the DMO method resulting from the heterogeneity of cells. When DMO is employed as the weak acid indicator for the determination of cell pH, analysis of extracellular fluid and tissue permits calculation of the mean concentration of the DMO anion ($\overline{A^-}$) in the tissues. Caldwell (11) has shown that if there are different hydrogen and hydroxyl ion activities* in subcellular compartments, the mean cellular ($\overline{DMO^-}$) will be given by the expression

$$(\overline{A^-}) = \frac{K_a}{K_w}(HA)\,(\overline{OH^-}), \qquad [1]$$

where HA is the concentration of undissociated acid (HDMO, in this case) throughout the system, ($\overline{OH^-}$) is the mean intracellular hydroxyl ion concentration, and K_a and K_w have their usual meaning.

It follows from Equation 1 that a method based on a weak acid indicator will theoretically permit accurate calculation of mean hydroxyl ion concentration. On the other hand, if the distribution of the anion of a weak acid is used to calculate the mean intracellular hydrogen ion concentration, one obtains a value different from that of the true mean concentration. The application of indicator methods depends on the mass action law, which when used to calculate the hydrogen ion concentration of a heterogeneous system, gives

$$(\overline{H^+}_{HA}) = \frac{K_a(HA)}{(\overline{A^-})} = \frac{K_a(HA)\Sigma V_n}{\Sigma A_n V_n}, \qquad [2]$$

where ($\overline{H^+}_{HA}$) = the apparent mean hydrogen ion concentration of the system, A_n = the concentration of the weak acid anion (DMO^-) in the n^{th} compartment, and V_n = the volume of the n^{th} compartment.

However, the true mean hydrogen ion concentration in the system is $(\overline{H^+}) = \Sigma H_n V_n / \Sigma V_n$, where H_n is the hydrogen ion concentration in the n^{th} compartment.

Now, since $H_n = (K_a)(HA)/A_n$, it follows that

$$(\overline{H^+}) = \frac{K_a(HA)\Sigma \frac{V_n}{A_n}}{\Sigma V_n}. \qquad [3]$$

It is therefore obvious from inspection of Equations 2 and 3 that $(\overline{H^+}) \neq (\overline{H^+}_{HA})$ and that the mean cell hydrogen ion activity will not be precisely determinable from the

* For the purposes of this discussion "activity" and "concentration" will be used synonymously.

DMO method, even assuming that this weak acid is ideal with respect to the relative permeabilities of the ionic and nonionic species.

To define more precisely what ($\overline{H^+}_{HA}$) does in fact measure, it is necessary only to combine Equations 1 and 2 to give

$$(\overline{H^+}_{HA}) = \frac{K_w}{(\overline{OH^-})}, \quad \text{or,} \quad \overline{pH}^+_{HA} = pK_w - \overline{pOH}^-. \quad [4]$$

Thus the apparent cellular hydrogen ion concentration, or the apparent pH, derived from the use of DMO or any weak acid indicator bears a constant relationship to the true mean hydroxyl ion concentration, or true mean pOH, and can therefore be used as an indirect but meaningful index of the acidity of the cell.

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