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Intracellular Acid-Base Regulation. II. The Interaction between CO_2 Tension and Extracellular Bicarbonate in the Determination of Muscle Cell pH *

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In the preceding paper (2) we showed that the acidity of resting skeletal muscle *in vitro* can be readily changed by variations in external $[HCO_3^-]$ as well as by variations in CO_2 tension. In those studies one factor was varied while the other was kept normal, and so we obtained no information about possible interactions between CO_2 tension and extracellular bicarbonate in the control of cell pH.

In acid-base disorders *in vivo*, primary disturbances in CO_2 tension or extracellular $[HCO_3^-]$ are usually modified by compensatory changes in the other quantity. Occasionally, a primary disturbance in one parameter is compounded by a superimposed disturbance in the other. In clinical acid-base disorders, as a consequence, cells are usually exposed to alterations in both CO_2 tension and extracellular $[HCO_3^-]$. It is therefore important to know to what extent each factor modifies the cellular effect of the other. At any given extracellular pH, how much can varying combinations of P_{CO_2} and extracellular $[HCO_3^-]$ change the intracellular pH?

To answer this question a series of further experiments has been carried out with the intact rat diaphragm preparation previously described (2–4). As before, C^{14}-labeled DMO [1] was used to measure cell pH in the steady state. In the present experiments, a large number of different combinations of P_{CO_2} and $[HCO_3^-]$ was established by setting one factor at a given elevated or reduced level and then systematically varying the other over a wide range.

The results show that cellular pH is a complex function of both P_{CO_2} and external $[HCO_3^-]$. It cannot be accurately predicted from the extracellular pH alone, for it depends upon the particular combination of CO_2 tension and extracellular bicarbonate that determines any given extracellular pH. The clinical and physiological implications of these findings are discussed.

Methods

All the experimental techniques and analytical methods used in this work were described, or referred to, in the preceding paper (2). Diaphragms were incubated at 37° C at a fixed external pH established by choosing the desired concentration of HCO_3^- in the medium and the percentage of CO_2 in the CO_2-O_2 gas mixture. Half the diaphragms were removed after 4 hours of incubation under given conditions and the remainder at 6 hours. In no instance was there a significant change [2] during the interval, and so observations at the two time intervals were always combined. In a few experiments, inulin space was not measured, and an assumed extracellular space (equal to that found under similar conditions in other experiments) was used in the calculation of cell pH. This was done only when the extracellular medium was very acidic, under which conditions relatively large variations in extracellular space have negligible effects on the calculated cell pH.

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[1] 5,5-Dimethyl-2,4-oxazolidinedione-2-C^{14}.

[2] Throughout this paper, differences between means that are described as "significant" have a p value (Student's *t* test) of < 0.01. When p is > 0.05, the difference is considered not significant.

TABLE I

*Effects of change in P_{CO_2} on muscle acidity when extracellular $[HCO_3^-]$ is held constant at 5.6 to 6.8 mEq per L**

No. of analyses	Medium				Muscle†	
	$[HCO_3^-]$	P_{CO_2}	$[H^+]$	pH	$[H^+]$	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
8	6.0	17	69	7.16	92 ± 7	7.03
8	6.1	18	69	7.16	82 ± 5	7.09
8	5.6	28	126	6.90	140 ± 9	6.85
8	6.5	29	105	6.98	124 ± 4	6.91
8	5.7	34	145	6.84	151 ± 22	6.82
8	5.8	36	146	6.84	162 ± 11	6.79
8	6.2	53	203	6.69	229 ± 29	6.64
8	6.8	119	417	6.38	456 ± 37	6.34

* nmoles = nanomoles (10^{-9} moles); ICW = intracellular water.
† In this and all subsequent Tables, data on muscle $[H^+]$ are given as mean ± standard deviation. The pH value shown is the negative log of the mean $[H^+]$.

Results

1) Effect of changes in CO_2 tension on muscle acidity at high or low external bicarbonate levels. In this group of experiments the external $[HCO_3^-]$ was fixed at one of three abnormal levels (5.6 to 6.8 mEq per L, 12.7 to 14.5 mEq per L, or 43 to 49 mEq per L), and the CO_2 tension was varied over a wide range to produce external pH's ranging from 6.38 to 8.22. The data are summarized in Tables I, II, and III; except for one experiment (last line, Table I) they are also depicted in Figure 1.

In the Figure, extracellular $[H^+]$ is plotted against intracellular $[H^+]$. Each point represents the data from one of the experiments in Tables I to III, given as the mean ± standard error of the mean of seven or eight muscle analyses. The experimental points are connected by dashed lines, drawn by inspection. The heavy solid line is the relationship for a "normal" extracellular HCO_3^- concentration of 22 to 23 mEq per L, as determined in the previous paper (2).

When external bicarbonate was very low (5.6 to 6.8 mEq per L), progressive increases in hydrogen ion concentration of the medium, produced by increasing P_{CO_2}, resulted in proportional increases in intracellular acidity over the entire range studied (Table I and Figure 1). As shown by the lower dotted line in Figure 1, there appeared to be direct linear relationship between cellular and extracellular $[H^+]$. At any given $[H^+]$ in the medium, cellular acidity was less at

TABLE II

*Effects of change in P_{CO_2} on muscle acidity when extracellular $[HCO_3^-]$ is held constant at 12.7 to 14.5 mEq per L**

No. of analyses	Medium				Muscle	
	$[HCO_3^-]$	P_{CO_2}	$[H^+]$	pH	$[H^+]$	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
8	13.7	18	30	7.52	59 ± 4	7.23
8	14.4	27	45	7.34	93 ± 8	7.03
8	14.5	28	46	7.34	89 ± 7	7.05
8	13.5	40	71	7.15	118 ± 12	6.93
8	14.1	48	81	7.09	141 ± 12	6.85
8	14.2	62	104	6.98	141 ± 12	6.85
8	12.7	65	123	6.91	141 ± 11	6.85
8	12.7	66	124	6.91	138 ± 9	6.86
8	12.9	66	123	6.91	147 ± 12	6.83
8	12.8	67	126	6.90	150 ± 8	6.82
8	14.1	88	150	6.82	198 ± 16	6.70
8	13.3	124	224	6.65	251 ± 23	6.60

* See footnote to Table I for abbreviations.

TABLE III

Effects of change in P_{CO_2} on muscle acidity when extracellular $[HCO_3^-]$ is held constant at 43 to 49 mEq per L

No. of analyses	Medium				Muscle	
	$[HCO_3^-]$	P_{CO_2}	$[H^+]$	pH	$[H^+]$	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
8	46.9	12	7	8.22	36 ± 6	7.44
8	43.4	17	10	8.02	46 ± 9	7.34
8	45.3	38	20	7.70	69 ± 14	7.16
8	45.4	38	20	7.69	67 ± 14	7.17
8	47.0	46	23	7.64	85 ± 10	7.07
8	45.0	68	36	7.45	117 ± 17	6.93
8	49.4	85	42	7.38	125 ± 15	6.90
8	45.0	124	66	7.18	164 ± 12	6.78
8	46.3	125	65	7.19	154 ± 13	6.81
8	49.0	187	91	7.04	175 ± 16	6.75
7	46.7	212	108	6.96	200 ± 20	6.70

an external $[HCO_3^-]$ of 6 to 7 mEq per L than at an $[HCO_3^-]$ of 22 to 23 mEq per L.

At a more moderately reduced bicarbonate level (12.7 to 14.5 mEq per L), the shape of the curve became triphasic again. As shown in Figure 1, the relationship between external and internal $[H^+]$ now closely resembled the normal, except that it was shifted to the right. The plateau oc-

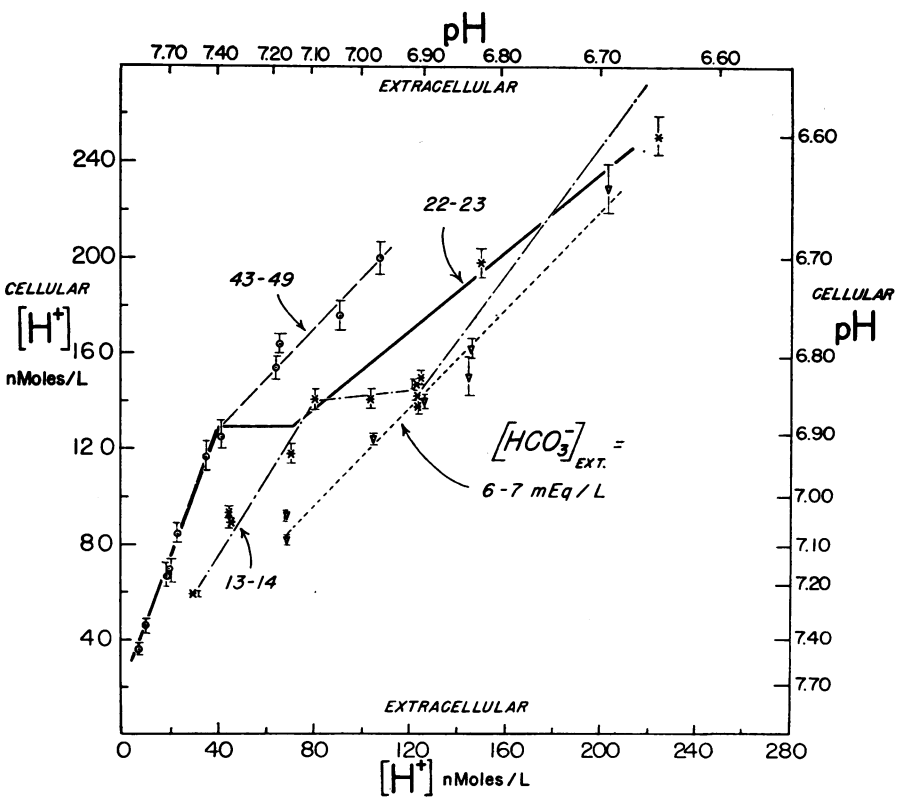

FIG. 1. THE RELATIONSHIP BETWEEN EXTRACELLULAR AND INTRACELLULAR ACIDITY AT DIFFERENT FIXED LEVELS OF EXTERNAL $[HCO_3^-]$, AS EXTERNAL $[H^+]$ IS VARIED BY CHANGING P_{CO_2}. The heavy solid curve indicates the relationship previously reported (2), at an external $[HCO_3^-]$ of 22 to 23 mEq per L. The three other curves represent the data in Tables I to III; the external $[HCO_3^-]$ is indicated. Each point is the mean ± standard error of the mean of seven or eight analyses. The curves are drawn through the experimental points by inspection.

curred at a more acid extracellular [H^+] and at a slightly more acid intracellular [H^+], but it extended over approximately the same range of P_{CO_2} (Table II).

When extracellular [HCO_3^-] was raised to 43 to 49 mEq per L (Table III), the relationship between external and internal [H^+] again deviated from normal and tended to become more linear. At all degrees of external alkalinity (external [H^+] < 40 nmoles per L), cellular [H^+] was the same at a bicarbonate of 43 to 49 mEq per L as at a normal level of 22 to 23 mEq per L. As the medium was acidified, however, cellular [H^+] tended to rise in a nearly proportional manner and was significantly higher at any given extracellular [H^+] than it was when the external [HCO_3^-] was normal (Figure 1).

To permit analysis of the results in more physiological terms, pertinent parts of the data from this group of experiments are displayed in a different manner in Figure 2. In this Figure, cell acidity is plotted against CO_2 tension. The straight lines crossing at right angles indicate the normal levels of P_{CO_2} and intracellular acidity. The upper two curves at low extracellular [HCO_3^-] include only those experiments in Tables I and II in which extracellular pH was below normal (7.40). In conventional clinical acid-base terminology, these curves define the conditions existing when there is extracellular "metabolic acidosis," and they are so labeled. The portions of the curves at CO_2 tensions to the left of the vertical line can therefore be considered as describing the effects of varying degrees of respiratory compensation on intracellular acidity. Those parts of the curves to the right of the line describe the intracellular effects of respiratory acidosis superimposed on metabolic acidosis.

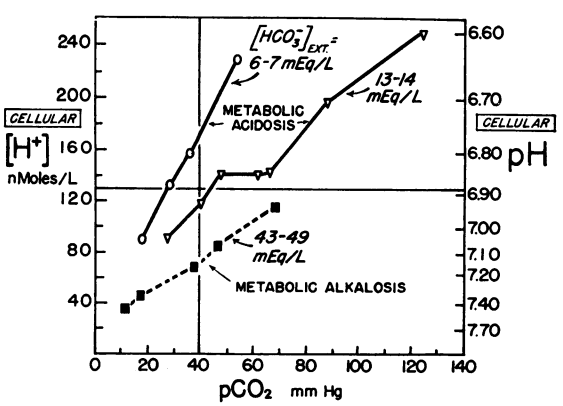

FIG. 2. THE RELATIONSHIP BETWEEN P_{CO_2} AND CELLULAR ACIDITY AT THREE DIFFERENT FIXED LEVELS OF EXTRACELLULAR [HCO_3^-]. See text for details.

The Figure shows that when there is no respiratory compensation, i.e., at a P_{CO_2} of 40 mm Hg, the cellular [H^+] is almost normal at an extracellular [HCO_3^-] of 13 to 14 mEq per L, but is definitely increased when the [HCO_3^-] is 6 to 7 mEq per L. Compensatory lowering of P_{CO_2} markedly reduces cell acidity under both conditions. At a P_{CO_2} of approximately 30 mm Hg, cell [H^+] is restored to normal at the lower bicarbonate level and is actually lower than normal at a bicarbonate of 13 to 14 mEq per L. Further reduction in P_{CO_2} results in intracellular alkalosis even at the lower bicarbonate level. Thus, partial respiratory compensation of extracellular metabolic acidosis can cause a paradoxical alkalosis within the muscle cell, despite a lower than normal external pH (*cf.* first two lines, Table I, and second and third lines, Table II). Elevation of P_{CO_2}, i.e., superimposition of a respiratory acidosis upon the metabolic acidosis, causes an even greater rise in cell acidity, as shown by the extension of the curves to the right. It is noteworthy, however, that at a HCO_3^- of 13 to 14 mEq per L, a rise in P_{CO_2} from 48 to 67 mm Hg (Table II) does not significantly increase cell acidity. As shown by the steep ascent of the topmost curve, cellular defense against a rising P_{CO_2} is much less effective at a very low external [HCO_3^-].

The lower curve in Figure 2 shows only those experiments at an elevated extracellular [HCO_3^-] in which the pH of the medium was above normal, and hence this curve can be considered descriptive of extracellular "metabolic alkalosis." The Figure shows that the cell is quite alkaline when there is no respiratory compensation. Compensatory elevation of P_{CO_2} progressively restores cell pH towards normal, but at the highest P_{CO_2} at which external pH is still above normal, cellular alkalosis has not been fully corrected (line 6, Table III). When respiratory alkalosis is superimposed on metabolic alkalosis, cellular alkalinity rapidly increases, as shown by the extension of the lower curve to the left. No plateau in cell pH is apparent over the entire range of P_{CO_2} studied.

TABLE IV

Effects of change in extracellular $[HCO_3^-]$ on muscle acidity when P_{CO_2} is held constant at 9 to 12 mm Hg

No. of analyses	Medium				Muscle	
	$[HCO_3^-]$	P_{CO_2}	$[H^+]$	pH	$[H^+]$	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
8	1.03	12	263	6.58	207 ± 13	6.68
8	3.38	12	83	7.08	87 ± 6	7.06
8	8.45	12	33	7.48	58 ± 6	7.24
8	20.7	9.8	11	7.95	43 ± 8	7.37
12	21.0	9.2	11	7.97	36 ± 6	7.45
8	46.9	12	7	8.22	36 ± 6	7.44

2) Effects of changes in extracellular bicarbonate concentration on muscle acidity at high or low CO_2 tensions. In a second group of experiments the CO_2 tension of the system was fixed at three different low levels (9 to 12, 17 to 19, and 27 to 29 mm Hg) and at two different high levels (62 to 69 and 119 to 129 mm Hg), while extracellular $[HCO_3^-]$ was varied to produce a wide range of external $[H^+]$ values. The data are summarized in Tables IV to VIII and are in part displayed in Figure 3. Experiments at a P_{CO_2} of 27 to 29 are omitted to simplify the Figure. The conventions used in the latter are the same as in Figure 1.

As shown in the Tables and the Figure, the relationship between cellular and extracellular $[H^+]$ at different fixed levels of P_{CO_2} varies considerably. At relatively low levels (9 to 12 and 17 to 19 mm Hg) the relationship is apparently linear. At 62 to 69 mm Hg, the curve is triphasic and closely resembles the normal. At the highest P_{CO_2} level studied (119 to 129 mm Hg) the curve has lost its plateau between an extracellular $[H^+]$ of 40 and 120 nmoles per L, but still bears a general resemblance to the normal. As clearly shown in Figure 3, cellular $[H^+]$ can vary widely, at any given extracellular $[H^+]$, depending upon the P_{CO_2}.

In Figure 4, relevant parts of the data from Tables IV, V, VII, and VIII are plotted as in Figure 2, except that extracellular $[HCO_3^-]$ is shown on the abscissa. The vertical line at an $[HCO_3^-]$ of 23 mEq per L defines an arbitrarily chosen "normal" level of bicarbonate at which there is no metabolic compensation. The two upper curves labeled "respiratory acidosis" show the data for all those experiments at high CO_2 tensions in which extracellular pH was normal or below. The two lower curves labeled "respiratory alkalosis" show those experiments at low CO_2 tension in which extracellular pH was elevated.

In the upper two curves, when external $[HCO_3^-]$ was held normal, cellular acidity was normal at a P_{CO_2} of 65 mm Hg, but significantly

TABLE V

Effects of change in extracellular $[HCO_3^-]$ on muscle acidity when P_{CO_2} is held constant at 17 to 19 mm Hg

No. of analyses	Medium				Muscle	
	$[HCO_3^-]$	P_{CO_2}	$[H^+]$	pH	$[H^+]$	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
4	2.09	18	214	6.67	190 ± 11	6.72
8	2.55	18	166	6.78	145 ± 10	6.84
8	2.87	18	147	6.83	144 ± 9	6.84
8	3.80	19	119	6.92	129 ± 9	6.89
8	3.47	17	119	6.92	120 ± 6	6.92
8	4.54	18	96	7.02	104 ± 11	6.98
8	5.97	17	69	7.16	92 ± 7	7.03
8	6.05	18	69	7.16	82 ± 5	7.09
8	13.7	18	30	7.52	59 ± 4	7.23
8	21.1	19	22	7.67	68 ± 6	7.17
8	21.1	18	21	7.68	64 ± 8	7.19
8	43.4	17	10	8.02	46 ± 9	7.34

TABLE VI

Effects of change in extracellular [HCO_3^-] on muscle acidity when P_{CO_2} is held constant at 27 to 29 mm Hg

No. of analyses	Medium				Muscle	
	[HCO_3^-]	P_{CO_2}	[H^+]	pH	[H^+]	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
8	4.40	28	148	6.83	168 ± 10	6.78
8	4.41	28	150	6.82	184 ± 11	6.73
8	5.51	29	126	6.90	140 ± 9	6.85
8	6.53	29	105	6.98	124 ± 4	6.91
8	7.72	28	88	7.05	140 ± 8	6.85
8	10.6	29	66	7.18	109 ± 11	6.96
8	14.4	27	45	7.34	93 ± 8	7.03
8	14.5	28	46	7.34	89 ± 7	7.05
8	21.2	28	31	7.51	95 ± 13	7.02
8	21.4	28	31	7.51	88 ± 21	7.06

increased at a P_{CO_2} of 122 mm Hg. Compensatory rises in extracellular bicarbonate tended to alkalinize the cell slowly, so that a very large increase was required to restore the cell [H^+] to normal at the highest P_{CO_2} level. However, the Figure clearly demonstrates that a sufficient rise in extracellular [HCO_3^-] can restore cellular pH to normal even in the face of severe hypercapnia. As shown in Table VIII, however, metabolic compensation must be complete, i.e., extracellular pH must be restored to normal, before cellular pH is fully corrected.

When extracellular [HCO_3^-] was lowered, i.e., when metabolic acidosis was superimposed on respiratory acidosis, cellular [H^+] rapidly and immediately increased, at the highest P_{CO_2} levels. However, at a P_{CO_2} of 65 mm Hg, cell [H^+] did not increase significantly until external [HCO_3^-] dropped below 10 mEq per L.

The two lower curves in Figure 4 show that cellular [H^+] is markedly reduced in uncompensated respiratory alkalosis and is only slightly, or not at all, improved by compensatory reduction in extracellular [HCO_3^-]. As shown in Tables IV and V, even complete metabolic compensation of the extracellular respiratory alkalosis fails to modify the alkalinity of the cells. Indeed, with these low levels of P_{CO_2}, intracellular alkalosis persists even when external [HCO_3^-] is lowered enough to cause moderate extracellular acidosis. When, on the other hand, extracellular metabolic alkalosis was superimposed on the pre-existing respiratory alkalosis, there was surprisingly little further increase in the alkalinity of the cell.

TABLE VII

Effects of change in extracellular [HCO_3^-] on muscle acidity when P_{CO_2} is held constant at 62 to 69 mm Hg

No. of analyses	Medium				Muscle	
	[HCO_3^-]	P_{CO_2}	[H^+]	pH	[H^+]	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
8	8.1	69	209	6.68	238 ± 17	6.62
8	9.4	67	172	6.76	222 ± 11	6.65
8	9.6	65	162	6.79	222 ± 16	6.65
8	12.6	65	123	6.91	141 ± 11	6.85
8	12.7	66	124	6.91	138 ± 9	6.86
8	12.8	67	126	6.90	150 ± 8	6.82
8	12.9	66	123	6.91	147 ± 12	6.83
8	14.2	62	104	6.98	141 ± 12	6.85
8	22.5	65	69	7.16	134 ± 15	6.87
8	22.6	68	72	7.14	121 ± 7	6.92
8	28.3	64	54	7.27	132 ± 8	6.88
8	36.8	62	40	7.40	119 ± 2	6.92
8	45.6	68	36	7.45	117 ± 17	6.93
7	54.1	62	28	7.56	95 ± 16	7.02

TABLE VIII

Effects of change in extracellular [HCO_3^-] on muscle acidity when P_{CO_2} is held constant at 119 to 129 mm Hg

No. of analyses	Medium				Muscle	
	[HCO_3^-]	P_{CO_2}	[H^+]	pH	[H^+]	pH
	mEq/L	*mm Hg*	*nmoles/L*		*nmoles/L ICW*	
8	6.83	119	417	6.38	456 ± 37	6.34
8	13.3	124	224	6.65	251 ± 23	6.60
8	15.9	129	194	6.71	231 ± 20	6.64
8	21.6	122	135	6.87	188 ± 16	6.72
8	21.6	121	134	6.87	179 ± 10	6.75
8	45.0	124	66	7.18	164 ± 12	6.78
8	46.3	125	65	7.19	154 ± 13	6.81
8	63.2	119	45	7.35	147 ± 16	6.83
8	72.3	119	38	7.42	131 ± 13	6.88
8	76.8	124	39	7.41	122 ± 19	6.92

3) The relationship between extracellular and intracellular acidity. In Figure 5, all of the data in Tables I to VIII plus those in Tables I and II of the preceding paper (2) are plotted to show the relationship between extracellular and intracellular acidity. The straight lines intersecting at right angles indicate the normal values for each parameter; the diagonal line defines equal intracellular and extracellular [H^+] values.

It is apparent from this Figure that at any

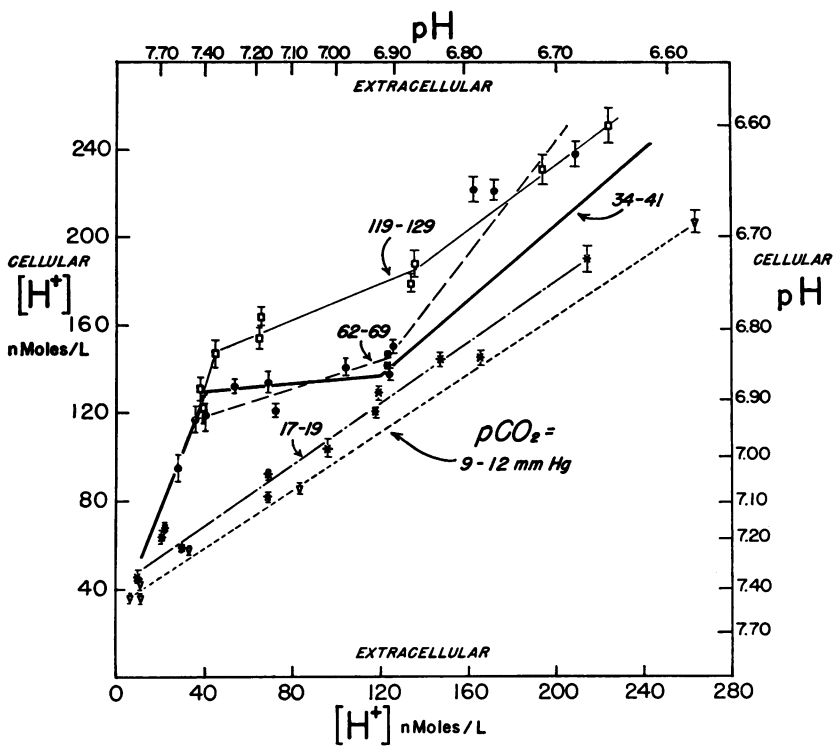

FIG. 3. THE RELATIONSHIP BETWEEN EXTRACELLULAR AND INTRACELLULAR ACIDITY AT DIFFERENT FIXED LEVELS OF P_{CO_2}, AS EXTERNAL [H^+] IS VARIED BY CHANGING [HCO_3^-]. The heavy solid line indicates the relationship previously reported (2), at a P_{CO_2} of 34 to 41 mm Hg. The four other curves, at different P_{CO_2} levels, represent the data in Tables IV, V, VII, and VIII. Each point is the mean ± standard error of the mean of four to 12 analyses. The curves are drawn through the experimental points by inspection.

given external [H^+], cellular [H^+] can vary widely; this variation, as shown in the Tables, depends upon the particular combination of P_{CO_2} and external [HCO_3^-] that determines the external [H^+]. At an external pH of approximately 7.20, for example, internal pH was found to vary from 6.80 to 7.10. All of the points falling in the right lower quadrant of the Figure ("extracellular acidosis" and "cellular alkalosis") represent conditions under which the cell was paradoxically alkaline while the extracellular fluid was more acid than normal. All of these represent the effects of partial respiratory compensation in metabolic acidosis, there being no instance in which partial metabolic compensation of respiratory acidosis produced a similarly paradoxical intracellular effect.

Although the acidity of the cell was thus relatively independent of the acidity of the extracellular medium, the Figure shows that whenever the extracellular fluid was definitely alkaline the muscle cell was also alkalotic, regardless of the particular level of P_{CO_2} or external [HCO_3^-]. It is also evident that whenever the extracellular fluid became very acidic the cell was similarly rendered acidotic; thus, cell pH was always found to be lower than normal when extracellular pH dropped below 6.85, regardless of P_{CO_2} or external [HCO_3^-].

A final fact demonstrated by the Figure is that the great majority of experimental points was found to be above the diagonal line of equality. In other words, the muscle cell was almost always more acid than its environment. There were,

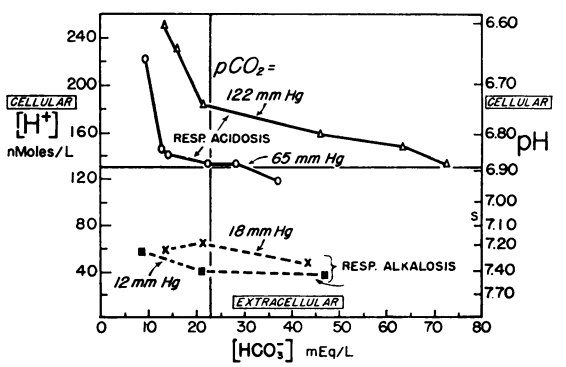

FIG. 4. THE RELATIONSHIP BETWEEN EXTRACELLULAR [HCO_3^-] AND CELLULAR ACIDITY AT FOUR DIFFERENT FIXED LEVELS OF P_{CO_2}. See text for details.

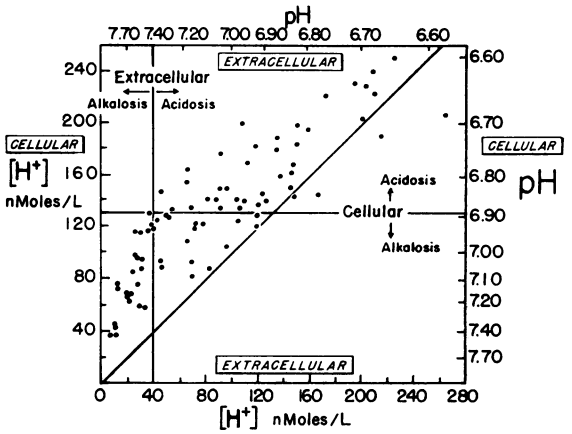

FIG. 5. MASS PLOT OF ALL THE DATA IN THIS AND PRECEDING PAPER (2), RELATING EXTRACELLULAR AND INTRACELLULAR ACIDITY. See text for details.

however, a few exceptions to this rule, as shown by the three points that are clearly below the diagonal. They represent examples of very severe extracellular metabolic acidosis in which there was enough compensatory fall in P_{CO_2} to make the cell more alkaline than the medium (line 1, Table IV; and lines 1 and 2, Table V).

Discussion

These observations demonstrate that the acidity of resting muscle cells cannot be accurately predicted from the extracellular pH alone. Intracellular pH appears to be a complex function of both P_{CO_2} and extracellular [HCO_3^-]; at any given extracellular pH, the acidity of the cell varies according to the particular combination of P_{CO_2} and [HCO_3^-] that determines the acidity of the medium.

In general, P_{CO_2} seems to have a relatively more important influence on cell pH than does the extracellular [HCO_3^-]. Thus, partial respiratory compensation of severe metabolic acidosis readily restores intracellular pH to normal (Table I), but nothing less than full metabolic compensation of severe respiratory acidosis suffices to correct cellular acidity (Table VIII). Furthermore, in respiratory alkalosis even complete metabolic compensation of extracellular pH fails to reduce significantly the alkalinity of the cell, whereas in metabolic alkalosis full respiratory compensation restores cell pH almost to normal.

The predominance of the cellular effect of CO_2 tension over that of external $[HCO_3^-]$ can sometimes lead to a paradoxical situation in which partial respiratory compensation of metabolic acidosis produces an intracellular alkalosis despite a lowered extracellular pH. For example, in the sixth line of Table VI, the extracellular $[HCO_3^-]$ is 10.6 mEq per L, the P_{CO_2} 29 mm Hg, and the pH 7.18. However, the muscle acidity in this example is significantly reduced, rather than increased.

Extrapolation of our experimental results to the intact organism is obviously fraught with uncertainty. Nevertheless, certain interesting comparisons and physiological implications deserve to be mentioned here. The acid-base condition of the extracellular medium in the example of the preceding paragraph can be considered fairly typical of that existing in the blood in many cases of clinical metabolic acidosis. If our *in vitro* preparation behaves like muscle cells *in vivo,* the implication of our findings would be that even partial respiratory compensation of metabolic acidosis suffices to prevent intracellular acidosis and may actually result in cellular alkalosis. If intracellular acidity is the stimulus to compensatory hyperventilation in the intact animal, obviously the pH of the cells of the respiratory center must respond in a somewhat different manner from that of skeletal muscle.

These data may shed some light on certain aspects of clinical acid-base disorders. One rarely sees patients surviving metabolic acidosis when the extracellular pH is much below 6.8 or 6.9. It may be significant that this is the level at which the cellular pH of the diaphragm muscle begins to decline sharply, regardless of whether or not there is some respiratory compensation. Furthermore, it is well known that patients seem to tolerate chronic hypercapnia fairly well until the P_{CO_2} reaches approximately 80 mm Hg; at or above these levels, disturbances of consciousness and other serious effects begin to appear. A possible explanation may reside in the observations of this and the preceding paper, which show that with or without partial metabolic compensation, cellular pH does not decrease very much until P_{CO_2} is elevated beyond 80 mm Hg. Finally, in view of the striking susceptibility of cells to extracellular alkalosis (Figure 5), it is interesting to consider that most clinical deviations of extracellular acid-base balance in the alkaline direction are relatively mild. Increases in blood pH above 7.6 are unusual, whereas equivalent and even greater degrees of acidosis are common. All these considerations suggest that intracellular phenomena play an important role in clinical acid-base disturbances and that closer examination of the cellular response will prove to be rewarding.

The present experiments appear to provide some clues to the nature of the mechanisms responsible for the control of cellular pH. The data demonstrate that the characteristic, triphasic curve describing the "CO_2 titration" of muscle under normal conditions is changed when the external $[HCO_3^-]$ is sufficiently altered. Thus, as shown in Figure 1, there appears to be intracellular "buffering" of CO_2 in the vicinity of pH 6.9 when extracellular $[HCO_3^-]$ is 22 to 23 or 13 to 14 mEq per L, but this completely disappears at an external $[HCO_3^-]$ of 43 to 49 mEq per L or 6 to 7 mEq per L. The plateau in cell pH also does not depend on a particular range of CO_2 tensions, as shown quite clearly in Figure 2. At an extracellular $[HCO_3^-]$ of 13 to 14 mEq per L a rise in P_{CO_2} from 50 to 70 mm Hg is completely neutralized, but when the external $[HCO_3^-]$ is 43 to 49 mEq per L, the same rise in P_{CO_2} causes a significant increase in cellular acidity. The response of the muscle to CO_2 is therefore not likely to be due to a purely physical process of intracellular buffering.

Similarly, as shown in Figure 3, the characteristic triphasic response of muscle pH to changes in external pH produced by bicarbonate is not determined solely by the external $[H^+]$, nor by the internal $[H^+]$ at which the cellular mechanisms are operating. Here again, it appears to be the particular combination of external $[HCO_3^-]$ and P_{CO_2} that is important. This fact provides further evidence against the idea that regulation of acid-base balance within cells is simply a matter of buffering. Our observations suggest, instead, that the complex acid-base behavior of the cell is more likely to be actively regulated by the metabolic activity of the cell. We have recently obtained more direct evidence in support of this suggestion by studying the temperature dependence of the muscle cell pH (5).

Summary

A systematic study has been made of the interaction between external pH, Pco_2, and external $[HCO_3^-]$ in the regulation of muscle cell pH in an intact rat diaphragm preparation. The results demonstrate that cell pH is a complex function, influenced by all three factors: external pH, external $[HCO_3^-]$, and Pco_2.

At any given external $[HCO_3^-]$ cell pH could be changed by Pco_2, and at any given Pco_2 cell pH varied with external $[HCO_3^-]$. At the same external pH, cell pH was dependent upon the absolute values of Pco_2 and external $[HCO_3^-]$.

CO_2 tension had a relatively greater influence on cell pH than did extracellular $[HCO_3^-]$. Partial respiratory compensation of extracellular metabolic acidosis readily prevented cellular acidosis and even caused paradoxical cellular alkalosis, despite moderately low external pH. Partial compensatory rise in external $[HCO_3^-]$ moderated but did not fully prevent cellular acidosis in severe hypercapnia. However, irrespective of absolute levels of Pco_2 or external $[HCO_3^-]$, whenever external pH fell below 6.85 the cell was acidotic. All degrees of extracellular alkalosis caused alkalinization of cells, regardless of compensatory changes in Pco_2 or external $[HCO_3^-]$. The muscle cell was usually more acid than its environment, but became more alkaline when Pco_2 was reduced in severe extracellular acidosis.

Some clinical implications of these experiments are discussed. We suggest that the regulation of the pH of resting skeletal muscle cannot be explained by simple buffer reactions and is probably linked to active metabolic processes.

References

1. Adler, S., A. M. Roy, and A. S. Relman. The nature of the extracellular factors determining cell pH. Clin. Res. 1964, **12**, 198.
2. Adler, S., A. Roy, and A. S. Relman. Intracellular acid-base regulation. I. The response of muscle cells to changes in CO_2 tension or extracellular bicarbonate concentration. J. clin. Invest. 1965, **44**, 8.
3. Relman, A. S., G. W. Gorham, and N. G. Levinsky. The relation between external potassium concentration and the electrolyte content of isolated rat muscle in the steady state. J. clin. Invest. 1961, **40**, 386.
4. Miller, R. B., I. Tyson, and A. S. Relman. pH of isolated resting skeletal muscle and its relation to potassium content. Amer. J. Physiol. 1963, **204**, 1048.
5. Adler, S., A. Roy, and A. S. Relman. Metabolic control of cell pH (abstract). J. clin. Invest. 1964, **43**, 1251.