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The Intracellular pH of the Human Platelet *

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A great number of cells are acidic compared to their environment (1). Changes in the pH of extracellular fluid are important primarily as they affect hydrogen ion activity within the cell. However, cellular hydrogen ion activity is dependent upon a number of factors, so that it is impossible to predict cellular pH from knowledge of extracellular pH alone (2, 3).

Since the interior of most cells is not homogeneous, measurements of cellular pH reflect an aggregate hydrogen ion activity (1). The present studies were performed to determine the aggregate intracellular pH of the human platelet and to characterize changes in cellular pH subsequent to changes of pH in extracellular fluid.

Methods

Platelets were isolated from healthy volunteers as previously described (4) and washed twice in 2 ml of KH_2PO_4–Na_2HPO_4 buffer, pH 7.4, ionic strength 0.2. The cells were then resuspended in Krebs-Ringer bicarbonate buffer, from which the magnesium and calcium salts had been omitted. In one group of experiments the bicarbonate concentration was constant, and pH was changed by varying the tension of carbon dioxide. In another group the carbon dioxide tension was constant, and the pH was changed by varying the concentration of bicarbonate. A constant osmolality was maintained, in the latter group, by appropriate changes in the concentration of chloride. The pH of these buffers was within 0.05 U of the predicted values and remained so throughout the duration of the experiments. The presence or absence of glucose (100 mg per 100 ml) in these buffers did not influence the results. CO_2 tension was calculated by the Henderson-Hasselbalch equation, taking pK_1 as 6.11 and CO_2 solubility as 0.0311 (5).

One-tenth volume of buffer containing approximately 0.17 μc of DMO (5,5-dimethyloxazolidine-2,4-dione-2-^{14}C), 7.42 μc per mmole,[1] was added to each suspension of washed platelets. The mixtures were incubated at 37° C in a Dubnoff metabolic shaker at 60 oscillations per minute. Preliminary experiments showed that the distribution of DMO had reached a steady state within 15 minutes of incubation. Measurements of distribution were therefore made at 15 minutes unless otherwise noted. The addition of carrier DMO [2] in a concentration 40 times that of the labeled compound had no effect on the distribution of the labeled compound.

After incubation, the platelets were sedimented, the supernates decanted, and the tubes swabbed with a cotton-tipped applicator stick. The samples were weighed and then lysed in 1 ml of distilled water. One-half ml of each lysate was added to 10 ml of *p*-dioxane containing 0.05 g POPOP [1,4-bis-2-(5-phenyloxazolyl)benzene], 7.0 g PPO (2,5-diphenyloxazole), and 50 g naphthalene per L. DMO-^{14}C in this medium was determined with a Packard Tri-Carb spectrometer. Counts per minute were eight to ten times background. The DMO in the lysate was expressed per milliliter of platelet water. Total platelet water, determined by drying identically treated platelets to a constant weight, was 76% of the wet weight plus or minus 5%. Extracellular water, determined with RISA,[3] was 27% of the wet weight plus or minus 5%. The intracellular pH of the platelet was calculated by the method of Waddell and Butler (6).

The effect of a number of agents on the pH of the platelet was measured. These agents included thrombin,[4] reserpine,[5] PCMB (*p*-chloromercuribenzoic acid sodium salt), dinitrophenol, iodoacetate, ouabain, hematoporphyrin,[6] cyanide, and fluoride. Final concentrations of these compounds were 1×10^{-3} mole per L unless otherwise specified. Thrombin, PCMB, and hematoporphyrin increased the total water of the platelets to 85 ± 5% of the wet weight (accountable by an increase in cell water).

In separate experiments, potassium in lysates of platelets was measured by flame photometry with an internal lithium standard.

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[1] New England Nuclear Corp., Boston, Mass.

[2] Kindly supplied by Abbott Laboratories, North Chicago, Ill.

[3] Radioiodinated (^{131}I) serum albumin (human) U.S.P., E. R. Squibb, New York, N. Y.

[4] Parke, Davis, Detroit, Mich.

[5] Kindly supplied by Ciba Pharmaceutical Co., Summit, N. J.

[6] Cosmos International Corp., New York, N. Y.

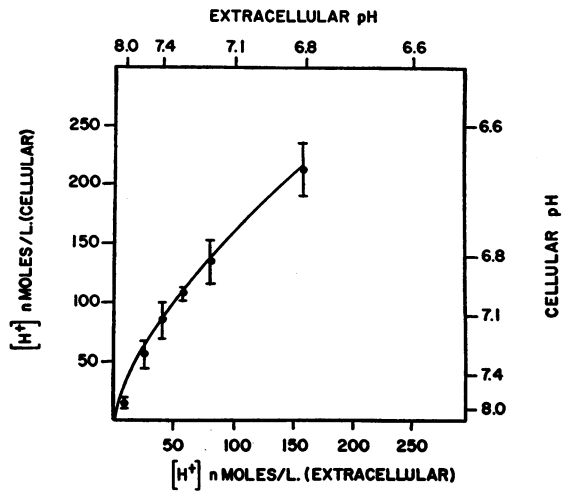

FIG. 1. THE pH OF THE PLATELET AS A FUNCTION OF EXTERNAL pH: EFFECT OF CO_2 TENSION. Washed platelets were incubated with DMO-^{14}C (5,5-dimethyloxazolidine-2,4-dione-2-^{14}C) in modified Krebs-Ringer bicarbonate buffer containing 25 mEq per L of bicarbonate for 15 minutes at 37° C. Extracellular pH was varied by varying CO_2 tension. The results are the mean values ±1 SD of 8 to 16 experiments.

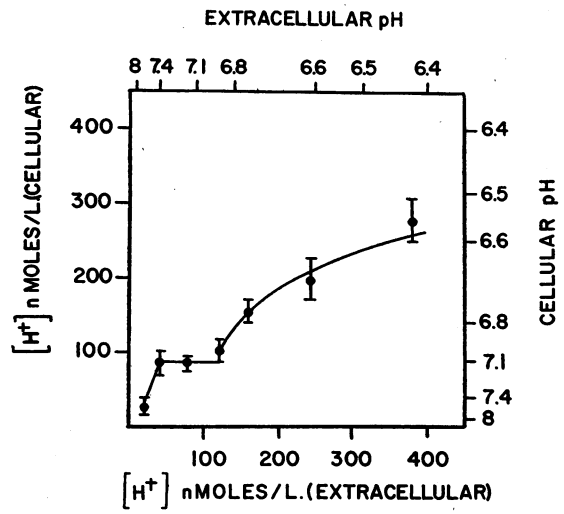

FIG. 2. THE pH OF THE PLATELET AS A FUNCTION OF EXTERNAL pH: EFFECT OF BICARBONATE CONCENTRATION. Washed platelets were incubated with DMO-^{14}C in modified Krebs-Ringer bicarbonate buffer for 15 minutes at 37° C. Constant P_{CO_2} = 40 mm Hg. Extracellular pH was varied by varying bicarbonate concentration. The results are the mean values ± 1 SD of 8 to 16 experiments.

Results

The pH of the platelet as a function of external pH

Effect of CO_2 tension. The relationship of the pH of the platelet to the pH of the medium, at a constant bicarbonate concentration (25 mEq per L), is shown in Figure 1. The cellular pH was acidic compared to the pH of the medium and decreased as the external pH was varied from 8.0 to 6.8 (10 to 158 nmoles per L) by changing CO_2 tension. A maximal ratio of internal to external hydrogen ion activities (2.1 to 1) occurred at an external pH of 7.4. In other experiments bicarbonate concentration was increased to 50 mEq per L, and external pH was again varied by changing CO_2 tension. Under these conditions cellular pH was the same at a given external pH as at a bicarbonate concentration of 25 mEq per L (Table I). In contrast, when bicarbonate concentration was decreased to 12.5 mEq per L, the intracellular pH remained constant when the external pH was varied from 7.4 to 7.1 (40 to 80 nmoles per L).

TABLE I

*The pH of the platelet as a function of external pH: changing CO_2 tension**

External pH	P_{CO_2}	HCO_3^-	Internal pH	Internal $[H^+]$ ±1 SD
	mm Hg	*mEq/L*		*nmoles/L*
7.4	20	12.5	7.10	80±18
7.1	40	12.5	7.09	81± 4
6.8	80	12.5	6.62	239±24
7.4	40	25	7.08	83±15
7.1	80	25	6.87	134±19
6.8	160	25	6.67	212±23
7.4	80	50	7.05	90±25
7.1	160	50	6.86	139± 5
6.8	320	50	6.58	265±53

* Washed platelets were incubated with DMO-^{14}C (5,5-dimethyloxazolidine-2,4-dione-2-^{14}C) in modified Krebs-Ringer bicarbonate buffer for 15 minutes at 37°C. Each value represents the mean of 8 to 16 experiments.

TABLE II

*The pH of the platelet as a function of external pH: changing bicarbonate concentration**

External pH	P_{CO_2}	HCO_3^-	Internal pH	Internal $[H^+]$ ±1 SD
	mm Hg	*mEq/L*		*nmoles/L*
7.4	40	25	7.08	83±15
7.1	40	12.5	7.09	81± 4
6.8	40	6.25	6.80	158±12
7.4	80	50	7.05	90±25
7.1	80	25	6.87	134±19
6.8	80	12.5	6.62	239±24
7.4	160	100	7.00	99±20
7.1	160	50	6.86	139± 5
6.8	160	25	6.67	212±23

* Washed platelets were incubated with DMO-^{14}C in modified Krebs-Ringer bicarbonate buffer for 15 minutes at 37° C. Each value represents a mean of 8 to 16 experiments.

TABLE III

*The effect of various agents on the pH of the platelet**

Agent	Concentration	Hydrogen ion activity of platelet	pH of platelet
		nmoles/L	
Control		84±15	$7.08^{+0.08}_{-0.08}$
Thrombin	100 U/ml	45± 9	$7.35^{+0.09}_{-0.08}$
PCMB†	1×10^{-4} M	52±12	$7.27^{+0.13}_{-0.08}$
Ouabain	1×10^{-3} M	64±14	$7.20^{+0.10}_{-0.09}$
Hematoporphyrin + light‡	6.7×10^{-6} M	65±16	$7.20^{+0.11}_{-0.11}$
Cyanide	1×10^{-3} M	63± 6	$7.20^{+0.04}_{-0.04}$
Dinitrophenol	1×10^{-3} M	75±18	$7.12^{+0.12}_{-0.09}$
Fluoride	2×10^{-3} M	134±42	$6.87^{+0.17}_{-0.12}$
Iodoacetate	1×10^{-3} M	83± 3	$7.08^{+0.02}_{-0.02}$
Reserpine	1.4×10^{-5} M	94±20	$7.03^{+0.10}_{-0.09}$

* Washed platelets were incubated with DMO-^{14}C in Krebs-Ringer bicarbonate buffer, pH 7.4, and the agents indicated. Preliminary experiments with each agent showed a steady state distribution of DMO to have been achieved within 15 minutes. Each value represents the mean plus or minus standard deviation of 8 determinations. In these experiments external hydrogen ion activity was 40 nmoles per L (pH 7.4), bicarbonate concentration was 25 mEq per L, and CO_2 tension was 40 mm Hg.
† PCMB = *p*-chloromercuribenzoic acid sodium salt.
‡ As described by Zieve, Solomon, and Krevans (4).

Effect of bicarbonate concentration. The relationship of the pH of the platelet to the pH of the medium, at a constant CO_2 tension (40 mm Hg), is shown in Figure 2. The cellular pH remained constant when the external pH was varied from 7.4 to 6.9 (40 to 126 nmoles per L) by changing bicarbonate concentration. Outside this range the pH of the platelet varied according to the pH of the medium.

In other experiments CO_2 tension was increased to either 80 or 160 mm Hg, and external pH was again varied by changing bicarbonate concentration. Under these conditions cellular pH did not remain constant but varied according to the pH of the medium (Table II).

The effect of various agents on the pH of the platelet

The effect of a number of agents on the pH of the platelet is shown in Table III. Under the conditions designated, thrombin, PCMB, and cyanide significantly altered the capacity of the platelet to maintain a gradient of hydrogen ion activity with respect to the incubation medium ($p \leq 0.01$).

The relationship of cellular potassium to intracellular pH of the platelet

The relationship of cellular potassium to intracellular pH of the platelet is shown in Table IV. There was no significant change in cellular potassium over a threefold range of intracellular hydrogen ion activities.

Discussion

The distribution of DMO, a weak organic acid, across cell membranes can be used to determine intracellular pH (6). The theoretical justification of this method has been considered by others (2, 6–8). In the present study, the distribution of DMO-^{14}C within the human platelet was unaffected by the presence of carrier in a concentration 40 times that of the labeled compound, indicating that neither active transport nor binding of DMO occurs in the platelet over this range of concentration.

Over a wide range of external pH, the platelet maintains a hydrogen ion gradient with respect to the medium. The platelet has a limited capacity to buffer changes in hydrogen ion activity produced by changes in P_{CO_2} and in this respect resembles many other cells (3, 9, 10), although buffering capacity apparently may vary from one type of cell to another (2). The present studies show that the intracellular pH of the platelet is much more sensitive to changes in external CO_2 tension than to changes in external bicarbonate concentration. The platelet is unable to maintain a constant pH, despite changes in bicarbonate concentration of 12.5 to 100 mEq per L, when external hydrogen ion activity is varied by increasing P_{CO_2} above 40 mm Hg.

It has been stated that cell membranes are rela-

TABLE IV

*The relationship of cellular potassium to intracellular pH of the platelet**

pH	[H⁺]	K⁺
	nmoles/L	*mEq/L*
7.08	84	98 ± 24
6.87	134	90 ± 16
6.67	212	108 ± 19

* Washed platelets were incubated for 15 minutes at 37° C in Krebs-Ringer bicarbonate buffer. The pH of the buffer was varied by changing CO_2 tension; bicarbonate concentration was 25 mEq per L. The potassium in lysates of these platelets was measured; the results are mean values ± 1 SD of 10 or more determinations.

tively impermeable to bicarbonate ions, since many cells are able to maintain a constant pH when external pH is varied over a wide range by changing bicarbonate concentration (3, 11, 12). On the other hand, external bicarbonate does influence intracellular pH of the platelet; for example, at an external pH of 7.4, over a range of CO_2 tension of 20 to 160 mm Hg, the internal pH is the same. It has been demonstrated that the pH of the rat diaphragm is also affected by changes in external bicarbonate concentration (2). Our methods did not allow us to determine whether the effect of changes of bicarbonate concentration on the pH of the platelet was due to movement of bicarbonate ions into the cell or of hydrogen ions out of the cell.

The pH of the platelet is apparently not dependent upon glycolytic metabolism of the cell since the hydrogen ion gradient is maintained in the absence of glucose and in the presence of fluoride and iodoacetate. However, thrombin and PCMB, agents that damage the membrane of the platelet, severely impair the ability of the cell to maintain a hydrogen ion gradient. This gradient may be caused by an accumulation of hydrogen ions within subcellular compartments. Indeed thrombin is known to degranulate platelets (13) and to release potassium from a compartment within the cell (14).

The changes in intracellular pH produced by variation in external pH are not reflected by changes in the concentration of intracellular potassium, suggesting that within this range of hydrogen ion activity there is no exchange of potassium for hydrogen ions.

Summary

The intracellular pH of the human platelet was measured in Krebs-Ringer bicarbonate buffer by use of 5,5-dimethyl-2,4-oxazolidine-dione-2-^{14}C (DMO). Extracellular pH was varied by changing CO_2 tension or bicarbonate concentration.

The platelet maintained a hydrogen ion gradient over a wide range of external pH. Cellular pH was much more sensitive to changes in external CO_2 tension than to changes in bicarbonate concentration, although the concentration of external bicarbonate did influence hydrogen ion activity within the cell.

Thrombin, *p*-chloromercuribenzoic acid sodium salt (PCMB), and cyanide impaired the ability of the platelet to maintain a hydrogen ion gradient. Fluoride, iodoacetate, and a number of other agents did not.

The changes in intracellular pH produced by variation in external pH were not accompanied by changes in the concentration of intracellular potassium.

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