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Intraerythrocytic pH variations during hemodialysis: A ^{31}P NMR study

J.P. MONTI, P. GALLICE, M. BAZ, A. MURISASCO, A. CREVAT, and R. ELSEN

Laboratoire de Biophysique, Faculté de Pharmacie, 13385 - Marseille Cedex 5; and Centre de Recherche en Néphrologie, Hôpital Sainte-Marguerite Marseille, France; and C.D. Medical Research, Brussels, Belgium

Intraerythrocytic pH variations during hemodialysis: A ^{31}P NMR study. Before hemodialysis, patients have an intraerythrocytic pH (pH_i) and an extracellular pH, measured in whole blood (pH_o), which are lower than those of healthy controls. During bicarbonate hemodialysis, pH_i values continuously increase, approaching a normal value at the end of the session. Concomitantly, pH_o values follow similar variations. During acetate hemodialysis, pH_i values exhibit a steep initial decrease, reaching a minimum after about 15 minutes. Concurrently, however, pH_o values decrease only slightly. This phenomenon seems to originate in the intraerythrocytic medium and might be due to a shift in intracellular CO_2/bicarbonate equilibrium. This drop in pH_i exhibits interpatient variability, suggesting that the magnitude of pH decrease would be correlated with the degree of the problems observed in some patients undergoing acetate hemodialysis.

Intracellular pH (pH_i) is a fundamental parameter of cells because it reflects cell equilibrium. Indeed, many enzymatic reactions and, consequently, the rate of in vivo biochemical reactions are highly dependent on H^+ activity. ^{31}P NMR allows direct measurement of pH_i, particularly in erythrocytes. The merits of the NMR method obviously lie in its non-invasiveness and non-perturbation, which permit direct investigation of the intact erythrocytes under physiological conditions. The values for pH_i obtained in NMR studies are comparable to those obtained with classical methods [1, 2]. In a previous study we showed that uremic patients exhibit a lower intraerythrocytic pH than healthy subjects, one which is later corrected by the hemodialysis session [3].

Disorders associated with acetate hemodialysis have been described [4] and are probably correlated with the initial loss of bicarbonate observed in acetate hemodialysis. Given that a loss of bicarbonate is expected to induce a decrease in pH, we sought to determine whether this decrease could be measured inside erythrocytes. For that purpose we monitored intra- and interdialytic pH_i variations obtained with acetate hemodialysis and compared them with those obtained with bicarbonate hemodialysis. Concomitantly, we measured the pH obtained in whole blood (pH_o), the bicarbonate concentration, and the pO_2 and pCO_2 pressures during the dialysis session.

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Methods

Hemodialysis procedure

We studied five patients for acetate (mean body weight 64 ± 9 kg; mean hematocrit level 24 ± 4%) and bicarbonate (mean body weight 58 ± 7 kg mean hematocrit level 26 ± 3%) hemodialysis, respectively.

The patients had been treated in a chronic hemodialysis program based on a sodium volume model [5] for at least two years. They underwent dialysis three times a week, for five hours, with conventional dialysis solution: Na^+ 135 to 150 mmol/liter; K^+ 2 mmol/liter; Mg^{2+} 0.75 mmol/liter; Ca^{2+} 1.6 mmol/liter; and glucose 1 g/liter. For acetate hemodialysis, this solution also contained acetate (41 mmol/liter); during bicarbonate hemodialysis, dialyzate bicarbonate concentration was 33 mmol/liter.

Capillary dialyzers (Models CDAK 4000 and 135 SCE; CD Medical Corp., Miami, Florida, USA) were used. For acetate hemodialysis, we used a bedside monitor (Gambro AK 10, Stockholm, Sweden) and for bicarbonate hemodialysis, Hospal Monitral 5 equipment (Lyon, France).

Sample preparation

Blood samples (2 ml) were drawn at the beginning of the hemodialysis session (acetate and bicarbonate), and after 5 min, 10 min, 15 min, 30 min, 1 hr, 1.5 hr, 2 hr, 3 hr, 4 hr and 5 hr, the end of the dialysis session. During the interdialytic period, samples were drawn after 30 min, 1 hr, 2 hr, 3 hr, 7 hr, 19 hr, 24 hr, 31 hr and 43 hr, the beginning of the next dialysis session.

The pH of seven healthy controls who had received no medication within two weeks of the study were measured.

Samples were prepared according to a technical procedure and chemical controls detailed in previous papers [3, 6]. Briefly, after centrifugation, the packed red blood cells are placed in a 5 mm NMR tube introduced into a 10 mm tube containing the external chemical shift reference.

To check for a possible non-ionic permeation of weak acid, we added a solution containing sodium acetate (final concentration 10 mM) and sodium chloride (final concentration 135 mM) buffered at pH = 7.45 to five samples.

pH_i determination by ^{31}P NMR

pH_i was classically determined by ^{31}P NMR [7, 8]. Chemical shifts of phosphorus compounds are pH sensitive and plots of

the chemical shift of intracellular phosphate versus pH provide a titration curve for determining pH_i. Briefly, standard titration curves are obtained from hemolyzates of erythrocytes, and the measurement of chemical shifts of phosphorus in intact erythrocytes makes it possible to obtain the pH_i values by referring to titration curves.

In the present paper, the kinetic studies required short spectra recording times. Indeed, in uremic patients, anemia precludes drawing large and repetitive volumes of blood. Since multiple sampling was necessary for our kinetic study, the classical use of P_i resonance to determine pH_i proved inappropriate. Therefore we developed a technique using 2 to 3 DPG resonances as a standard for pH_i determination, which allows rapid measurement with small blood samples [6].

The ^{31}P NMR spectra were recorded at 37 ± 0.5°C on a Brucker AM 200 spectrometer (Service Interuniversitaire de RMN, Faculté de Pharmacie, Marseille, France) operating at 81.02 MHz. Spectra were recorded in the Fourier transform mode and a broad band proton noise decoupling was used. The experimental conditions were: sweep width 6250 Hz; acquisition time 0.655 s; number of scans 500. An artificial line broadening of 10 Hz was used to improve the spectral signal-to-noise ratio.

Whole blood pH, bicarbonate concentration, pO_2 and pCO_2 measurement

In all samples, these various parameter values were classically determined with a blood gas analyzer (Model 178, Corning Medical and Scientific, Nedfield, Massachusetts, USA). This method of pH determination measures the steady state extracellular fluid pH in blood.

Results

Acetate hemodialysis. Table 1 shows whole blood pH and intraerythrocytic pH variations, blood bicarbonate concentration, pO_2 and pCO_2 during and after the dialysis session for the 5 patients studied.

Bicarbonate hemodialysis. Table 2 shows whole blood pH and intraerythrocytic pH variations, blood bicarbonate concentration, pO_2 and pCO_2 during and after the dialysis session for the five patients studied. pH values are given with an experimental error of 0.03 to 0.07 pH unit.

To facilitate comparison between these results, we plotted the variations of mean values of intraerythrocytic and whole blood pH versus time in Figure 1 (acetate hemodialysis) and in Figure 2 (bicarbonate hemodialysis). As previously described [3], the patients have a lower than normal intracellular pH before dialysis. Herein, the patients had a mean pH_i of 7.17 (SD 0.05) before dialysis which is lower than that of the seven healthy controls, 7.28 (SD 0.04). Similarly, mean pH_o was 7.31 (SD 0.03) for patients and 7.42 (SD 0.04) for healthy subjects.

During acetate hemodialysis, pH_i exhibited a steep initial decrease, reaching a minimum value after about 15 minutes (Fig. 1). The amplitude of minimum values varied among patients. For pH_o, the initial decrease was much less pronounced than for pH_i (Fig. 1). During the hemodialysis session, and after the initial drop, however, pH_o and pH_i rapidly recovered similar patterns of pH values. During the postdialytic period, when pH values decreased, similar pH_o and pH_i patterns were also obtained (Fig. 1). During these first 15 minutes a decrease in bicarbonate concentration and in pCO_2 pressure were observed (Table 1).

During bicarbonate hemodialysis, pH_i values continuously increased, approaching a normal value at the end of the session (Fig. 2); concomitantly pH_o values followed similar variations (Fig. 2). pO_2 and pCO_2 remained constant within experimental error and HCO_3^- concentration classically exhibited a slight increase (Table 2) [9]. During this dialysis in which no drop was

Table 1. Intraerythrocytic pH, whole blood pH, blood bicarbonate concentrations, CO_2 and O_2 pressures during acetate hemodialysis ($N = 5 \pm SD$)

Time	pH_i	pH_o	HCO_3 mM/liter	pCO_2 mm Hg	pO_2 mm Hg
0	7.18 ± 0.05	7.32 ± 0.03	19.6 ± 2.3	35.7 ± 2.3	92.6 ± 8.6
5 min	7.16 ± 0.04	7.31 ± 0.04	19.4 ± 1.0	36.3 ± 2.4	91.9 ± 6.0
10 min	7.13 ± 0.03	7.31 ± 0.05	18.2 ± 2.4	31.4 ± 1.5	89.5 ± 9.2
15 min	7.07 ± 0.03	7.30 ± 0.04	14.5 ± 3.3	28.7 ± 3.6	85.3 ± 7.8
30 min	7.14 ± 0.05	7.31 ± 0.04	18.0 ± 2.9	34.0 ± 1.5	94.0 ± 7.0
1 hr	7.18 ± 0.05	7.33 ± 0.05	18.1 ± 1.8	32.3 ± 2.1	90.1 ± 7.2
1.5 hr	7.25 ± 0.05	7.35 ± 0.03	18.4 ± 2.0	33.0 ± 3.1	92.5 ± 8.1
2 hr	7.29 ± 0.02	7.39 ± 0.03	19.9 ± 2.9	36.3 ± 2.9	91.0 ± 4.2
3 hr	7.30 ± 0.02	7.38 ± 0.02	18.2 ± 0.9	35.0 ± 2.6	94.2 ± 6.4
4 hr	7.31 ± 0.02	7.39 ± 0.02	21.0 ± 3.3	34.8 ± 1.5	93.1 ± 9.0
5 hr	7.30 ± 0.02	7.39 ± 0.03	21.3 ± 2.8	33.7 ± 1.7	94.0 ± 4.8
End of dialysis					
5.5 hr	7.32 ± 0.02	7.38 ± 0.02			
6 hr	7.31 ± 0.03	7.39 ± 0.02			
7 hr	7.29 ± 0.02	7.38 ± 0.03			
8 hr	7.32 ± 0.02	7.38 ± 0.02			
12 hr	7.28 ± 0.02	7.37 ± 0.03			
24 hr	7.24 ± 0.01	7.31 ± 0.03			
29 hr	7.23 ± 0.02	7.30 ± 0.02			
36 hr	7.20 ± 0.02	7.30 ± 0.02			
48 hr	7.19 ± 0.03	7.29 ± 0.03			

Table 2. Intraerythrocytic pH, whole blood pH, blood bicarbonate concentrations, CO_2 and O_2 pressures during acetate hemodialysis ($N = 5 \pm SD$)

Time	pH_i	pH_o	HCO_3 mM/liter	pCO_2 mm Hg	pO_2 mm Hg
0	7.15 ± 0.05	7.30 ± 0.03	17.0 ± 1.1	33.8 ± 3.0	96.5 ± 6.2
5 min	7.16 ± 0.04	7.30 ± 0.04	17.5 ± 2.4	33.0 ± 2.1	95.1 ± 7.0
10 min	7.15 ± 0.05	7.31 ± 0.04	17.2 ± 1.2	33.2 ± 2.5	97.0 ± 8.6
15 min	7.15 ± 0.05	7.31 ± 0.05	17.5 ± 2.1	32.5 ± 3.5	99.9 ± 6.8
30 min	7.17 ± 0.04	7.34 ± 0.03	17.4 ± 0.7	32.4 ± 0.8	99.4 ± 9.0
1 hr	7.19 ± 0.05	7.35 ± 0.04	18.6 ± 3.5	30.0 ± 2.2	99.1 ± 5.1
1.5 hr	7.23 ± 0.04	7.37 ± 0.02	19.0 ± 2.7	29.9 ± 3.8	95.5 ± 6.9
2 hr	7.23 ± 0.02	7.40 ± 0.02	21.1 ± 1.0	34.0 ± 2.8	98.5 ± 6.4
3 hr	7.27 ± 0.01	7.41 ± 0.02	20.4 ± 2.1	32.0 ± 1.0	96.3 ± 6.8
4 hr	7.27 ± 0.03	7.42 ± 0.02	22.0 ± 0.3	31.5 ± 2.1	92.5 ± 8.1
5 hr	7.29 ± 0.02	7.41 ± 0.03	22.5 ± 1.9	33.1 ± 1.4	96.3 ± 5.8
End of dialysis					
5.5 hr	7.29 ± 0.02	7.40 ± 0.02			
6 hr	7.30 ± 0.01	7.39 ± 0.02			
7 hr	7.29 ± 0.01	7.40 ± 0.02			
8 hr	7.27 ± 0.02	7.39 ± 0.02			
12 hr	7.25 ± 0.03	7.35 ± 0.01			
24 hr	7.18 ± 0.01	7.31 ± 0.02			
29 hr	7.18 ± 0.01	7.32 ± 0.02			
36 hr	7.15 ± 0.02	7.30 ± 0.03			
48 hr	7.16 ± 0.01	7.29 ± 0.02			

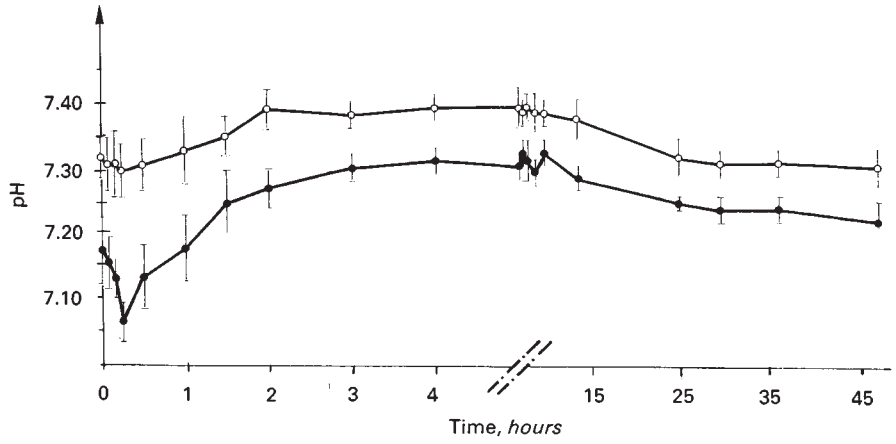

Fig. 1. *Whole blood pH and intraerythrocytic pH variations during and after acetate hemodialysis.* Each point represents the mean ± SD from 5 patients. Symbols are (○) whole blood pH (pH_o); (●) intraerythrocytic pH (pH_i).

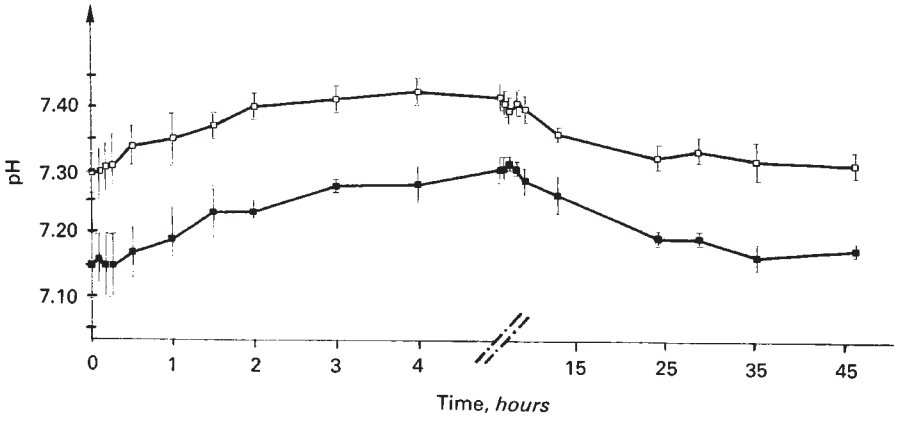

Fig. 2. *Whole blood pH and intraerythrocytic pH variations during and after bicarbonate hemodialysis.* Each point represents the mean ± SD from 5 patients. Symbols are: (□) whole blood pH (pH_o); (■) intraerythrocytic pH (pH_i).

observed, whole blood pH and intraerythrocytic pH curves also exhibited similar profiles (Fig. 2).

During the interdialytic period, both pH_i and pH_o values decreased regularly after bicarbonate and acetate hemodialysis (Figs. 1, 2), so that patients returned to pH values which were lower than those of normal subjects.

The addition of sodium acetate (final concentration 10 mM) in five samples led to a non-significant drop in pH_i of 0.01 pH unit (SD 0.02).

Discussion

Classically, intraerythrocytic pH is lower than extracellular pH [1]. pH_i values obtained from NMR studies are comparable to those obtained by classical techniques, such as distribution of weak acids or bases, colorimetry or microelectrode methods [1, 2]. Thus the difference between whole blood pH which is the plasma pH in steady state and intraerythrocytic pH is physiologically real and not an artefact related to the difference in methodologies.

The results obtained with bicarbonate hemodialysis were to be expected, whereas acetate hemodialysis results lead to the following questions: is the acute pH_i drop typical and limited to erythrocytes, or is it a general phenomenon occurring in all cells during the first 15 minutes of acetate hemodialysis? How may it be explained? Is it due to a non-ionic permeation of weak acid?

The non-ionic permeation of weak acid, such as acetic acid, is one of the techniques for measuring intracellular pH [1]. This method can induce a drop in pH_i because the neutral form enters the cells much faster. To discard this possibility we verified that addition of acetate to a suspension of erythrocytes does not modify the intraerythrocytic pH. We chose a concentration of 10 mM acetate in accordance with the results of Tolchin et al [10] who showed that during the hemodialysis session, the acetate concentration continuously increases to reach this final concentration. Other authors [11] given similar results but indicate lower final concentrations, but we preferred to operate in more unfavorable experimental conditions. Our results show a nonsignificant drop in pH_i: 0.01 pH unit (SD 0.02) which is most likely due to the high intracellular buffer power (mainly Hb) of erythrocytes. Thus this result clearly indicates that the decrease in pH_i during hemodialysis is not due to the non-ionic permeation of acetic acid. But even if this effect is not predominant, it will help to diminish the intraerythrocytic buffer power and thus it will render much more evident the effect of another acidification mechanism.

Any hemodialysis procedure has its immediate effect on the plasma and extracellular environments. There is no reason to believe that the general phenomena regulating acid-base balance early on and late in the hemodialysis procedure are different. Since our results show that pH_o and pH_i curves differ markedly in the first 15 minutes but that their profiles are similar after this time period, one may conclude that this drop origi-

nates in the intraerythrocytic medium. To better understand the mechanism of action, let us follow an erythrocyte through a tissue-lung cycle.

Basically, at the level of tissue cells, metabolic CO_2 enters the extracellular environment and plasma and is stored in the form of dissolved CO_2 and bicarbonate HCO_3^-. The H^+ generated is buffered by plasma protein anions, CO_2 moves into the erythrocyte and is stored as: dissolved CO_2, carbamino compound, resulting from the reaction with protonated deoxyhemoglobin, and bicarbonate, after conversion of CO_2 by carbonic anhydrase; the proton generated is taken up by the oxyhemoglobin anion which gives up O_2 and is converted into the less acidic deoxyhemoglobin. Bicarbonate diffuses out of the erythrocytes and Cl^- ion moves into the erythrocyte (chloride shift). This whole process is isohydric but not isoosmolar, (polyvalent anions are replaced by monovalent anions), thus a little water will also move into the cell.

At the level of lung alveoli, oxygen is transported into the erythrocyte and carried by oxyhemoglobin. CO_2 transported as bicarbonate, carbamino compounds and dissolved CO_2, leaves the cell and is exhaled. Bicarbonate moves into the erythrocyte to buffer the H^+ generated by the more acidic oxyhemoglobin. Cl^- ion and H_2O move out.

During acetate hemodialysis, a significant loss of bicarbonate occurs during the whole session. Until a sufficient amount of metabolic HCO_3^- ions is generated from acetate conversion, there is a net loss of bicarbonate (Table 1). It may be thought that during transit through the dialyzer, some bicarbonate diffuses out of the erythrocyte to compensate for this loss of plasmatic bicarbonate. Consequently, CO_2/bicarbonate equilibrium inside the cell will shift to bicarbonate formation, with equivalent generation of H^+. Unlike at the level of the tissue cells, these protons cannot be immediately buffered by the hemoglobin buffer system since no oxygen is released (pO_2 too high). There is a "proton bottleneck", which we believe may be responsible for the decrease in pH_i observed. Obviously, the non-ionic permeation of acetic acid which lowers the hemoglobin buffer power will certainly contribute to this decrease.

To verify this hypothesis we intend to measure, in a further study, the following parameters: pH_i, pH_o, bicarbonate concentration, pO_2 and pCO_2, after blood passage through the dialyzer.

Further conversion of CO_2 into bicarbonate will reduce CO_2 pressure and thus lower pCO_2, which is in agreement with our and classical results (Table 1). In addition, a small amount of dissolved CO_2 might be directly lost by transport through the dialyzer membrane.

The mechanism of pO_2 reduction might be more complicated. A survey of the literature indicates several possibilities [12]. In light of our observations, we are inclined to favor the hypothesis of hypoventilation occurring after the drop in pCO_2, thus reducing pO_2.

The mechanisms described are necessarily intraerythrocytic. But as the body buffer systems are complex and interconnected, it is not unreasonable to expect them to influence other body cells, and thus affect patient welfare.

The effects described might be at least in part responsible for the so-called "acetate intolerance" of some patients. We are presently studying a model describing intraerythrocytic and whole blood pH variations both during hemodialysis and in the post-dialytic interval by using bicarbonate [13] and acetate buffers.

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Reprint requests to A. Crevat, Laboratoire de Biophysique, Faculté de Pharmacie, 27 Bd Jean Moulin, 13385–Marseille Cedex 5, France.

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