Effects of metabolic alkalosis, metabolic acidosis and uraemia on whole-body intracellular pH in man

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Summary
1. Whole-body intracellular pH (pHi) was measured by the 14C-labelled DMO method in twenty-four control subjects, eighteen normal subjects with induced acute metabolic alkalosis, ten normal subjects with induced acute metabolic acidosis, twelve normal subjects with chronic acidosis and in fifteen patients with chronic renal insufficiency and acidosis.

2. The change in pHi per unit change in extracellular pH is significantly larger in acute metabolic alkalosis than in acute metabolic acidosis. In chronic metabolic acidosis, pHi decreased in proportion to the total amount of ammonium chloride administered; pHi was normal in patients with uraemic acidosis.

3. These observations confirm the role that tissue buffers play in the protection of the cellular environment in some forms of acidosis. When the acid load overcomes tissue buffer capacity, pHi becomes a function of extracellular pH.

4. Cells seem more protected from acute acidosis than from acute alkalosis.

Key words: acidosis, alkalosis, DMO, intracellular pH, uraemia.

Abbreviations: DMO, 5,5-dimethyl-2,4-oxazolidinedione; ECF, extracellular fluid; ICF, intracellular fluid.

Introduction
Numerous studies have shown that tissues play an important role in acid–base regulation. Swan & Pitts (1955) and Schwartz, Ornì & Porter (1957) administered large acid loads to animals and demonstrated the presence of readily available buffers in tissues. Similar conclusions have been reached in human subjects (Schwartz, Jenson & Relman, 1954). Soft tissue and the reactive surfaces of connective tissues and bone, and the extracellular fluid, are the components of the readily available buffers pool, which can provide 15 mmol of alkali/kg body weight (Swan & Pitts, 1955; Schwartz et al., 1957) during acute acid-loads.

Lemann, Lennon, Goodman & Relman (1965) and Goodman, Lemann, Lennon & Relman (1965) measured the net external acid balance in normal subjects after prolonged administration of NH4Cl and in patients with chronic renal failure and acidosis. They concluded that there was another large buffer store which comes into play during chronic proton retention: this may be bone salts. This store, estimated at about 500 mmol of alkali/kg body weight, slowly exchanges during long-term retention of acid.

The existence of non-extracellular compartments involved in acid–base regulation is also suggested by experiments in man and animals (Singer, Clark, Barker, Crosley & Elkinton, 1955; Swan, Axelrod, Seip, Pitts & Madsso, 1955), where 20–25% of intravenously injected alkali diffused into non-extracellular compartments.

The purpose of this study was to examine the whole-body intracellular pH (pHi) in man with metabolic alkalosis and acidosis, in the light of tissue participation in acid–base balance. The
various experimental conditions (Waddell was the best available for studies of this sort. For the measurement of skeletal muscle pH, this method was subsequently used for the determination of pH in different tissues and in various experimental conditions (Waddell & Bates, 1969). Robin, Wilson & Bromberg (1961b) applied the DMO method to the measurement of the whole-body pH.

Methods

Subjects

Volunteers for the study were informed of the nature and the purposes of the procedures.

Control group. Twenty-four normal subjects between the ages of 23 and 69 years, with normal plasma acid–base and electrolyte concentrations, were used as controls. In this group, pH measurement was performed 3 and 4 h after the injection of the 14C-labelled DMO, the distribution of which was calculated both from arterial and venous blood concentrations. Fourteen subjects were used for acute metabolic alkalosis studies and ten subjects for acute acidosis studies.

Normal subjects with acute induced metabolic alkalosis. Eighteen normal subjects (23–53 years of age) were studied. After obtaining samples for the base-line values 3 and 4 h after the injection of the 14C-DMO, intravenous sodium bicarbonate was administered over 30 min as a 595 mmol/l solution so that 4-8, 2-4, 1-6 and 1-2 mmol/kg body weight were given to ten, four, two and two subjects respectively. At 90 and 120 min from the beginning of the infusion, blood and urine samples for radioactivity counting, acid–base and electrolyte determinations were taken. Titratable acidity, ammonium excretion, pH and Pco2 were determined in the urine. Bicarbonate balance was calculated according to Singer et al. (1955).

Normal subjects with acute induced metabolic acidosis. After samples were obtained for the base-line values 3 and 4 h after the injection of isotopes, ten normal subjects (23–69 years of age) received a drip infusion of NH4Cl solution (374 mmol/l): 1-0-5-8 mmol/kg body weight was administered over periods of 2-8 h (about 0.9 mmol/min). Since it is not advisable to infuse NH4Cl into man at a rate higher than 1 mmol/min, an acute severe metabolic acidosis can be induced only by extending the infusion period. At the end of the infusion, blood and urine samples for the above-mentioned determinations were obtained. Hydrogen ion balance was calculated in these subjects according to Schwartz et al. (1954).

Normal subjects with chronic induced metabolic acidosis. Twelve normal subjects (21–62 years of age) were given NH4Cl orally, four times daily over periods of 4–8 days. The total amount administered was between 10-0 and 38-7 mmol/kg body weight. During the entire period titratable acidity, ammonium and bicarbonate excretion were measured. The tracers were injected 4 h after the last administration of NH4Cl. Three hours later, blood and urine samples were taken. Hydrogen ion balance was also calculated for this group.

Uraemic patients. Fifteen patients, between the ages of 18 and 67 years, with severe chronic renal failure and acidosis were studied. Their blood urea values ranged between 23.3 and 46.6 mmol/l. Chronic glomerulonephritis was present in ten patients (two with nephrotic syndrome) and interstitial nephritis in three cases. Uraemia had been present for 6 months to 4 years and chronic acidosis was likely (Giovannetti & Bigalli, 1956; Elkinton, Squires & Singer, 1962; Migone & Borghetti, 1968). For not less than 3 weeks before this study all these patients were given the Giovannetti diet, which provided them with about 30 mmol of fixed acid daily. During the same period titratable acidity, ammonium and bicarbonate excretion were measured daily. There was no exacerbation of uraemia, respiratory tract disease or congestive cardiac failure at the time of this study.

In four patients, pH was measured before and 120 min after the beginning of bicarbonate infusion (4.8 mmol/kg body weight in 30 min). In seven patients, about to undergo their first haemodialysis, pH and acid–base values were determined immediately before this treatment and repeated just after. At the end of haemodialysis, samples for radioactivity blanks were taken and a new tracer was given. pH and the other measurements were then determined at 150 and 300 min after the second administration of tracer. Haemodialysis was carried out with a Kill apparatus for 8 h against a bath containing sodium acetate (38 mmol/l).

Measurement of intracellular pH

The method used for pH determination is the
Intracellular pH in man

The modification of the method of Waddell & Butler (1959), described by Schloerb & Grantham (1965). Total body water was measured with tritiated water (3H2O) and extracellular water was determined with 36Cl (Na36Cl). Intracellular fluid was calculated from the difference between total body water and ECF. pH1 has been calculated according to the formula proposed by Waddell & Butler (1959) and used by Robin, Wilson & Bromberg (1961a) for whole-body pH1 measurement.

14C-DMO (specific radioactivity: 1 mCi/16.2 mg of DMO) was supplied by New England Nuclear Corp. (Boston, U.S.A.) as the ethyl acetate solution. Before use the solvent was removed by evaporation at 80°C (P. R. Schloerb, personal communication). A mixture of the tracers, diluted in NaCl solution (150 mmol/l), was injected in the following amounts (μCi/kg body weight): 14C-DMO, 0.7; 3H2O, 7.3; 36Cl, 0.3. The total dose of radioactivity injected into a 70 kg man was 0.19 rad/h, within the limit recommended by the International Commission on Radiological Protection (1960).

Subjects, who fasted and rested for 12 h before the experiments, received the isotope in an antecubital vein. Three hours after the injection, venous and arterial blood samples were obtained in heparinized syringes and used for measurement of body fluid compartments, DMO distribution, pH1, Pco2, electrolyte concentration and plasma osmolarity. Arterial blood was obtained through a catheter previously placed percutaneously in a radial artery. The plasma concentration of the three isotopes reached equilibrium in 2 h and remained stable for at least the subsequent 2 h. When NaHCO3 was infused, radioactivity was counted at the equilibrium point 30 min after the end of the infusion. On the other hand, after NH4Cl infusion equilibrium was already established at the end of administration, when radioactivity was counted. Urine, collected under mineral oil at the time of the isotope injection and subsequently at each sampling time, was utilized for the determination of isotopes, pH, PaCO2, ammonium, titratable acidity, electrolyte excretion and osmolality.

Determination of radioactive isotope

Duplicate samples of plasma deproteinized with trichloroacetic acid (0-6 mol/l) were added to vials containing 15 ml of scintillation liquid (naphthalene, 9%, w/v, PPO, 7%, w/v, and dimethyl-POPOP, 0.03%, w/v, dissolved in dioxan). Samples of urine, diluted with deionized water, were used also for the radioactivity count. The differential count of the three isotopes was carried out on a three-channel β-counter (Packard Tricarb type 3320). External standardization was used for correction of quenching. Efficiencies of about 35% for 3H, 58% for 14C and 60% for 36Cl were obtained. Samples were counted for radioactivity for 5 min with a statistical error not higher than 2% for 14C and 36Cl and not higher than 1% for 3H. 36Cl and 14C as d.p.m. in plasma water were calculated by dividing by 0.93 and corrected for the Donnan factor by multiplying by 1.05.

Acid-base and electrolyte measurements

Blood and urine pH and Pco2 were determined at 37°C with an Echweiler type Combi apparatus (Kiel, West Germany) and [HCO3-] was calculated from the Henderson-Hasselbalch equation. Plasma and urine concentration of Na+ and K+ were measured with a EEL 450 flame photometer (Evans Electronics Ltd, Halstead, Essex, U.K.), Cl- with a titrimetric method (Schales) and osmolality with a Fiske osmometer (Fiske Ass., Uxbridge, Mass., U.S.A.). Titratable acidity was measured by titrating urine with NaOH (0.1 mol/l) up to arterial pH; ammonium was determined with Nessler reagent.

Statistical methods

All data were evaluated with standard statistical methods. Completely randomized design or randomized block design was applied to the analysis of variance among the variables considered. Relationships between pH1 and pH2, [HCO3-], and PaCO2 were studied by analysis of linear regression and correlation (Dixon & Massey, 1957). The regressions between pH1 and pH2 in acute alkalosis and acidosis were compared by an analysis of variance of unbalanced lines (Bliss, 1967).

Since pH1 is calculated also from pH2, the correlation coefficient (r) between these two variables may be inflated. Therefore r may seem statistically significant even if the two
variables are not correlated. In order to overcome such a possibility, the significance of $r$ was assessed by values within the ranges covered by the present study were used for pH., body fluid compartments and $1^4$-C-DMO extracellular concentration; pH$_1$ was calculated from these values. The correlation coefficient between pH$_1$ and pH$_x$ of 100 groups of $n$ pairs of pH$_1$ and pH$_x$ was then calculated. In this manner the correct value of $r$ corresponding to $P < 0.05$ and $P < 0.01$, for a number of degrees of freedom of the various groups studied, was obtained. Values of $r$ obtained in this way are higher than the values reported in the standard tables. (For instance: $r = 0.702$ and 0.792 respectively for $P < 0.05$ and $P < 0.01$ if the degrees of freedom are 8.) Such values were used in this study when pH$_1$ and pH$_x$ had to be correlated. A similar approach was followed by Albert, Dell & Winters (1967) in evaluating the relationship between Pa$_{CO_2}$ and [HCO$_3^-$].

Results

Control group

In normal subjects pH$_1$ was between 6.67 and 6.84 (mean 6.74 ± 0.009); pH$_x$ was in the range 7.38–7.48 (mean 7.43 ± 0.005); Pa$_{CO_2}$ values were from 4.59 to 5.85 kPa (mean 5.13 ± 0.063); [HCO$_3^-$]$_x$ was between 24.8 and 29.0 mmol/l (mean 26.6 ± 0.22); [HCO$_3^-$]$_x$ was 5.8 ± 0.11 mmol/l; the ratio [H$^+$]/[H$^+$], was 4.98 ± 0.106 mmol/l; total body water was 52.74 ± 1.29% of body weight, and ECF was 22.41 ± 0.40% and ICF was 30.33 ± 1.11% of body weight.

Normal subjects with acute induced metabolic alkalosis (Table 1)

The intravenous infusion of NaHCO$_3$ causes a significant intracellular alkalosis as well as an extracellular alkalosis. When 4.8 mmol of NaHCO$_3$/kg body weight was administered, 90 and 120 min after the beginning of the infusion, there was an increase of 0.09 and 0.10 unit in pH$_x$, of 0.55 and 0.41 kPa in Pa$_{CO_2}$, of 10.3 and 9.6 mmol/l in [HCO$_3^-$]. pH$_1$ rose from a baseline value of 6.73 ± 0.017 to 6.90 ± 0.028 after 90 min, and to 6.97 ± 0.38 after 120 min. [HCO$_3^-$] increased to 4.1 and 5.0 mmol/l at those times. pH$_1$ and pH$_x$ did not increase to the same extent, as suggested by the decrease of the ratio [H$^+$]/[H$^+$]. The infusion of 2.4, 1.6 and 1.2 mmol of NaHCO$_3$/kg body weight induced linear and proportional variations in the same direction ($r = +0.66$; $P = 0.05$). Highly significant correlations ($P < 0.01)$ were found between pH$_1$ and pH$_x$ ($r = +0.80$) (Fig. 1) and between pH$_1$ and [HCO$_3^-$]$_x$ ($r = +0.82$), in the presence of fairly constant Pa$_{CO_2}$ values. The calculation of bicarbonate distribution indicated that 20.7 ± 4.1% and 30.7 ± 4.7% of the infused alkalai had been excreted in the urine at 90 and 120 min after the infusion, whereas 23.1 ± 1.9% and 15.7 ± 1.9% of the injected bicarbonate could not be found in ECF. When 4.8 mmol of NaHCO$_3$/kg body weight was administered, variations of body fluid spaces at 90 min were consistent with an expansion both of total body water and of ECF, without significant changes in ICF. At 120 min after the beginning of the infusion, total body water and ECF returned to close to base-line values; the slight increase of ICF was not statistically significant. After the administration of bicarbonate, a slight increase of serum Na$^+$ and osmolarity was observed, associated with a moderate drop of serum K$^+$ and Cl$^-$.

Normal subjects with acute induced metabolic acidosis (Table 2)

At the end of NH$_4$Cl infusion an extracellular metabolic acidosis occurred: [HCO$_3^-$]$_x$ was 9.9 mmol/l below the base-line value, with a decrease of 1.08 kPa in Pa$_{CO_2}$ and of 0.11 unit in pH$_x$, whereas pH$_1$ was not significantly changed (6.70 ± 0.021). pH$_1$ showed no significant correlation with pH$_x$ (Fig. 2), with [HCO$_3^-$]$_x$ or with Pa$_{CO_2}$. Hydrogen ion balance showed an accumulation of 1–9 mmol of H$^+$/l of ICF. Small variations of total body water and ECF seemed to be related to the infused NH$_4$Cl solution (500–1000 ml). The comparison of the regressions of pH$_1$ on pH$_x$ in acute acidosis and alkalosis shows a highly significant difference ($P < 0.001$) between the slopes.

Normal subjects with chronic induced metabolic acidosis (Table 3)

The long-term administration of NH$_4$Cl caused an extracellular metabolic acidosis (pH$_x$ 7.32 ± 0.018, [HCO$_3^-$]$_x$ 14.7 ± 1.09 mmol/l,
### Table 1. Acid–base balance in acute experimental metabolic alkalosis

* $P<0.05$, compared with basal values; ** $P<0.01$, compared with basal values. The comparison was carried out by analysis of variance with a randomized block design.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No. of cases</th>
<th>$\text{pH}_e$</th>
<th>$P_{a,\text{CO}_2}$ (kPa)</th>
<th>$[\text{HCO}_3^-]_e$ (mmol/l)</th>
<th>$\text{pH}$</th>
<th>$[\text{HCO}_3^-]_i$ (mmol/l)</th>
<th>$[\text{H}^+]_i/[\text{H}^+]_e$</th>
<th>Total body water (% body wt.)</th>
<th>ECF (% body wt.)</th>
<th>ICF (% body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Infusion of $\text{NaHCO}_3$ (4.8 mmol/kg body wt.)</td>
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</tr>
<tr>
<td>0</td>
<td>10</td>
<td>7.42±0.010</td>
<td>5.0±0.119</td>
<td>25.6±0.30</td>
<td>6.73±0.017</td>
<td>5.6±0.24</td>
<td>5.06±0.224</td>
<td>52.1±1.52</td>
<td>22.9±0.46</td>
<td>29.2±1.34</td>
</tr>
<tr>
<td>90 (after start of infusion)</td>
<td>6</td>
<td>7.51±0.010**</td>
<td>5.8±0.119*</td>
<td>36.4±0.80**</td>
<td>6.90±0.028**</td>
<td>9.7±0.59**</td>
<td>4.10±0.225**</td>
<td>53.6±1.05*</td>
<td>23.8±0.66*</td>
<td>29.8±0.66</td>
</tr>
<tr>
<td>120 (after start of infusion)</td>
<td>8</td>
<td>7.52±0.020**</td>
<td>5.37±0.173*</td>
<td>34.9±0.70**</td>
<td>6.98±0.038**</td>
<td>10.6±0.76**</td>
<td>3.63±0.191**</td>
<td>52.7±1.99*</td>
<td>22.8±0.84</td>
<td>29.9±1.89</td>
</tr>
<tr>
<td>(b) Infusion of $\text{NaHCO}_3$ (2.4 mmol/kg body wt.)</td>
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<td></td>
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</tr>
<tr>
<td>0</td>
<td>4</td>
<td>7.45±0.007</td>
<td>5.03±0.160</td>
<td>26.9±0.60</td>
<td>6.76±0.010</td>
<td>6.1±0.12</td>
<td>4.84±0.119</td>
<td>51.4±2.24</td>
<td>21.8±0.51</td>
<td>29.6±1.87</td>
</tr>
<tr>
<td>120 (after start of infusion)</td>
<td>4</td>
<td>7.51±0.010**</td>
<td>5.35±0.198*</td>
<td>34.0±1.88**</td>
<td>6.89±0.037**</td>
<td>8.8±0.96**</td>
<td>4.20±0.273**</td>
<td>53.2±2.37</td>
<td>23.1±0.82</td>
<td>30.1±1.59</td>
</tr>
</tbody>
</table>

### Table 2. Acid–base balance in acute experimental metabolic acidosis

* $P<0.05$, compared with basal values; ** $P<0.01$, compared with basal values. The comparison was carried out by analysis of variance with randomized block design.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>$\text{pH}_e$</th>
<th>$P_{a,\text{CO}_2}$ (kPa)</th>
<th>$[\text{HCO}_3^-]_e$ (mmol/l)</th>
<th>$\text{pH}_i$</th>
<th>$[\text{HCO}_3^-]_i$ (mmol/l)</th>
<th>$[\text{H}^+]_i/[\text{H}^+]_e$</th>
<th>Total body water (% body wt.)</th>
<th>ECF (% body wt.)</th>
<th>ICF (% body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>10</td>
<td>7.45</td>
<td>4.84</td>
<td>26.4</td>
<td>6.74</td>
<td>5.6</td>
<td>5.17</td>
<td>50.9</td>
<td>22.2</td>
</tr>
<tr>
<td>NH$_4$Cl infusion</td>
<td>±0.007</td>
<td>±0.166</td>
<td>±0.65</td>
<td>±0.013</td>
<td>±0.21</td>
<td>±0.135</td>
<td>±1.77</td>
<td>±0.73</td>
<td>±1.29</td>
</tr>
<tr>
<td>After</td>
<td>10</td>
<td>7.34**</td>
<td>3.76**</td>
<td>16.5**</td>
<td>6.70</td>
<td>4.0*</td>
<td>4.39*</td>
<td>51.7**</td>
<td>23.2**</td>
</tr>
<tr>
<td>NH$_4$Cl infusion</td>
<td>±0.025</td>
<td>±0.239</td>
<td>±1.76</td>
<td>±0.021</td>
<td>±0.38</td>
<td>±0.200</td>
<td>±1.70</td>
<td>±0.65</td>
<td>±1.22</td>
</tr>
</tbody>
</table>
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FIG. 1. Correlation between pH₁ and pHₑ in acute alkalosis. The area resulting from the overlapping of normal values (P<0.05) of pH₁ and pHₑ represents the normal population.

FIG. 2. Correlation between pH₁ and pHₑ in acute acidosis. Normal population is reported as in Fig. 1. n.s. = not significant.

\( \text{pH}₁ = 6.469 + 1.782 \text{pHₑ} \\
\text{r} = 0.890 \) (P<0.05)

\( r = 0.65 \) (n.s.)

\( \text{Pa,CO}_₂ 3.55 \pm 0.168 \text{ kPa} \) associated with a severe intracellular acidosis (pH₁ 6.46 \pm 0.036). In all subjects intracellular acidosis appeared more severe than extracellular acidosis (\([\text{H}⁺]\)/[\text{H}⁺]ₑ = 7.32 \pm 0.457). The fall of pH₁ was correlated with the total amount of \( \text{NH}_₄\text{Cl} \) administered (\( r = -0.96 \)). Hydrogen ion balance indicated an accumulation of 16–54 mmol of \( \text{H}⁺/l \) of ICF in 4–8 days, or 4–7 mmol of \( \text{H}⁺/l \) of ICF daily. In contrast with the subjects with acute induced acidosis, pH₁ showed a significant correlation with pHₑ (\( r = +0.87 \)) (Fig. 3) and \([\text{HCO}_₃]ₑ \) (\( r = +0.93 \)). Body fluid compartments were within the normal range of values.

**Uraemic patients**

In this group of patients there was a metabolic acidosis (pHₑ 7.34 ± 0.031, \( \text{Pa,CO}_₂ 3.79 ± 0.232 \text{ kPa}, [\text{HCO}_₃]ₑ 16.5 ± 1.54 \text{ mmol} \)). The mean value of pH₁ (6.74 ± 0.030) was not different from the value of the control group as shown by the analysis of variance. In three patients, however, a lower pH₁ (6.57, 6.58 and 6.59) was observed. Although such values were below the mean value of the control group, on the basis of statistical evaluation they cannot be considered a separate group. An accumulation of 0.7–0.9 mmol of \( \text{H}⁺/l \) of ICF daily was calculated.

Fluid compartments showed a significant increase in total body water (56.8 ± 2.04% body weight) as well as in ECF (29.2 ± 2.16% body weight), whereas ICF decreased (27.6 ± 1.12% body weight). Plasma Na⁺ values ranged from 127.1 to 149.8 mmol/l, K⁺ from 2.6 to 5.8 mmol/l, Cl⁻ from 97.3 to 108.3 mmol/l. No significant correlation was found by plotting pH₁ against pHₑ (Fig. 4) or \([\text{HCO}_₃]ₑ \), even if the distribution of most values seems to suggest a tendency towards a linear relationship. Furthermore no correlation was detected between pH₁ and blood urea. The \( \text{Pa,CO}_₂ \), which in these patients is an index of alveolar ventilation, was significantly correlated to pH₁ (Fig. 5a), whereas it seemed not to depend on pHₑ (Fig. 5b).

In the uraemic patients, who were given NaHCO₃ intravenously (Table 4a), extracellular acidosis was partially corrected (pHₑ 7.40 ± 0.022, \([\text{HCO}_₃]ₑ 19.3 ± 2.15 \text{ mmol} /l, \text{Pa,CO}_₂ 3.96 ± 0.498 \text{ kPa} \). There was no effect on pH₁ (6.72 ± 0.044). Haemodialysis (Table 4b) corrected extracellular acidosis, in some cases to an excessive degree, owing to the persistent hypocapnia (2.50 h after the end of dialysis: pHₑ 7.48 ± 0.017, \( \text{Pa,CO}_₂ 4.34 ± 0.291 \text{ kPa), [HCO}_₃]ₑ 25.5 ± 1.64 \text{ mmol/l} \). At the end of the dialysis,
**Table 3. Acid-base balance in chronic experimental metabolic acidosis**

**P < 0.01**, compared with control. The comparison was carried out by analysis of variance with a completely randomized design.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>pH_e</th>
<th>$P_{\text{a}CO_2}$ (kPa)</th>
<th>$[\text{HCO}_3^-]_e$ (mmol/l)</th>
<th>pH_t</th>
<th>$[\text{HCO}_3^-]_t$ (mmol/l)</th>
<th>$[\text{H}^+/\text{H}^+]_e$ (nmol/l)</th>
<th>Total body water (%) body wt.</th>
<th>ECF (% body wt.)</th>
<th>ICF (% body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>7.43 ± 0.005</td>
<td>5.13 ± 0.063</td>
<td>26.6 ± 0.22</td>
<td>6.74 ± 0.009</td>
<td>5.8 ± 0.11</td>
<td>4.98 ± 0.106</td>
<td>52.7 ± 1.29</td>
<td>22.4 ± 0.40</td>
</tr>
<tr>
<td>Chronically acidotic subjects</td>
<td>12</td>
<td>7.32 ± 0.018**</td>
<td>3.55 ± 0.168**</td>
<td>14.7 ± 1.09**</td>
<td>6.46 ± 0.036**</td>
<td>2.4 ± 0.30**</td>
<td>7.32 ± 0.457**</td>
<td>53.6 ± 1.38</td>
<td>23.3 ± 0.60</td>
</tr>
</tbody>
</table>

**Table 4. Acid-base balance in uraemic patients before and after treatment**

*P < 0.05 compared with basal values. The comparison was carried out by analysis of variance with a randomized block design.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No. of cases</th>
<th>pH_e</th>
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<th>$[\text{HCO}_3^-]_t$ (mmol/l)</th>
<th>$[\text{H}^+/\text{H}^+]_e$ (nmol/l)</th>
<th>Total body water (%) body wt.</th>
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<tr>
<td>(a) Infusion of NaHCO₃ (4.8 mmol/kg body wt.)</td>
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<td></td>
</tr>
<tr>
<td>0 (before start of infusion)</td>
<td>4</td>
<td>7.22 ± 0.038</td>
<td>3.46 ± 0.514</td>
<td>11.2 ± 2.23</td>
<td>6.72 ± 0.044</td>
<td>4.0 ± 0.86</td>
<td>3.20 ± 0.696</td>
<td>56.6 ± 3.09</td>
<td>26.9 ± 1.46</td>
<td>29.7 ± 2.48</td>
</tr>
<tr>
<td>120 (after start of infusion)</td>
<td>4</td>
<td>7.40 ± 0.022*</td>
<td>3.96 ± 0.498</td>
<td>19.3 ± 2.15*</td>
<td>6.77 ± 0.059</td>
<td>5.2 ± 1.27</td>
<td>4.38 ± 0.572</td>
<td>57.3 ± 3.09</td>
<td>28.0 ± 1.44*</td>
<td>29.3 ± 2.36</td>
</tr>
<tr>
<td>(b) Haemodialysis (8 h against acetate bath)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 (before haemodialysis)</td>
<td>7</td>
<td>7.38 ± 0.028</td>
<td>4.03 ± 0.334</td>
<td>18.7 ± 1.84</td>
<td>6.76 ± 0.050</td>
<td>5.2 ± 0.80</td>
<td>4.39 ± 0.636</td>
<td>55.7 ± 2.16</td>
<td>27.0 ± 2.08</td>
<td>28.7 ± 1.40</td>
</tr>
<tr>
<td>150 (after the end)</td>
<td>7</td>
<td>7.48 ± 0.017*</td>
<td>4.34 ± 0.291</td>
<td>25.5 ± 1.64*</td>
<td>6.83 ± 0.028</td>
<td>6.2 ± 0.63</td>
<td>4.60 ± 0.327</td>
<td>53.0 ± 1.64</td>
<td>24.6 ± 1.46</td>
<td>28.4 ± 0.99</td>
</tr>
<tr>
<td>300 (after the end)</td>
<td>4</td>
<td>7.49 ± 0.543*</td>
<td>3.92 ± 0.690</td>
<td>23.4 ± 3.37*</td>
<td>6.83 ± 0.010</td>
<td>5.6 ± 1.08</td>
<td>4.71 ± 0.696</td>
<td>55.1 ± 0.82</td>
<td>24.8 ± 1.58</td>
<td>30.3 ± 0.76</td>
</tr>
</tbody>
</table>
pH$_1$ (6.83 ± 0.028) was not significantly different from the mean value obtained in the same group before haemodialysis (6.76 ± 0.050). No significant change in these data was observed 5 h after the end of haemodialysis. Haemodialysis restored pH$_1$ to the normal range in the three patients who had lower values before treatment.

**Discussion**

Whole-body pH$_1$ measured by the DMO method may be considered as a volume-weighted mean pH of the intracellular compartments, which differ in pH within individual cells and from organ to organ.

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**Fig. 3.** Correlation between pH$_1$ and pH$_e$ in chronic experimental acidosis. Normal population is reported as in Fig. 1.

**Fig. 4.** Correlation between pH$_1$ and pH$_e$ in uraemic patients with acidosis. Normal population is reported as in Fig. 1. n.s. = not significant.

**Fig. 5.** Correlation between (a) $P_{a, CO_2}$ and pH$_1$ and (b) $P_{a, CO_2}$ and pH$_e$ in uraemic patients with acidosis. n.s. = not significant.
The mean value of $\text{pH}_1$ obtained in normal subjects studied here ($\text{pH}_1 = 6.74$) is lower than the values reported by other authors who also used the DMO method in man (Robin et al., 1961a; Manfredi, 1963; Grantham & Schloerb, 1964; Lambie, Anderton, Cowie, Simpson, Tothill & Robson, 1965; Acquarone, Bertocchi, Bottino, De Ferrari, Faravelli, Gurreri & Sardini, 1970; Acquarone, Bertocchi, De Ferrari, Fresco, Gurreri, Menozzi & Tizianello, 1970). The difference seems to be related to the measurements of ECF by $^{36}\text{Cl}$. In fact, if data of DMO distribution obtained in our control group are related to values of ECF measured in normal subjects studied here ($\text{pH}_1 = 6.74$) is lower than the distribution obtained with different experimental models (Waddell & Butler, 1959; Kibler, O’Neil & Robin, 1964; Adler, Roy & Relman, 1965a, b; Irvine & Dow, 1966; Cohen, Simpson, Goodwin & Strunin, 1967; Burnell, 1968; Adler, 1972), confirm an increased $\text{pH}_1$ when, keeping $\text{Pa}_\text{CO}_2$ within a physiological range, $[\text{HCO}_3^-]$ is raised at least 8–9 mmol/l and when measurements are performed after equilibrium has been reached. In different situations, when $\text{Pa}_\text{CO}_2$ is markedly increased after bicarbonate infusion (Wallace & Hastings, 1942; Brown & Goott, 1963; Robin, 1963), or when $[\text{HCO}_3^-]$ is raised slightly (Manfredi, 1963), $\text{pH}_1$ shows no significant variations. In summary, cells appear capable of preserving a steady $\text{pH}_1$ only when there is a small increase in $[\text{HCO}_3^-]$. Beyond a certain degree of extracellular alkalosis (in acute metabolic alkalosis beyond approximately 30 mmol of $[\text{HCO}_3^-]/l$ with a normal $\text{Pa}_\text{CO}_2$), $\text{pH}_1$ changes proportionally to $[\text{HCO}_3^-]$.

Our findings in acute acidosis in normal subjects are consistent with those of other authors, who studied muscular tissues and whole animals (Waddell & Butler, 1959; Brown & Goott, 1963; Robin, 1963; Adler et al., 1965b, c; Burnell, 1968; Adler, 1972). In our study no significant change of $\text{pH}_1$ was obtained by lowering $\text{pH}_1$, to 7.24 or $[\text{HCO}_3^-]$, to 8.8 mmol/l. Only a more severe metabolic acidosis could induce a fall of $\text{pH}_1$. This can be compared with a critical figure of $\text{pH}_1$, 6.90 in isolated rat diaphragm (Adler et al., 1965b, c; Adler, 1972), $\text{pH}_1$, 7.17 in dog muscle (Waddell & Butler, 1959; Brown & Goott, 1963; Burnell, 1968) or $\text{pH}_1$, 7.20 in whole dog (Robin, 1963). However, even in the presence of such severe extracellular acidosis, $[\text{H}^+]$ increased only slightly and always to a lesser extent than the increase in $[\text{H}^+]$, as shown by the drop of $[\text{H}^+]/[\text{H}^+]$. In experiments with acute acid-loads, a large amount of infused protons was titrated by extracellular bicarbonate. However, this mechanism, by itself, cannot account for normal values of $\text{pH}_1$, measured in such a condition where an accumulation of 1–9 mmol of $\text{H}^+/l$ of ICF occurs. Thereby normal $\text{pH}_1$ must also be explained by tissue buffering and/or by metabolic factors (Adler et al., 1965a; Hudson, 1966).

Markedly different findings were obtained in chronic metabolic acidosis. Respiratory and renal compensation kept $\text{pH}_1$ and $[\text{HCO}_3^-]$ in
TABLE 5. Acid-base balance in experimental metabolic acidosis and in uraemia

**P<0.01**, compared with acute experimental acidosis and with chronic uraemia. The comparison was carried out by analysis of variance with a randomized block design.

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>pH_1</th>
<th>P_aCO_2 (kPa)</th>
<th>[HCO_3^-]_1</th>
<th>pH_1 Titr. role of soft tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental acidosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>10</td>
<td>7.34±0.025</td>
<td>3.76±0.239</td>
<td>16.5±1.76</td>
<td>6.70±0.021 (+)</td>
</tr>
<tr>
<td>Chronic</td>
<td>12</td>
<td>7.32±0.018</td>
<td>3.55±0.168</td>
<td>14.7±1.09</td>
<td>6.46±0.036** (+ + +)</td>
</tr>
<tr>
<td>Chronic uraemia</td>
<td>13</td>
<td>7.34±0.031</td>
<td>3.79±0.232</td>
<td>16.5±1.54</td>
<td>6.74±0.030 (+)</td>
</tr>
</tbody>
</table>

the same range as that in acute induced acidosis (Table 5) in spite of the larger load of H^+, whereas pH_1 decreased significantly, owing to the exhaustion of tissue reserves. When uraemic patients and chronic NH_4Cl-loaded subjects are compared, extracellular acid-base values overlap. However, intracellular composition shows important differences with a normal pH_1 in uraemic patients. In these patients the amount of H^+ daily moving into compartments not accessible to ECF tracers is lower than that in subjects with chronic induced metabolic acidosis, and this may account for the differences observed between these two groups of metabolic acidosis. Normal values of pH_1 in uraemic patients support the conclusions presented by Lemann, Litzow & Lennon (1966), who outlined the presence of a large store of potential alkali acting at a slow rate and able to buffer about 25 mmol of H^+ daily. When larger loads of acid come into play, as in the experiments here reported with oral NH_4Cl administration, bone titration capacity is overwhelmed and pH_1 drops. The normal mean value of pH_1 in chronic renal disease with uraemia is largely supported by the literature (Bittar, Watt, Pateras & Parrish, 1962; Lambie et al., 1965; Maschio, Bertaglia, Sardini, Bazzato, Riz, Gambaro & Mioni, 1968). Only a few cases showed lower pH_1 (Bittar et al., 1962: one case; Lambie et al., 1965: three cases; Maschio et al., 1968: one case). Three patients within the group reported here had a lower pH_1; in all of them marked hypokalaemia and electrocardiographic signs of K^+ depletion were present. Muscle K^+ depletion (Lemann et al., 1966) may therefore play a major role in lowering pH_1 in chronic uraemia. A direct correlation between K^+ content in skeletal muscle and pH_1 has been found (Maschio et al., 1968).

In uraemic patients P_aCO_2 is directly correlated with pH_1 (Fig. 5a). Such correlation probably outlines a relationship between whole-body pH_1 and the pH of the respiratory centre: when the respiratory centre pH decreases, there is a greater stimulation of the alveolar ventilation. This hypothesis seems to be confirmed by the response to an alkalizing treatment (bicarbonate administration or haemodialysis) observed in these patients. The lower the initial pH_1, the greater the increase in P_aCO_2 after treatment where pH_1 becomes normal.

Our results support the concept of different tissue buffer stores coming into play when [HCO_3^-]_1 is reduced and acting as 'readily available' and 'slow rate' buffers. The different pH_1 values observed in acute alkalosis and acidosis indicate that systems of cellular protection are more effective in acidosis than in alkalosis. When acid-loads overwhelm such systems, intracellular proton concentration becomes a function of extracellular hydrogen ion content.

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