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Analysis of the Metabolic Changes in pH, $[HCO_3^-]$ and Pco_2 in Blood Plasma at Steady State *in vivo*

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ABSTRACT

Previously we analysed $[HCO_3^-]$ in blood plasma, by dividing it into a respiratory component, $[HCO_3^-]^*$, and a metabolic component, $[HCO_3^-]^o$. Assuming $[HCO_3^-]^o$ to be independent of Pco_2, an exponential function of Pco_2 was obtained for $[HCO_3^-]^*$. More recently, we found, through regression analysis of the difference in pH from 7.4, that the metabolic component of pH−7.4, designated by pH−pH*, could be evaluated by using the ratio $[HCO_3^-]^o/[HCO_3^-]^*$, and that this ratio, not the value for $[HCO_3^-]^o$, was independent of Pco_2. $[HCO_3^-]^o$ at pH = 7.4, designated by $s[HCO_3^-]^o$, was calculated to test the validity of $[HCO_3^-]^*$, the regression functions of $[HCO_3^-]^o$ and $s[HCO_3^-]^o$ in venous blood, against those in arterial blood, were then calculated. Both the correlation and regression coefficients were close to unity, though the regression coefficient of $[HCO_3^-]^o$ was slightly higher than that of $s[HCO_3^-]^o$. Further, in arterial and venous blood, the regression lines of pH and Pco_2 against $s[HCO_3^-]^o$ were obtained in a number of acidotic and alkalotic patients, and the change in Pco_2, compensating for pH, was quantified. Designating $[H^+]$ in normal blood, where $s[HCO_3^-]^o = 0$, by $[H^+]^*$, the ratio $[H^+]/[H^+]^*$ was obtained in a number of anemic patients to check the effect of the hemoglobin concentration [Hb] on the metabolic change in pH. $[H^+]$ agreed well with $[H^+]^*$, and no significant effect of [Hb] on the metabolic change in pH was found.

Key words : Acid-base imbalance, Components of $[HCO_3^-]$, Change in pH, Metabolic change in Pco_2, Hemoglobin concentration.

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INTRODUCTION

The bicarbonate concentration $[HCO_3^-]$ in blood plasma at steady state in vivo has a respiratory component $[HCO_3^-]^*$ and a metabolic component $[HCO_3^-]^o$[1,2]. Through regression analysis of $[HCO_3^-]$ in simultaneously sampled arterial and venous blood, we found that when $[HCO_3^-]^*$ was given by a specific exponential function of Pco_2, $[HCO_3^-]^o$ became independent of Pco_2. Recently, we have analysed the difference of pH from 7.4, i.e., pH -7.4 by dividing it into a respiratory component, designated by $pH^*-7.4$, and a metabolic component, designated by $pH-pH^*$[3].

$[HCO_3^-]^*$ represents $[HCO_3^-]$ in normal plasma, where $[HCO_3^-]^o = 0$, and is calculated by setting the measured Pco_2 in the known function for $[HCO_3^-]^*$. Hence, $[H^+]$ in normal plasma ($[H^+]^*$) was easily obtained by setting $[HCO_3^-]^*$ in the Henderson equation[4]. Since $[H^+]$ at pH = 7.4 is 39.81 nEq, $pH^*-7.4$ was obtained from the ratio $[H^+]^*/39.81$. By subtracting $pH^*-7.4$ from $pH-7.4$, $pH-pH^*$ was readily obtained. Additionally, since $pH-pH^*$ is equal to $\log([H^+]^*/[H^+])$, the ratio $[H^+]^*/[H^+]$ was given by setting $[HCO_3^-]/[HCO_3^-]^*$ $(= 1 + [HCO_3^-]^o/[HCO_3^-]^*)$ in the Henderson equation. Essentially, $pH-pH^*$ and $[HCO_3^-]/[HCO_3^-]^*$ depended on the the concentrations of fixed acids and bases and were independent of Pco_2.

The total concentration of buffer bases in blood remains unchanged despite a change in Pco_2, since the change in $[HCO_3^-]$ caused by CO_2 reactions is counterbalanced by the opposite change in concentration of conjugate bases of buffer proteins.[5,6,7] Thus, when the equation for $[HCO_3^-]^*$ was derived, it was considered that $[HCO_3^-]^o$ remained unchanged regardless of the change in Pco_2, as far as the concentrations of fixed acids and bases remained constant. According to the Henderson equation, when $[H^+]^*$ and $[HCO_3^-]^*$ are calculated by using the same Pco_2 as measured in the sampled blood, the Pco_2 is eliminated from ratios $[H^+]^*/[H^+]$ and $[HCO_3^-]/[HCO_3^-]^*$, and they become equal, irrespective of the Pco_2 used for the calculation. Since $[HCO_3^-]$ is the sum of $[HCO_3^-]^*$ and $[HCO_3^-]^o$, the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ also becomes independent of the Pco_2. Thus, it was recognized that the value for the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ at any Pco_2 could be replaced by the ratio calculated at a specific Pco_2. When $pH^* = 7.4$, Pco_2 became 42.67 mmHg and $[HCO_3^-]^*$ was 26.2 mEq. Designating $[HCO_3^-]^o$ at $Pco_2 = 42.67$ mmHg by $s[HCO_3^-]^o$, $s[HCO_3^-]^o$ could be calculated by multiplying the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ by 26.2. The same value for $s[HCO_3^-]^o$ was calculated from the value for $pH-pH^*$, which was given by $\log(1+ s[HCO_3^-]^o/26.2)$.

To examine whether the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ was independent of Pco_2 or not, we have measured $[HCO_3^-]$ and $[HCO_3^-]^*$ in simultaneously sampled arterial and venous blood, and calculated the regression functions of $[HCO_3^-]^o$ and $s[HCO_3^-]^o$ in venous blood against those in arterial blood. Both the correlation and regression coefficients of $[HCO_3^-]^o$ and $s[HCO_3^-]^o$ were very close to unity, demonstrating that the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ was independent of Pco_2 and that the equation for $[HCO_3^-]^*$ was justified.

The base excess has mainly been evaluated by using the buffer value, or the slope of $[HCO_3^-]$ to pH obtained in normal blood *in vitro*[7]. However, this value is greatly different

from that obtained in blood at steady state *in vivo* [8,9,10]. Furthermore, even though the base excess would be evaluated, the value for pH − pH* could not be quantified theoretically. Thus, to develop a precise new method for evaluating the metabolic change in $[HCO_3^-]$, we measured the regression functions of pH and Pco_2 against $s[HCO_3^-]^o$ in arterial and venous blood sampled from a number of acidotic and alkalotic patients, and investigated the compensatory effect of Pco_2 on the metabolic change in pH. Since the metabolic change in pH has been considered to depend on the hemoglobin concentration ([Hb]), the relationship between $s[HCO_3^-]^o$ and [Hb] was also tested through regression analysis of $[H^+]$ against $[H^+]^*$ in anemic patients.

THEORY —— The acid - base status at steady state *in vivo*.

As reported previously, $[HCO_3^-]$ at steady state in vivo has a Pco_2-dependent respiratory component, $[HCO_3^-]^*$ and a metabolic component, $[HCO_3^-]^o$. The former component was given by[1]

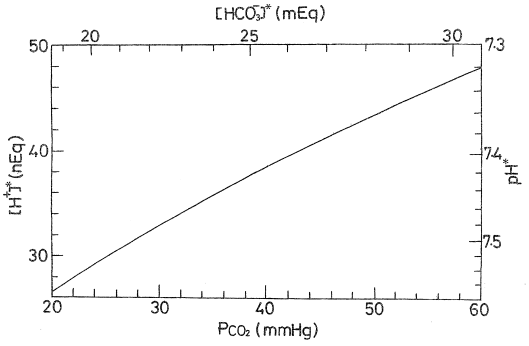

Fig. 1.
$[H^+]^*$ and pH* plotted against Pco_2 and $[HCO_3^-]^*$ in blood plasma. (Calculated by using Eqs. (1) and (2)).

$$[HCO_3^-]^* = 4.717\ Pco_2^{0.457}, \text{(mEq)}. \quad (1)$$

If, at the normal state, where $[HCO_3^-]^o$ is ignored, $[H^+]$ is designated by $[H^+]^*$, this is expressed from the Henderson equation as follows[4]:

$$[H^+]^* = 24.465\ Pco_2/[HCO_3^-]^*, \text{(nEq)}. \quad (2)$$

Designating − log $[H^+]^*$ by pH*, the relationship between Pco_2, $[HCO_3^-]^*$, $[H^+]^*$ and pH* is easily calculated as shown in Fig.1. $[H^+]^*$ increases and pH* decreases with an increase in Pco_2. Setting the measured Pco_2 value in Eq. (2), the ratio of $[H^+]^*$ to the measured $[H^+]$ is given from the Henderson equation by

$$[H^+]^*/[H^+] = [HCO_3^-]/[HCO_3^-]^*. \quad (3)$$

Since $[HCO_3^-]$ is the sum of $[HCO_3^-]^*$ and $[HCO_3^-]^o$, the ratio of Eq. (3) is rewritten as

$$[H^+]^*/[H^+] = 1 + [HCO_3^-]^o/[HCO_3^-]^*. \quad (4)$$

The above equation holds true regardless of the measured Pco_2. When Pco_2 varies, $[HCO_3^-]^o$ changes together with $[HCO_3^-]^*$, maintaining the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ at a constant level defined by the concentration of fixed acids and bases. When Eq. (1) for $[HCO_3^-]^*$ was elaborated, $[HCO_3^-]^o$, not the ratio $[HCO_3^-]^o/[HCO_3^-]^*$, was assumed to be independent of Pco_2. Thus, it became necessary to show in measured data that $[HCO_3^-]^o$ is proportional to $[HCO_3^-]^*$ and to clarify the validity of Eq. (1).

As shown in Fig. 1, $[HCO_3^-]^*$ at Pco_2 = 42.67 mmHg obtained from Eq. (1) is 26.2 mEq and pH* calculated from Eq. (2) is 7.4. Hence,

designating $[HCO_3^-]^o$ at pH = 7.4 by $s[HCO_3^-]^o$, $[H^+]^*/[H^+]$ of Eq. (4) is replaced by the following equation:

$$[H^+]^*/[H^+] = 1 + s[HCO_3^-]^o/26.2. \quad (5)$$

Since $[HCO_3^-]^*$ is calculated using measured Pco_2, and $[HCO_3^-]^o$ is readily obtained by subtracting $[HCO_3^-]^*$ from $[HCO_3^-]$, $s[HCO_3^-]^o$ is easily obtained from $[HCO_3^-]^o$ and $[HCO_3^-]^*$ as follows:

$$s[HCO_3^-]^o = 26.2\ [HCO_3^-]^o/[HCO_3^-]^*, \text{ (mEq)}. \quad (6)$$

Thus, the theoretical evidence that $[HCO_3^-]^o/[HCO_3^-]^*$ is independent of Pco_2 was examined by regression analysis of $[HCO_3^-]^o$ and $s[HCO_3^-]^o$ in venous blood against the same parameters in arterial blood.

Taking the logarithm of both sides of Eq. (5), the following equation is derived:

$$pH - pH^* = \log(1 + s[HCO_3^-]^o/26.2). \quad (7)$$

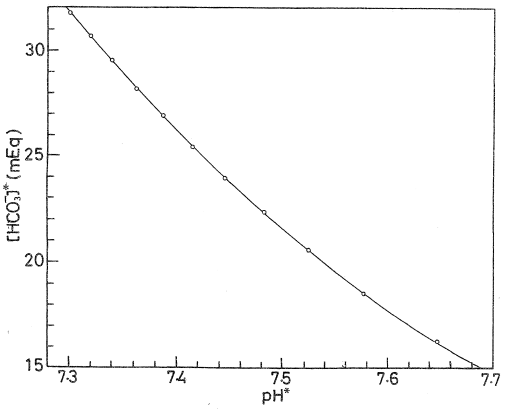

Fig. 2.
$[HCO_3^-]^*$ plotted against pH* calculated by using Eqs. (1) and (8).

As described previously[1], pH* is given from Eqs. (1) and (2) by

$$pH^* = 8.285 - 0.543 \log Pco_2. \quad (8)$$

Thus, Pco_2 is calculated pecisely from pH and $s[HCO_3^-]^o$ using Eqs. (7) and (8). Fig. 2 shows $[HCO_3^-]^*$ plotted against pH* obtained from Eqs. (8) and (1). $[HCO_3^-]^*$ of Fig. 2 was given approximately by the following equation:

Table 1. Summarized data for pH, Pco_2, $[HCO_3^-]$ and concentrations of other constituent ions (mEq) in simultaneously sampled arterial and venous blood of 56 elderly patients.

Samples and patients		
	male	female
No of samples	25	61
No of subjects	16	40
Mean age ± SD	83.5 ± 7.1	84.2 ± 6.3
Measured data		
Parameters	Arterial blood	Venous blood
pH	7.446 ± 0.044	7.410 ± 0.048
Pco_2 (mmHg)	37.91 ± 5.41	44.39 ± 6.80
$[Na^+]$	135.43 ± 6.2	136.61 ± 6.94
$[K^+]$	3.47 ± 0.50	3.51 ± 0.48
$[Cl^-]$	100.20 ± 4.55	99.84 ± 4.78
Parameters obtained from measured data of pH, Pco_2 and constituent ions		
$[HCO_3^-]$	26.13 ± 4.64	27.97 ± 4.73
$[HCO_3^-]^*$	24.78 ± 1.56	26.62 ± 1.85
$[HCO_3^-]^o$	1.30 ± 3.67	1.33 ± 3.48
$s[HCO_3^-]^o$	1.28 ± 3.37	1.23 ± 3.33
pH − pH*	0.018 ± 0.053	0.017 ± 0.051
$[H_2O](\%)$	100.9 ± 4.1	100.8 ± 3.8

$[H_2O]$ is the percentage of the water content in plasma to that at the standard state, where Pco_2 is 41.01 mmHg and $[HCO_3^-]^o = 0$[8].

$$[HCO_3^-]^* = 26.2 - 51.5\,(pH^* - 7.4) + 47.3\,(pH^* - 7.4)^2, \text{(mEq)}. \quad (9)$$

The above equation will be applied for obtaining $[HCO_3^-]^*$ at any pH* levels.

METHODS

The proportional relationship between $[HCO_3^-]^o$ and $[HCO_3^-]^*$, and a change in Pco_2 compensating for the metabolic change in pH were tested on blood sampled from a total of 347 elderly patients (Tables 1, 2, 3 and 4). Their ages ranged from 64 to 98 with a mean ± SD of 83.5 ± 6.82. In 56 patients arterial and venous blood was sampled simultaneously, as shown in Table 1. An acidotic group, where pH−pH* was less than −0.02, an alkalotic group, where pH−pH* was greater than 0.02, and a normal group were selected from 182 patients. Summarized data for pH, Pco_2, $[HCO_3^-]$ and other constituent ions (mEq) in arterial blood and venous blood are tabulated in Tables 2 and 3, respectively. An anemic group (32 patients), with a hemoglobin concentration [Hb] of less than 9 g/dl and a

Table 2. Summarized data for pH, Pco_2, $[HCO_3^-]$ and concentrations of other constituent ions (mEq) in arterial blood sampled from 85 acidotic, normal and alkalotic elderly patients.

	Acidotic group		Normal pH group		Alkalotic group	
Samples and patients						
	male	female	male	female	male	female
No of samples	8	21	13	24	10	32
No of subjects	8	21	9	19	6	22
Mean age ± SD	85.5 ± 5.9	84.6 ±6.2	81.2 ± 7.9	83.4 ±5.2	86.7 ± 3.9	83.7 ± 5.8
Measured data						
pH	7.404 ± 0.028		7.438 ± 0.025		7.495 ± 0.045	
Pco_2 (mmHg)	32.48 ± 4.61		36.72 ± 3.70		40.72 ± 6.16	
$[Na^+]$	133.0 ± 8.57		135.7 ± 6.03		134.3 ± 7.30	
$[K^+]$	4.01 ± 0.68		3.58 ± 0.30		3.13 ± 0.54	
$[Cl^-]$	100.3 ± 5.66		101.4 ± 4.19		96.3 ± 5.23	
[Hb] (g/dl)	12.4 ± 1.79		13.6 ± 2.03		13.6 ± 2.51	
Parameters obtained from measured data of pH, Pco_2 and constituent ions						
$[HCO_3^-]$	20.13 ± 2.75		24.53 ± 1.33		31.13 ± 4.61	
$[HCO_3^-]^*$	23.09 ± 1.56		24.45 ± 1.11		25.59 ± 1.75	
$[HCO_3^-]^o$	−2.96 ± 1.49		0.08 ± 0.64		5.54 ± 3.48	
$s[HCO_3^-]^o$	−3.45 ± 1.89		0.06 ± 0.65		5.57 ± 3.27	
pH−pH*	−0.063 ± 0.037		0.001 ± 0.011		0.082 ± 0.043	
pH*	7.467 ± 0.037		7.437 ± 0.023		7.414 ± 0.035	
$[H_2O]$ (%)	104.3 ± 6.0		101.1 ± 3.9		100.7 ± 4.8	

control group with normal [Hb] (77 patients) were selected, as shown in Table 4.

Blood gas and the ionic concentrations were measured using a blood gas analyser (Ciba Corning 188). $[HCO_3^-]$ was calculated from $[H^+]$ and Pco_2 using the Henderson equation. The anion gap concentration [AG] was obtained by subtracting $[HCO_3^-]$ and $[Cl^-]$ from the sum of $[Na^+]$ and $[K^+]$. $[HCO_3^-]$ was divided into a respiratory component $[HCO_3^-]^*$ and a metabolic component $[HCO_3^-]^o$. Setting Pco_2 in Eq. (1), $[HCO_3^-]^*$ was calculated and $[HCO_3^-]^o$ was obtained by subtracting $[HCO_3^-]^*$ from $[HCO_3^-]$, subsequently, $s[HCO_3^-]^o$ was obtained from Eq. (6).

Using the measured values for $[H^+]$ and $[H^+]^*$ of Eq. (2), $s[HCO_3^-]^o$ was obtained from Eq. (5), as follows:

$$s[HCO_3^-]^o = 26.2\ ([H^+]^*/[H^+]-1),\ (mEq). \quad (10)$$

The agreement of the values for $s[HCO_3^-]^o$ given by Eqs. (6) and (10) was confirmed in each blood sample.

After calculating $s[HCO_3^-]^o$, the regression functions of $[HCO_3^-]^o$ and $s[HCO_3^-]^o$ in venous blood, against those in arterial blood, were calculated to check the validity of $[HCO_3^-]^*$ and to clarify whether the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ was independent of Pco_2. Furthermore, the relationship between the change in Pco_2 and

Table 3. Summarized data for pH, Pco_2, $[HCO_3^-]$ and concentration of other constituent ions (mEq) in venous blood from 97 acidotic, normal and alkalotic elderly patients.

	Acidotic group		Normal pH group		Alkaloticgroup	
Samples and patients						
	male	female	male	female	male	female
No of samples	21	28	20	41	10	29
No of subjects	17	19	14	32	4	11
Mean age ± SD	83,5 ± 5.7	85.2 ± 7.5	82.5 ± 7.1	84.0 ± 6.4	90.0 ± 1.7	82.5 ± 5.4
Measured data						
pH	7.341 ± 0.054		7.398 ± 0.036		7.456 ± 0.032	
Pco2 (mmHg)	39.30 ± 9.74		42.76 ± 7.42		48.77 ± 7.72	
$[Na^+]$	137.6 ± 6.91		136.6 ± 4.90		138.2 ± 7.66	
$[K^+]$	4.28 ± 0.61		3.64 ± 0.35		3.02 ± 0.44	
$[Cl^-]$	102.5 ± 5.3		101.0 ± 3.83		96.5 ± 4.26	
[Hb] (g/dl)	12.4 ± 1.84		13.3 ± 2.02		13.6 ± 2.29	
Parameters obtained from measured data of pH, Pco_2 and constituent ions						
$[HCO_3^-]$	21.24 ± 3.23		26.42 ± 2.13		34.29 ± 4.40	
$[HCO_3^-]^*$	25.31 ± 2.56		26.37 ± 1.57		27.84 ± 2.00	
$[HCO_3^-]^o$	−4.13 ± 1.63		0.07 ± 1.32		6.43 ± 2.78	
$s[HCO_3^-]^o$	−4.33 ± 1.83		−0.01 ± 1.37		5.98 ± 2.25	
pH−pH*	−0.080 ± 0.039		0.001 ± 0.022		0.088 ± 0.030	
pH*	7.422 ± 0.053		7.399 ± 0.031		7.371 ± 0.037	
$[H_2O]$ (%)	102.6 ± 6.0		101.0 ± 0.031		98.8 ± 0.048	

the metabolic change in pH was examined by analysing the regression functions of pH, Pco_2 and pH* against $s[HCO_3^-]^o$ in arterial and venous blood from the patients shown in Tables 2 and 3. The influence of the hemoglobin concentration on the metabolic change in $[H^+]$ was then tested by obtaining the relationship between $[H^+]$ and $[H^+]^*$ in the patients shown in Table 4.

The water content $[H_2O]$ in plasma (%) was obtained by using the method reported in a previous paper [8]. In the normal blood plasma, where $[HCO_3^-]^* = 25{,}75$ mEq and $[HCO_3^-]^o = 0$, the concentration difference between $[Na^+]$ and $[Cl^-]$ was about 36.2 mEq. Thus, taking the water content of normal plasma to be 100%, and designating the concentrations for $[Na^+]$ and $[Cl^-]$, depending mainly on $[H_2O]$, by $[Na^+]''$ and $[Cl^-]''$, respectively, $[H_2O]$ was given by the following equation:

$$[H_2O] = 100/(1 + ([Na^+]'' - [Cl^-]'')/36.2), (\%). \quad (11)$$

$[Na^+]''$ and $[Cl^-]''$ were evaluated by excluding the influence of the changes in $[HCO_3^-]^*$ and

Table 4. Summarized data for [Hb], pH, Pco_2, $[HCO_3^-]$ and concentration of other constituent ions (mEq) in 32 anemic and 77 normal elderly patients.

	Anemic group		Normal group	
Samples and patients				
	male	female	male	female
No of samples	18	60	22	55
No of subjects	8	24	22	55
Mean age ± SD	82.4 ± 6.7	83.8 ± 7.6	81.1 ± 6.9	82.4 ± 6.3
Measured data				
[Hb] (g/dl)	7.65 ± 1.01		13.78 ± 1.54	
pH	7.421 ± 0.044		7.393 ± 0.035	
Pco_2 (mmHg)	40.39 ± 5.69		44.55 ± 6.43	
$[Na^+]$	133.92 ± 5.43		139.32 ± 3.18	
$[K^+]$	3.82 ± 0.69		3.80 ± 0.37	
$[Cl^-]$	98.08 ± 4.72		102.04 ± 2.33	
Parameters obtained from measured data of pH, Pco_2 and constituent ions				
$[HCO_3^-]$	25.88 ± 2.53		26.74 ± 1.81	
$[HCO_3^-]^*$	25.51 ± 1.63		26.70 ± 1.74	
$[HCO_3^-]^o$	0.38 ± 1.90		0.04 ± 0.45	
$s[HCO_3^-]^o$	0.38 ± 1.89		0.03 ± 0.44	
$[H^+]$(nEq)	38.38 ± 3.94		40.65 ± 3.21	
$[H^+]^*$(nEq)	38.66 ± 2.95		40.61 ± 3.24	
$[H^+]^* - [H^+]$(nEq)	0.28 ± 2.71		0.04 ± 0.62	
$[H_2O]$ (%)	102.9 ± 3.6		99.8 ± 2.0	

$[HCO_3^-]^o$ from their measured values. The respiratory and metabolic components of $[HCO_3^-]$, $[H^+]$ and pH were calculated through an Excel computer program on a DELL personal computer. The regression functions were calculated using Kaleid Graph Software (Synergy).

RESULTS

1) The regression coefficients of $[HCO_3^-]^o$ and $s[HCO_3^-]^o$ in venous blood against those in arterial blood.

In the arterial and venous blood samples shown in Table 1, values for $[HCO_3^-]^o$ were distributed between − 5.94 and 14.1 (mEq). The mean ± SD of $[HCO_3^-]^o$ in arterial blood was 1.30 ± 3.67 mEq and in venous blood 1.33 ± 3.48 mEq. In Fig. 3 values for $[HCO_3^-]^o$ obtained in venous blood are plotted against those in arterial blood. The correlation coefficient of 0.993 and the regression coefficient of 1.028 indicate that $[HCO_3^-]^o$ in venous blood was almost equal to those in arterial blood, though there is a difference in Pco_2 between arterial and venous blood. The correlation and regression coefficients of Fig. 3 make it clear that $[HCO_3^-]^o$ is almost independent of Pco_2.

To ascertain the difference between $[HCO_3^-]^o$ and $s[HCO_3^-]^o$, $s[HCO_3^-]^o$ in venous blood was plotted against that in arterial blood (Fig. 4). According to Eq. (4) the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ should remain constant regardless of the difference in Pco_2. Since Pco_2 is higher in venous than arterial blood, $[HCO_3^-]^*$ is generally higher in venous than arterial blood as given by Eq. (1). Thus, $[HCO_3^-]^o$ in venous blood also becomes slightly higher than that in arterial blood. However, since $s[HCO_3^-]^o$ is independent of $[HCO_3^-]^*$, the regression coefficient of $s[HCO_3^-]^o$ in venous blood against that in arterial blood becomes lower

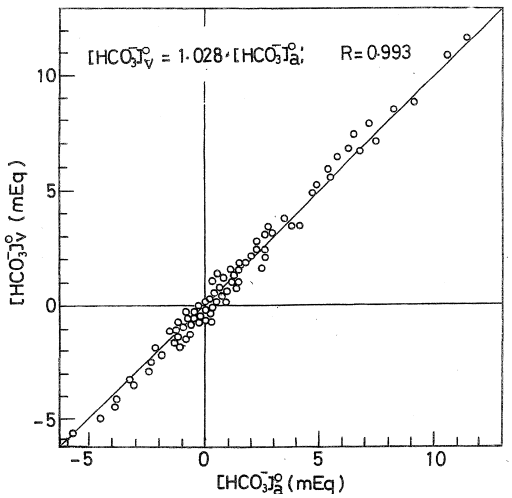

Fig. 3.
$[HCO_3^-]^o$ obtained in venous blood plotted against that in arterial blood sampled from 56 patients shown in Table 1. R is the correlation coefficient.

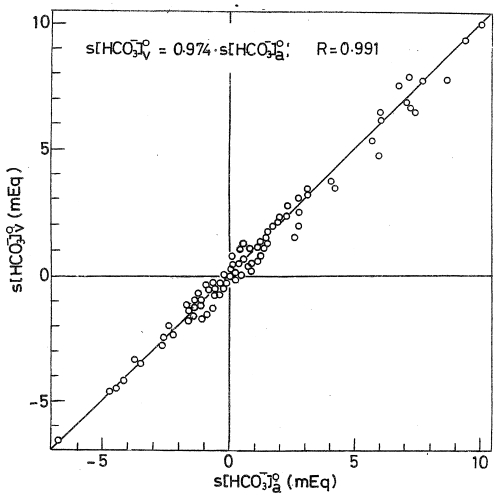

Fig. 4.
The standard $[HCO_3^-]^o$, designated by $s[HCO_3^-]^o$, in venous blood plotted against that in arterial blood. $s[HCO_3^-]^o$ was obtained by multiplying $[HCO_3^-]^o$ by the ratio $26.2/[HCO_3^-]^*$, 26.2 (mEq) being the $[HCO_3^-]^*$ at pH = 7.4.

than that of $[HCO_3^-]^o$ shown in Fig. 3. The correlation coefficient of $s[HCO_3^-]^o$ of Fig. 4 was 0.991 and the regression coefficient was 0.974, being slightly lower than that for $[HCO_3^-]^o$ in Fig.3. The mean ± SD of the difference of individual points from the regression lines in both Figs. 3 and 4 was 0.01 ± 0.46 mEq. The mean ± SD of the arterial - venous difference in Pco_2 was 6.42 ± 5.20 mmHg and that of the ratio of $[HCO_3^-]^*$ in venous blood to that in arterial blood was 1.077 ± 0.065. The mean arterial - venous difference of $[HCO_3^-]^o$ in Fig. 3 and that of $s[HCO_3^-]^o$ in Fig. 4 did not differ significantly. Thus, the assumption made in the previous study[1] that $[HCO_3^-]^o$ is independent of Pco_2, seems justifiable.

2) The regression functions of pH, and Pco_2 against $s[HCO_3^-]^o$ in arterial blood.

To confirm whether the respiratory change in Pco_2 compensates for the metabolic change in pH, the correlations of pH, Pco_2 and pH* against $s[HCO_3^-]^o$ were calculated in arterial blood sampled from the acidotic, normal and alkalotic patients shown in Table 2. Fig. 5 shows pH plotted against $s[HCO_3^-]^o$ in arterial blood. The correlation coefficient was 0.807 and the regression function ($\overline{pH}$) was given by

$$\overline{pH} = 7.439 + 0.01\ s[HCO_3^-]^o, \qquad (12)$$

where the mean ± SD of the difference of individual points from the regression line was 0.002 ± 0.029. Fig. 6 shows Pco_2 plotted against $s[HCO_3^-]^o$. The correlation coefficient was 0.623 and the regression function (Pco_2) was approximately given by the following hyperbolic equation:

$$\overline{Pco_2} = 36.5 + 25(s[HCO_3^-]^o/26.2)/(1 + s[HCO_3^-]^o/26.2), (mmHg). \qquad (13)$$

The above equation was calculated from $\overline{pH}$ and $s[HCO_3^-]^o$ of Eq. (12), using Eqs. (7) and (8). The mean ± SD of the difference of individual points from the regression line was −0.103 ± 4.60 mmHg. From pH and $s[HCO_3^-]^o$ shown in Fig. 5, the value for pH* was calculated, according to Eq. (7), as follows:

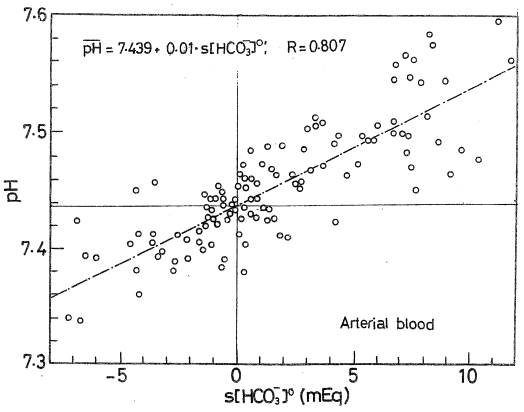

Fig. 5.
pH plotted against $s[HCO_3^-]^o$ in arterial blood sampled from 85 patients shown in Table 2.

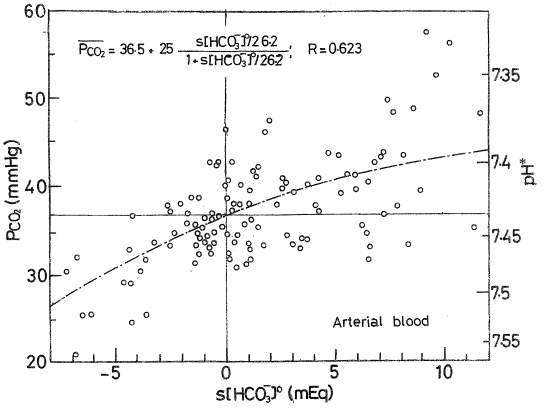

Fig. 6.
Pco_2 plotted against $s[HCO_3^-]^o$ in arterial blood sampled from the 85 patients shown in Table 2. The interrupted line is the regression function calculated from the regression line of pH, shown in Fig. 5 by using Eqs. (7) and (8). (See text)

$$pH^* = pH - \log(1 + s[HCO_3^-]^o/26.2). \quad (14)$$

pH* increases with a decrease in Pco_2, as given by the right - hand ordinate in Fig. 6. Fig. 7 shows pH* plotted against $s[HCO_3^-]^o$. The interrupted line represents the regression function of pH* ($\overline{pH^*}$) calculated from $\overline{pH}$ of Fig. 5, using Eq. (14). The difference of the individual points of pH* from $\overline{pH^*}$ was equal to $pH - \overline{pH}$, indicating that the error components in measured values of pH and pH* become equal. Fig. 8 shows the regression functions of pH* and pH against $s[HCO_3^-]^o$ together with the metabolic change in pH, which is given by $7.439 + \log(1 + s[HCO_3^-]^o/26.2)$, in arterial blood. The difference, $pH - pH^*$, measured in individual samples is always equal to that of their regression functions, $\overline{pH} - \overline{pH^*}$, shown in Fig. 8. As given by Eq. (14), pH is the sum of pH* and $\log(1 + s[HCO_3^-]^o/26.2)$, and, in addition, the change in pH* is opposite in sign to the $\log(1+s[HCO_3^-]^o)$. Hence, it is clear that in each blood sample, the metabolic change in pH is greatly minimized by the reciprocal change in pH*, as shown in Fig. 8.

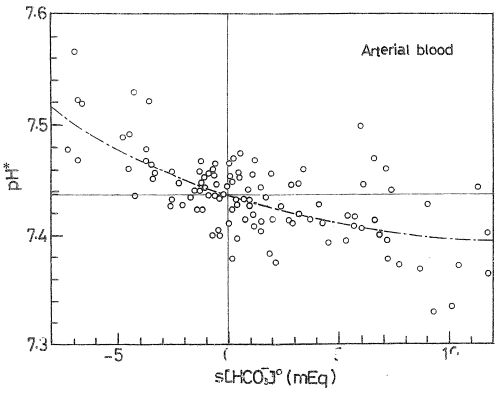

Fig. 7.
The correlation of pH* against $s[HCO_3^-]^o$ in arterial blood, where pH* was calculated from pH and $s[HCO_3^-]^o$ of Fig. 5 by using Eq. (7).

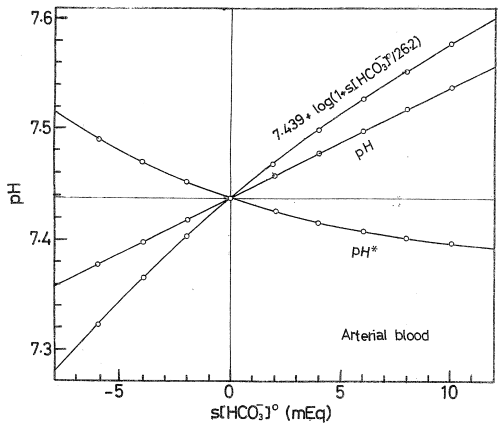

Fig. 8.
The regression functions of pH and pH* against $s[[HCO_3^-]^o$ in arterial blood given by Eqs. (12) and (14), respectively, and the value for $7.439+\log(1+s[[HCO_3^-]^o/26.2)$.

3) The regression functions of pH and Pco_2 against $s[HCO_3^-]^o$ in venous blood.

To confirm the validity of the correlations of pH and Pco_2 against $s[[HCO_3^-]^o$ in arterial blood, regression analysis was applied to the

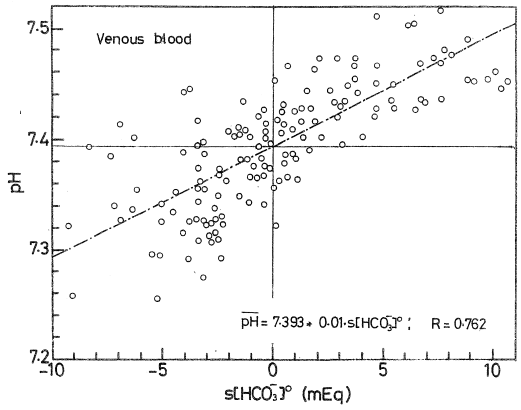

Fig. 9.
pH plotted against $s[HCO_3^-]^o$ in venous blood sampled from the 97 patients shown in Table 3. (See text for correlation coefficient)

values in venous blood shown in Table 3. Fig. 9 shows the pH value plotted against $s[HCO_3^-]^o$ in venous blood, where the correlation coefficient was 0.762 and the regression function was given by

$$\overline{pH} = 7.393 + 0.01\ s[HCO_3^-]^o. \qquad (15)$$

The mean ± SD of the difference of individual points from the regression line was 0.002 ± 0.039. In Fig. 10, Pco_2 in venous blood is plotted against $s[HCO_3^-]^o$. The correlation coefficient was 0.540 and the regression function ($\overline{Pco_2}$) was approximately given by the following hyperbolic function:

$$\overline{Pco_2} = 43.93 + 30\ (s[HCO_3^-]^o/26.2)/(1 + s[HCO_3^-]^o/26.2), (mmHg). \qquad (16)$$

$\overline{Pco_2}$ in Eq. (16) was calculated from $\overline{pH}$ and $s[HCO_3^-]^o$ of Eq. (15), using Eqs (7) and (8). The mean ± SD of the difference of individual points from the regression line was 0.35 ± 6.76 mmHg. From $\overline{pH}$ of Eq. (15) the regression function of pH* in venous blood was calculated by using Eq. (14). Because Pco_2 is higher in venous blood than arterial blood, the regression lines of pH and pH* in venous blood were lower than those in arterial blood, but parallel to them (Fig. 11).

4) Relationship between $s[HCO_3^-]^o$ and the hemoglobin concentration.

The buffering capacity in true plasma decreases with a decrease in hemoglobin concentration. Hence, it has been thought likely that a decrease in [Hb] would accompany metabolic acidosis. Thus, the relationship between $[H^+]$ and $[H^+]^*$ of Eq. (5) was studied in the anemic and normal patients shown in Table 4. Since $[H^+]$ and $[H^+]^*$ were measured in both arterial and venous blood, the SD of their distribution around the mean was in a range of 3 to 4 nEq even in normal blood. However, since $s[HCO_3^-]^o$ in normal blood was close to zero, the SD of the difference $[H^+]^* - [H^+]$ was reduced to 0.62 nEq. In the anemic patients the distribution of the difference $[H^+]^*$

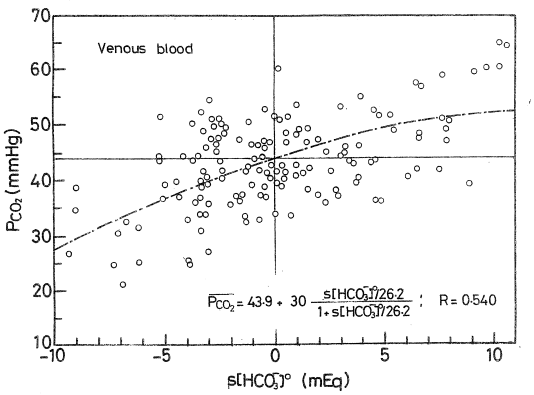

Fig. 10.
Pco_2 plotted against $s[HCO_3^-]^o$ in venous blood sampled from the same patients as shown in Fig. 9.

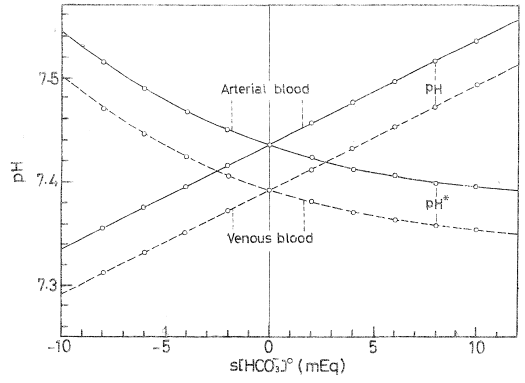

Fig. 11.
The regression functions of pH and pH* against $s[HCO_3^-]^o$ in arterial and venous blood. pH* in venous blood was obtained from Eq. (15) by using Eq. (7).

$-[H^+]$ was, nevertheless, greater than that of the normal group, the mean of 0.28 nEq being close to zero. In Fig. 12 values for $[H^+]$ are plotted against $[H^+]^*$, where the regression coefficient was almost equal to unity, demonstrating that $s[HCO_3^-]^o$ was close to zero even in the anemic patients. This makes it clear that [Hb] is independent of the metabolic change in pH.

DISCUSSION

According to the Henderson equation, when $[HCO_3^-]^*$ and $[H^+]^*$ in normal blood are evaluated using the measured Pco_2, Pco_2 is eliminated from the ratios $[H^+]^*/[H^+]$ and $[HCO_3^-]/[HCO_3^-]^*$, and these ratios become equal and independent of Pco_2. Since $[HCO_3^-] = [HCO_3^-]^* + [HCO_3^-]^o$, the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ becomes also independent of Pco_2, as shown in Eq. (4). From this theoretical evidence, the ratio $[HCO_3^-]^o/[HCO_3^-]^*$ could be replaced by $s[HCO_3^-]^o/26.2$, 26.2 (mEq) being $[HCO_3^-]^*$ at pH = 7.4. In other words, when $[HCO_3^-]^*$ and $[HCO_3^-]$ are evaluated at the same Pco_2, $s[HCO_3^-]^o$, becoming free from Pco_2, represents the metabolic change in $[HCO_3^-]$. Similarly, since $\log [H^+]^*/[H^+] = pH - pH^*$, the metabolic component of pH is given by $pH - pH^*$[3]. As far as the concentrations of fixed acids and bases are constant, $s[HCO_3^-]^o$ and $pH - pH^*$ remain constant. Actually, in simultaneously sampled arterial and venous blood, no significant difference in $s[HCO_3^-]^o$ was found between venous and arterial blood, regardless of the difference in Pco_2 (Fig. 4). When Eq. (1) for $[HCO_3^-]^*$ was derived, $[HCO_3^-]^o$ was presumably taken to be independent of Pco_2. However, because the difference in $[HCO_3^-]^*$ between venous and arterial blood was small, the regression coefficient of $[HCO_3^-]^o$ in venous blood, against that in

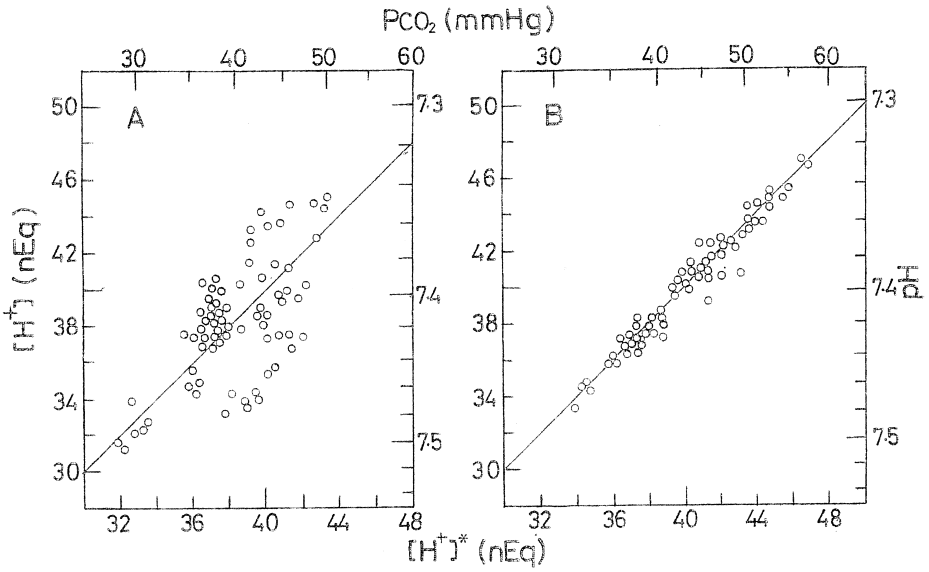

Fig. 12.
$[H^+]$ plotted against $[H^+]^*$ obtained from 32 anemic patients shown in Table 4 (A) and from 77 patients with normal [Hb] (B).

arterial blood, was close to unity. Thus, the derivation of Eq. (1) for $[HCO_3^-]^*$ is justified.

The acid - base imbalance has been quantified in terms of the base excess (BE), i.e., the deviation of $[HCO_3^-]$ from the standard value, which was approximated by using the regression coefficient of $[HCO_3^-]$ against pH in normal blood *in vitro*[9,10]. In addition, the deviation of $[HCO_3^-]$ from its standard value was evaluated at the level of pH = 7.4. However, to evaluate the metabolic change in $[HCO_3^-]$, i.e., $[HCO_3^-]^o$, the values for $[HCO_3^-]^*$ and $[H^+]^*$ must be evaluated at the Pco_2 level measured in the sampled blood by using Eq. (1). If $[HCO_3^-]^*$ is calculated by using any other Pco_2 different from the measured value, the metabolic changes in $[H^+]$, pH and $[HCO_3^-]$ will undergo the influence of the difference in Pco_2. In other words, for obtaining the metabolic changes of $[H^+]$ and $[HCO_3^-]$, $[H^+]^*$ and $[HCO_3^-]^*$ must be obtained using the same Pco_2, not the same pH, as measured in the sampled blood.

Fig. 13 shows the relationship between $[HCO_3^-]$ and pH at three different levels of $s[HCO_3^-]^o$ and Pco_2. Points A and A' represent the measured values for $[HCO_3^-]$, pH and Pco_2 at two levels of $s[HCO_3^-]^o$ of 5 and −5 mEq. B and B' represent $[HCO_3^-]^*$ and pH* in the normal plasma at the measured Pco_2 levels; C and C', $[HCO_3^-]^*$ and Pco_2 in the normal plasma at the measured pH levels. Designating Pco_2 and $[HCO_3^-]$ at C and C' by Pco_2" and $[HCO_3^-]$", respectively, the following equation is derived from the Henderson equation:

$$[H^+]\,[HCO_3^-]'' = K'\,Pco_2''. \qquad (17)$$

On the other hand, from Pco_2, $[H^+]$ and $[HCO_3^-]$ at A and A', the Henderson equation is written as follows:

$$[H^+]\,[HCO_3^-] = K'\,Pco_2. \qquad (18)$$

Eliminating $[H^+]$ from Eqs. (17) and (18), the

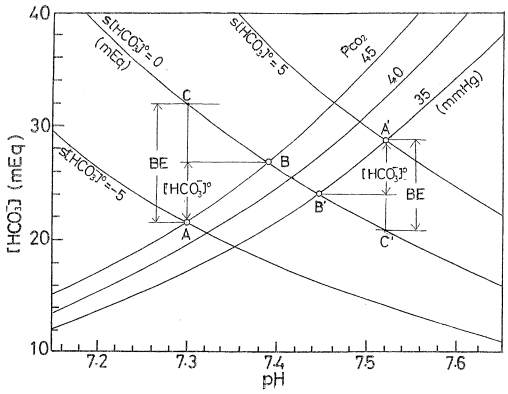

Fig. 13
The relationship between $[HCO_3^-]$ and pH at three levels of $s[HCO_3^-]^o$ and Pco_2. A and A' represent the measured values for pH, $[HCO_3^-]$ and Pco_2, B and B', $[HCO_3^-]^*$ and pH* at the measured Pco_2, and C and C', the normal values for $[HCO_3^-]$ and Pco_2 at the measured pH level.

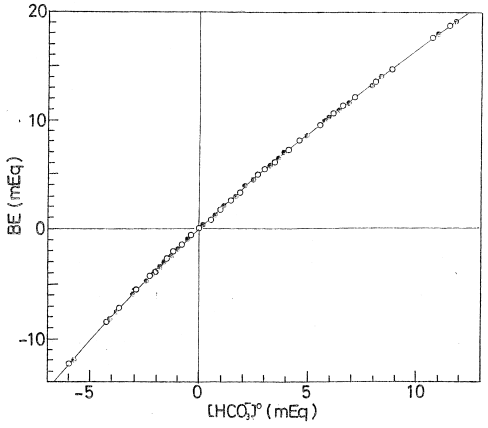

Fig. 14
The regression functions of BE against $[HCO_3^-]^o$ obtained in simultaneously sampled arterial and venous blood shown in Table 1. Open circles indicate arterial blood and the filled circles, venous blood.

following equation is derived:

$$[HCO_3^-]/ [HCO_3^-]'' = Pco_2/Pco_2''. \quad (19)$$

Subtracting unity from the both sides of Eq. (19), we obtain the following equation:

$$([HCO_3^-]-[HCO_3^-]'')/[HCO_3^-]''= (Pco_2-Pco_2'')/Pco_2'' \quad (20)$$

Designating $[HCO_3^-]-[HCO_3^-]''$ by BE, Eq. (20) is rewritten as follows:

$$BE/ [HCO_3^-]'' = (Pco_2-Pco_2'')/Pco_2''. \quad (21)$$

Eq. (21) makes it clear that BE depends on the ratio $(Pco_2-Pco_2'')/Pco_2''$. Setting the measured pH value in Eq. (9), $[HCO_3^-]''$ of Eq. (21) is evaluated, and, setting $[HCO_3^-]''$ in Eq. (1), Pco_2'' is obtained. Thus, the validity of Eq. (21) is readily ascertained.

Setting the measured Pco_2 in Eq. (1), $[HCO_3^-]^*$ is obtained, and $[HCO_3^-]^o$ is obtained, subtracting $[HCO_3^-]^*$ from $[HCO_3^-]$. Thus, the numerical relationship between $[HCO_3^-]^o$ and BE was easily calculated. Fig. 14 illustrates the relationship between BE and $[HCO_3^-]^o$ obtained in simultaneously sampled arterial and venous blood from the patients shown in Table 1. BE was about twice as great as $[HCO_3^-]^o$ and no difference in BE was observed between venous and areterial blood, where BE was approximately given by the following quadratic equation of $[HCO_3^-]^o$:

$$BE = 1.9\,[HCO_3^-]^o - 0.028\,[HCO_3^-]^{o\,2}, \quad (mEq). \quad (22)$$

As shown in Fig. 8, in a negative range of $s[HCO_3^-]^o$ the change in pH*, given by pH*—7.439, was about a half of the metabolic change, given by $\log(1+s[HCO_3^-]^o/26.2)$. However, in the positive range of $s[HCO_3^-]^o$, pH*—7.439 was about a quarter of the metabolic change given by $\log(1+s[HCO_3^-]^o/26.2)$. Furthermore, as shown in Figs. 5, 6, 9 and 10, the correlation coefficients of pH and Pco_2 against $s[HCO_3^-]^o$ were lower in venous than arterial blood, suggesting that the above compensatory changes in Pco_2 or pH* were attributable to the CO_2 transfer across the lung capillaries. From the fact that the regression lines of pH and pH* in venous blood were parallel to the equivalent lines in arterial blood (Fig. 11), it was suggested that the respiratory control of Pco_2 remained unchanged in blood during the transit through the capillaries in the tissue.

Figure 12 shows that the regression coefficient of $[H^+]$ against $[H^+]^*$ calculated in the anemic patients (Table 4) was close to unity. From this fact it was suggested that the relationship between Pco_2 and $[HCO_3^-]^*$, given by Eq. (1), was dependent not on [Hb], but on the balance between the hydration rate of CO_2 and the dehydration rate of HCO_3^- via carbonic anhydrase, as described before[1]. At all events, these measured data make it important that the relationship between $[HCO_3^-]^o$ and the concentration of fixed acids and bases is analysed quantitatively in near future.

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