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Analysis of Strong Ion Difference and Anion Gap in Blood Plasma of Elderly Patients at Steady State *in vivo*

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ABSTRACT

The amount of positively charged ions in blood plasma is given by the strong ion difference, i.e. $[Na^+]+[K^+]-[Cl^-]$ designated by [SID]. [SID] is balanced by the sum of $[HCO_3^-]$ and an anion gap, i.e. $[SID]-[HCO_3^-]$ designated by [AG]. Since $[HCO_3^-]$ has a Pco_2−dependent component $[HCO_3^-]^*$ and a metabolic component $[HCO_3^-]^\circ$, it was proposed that [SID] and [AG] also have Pco_2−dependent and metabolic components. This hypothesis has been tested in elderly patients with blood of a normal pH, where $[HCO_3^-]^\circ$ was distributed closely around zero, the correlation coefficient of [SID] against $[HCO_3^-]^*$ was 0.74, thus, the Pco_2−dependent component of [SID], [SID]* was derived from its regression function. The same component of [AG], [AG]* was obtained by subtracting $[HCO_3^-]^*$ from [SID]*. In acidotic and alkakotic patients, the correlation coefficient of [AG]−[AG]* against $[HCO_3^-]^\circ$ was 0.9, thus, the metabolic component of [AG], $[AG]^\circ$ was obtained from its regression function. The metabolic component of [SID], $[SID]^\circ$ was then derived by adding $[HCO_3^-]^\circ$ to $[AG]^\circ$. Designating the error component of [SID] and [AG], respectively, by i[SID] (= $[SID]-[SID]^*-[SID]^\circ$) and i[AG] (= $[AG]-[AG]^*-[AG]^\circ$), these became equal to each other. The Pco_2−dependent and metabolic components of $[H^+]$ and pH were not linear against either $[HCO_3^-]^*$ and $[HCO_3^-]^\circ$.

Key words : Electroneutrality, Correlation coefficients, Regression analysis, Chloride shift, Metabolic CO_2 reaction.

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INTRODUCTION

Previously we found that [HCO_3^-] in blood plasma *in vivo* has a Pco_2−dependent component, [HCO_3^-]* and a metabolic component, [HCO_3^-]°[1]. In blood of normal pH, where [HCO_3^-]° can be ignored, CO_2 reactions mainly occur within the red blood cell (RBC), not in the blood plasma. In the RBC, however, the change in [HCO_3^-] is much reduced via the bicarbonate shift across the RBC membrane in exchange for Cl^-[2]. As a result of this shift, the change in [HCO_3^-] in the plasma becomes the reciprocal of that of [Cl^-]. Since [Na^+] and [K^+] are not diffusible across the RBC membrane, the change in [HCO_3^-] becomes linearly proportional to that of the strong ion difference, i.e. [SID] = [Na^+]+[K^+]−[Cl^-][3]. In circulating blood *in vivo*, Pco_2 changes with oxygenation or deoxygenation, thus, the change in [HCO_3^-] complies with the Haldane effect[4]. The value for [HCO_3^-]*, used in the present study, was obtained from normal blood *in vivo* taking the chloride shift and the Haldane effect into account, hence it can be applied to the analysis of both arterial and venous blood.

When the hydration reaction of CO_2 occurs in the blood plasma, the H^+ ions produced are bound to ionized buffer anions (A^-) to form the unionized acid molecule (HA). When HCO_3^- is dehydrated in plasma, H^+ is freed from HA, increasing [A^-][5]. The change in [A^-] and [HA] in blood plasma is given by the change in the anion gap [AG]. When no CO_2 reaction occurs in the plasma, [AG] will not change.

When [H^+] or pH change in blood plasma due to some metabolic process, CO_2 reactions occur together with a change in [AG]. When [H^+] decreases in alkalotic plasma, hydration of CO_2 occurs, increasing [HA]. Thus, [HCO_3^-] becomes higher than [HCO_3^-]* and [AG] becomes lower than its Pco_2−dependent component [AG]*. In acidotic blood plasma, on the other hand, [HCO_3^-] falls below [HCO_3^-]* and [AG] increases above [AG]*. When CO_2 reactions take place following a change in Pco_2, this results mainly on a change in [SID]. In contrast when CO_2 reactions occur following a change in pH, the main change is in [AG].

The difference in concentration between [SID] and [AG] is always equal to [HCO_3^-] to maintain electroneutrality. Moreover, since [HCO_3^-] has respiratory and metabolic components, it seemed possible that [SID] and [AG] could also be divided into these two components. The correlation coefficient of [SID] against [HCO_3^-]* was high (0.74) in blood plasma sampled from elderly patients and volunteers, hence the respiratory component of [SID], [SID]* was evaluated from its linear regression function. The respiratory component of [AG], [AG]* was evaluated by subtracting [HCO_3^-]* from [SID]*. The correlation coefficient of [AG]−[AG]* against [HCO_3^-]° was also high (0.90). Thus, the metabolic component of [AG], [AG]° was evaluated from the regression function of [AG]−[AG]* against [HCO_3^-]°. The metabolic component of [SID], [SID]° was then obtained by adding [HCO_3^-]° to [AG]°.

METHODS

The correlations of [SID] and [AG] against [HCO_3^-]* were calculated in blood plasma from a group of elderly patients with normal pH (n = 96) and volunteers (n = 59), and in plasma from acidotic (n = 43) and alkalotic (n = 24)

Table 1. Summarized data for pH, $[H^+]$ (nEq), Pco_2, $[HCO_3^-]$ and other constituent ions (mEq) in arterial and venous blood of normal elderly patients and volunteers.

Samples and patients	Elderly patients		Volunteers	
	Male	Female	Male	Female
No of samples	33	79	36	31
No of subjects	30	66	29	30
Mean age ± SD	81.1 ± 8.7	83.3 ± 6.8	32.2 ± 11.4	36.0 ± 10.4
Age range	54 − 96	64 − 95	19 − 57	24 − 55
Measured data				
pH	7.410 ± 0.039		7.382 ± 0.027	
Pco_2 (mmHg)	41.57 ± 6.60		46.97 ± 5.79	
$[Na^+]$	138.18 ± 3.86		139.84 ± 1.89	
$[K^+]$	3.72 ± 0.36		3.83 ± 0.29	
$[Cl^-]$	101.69 ± 2.21		102.05 ± 1.85	
Parameters obtained from measured data				
$[HCO_3^-]$	25.86 ± 1.88		27.59 ± 2.14	
$[HCO_3^-]$	25.85 ± 2.11		27.30 ± 1.57	
$[HCO_3^-]$	0.06 ± 0.54		0.25 ± 0.98	
$[H^+]$	39.21 ± 3.19		41.50 ± 2.60	
$[H^+]$*	39.28 ± 3.19		41.42 ± 4.97	
$[H^+]^o$	−0.07 ± 0.77		−0.36 ± 1.54	
[SID]	40.21 ± 2.15		41.61 ± 1.79	
[SID]*	41.03 ± 1.66		39.83 ± 2.00	
$[SID]^o$	−0.10 ± 0.04		−0.08 ± 0.08	
[AG]	14.28 ± 1.49		13.84 ± 2.28	
[AG]*	14.66 ± 0.25		14.47 ± 0.20	
$[AG]^o$	−0.13 ± 0.52		−0.37 ± 0.99	
Error components of [SID], i[SID] and [AG], i[AG]:				
i[SID] = i[AG]	−0.14 ± 1.40		−0.12 ± 1.43	

elderly patients. The blood samples were obtained with consent of the patients. Table 1 gives information about the patients with normal pH and the volunteer control subjects. Measured values and the parameters calculated from the data are shown. Table 2 gives the same information about patients in the acidotic and alkalotic groups.

pH, Pco_2, $[Na^+]$, $[K^+]$ and $[Cl^-]$ were measured using a blood gas analyser (Ciba Corning 188). $[HCO_3^-]$ was calculated from $[H^+]$ and Pco_2 using the Henderson equation[6]. The Pco_2−dependent component of $[HCO_3^-]$, $[HCO_3^-]^*$ was calculated by setting Pco_2 into the following equation[1]:

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

The metabolic component of $[HCO_3^-]$, $[HCO_3^-]^o$ was obtained by subtracting $[HCO_3^-]^*$ from the measured value of $[HCO_3^-]$.

The correlation coefficients and regression functions were obtained using Kaleida Graph Software (Synergy). The correlation coefficient of [SID] against $[HCO_3^-]^*$ was first calculated in blood samples of all the groups of elderly

Table 2. Summarized data for pH, $[H^+]$(nEq), Pco_2, $[HCO_3^-]$ and other constituent ions (mEq) in arterial blood sampled from 65 acidotic and alkalotic elderly patients.

Samples and patients	Acidotic group		Alkalotic group	
	Male	female	male	female
No of samples	22	35	15	47
No of subjects	15	28	5	19
Mean age ± SD	84.9 ± 5.5	84.3 ± 7.6	89.0 ± 2.5	83.5 ± 5.2
Age range	74 − 95	69 − 96	85 − 91	72 − 94
Measured data				
pH	7.343 ± 0.057		7.488 ± 0.047	
Pco_2	39.78 ± 7.99		44.31 ± 7.39	
$[Na^+]$	137.81 ± 4.28		134.11 ± 5.72	
$[K^+]$	4.18 ± 0.50		3.06 ± 0.55	
$[Cl^-]$	102.32 ± 3.85		94.70 ± 4.29	
Parameters calculated from measured data				
$[HCO_3^-]$	21.19 ± 2.97		33.04 ± 3.39	
$[HCO_3^-]$*	25.26 ± 2.37		26.58 ± 2.04	
$[HCO_3^-]^\circ$	−4.07 ± 1.76		6.46 ± 2.15	
$[H^+]$	45.82 ± 6.14		32.70 ± 3.46	
$[H^+]$*	38.12 ± 4.25		40.50 ± 3.68	
$[H^+]^\circ$	7.70 ± 4.44		−7.80 ± 2.20	
[SID]	39.65 ± 2.52		42.98 ± 2.85	
[SID]*	40.00 ± 2.06		41.22 ± 1.83	
$[SID]^\circ$	−0.39 ± 0.12		0.35 ± 0.15	
[AG]	18.47 ± 2.98		9.43 ± 2.77	
[AG]*	14.73 ± 0.31		14.57 ± 0.26	
$[AG]^\circ$	3.69 ± 1.64		−6.11 ± 2.00	
Error components of [SID], i[SID] and [AG], i[AG]:				
i[AG] = i[SID]	−0.14 ± 1.40		−0.12 ± 1.43	

patients and volunteers to validate the significance of the regression function, and [SID]* was then given by the regression function. Since [SID]* was linear against $[HCO_3^-]$*, [AG]* was obtained by subtracting $[HCO_3^-]$* from [SID]*. Next, the correlation coefficient of [AG]−[AG]* against $[HCO_3^-]^\circ$ was calculated. Since the above correlation coefficient was high (0.9), $[AG]^\circ$ was evaluated from its linear regression function. Finally, the metabolic component of [SID], $[SID]^\circ$ was evaluated by adding $[HCO_3^-]^\circ$ to $[AG]^\circ$.

RESULTS

Figs. 1, 2 and 3 show $[Na^+]$, $[K^+]$ and $[Cl^-]$ plotted againt $[HCO_3^-]$* in plasma from normal elderly patients (open circles) and volunteers (filled circle) as given in Table 1. In neither group was a significant correlation observed between the ionic concentrations and $[HCO_3^-]$*. The correlation coefficients of $[Na^+]$, $[K^+]$ and $[Cl^-]$ were respectively 0.43, 0.32 and 0.01. The regression functions of $[Na^+]$, $[K^+]$ and $[Cl^-]$ were given, respectively by the

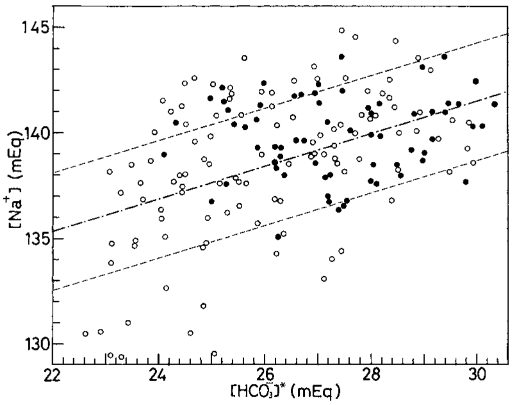

Fig. 1. [Na^+] plotted against [HCO_3^-]* in blood plasma from a number of patients with normal pH (open circles) and volunteers (filled circles) (Table 1). The interrupted line is the regression line and the dashed lines show the standard deviation of individual points for [Na^+] from the regression line.

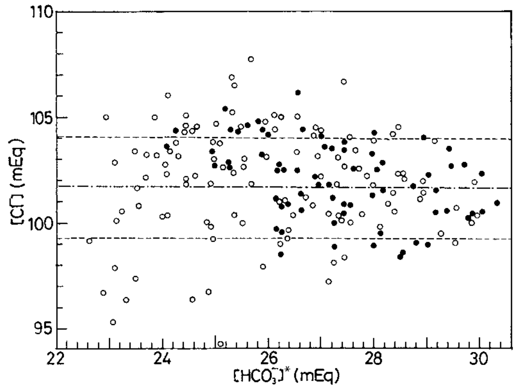

Fig. 3. [Cl^-] plotted against [HCO_3^-]* in samples of the same blood plasma used for the analysis shown in Fig. 1. Normal patients, open circles, and volunteers, filled circles. The interrupted line is the regression line and the dashed lines show the standard deviation of individual points for [Cl^-] from the regression line.

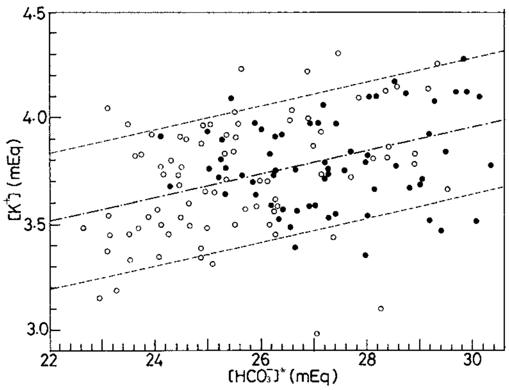

Fig. 2. [K^+] plotted against [HCO_3^-]* in samples of the same blood plasma used for the analysis shown in Fig. 1. Normal patients, open circles, and volunteers, filled circles. The interrupted line is the regression line and the dashed lines show the standard deviation of individual points for [K^+] from the regression line.

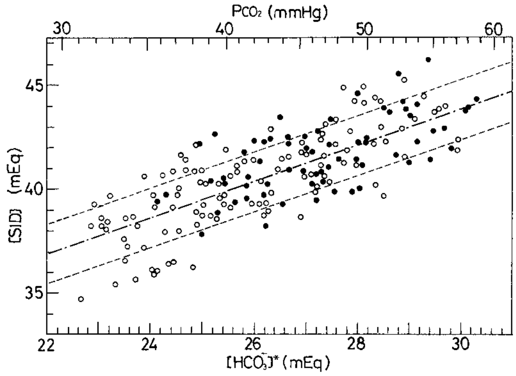

Fig. 4. [SID] plotted against [HCO_3^-]* in samples of the same blood plasma used for the analysis shown in Fig. 1. Normal patients, open circles, and volunteers, filled circles. The interrupted line is the regression line and the dashed lines show the standard deviation of individual points for [SID] from the regression line.

following equations:

$$[Na^+]=137.35+0.80([HCO_3^-]^*-25),(mEq),(2)$$

$$[K^+]=3.66+0.06([HCO_3^-]^*-25),(mEq),\quad(3)$$

$$[Cl^-]=101.64-0.02([HCO_3^-]^*-25),(mEq).(4)$$

The mean ± SD of the deviations (mEq) of individual points from the regression lines of [Na^+], [K^+] and [Cl^-] were respectively −0.01

$\pm$ 2.78, 0.00 $\pm$ 0.32 and 0.27 $\pm$ 3.72 mEq.

The correlation coefficient of [SID] against $[HCO_3^-]^*$ was greater than that of $[Na^+]$, $[K^+]$ and $[Cl^-]$. Fig. 4 illustrates [SID] plotted against $[HCO_3^-]^*$ in the group of patients with normal pH and volunteers. The correlation coefficient was 0.74. Thus, the P_{CO_2}−dependent component of [SID], [SID]* was given by its regression function as follows:

$$[SID]^* = 39.77 + 0.87([HCO_3^-]^* - 25), (mEq). (5)$$

The mean $\pm$ SD of the deviation of individual points from the regression line was 0.22 $\pm$ 1.44 mEq, the SD being much smaller than that of $[Na^+]$ and $[Cl^-]$.

Figs. 5 and 6 show [SID] plotted against $[HCO_3^-]^*$ in the alkalotic and acidotic groups, respectively. The correlation coefficient was similar to that of the normal group (Fig. 4). The regression function of the acidotic group (Fig. 6) was given by Eq. (5), but that of the alkalotic group (Fig. 5) was given by the following equation:

$$[SID]^* = 40.20 + 1.20([HCO_3^-]^* - 25), (mEq), (6)$$

where the mean $\pm$ SD of the deviation of the individual points of [SID] from the regression line was 0.34 $\pm$ 2.10 mEq.

Since [AG]* is given by subtracting $[HCO_3^-]^*$ from [SID]*, the equation for [AG]* in the acidotic group (Table 2) was give from Eq. (5) by

$$[AG]^* = 14.77 - 0.13([HCO_3^-]^* - 25), (mEq). (7)$$

[AG]* in the alkalotic group was given by subtracting $[HCO_3^-]^*$ from [SID]* of Eq. (6) as follows:

$$[AG]^* = 15.20 + 0.20([HCO_3^-]^* - 25), (mEq). (8)$$

The metabolic component of [AG], $[AG]^\circ$ was obtained from the regression function of [AG] −[AG]* calculated against $[HCO_3^-]^\circ$ in samples of the blood plasma of normal pH, acidotic and alkalotic groups. Fig. 7 shows [AG]−[AG]* plotted against $[HCO_3^-]^\circ$, where the

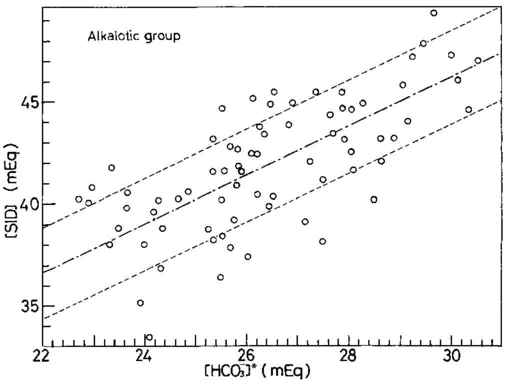

Fig. 5. [SID] plotted against $[HCO_3^-]^*$ in blood plasma from a number of alkalotic patients (Table 2). The interrupted line is the regression line and dashed lines show the standard deviation of individual points for [SID] from the regression line.

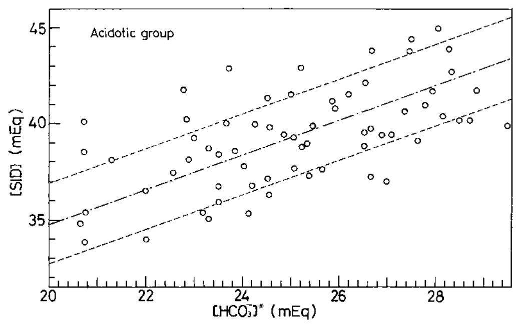

Fig. 6. [SID] plotted against $[HCO_3^-]^*$ in blood plasma from a number of acidotic patients (Table 2). The interrupted line is the regression line and the dashed lines show the standard deviation of individual points for [SID] from the regression line.

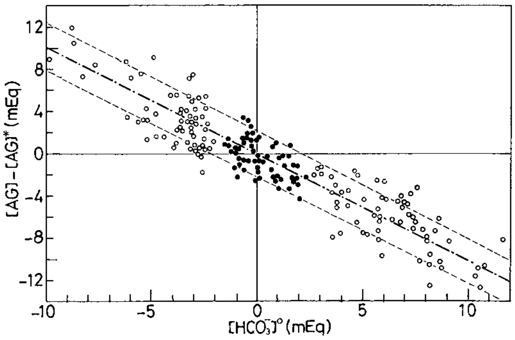

Fig. 7. [AG]−[AG]* plotted against [HCO_3^-]° in blood plasma from a number of acidotic and alkalotic patienst (open circles) and volunteers (filled circles) (Table 2). The interrupted line is the regression line and the dashed lines show the standard deviation of individual points for [AG]−[AG]*.

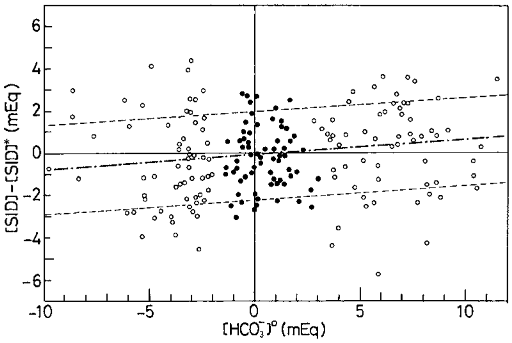

Fig. 8. [SID]−[SID]* plotted against [HCO_3^-]° in the same blood plasma used for the analysis shown in Fig. 7. The interrupted line is the regression line given by Eq. (10) and dashed lines show the standard deviation of individual points for [SID]−[SID]*.

correlation coefficient was 0.90 and the regression function, [AG]° was given by the following equation,

$$[AG]^\circ = -0.10 - 0.93\,[HCO_3^-]^\circ, \text{(mEq)}. \quad (9)$$

To maintain electroneutrality, the metabolic component of [SID], [SID]° is always balanced by the sum of [HCO_3^-]° and [AG]°, thus, [SID]° was given by adding [HCO_3^-]° to [AG]° of Eq. (9) as follows:

$$[SID]^\circ = -0.10 + 0.07\,[HCO_3^-]^\circ, \text{(mEq)}. \quad (10)$$

Fig. 8 shows [SID]−[SID]* plotted against [HCO_3^-]°, where the interrupted line shows the value of [SID]° of Eq. (10).

The deviation of individual points of [AG]−[AG]* from [AG]° shown in Fig. 7 was an error component of [AG], i[AG] given by subtracting [AG]° from [AG]−[AG]*. The error component of [SID], i[SID] was also given by subtracting [SID]° from [SID]−[SID]*. Since [HCO_3^-]−[HCO_3^-]*−[HCO_3^-]° = 0, the difference, i[SID] −i[AG], becomes zero, that is, i[SID] = i[AG]. The mean ± SD of the deviations of individual points of [AG]−[AG]* from [AG]°, that is, i[AG] = i[SID] was −0.13 ± 1.43 mEq, as summarized in Tables 1 and 2.

DISCUSSION

Hitherto, we have established that [HCO_3^-] in blood plasma could be divided into a Pco_2−dependent component, [HCO_3^-]* and a metabolic component, [HCO_3^-]°. [HCO_3^-]* was given by Eq. (1) and [HCO_3^-]° was obtained by subtracting [HCO_3^-]* from [HCO_3^-][1]. Furthermore, we found that [SID] in a group of elderly patients with normal pH was linearly correlated with [HCO_3^-]*, and that under normal respiratory conditions, [AG] in acidotic and alkalotic patients was also linear against [HCO_3^-]°. In the present study we have shown that [SID] and [AG], like [HCO_3^-], could be divided into the Pco_2−dependent respiratory components and the metabolic components. Through regression analysis of [SID] against

$[HCO_3^-]^*$, we have derived the respiratory component $[SID]^*$. Subtracting $[HCO_3^-]^*$ from $[SID]^*$, $[AG]^*$ was obtained and through analysis of $[AG]-[AG]^*$ against $[HCO_3^-]^\circ$, $[AG]^\circ$ was obtained; by adding $[HCO_3^-]^\circ$ to $[AG]^\circ$, $[SID]^\circ$ was formulated. Since the regression coefficient of $[SID]^*$ against $[HCO_3^-]^*$ was 0.87 as shown in Fig. 4 and Eq. (5), it was suggested that about 87% of CO_2 reactions following a change in Pco_2 took place via the chloride shift across the RBC membrane, and that about 13% of those reactions occurred in the blood plasma, since $[HCO_3^-]^* = [SID]^* - [AG]^*$. Moreover, about 93% of CO_2 reactions due to a metabolic change in pH occurred via the buffering action of weak acids in the plasma.

The Pco_2-dependent component of $[H^+]$, $[H^+]^*$ was derived by setting $[HCO_3^-]^*$ of Eq. (1) into the Henderson equation as follows[6),7)] :

$$[H^+]^* = 5.187\ Pco_2^{0.543},\ (nEq). \qquad (11)$$

Fig. 9 shows $[H^+]^*$ and $[HCO_3^-]^*$ calculated against Pco_2 by using Eqs. (11) and (1), where $[H^+]^*$ was approximately parallel to $[HCO_3^-]^*$. From Eqs. (1) and (11), the ratio $[H^+]^*/[HCO_3^-]^*$

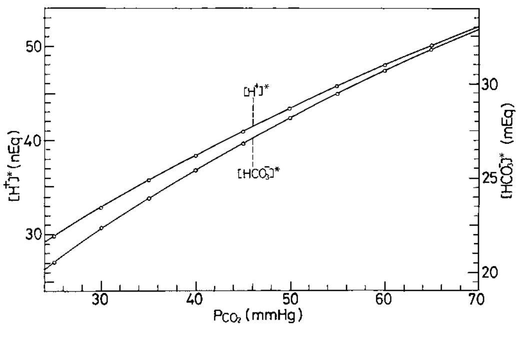

Fig. 9. $[H^+]^*$ and $[HCO_3^-]^*$ calculated against Pco_2 using Eqs. (11) and (1), respectively.

was obtained as

$$[H^+]^*/[HCO_3^-]^* = 1.1 \times 10^{-6}\ Pco_2^{0.086}. \qquad (12)$$

In the Pco_2 range from 25 to 70mmHg, the above ratio increased from 1.451×10^{-6} to 1.585×10^{-6}. Therefore, if CO_2 reactions occur in the plasma following a change in Pco_2, $[H^+]^*$ must change in parallel to $[HCO_3^-]^*$. Furthermore, it was supposed that the change in $[AG]^*$ would follow that of $[HCO_3^-]^*$, similarly to the relationship between $[AG]^\circ$ and $[HCO_3^-]^\circ$ as shown in Fig. 7 and Eq. (9). However, the change in $[AG]^*$ was only about 13% of that of $[HCO_3^-]^*$, as shown in Eq. (7). It was then realized that CO_2 reactions due to the change in Pco_2 occurred mainly in the RBC, not in the plasma and the change in $[HCO_3^-]^*$ in the plasma was caused by the chloride shift across the RBC membrane.

The metabolic component of $[H^+]$, $[H^+]^\circ$ was then evaluated by subtracting $[H^+]^*$ from the measured value for $[H^+]$. At any Pco_2, the product of $[H^+]$ and $[HCO_3^-]$ is equal to that of $[H^+]^*$ and $[HCO_3^-]^*$, as given by the Henderson equation. Thus, setting $[H^+] = [H^+]^* + [H^+]^\circ$ and $[HCO_3^-] = [HCO_3^-]^* + [HCO_3^-]^\circ$ into the Henderson equation, $[HCO_3^-]^\circ$ was given by the following hyperbolic equation of $[H^+]^\circ$

$$[HCO_3^-]^\circ = -[HCO_3^-]^*[H^+]^\circ/([H^+]^* + [H^+]^\circ),\ (mEq). \qquad (13)$$

To test the individual variation of $[HCO_3^-]^\circ$ against $[H^+]^\circ$, $[HCO_3^-]^\circ$ was plotted against $[H^+]^\circ$ in blood samples from a number of elderly patients (Tables 1 and 2). As shown in Fig. 10, $[HCO_3^-]^\circ$ was distributed closely along the following regression function:

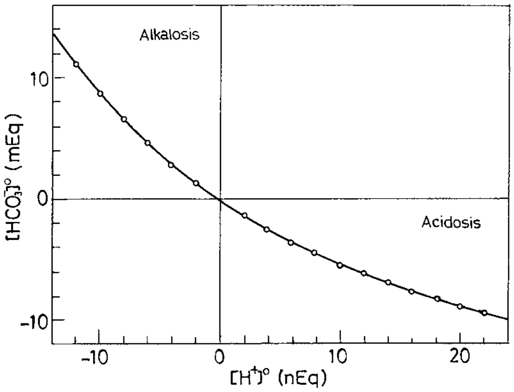

Fig. 10. Regression line of $[H^+]^\circ$ against $[HCO_3^-]^\circ$ in samples from the same blood plasma used for analysis shown in Figs. 7 and 8.

$$[HCO_3^-]^\circ = -26.65\,[H^+]^\circ / (40.61 + [H^+]^\circ),\ (mEq). \quad (14)$$

The mean ± SD of the deviations of individual points from the regression line was 0.01 ± 0.13 mEq. $[H^+]^*$ and $[HCO_3^-]^*$ of Eq. (13) are the functions of P_{CO_2}, however, the effect of P_{CO_2} on $[HCO_3^-]^\circ$ seems fairly small.

From the data of Fig. 10, it was clear that in alkalotic blood plasma $[HCO_3^-]^\circ$ increased above zero, that is, the hydration reaction of CO_2 occurred, when $[H^+]^\circ$ decreased. In contrast, $[HCO_3^-]^\circ$ decreased below zero in acidotic blood plasma, indicating the dehydration reaction of HCO_3^- took place with an increase in $[H^+]^\circ$. The relationship between $[AG]^\circ$ and $[HCO_3^-]^\circ$ shown in Fig. 7 confirms that the hydration reaction of CO_2 occurred in the alkalotic blood and dehydration of HCO_3^- occurred in the acidotic blood.

To widen our knowledge on the chemistry of the CO_2 reactions due to the changes in P_{CO_2} and $[H^+]$, it is vital to know the quantitative relationship between P_{CO_2}, $[H^+]$, $[HCO_3^-]$ and other ionic concentrations. The regression coefficient of $[SID]^*$ against $[HCO_3^-]^*$ (Fig. 4) was much greater than that of $[AG]^*$ (Eq, 7). As shown in Fig. 7, $[AG]^\circ$ was well correlated linearly against $[HCO_3^-]^\circ$, where the regression coefficient was −0.93 as given by Eg. (10). Taking the above results into account, it was thought useful to divide [SID] and [AG] into the respiratory and metabolic components.

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REFERENCES

1. Mochizuki M: Analysis of bicarbonate concentration in human blood plasma at steady state *in vivo*. Yamagata Med J. 2004: 22; 9-24
2. Hamburger HJ: Anionen Wanderungen in Serum und Blut unter dem Einfluss von CO_2, Säuren und Alakali. Biochem Z: 1918; 86: 309-324
3. Stewart PA: Modern quantitative acid-base chemistry. Can J Physiol Pharmacol. 1983: 61: 1444-146
4. Christiansen C, Douglas CG, Haldane JS: The absorption and dissociation of carbon dioxide by human blood. J. Physiol: 1914: 48: 244-271
5. Roughton FJW: Transport of oxygen and carbon dioxide. In: Fenn WO and Rahn H Eds. Handbook of Physiology, Respiration: Washington DC, Am Physiological Society, 1964: 767-825
6. Henderson LJ: Das Gleichgewicht zwischen Basen und Säuren im tierischen Organismus. Ergebn Physiol: 1909: 8: 254-325
7. Mochizuki M: Analysis of the metabolic changes in pH, $[HCO_3^-]$ and P_{CO_2} in blood

plasma at steady state *in vivo*. Yamagata Med J: 2007: 25(2): 33-47