Temperature Coefficient of and Oxygen Effect on the Antimony Microelectrode

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Abstract With regard to pH measurement of biological fluids *in vivo* with metal-metal oxide microelectrodes, the effect of temperature and partial pressure of oxygen on antimony (Sb) microelectrodes was examined, and pH of blood was estimated in the bullfrog.

The temperature coefficient (dE/dt) of electromotive force (EMF) of Sb-microelectrodes in the range of 7 to 37°C was -1.18 ± 0.113 mV/°C (mean $\pm$ SEM) in Ringer solution, whereas that of the pH glass electrode in the same solution was -0.43 ± 0.035 mV/°C. When estimated in Tris buffer solution, it was -0.06 ± 0.063 mV/°C for Sb-microelectrodes and 1.05 ± 0.036 mV/°C for glass electrodes.

The change of slope constant (α in $-mV/pH$) in the Sb-microelectrode due to temperature change could be predicted empirically from: $\alpha = 0.40(t - 25) + 55.3$, where t represents the measuring temperature in °C.

The resultant deviation of pH readings between Sb and glass electrodes, $\Delta pH_{Sb-Glass}$, may be expressed by:

$$\Delta pH_{Sb-Glass} = 0.00183(t - 25) + 0.016.$$

In the range of 45 to 760 mmHg of oxygen partial pressure at fixed pH, the EMF increased linearly with the increase of P_{O_2} , the slope ($dE/d \log(P_{O_2})$) being 11.7 ± 0.42 (SEM) mV ($n = 13$, $t = 25^\circ C$).

In consideration of the above effects, the blood pH of bullfrog was estimated to be 7.697 ± 0.092 (SD) and 7.729 ± 0.111 with glass and Sb-microelectrodes respectively, the difference between the two being relatively minor.

Application of the antimony (Sb) microelectrode for renal micropuncture study was first attempted by VIEIRA and MALNIC (1968). Since then, a number of investigators have carried out the experiment for studying the mechanism of hydrogen ion transport across the tubular borders (MALNIC *et al.*, 1972; KARLMARK and DANIELSON, 1974; GREEN and GIEBISCH, 1975; SOHTELL and KARLMARK 1976; KOTERA *et al.*, 1977), but the detailed electrochemical behavior of the antimony

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electrode is not yet fully understood, numerous conflicting data existing concerning the disturbances of their electrical potential by various physicochemical parameters (GREEN and GIEBISCH, 1974; PUSCHETT and ZURBACH, 1974). PERLEY (1939) and STOCK *et al.* (1958) pointed out that the change of temperature would have a considerable effect on the pH reading of the antimony electrode, its temperature coefficient varying with the pH and type of buffer used and reference electrode in the system. However, details remained obscure as to whether the temperature affects the electromotive force of the electrode system or sensitivity of the pH electrode. In view of the fact that the electrode shows a great deal of instability, we have to establish a method of measurement with the least trouble in stability of pH reading.

Concerning the influence of partial pressure of oxygen, several authors have reported that the potential of the Sb-electrode varied with the concentration of dissolved oxygen (KAUKO and KNAPPSBERG, 1939; TOURKY and MOUSSA, 1948; EL WAKKAD, 1950). VIEIRA and MALNIC (1968) examined the effect of oxygen on pH readings of pre-equilibrated buffers between pH 5.05 and 7.54 at 37°C with the P_{O_2} ranging from 35 to 138 mmHg, and they showed that no significant effect was observed with changes in oxygen tensions. On the other hand, in a recent report by QUEHENBERGER (1977), a considerably large value of $dE/d\log(P_{O_2})$, 58.5 mV, under conditions of constant pH (=7.1) and P_{CO_2} (=33.2 mmHg) has been described. Accordingly, we need to reconsider the effect of oxygen, as well as of temperature, on the Sb-microelectrode and examine the practical applicability of this electrode to the pH measurement of biological fluid, such as blood. This report deals with the above problem. The protein effect on the Sb-electrode will be discussed in a subsequent report (SATAKE *et al.*, 1980).

METHODS

1) *pH-Electrodes.* The antimony microelectrode (Sb-microelectrode) was manufactured by the method reported previously (FUJIMOTO *et al.*, 1980). The tip of the microelectrode was ground to about 2–5 μm in diameter, and it was calibrated with Tris buffer and frog Ringer bicarbonate solution. The details of its calibration have been described elsewhere (MATSUMURA *et al.*, 1980). Since it is known that the Sb-electrode is susceptible to the effects of various buffers, especially phosphate and citrate buffers (YOSHIMURA, 1936; GREEN and GIEBISCH, 1974) these solutions may not be used for the calibration of the Sb-electrode. In search of the most suitable buffer for this purpose, we concluded that Tris was the most appropriate among the various buffers tested. The possible effect of ionic strength on the Sb-electrode (the "salt effect") was also discussed in the preceding paper (MATSUMURA *et al.*, 1980), where it was shown that, in the practical use of the Sb-microelectrode, special care must be taken regarding the following points: (1) For electrode calibration, Tris buffer solution should be

used, (2) Phosphate concentration should be maintained constant during all measurements, and (3) The ionic strength of samples should be the same as those of calibration solutions. The electrodes with slope constants of more than -50 mV/pH and $\Delta pH_{Sb-Glass}$ of less than 0.02 units, calibrated with frog Ringer solution, were used for the present study. The pH glass electrode used for the general test was a commercial electrode of an immersion type (Toa HG-2005) and that for the blood pH measurement was a syringe type (Toa HG-5005).

The electrical measurements were carried out with an electrometer (Keithley 610C) and digital pH meter (Toa HM-15A) at room temperature ($20 \pm 2.5^\circ C$), where a saturated calomel electrode (Toa HC-305D) was used as the reference half-cell.

2) *Measurement of temperature and P_{O_2} effect.* Effects of change in temperature and partial pressure of oxygen (P_{O_2}) on the electromotive force (EMF) of Sb-microelectrodes were examined with the apparatus shown in Fig. 1.

The temperature of the total assembly of the electrode system including pH and reference electrodes was maintained constant over the range of 7 to $37^\circ C$ by a thermoregulator with external circulation (Yamato, Coolnics). The chamber containing the sample was placed on a heat-insulating plate over a magnetic stirrer and the sample was stirred with this "cool-mixer" while monitoring with a thermistor. The Sb-microelectrode or glass electrode was inserted into the sample from the top of the chamber. A reference calomel electrode using 3 M KCl solution was connected with the sample through a 3 M KCl agar bridge. This

Apparatus for temperature and PO_2 calibration of Sb-microelectrode

Fig. 1. Experimental apparatus for measuring the effects of temperature and partial pressure of oxygen (P_{O_2}) on the electromotive force (EMF) of Sb-microelectrode.

Table 1. Composition of test solutions for the experiments of temperature and P_{O_2} effect.

	Tris buffer solution	Frog Ringer bicarbonate
NaCl	2.0	92.0
CaCl ₂	1.8	1.8
KCl	3.5	3.5
MgSO ₄	1.2	1.2
Na ₂ HPO ₄	1.58	1.58
NaH ₂ PO ₄	0.5	0.5
NaHCO ₃	15.6	15.6
Tris	90.0	—
<i>I</i> (Ionic strength)	0.127	0.127
Osmolality (mOsm/kg)	217	217

Values of electrolytes are shown in mM. The Tris buffer solution has the same composition as frog Ringer solution except for the substitution Tris for NaCl. Ionic strength and osmolality are the same.

buffer and Ringer bicarbonate (RB) solution were used for the test samples. Their compositions are shown in Table 1.

In the observation of P_{O_2} change, the test solutions were kept stirred at a fixed temperature of 25°C and the P_{O_2} was controlled by a continuous supply of oxygen gas with an oxygen pump. The P_{O_2} was measured with an oxymeter (Kyusui Chemical Institute, Bioxygraph) using a P_{O_2} electrode which was inserted into the sample near the bottom thereof.

3) *Blood pH measurement of bullfrog (Rana catesbeiana)*. Arterial blood was collected from a large abdominal vessel or the aorta with a heparinized syringe under anaerobic conditions, and transferred into a 10 ml beaker under mineral oil. The measurement of blood pH was performed both with the Sb-microelectrode and glass electrode simultaneously at room temperature.

RESULTS

1) *The change of EMF at different temperatures*

The change of electromotive force (EMF) of Sb-micro and glass electrodes was examined over the range of 7 to 37°C. The result is shown in Fig. 2. The magnitude in EMF change from the value at 25°C was plotted against the temperature in °C. The upper panel shows the data using Tris buffer solution. The open circles (○) are those from solution of pH 7.2 and the open triangles (△) those from solution of pH 7.8. The values were presented as mean ± SEM of 9 samples. It was found that in the Sb-microelectrodes calibrated with Tris buffer the change of EMF was relatively minor, whereas in the glass electrode the EMF increased

Fig. 2. Effect of temperature on the EMF of Sb-micro and glass electrodes in Tris buffer and frog Ringer bicarbonate solutions. Changes of EMF from the value at 25°C are plotted against temperature in °C.

Table 2. Temperature coefficients of EMF (dE/dt) in pH measurement by Sb-micro and glass electrodes in both Tris buffer and frog Ringer solutions.

		Temperature (°C)			Total range
		7-17	12-27	27-37	
Sb-microelectrode	Tris buffer	0.02 ±0.075	0.01 ±0.059	-0.02 ±0.055	-0.06 ±0.063
	Frog Ringer	-0.91 ±0.116	-1.21 ±0.106	-1.41 ±0.118	-1.18 ±0.113
Glass electrode	Tris buffer	1.42 ±0.055	0.91 ±0.022	0.84 ±0.028	1.05 ±0.036
	Frog Ringer	-0.08 ±0.037	-0.56 ±0.017	-0.65 ±0.050	-0.43 ±0.035

Values are presented in mV/°C as mean±SEM of 9 examples. Note that the temperature coefficient was negligibly small (*i.e.*, -0.06 ± 0.063 mV/°C) for Sb-micro-electrodes in Tris buffer solution.

almost linearly with the increase of temperature. The lower panel shows the data from frog Ringer bicarbonate solution. The solid circles (●) are the data of the glass electrode, while the solid triangles (▲) are those of the Sb-micro-

electrode. The degree of EMF change decreased with the increase of temperature in both electrodes, but the slope of Sb-microelectrodes was larger than that of glass electrodes.

Temperature coefficient of EMF. The temperature coefficient (dE/dt) of EMF for both electrodes was calculated in the range of 7 to 37°C, the data being listed in Table 2. The values were presented in mV/°C as mean $\pm$ SEM of 9 samples. The temperature coefficient was -1.18 ± 0.113 mV/°C for Sb-microelectrodes in Ringer solution, and -0.43 ± 0.035 mV/°C for glass electrodes. When estimated in Tris buffer solution, however, it was -0.06 ± 0.063 mV/°C for Sb-microelectrodes and 1.05 ± 0.036 mV/°C for glass electrodes.

2) The change of slope constant due to temperature change

The temperature may affect not only the EMF, but also the slope constant or sensitivity to pH in both Sb-micro and glass electrodes. Figure 3 shows the change of slope constant (α in $-mV/pH$) for the Sb-microelectrode due to temperature change. The slope constant of the Sb-microelectrode presented on the vertical axis was related to the temperature (°C) on the abscissa. The values are presented as mean $\pm$ SEM of samples of parenthesized numbers. As is shown in Fig. 3, the slope increased with the increase of temperature. The general trend was shown by a dashed line drawn by eye. The dotted line indicates the theoretical line which is derived from $2.303 RT/F$ (where R is the gas constant, T the absolute

Fig. 3. Change of slope constant (α in $-mV/pH$) for the Sb-microelectrode due to temperature change.

Fig. 4. A temperature-dependent deviation of pH readings by Sb-microelectrode from glass electrode, $\Delta \text{pH}_{\text{Sb-Glass}}$, produced by a combined effect of temperature on EMF and slope constant.

temperature and F the Faraday constant). The solid line shows the calculated regression line of the observed data, whose correlation coefficient is 0.975. Thus, one can predict the slope constant for the Sb-microelectrode from the empirical equation, as

$$\alpha = 0.40(t - 25) + 55.3, \quad (1)$$

where t represents the measuring temperature in °C.

3) The change of $\Delta \text{pH}_{\text{Sb-Glass}}$

Combining the temperature effects on the EMF and slope constant, the deviation of pH readings between Sb-micro and glass electrode, $\Delta \text{pH}_{\text{Sb-Glass}}$, was estimated, the result being shown in Fig. 4. The dashed line gives a general trend and the solid line the regression line, the latter being represented over the range of 7 to 37°C, as

$$\Delta \text{pH}_{\text{Sb-Glass}} = 0.00183(t - 25) + 0.016. \quad (2)$$

4) P_{O_2} effect

Effect of P_{O_2} on the EMF of the Sb-microelectrode was examined on 13 different microelectrodes, and the data are shown in Fig. 5. The EMF of the Sb-microelectrode in mV was plotted against the partial pressure of oxygen in mmHg on a logarithmic scale. The pH and temperature of solution were maintained at a constant level in each experiment. As seen in Fig. 5, the EMF log-linearly in-

Fig. 5. Effect of oxygen on EMFs of individual Sb-microelectrodes.

Table 3. Ratios of changes in EMF of Sb-microelectrode to oxygen partial pressure.

Exp't number	$\frac{dE}{d \log (P_{O_2})}$
1	12.5
2	10.2
3	11.4
4	8.6
5	10.7
6	12.7
7	12.3
8	13.4
9	14.4
10	10.9
11	12.5
12	12.2
13	10.9
Mean $\pm$ SEM (n)	11.7 $\pm$ 0.42 (13)

creased with the increase of P_{O_2} in the range of *ca.* 45 to 750 mmHg.

The ratio of changes in EMF to oxygen partial pressure was calculated for each electrode, the results being summarized in Table 3. The measuring temperature was 25°C. In the range of *ca.* 45 to 750 mmHg of P_{O_2} , the electrodes showed a response of $dE/d \log(P_{O_2}) = 11.7 \pm 0.42$ mV (mean $\pm$ SEM, $n=13$). This value is close to the theoretical value, 14.8 mV.

5) pH measurement of bullfrog blood

Using both Sb-micro and glass electrodes, the blood pH of bullfrog was measured at P_{CO_2} of 15 mmHg at various temperatures. The results are shown in Fig. 6 and Table 4, indicating that the blood pH decreased with the increase of temperature. In the former, the solid circles (●) were the data of the glass electrode, while the open triangles (△) those of the Sb-microelectrode. The mean temperature coefficient for frog blood pH under our experimental conditions (in temperature range of 21 to 25°C) was calculated to be $-0.0513/^\circ\text{C}$ (correlation coefficient, $r=0.644$, $p<0.01$, $n=15$) for the glass electrode and $-0.0610/^\circ\text{C}$ ($r=0.641$, $p<0.01$, $n=15$) for the Sb-microelectrode. The mean pH value of 15 different blood samples was 7.697 ± 0.092 (SD) with the pH glass electrode and 7.741 ± 0.110 with the Sb-microelectrode. Therefore, $\Delta\text{pH}_{\text{Sb-Glass}}$ was 0.044 ± 0.043 at the mean temperature of $23.1 \pm 1.2^\circ\text{C}$. When corrected for the effect of temperature by the use of the empirical formula (2), it was reduced to 0.032 ± 0.043 . This would suggest that an accurate frog blood pH could be estimated by Sb-microelectrode if the correction for temperature could properly be made.

Fig. 6. Relationship between the blood pH of bullfrog and the measuring temperature.

Table 4. pH measurement of bullfrog blood at various temperatures.

Sample	pH _{Glass}	Temp. (°C)	Δ pH _{Sb-Glass}	Δ pH corrected for temp.
1	7.731	21.0	0.085	0.076
2	7.740	21.9	0.000	-0.010
3	7.790	22.0	0.130	0.119
4	7.798	22.0	0.050	0.039
5	7.817	22.1	0.064	0.054
6	7.687	22.4	-0.015	-0.026
7	7.735	22.4	0.064	0.053
8	7.736	23.0	0.020	0.008
9	7.529	24.0	0.070	0.056
10	7.582	24.0	0.040	0.026
11	7.599	24.0	0.080	0.066
12	7.613	24.0	-0.040	-0.054
13	7.818	24.0	0.050	0.036
14	7.660	24.0	0.030	0.016
15	7.625	25.0	0.030	0.014
Mean	7.697	23.1	0.044	0.032
$\pm$ SD	± 0.092	± 1.2	± 0.043	± 0.043

DISCUSSION

Temperature coefficient. Generally, the electromotive force (EMF) of the pH calomel electrode assembly, E , is expressed by

$$E = E^\circ + \alpha \text{ pH}, \quad (3)$$

where, E° is the standard potential (mV), and α the sensitivity of electrode (slope) expressed in -mV per pH. By differentiating Eq. (3) with respect to temperature (t), one can derive

$$\frac{dE}{dt} = \frac{dE^\circ}{dt} + \text{pH} \frac{d\alpha}{dt} + \alpha \frac{d\text{pH}}{dt}. \quad (4)$$

(I) (II) (III)

Since (I) and (II) are considered to be particular constant values for the electrode system employed, they are combined and expressed as dE^*/dt .

Thus,

$$\frac{dE^*}{dt} \equiv \frac{dE^\circ}{dt} + \text{pH} \frac{d\alpha}{dt}. \quad (5)$$

The value of dE^*/dt for the Sb-microelectrode was determined by testing 2 solutions: i.e., (a) Tris buffer and (b) RB solution. As is shown in Fig. 2 and Table 2, the average of measured values of dE/dt for Tris and RB by Sb-microelectrode, was -0.06 and -1.18 mV/°C, respectively. Using Eqs. (4) and (5), one can calculate

the values of dE^*/dt for the Sb-microelectrode in Tris and RB solution, as (see APPENDIX)

$$\left(\frac{dE^*}{dt}\right)_{\text{Sb} \cdot \text{Tris}} = \frac{dE}{dt} - \alpha \frac{dpH}{dt} = -0.06 - 1.63 = -1.69 \text{ mV}/^\circ\text{C},$$

$$\left(\frac{dE^*}{dt}\right)_{\text{Sb} \cdot \text{RB}} = \frac{dE}{dt} - \alpha \frac{dpH}{dt} = -1.18 - 0.15 = -1.33 \text{ mV}/^\circ\text{C}.$$

The difference in these two values, unless due to that of liquid junction potential, would probably depend on the error concerning $d\alpha/dt$ (the component (II)), because the Sb-microelectrode which behaves non-ideally might react differently to Tris or RB even during the calibration stage where all the physicochemical properties (ionic strength, osmolality, *etc.*) are maintained identical in both solutions. Since we were unable to decide which was more suitable for the value of $(dE^*/dt)_{\text{Sb}}$ in general, we regarded $-1.51 \text{ mV}/^\circ\text{C}$, the mean value of the two, as the real value of dE^*/dt for the Sb-microelectrode for the sake of convenience.

When the Sb-microelectrode is calibrated with Tris buffer, the calculation of Eqs. (4) and (5) yields

$$\left(\frac{dE}{dt}\right)_{\text{Sb} \cdot \text{Tris}} = -1.69 + 1.63 = -0.06 \text{ mV}/^\circ\text{C}.$$

It is noted that the two components, dE^*/dt and $\alpha \cdot dpH/dt$ of the temperature coefficient for the Sb-microelectrode cancel each other in Tris buffer solution. This suggests that changes of temperature may have little effect on the EMF of the Sb-microelectrode when calibrated with Tris buffer solution.

The temperature coefficient in terms of $d\alpha/dt$ for the Sb-microelectrode was shown to be $-0.40 \text{ mV/pH}/^\circ\text{C}$, as indicated in the regression equation in Fig. 3. It is about twice as great as the theoretical value of thermodynamic response, $0.20 \text{ mV/pH}/^\circ\text{C}$, as given by the dotted line in Fig. 3. The reason for this difference is not clear in detail, but it might be related to the fact that the reactions underlying the antimony electrode system are not so simple, because of the complicated participation of several components including metal oxides, oxyanions and oxycations (see Fig. 7 below).

Table 5 summarizes all the data stated above which concerns the temperature coefficient for both the glass and Sb-microelectrode measured in Tris and RB solutions.

From a practical point of view, the temperature coefficient of $\Delta pH_{\text{Sb-Glass}}$ would become simpler as far as we could use $0.00183 \text{ pH}/^\circ\text{C}$ as an empirical value (Fig. 4). In other words, it may be stated that in practice the Sb-microelectrode, calibrated with Tris buffer, should be corrected for the temperature effect by means of Eq. (2), as was described previously.

Oxygen effect. In the antimony-antimony oxide electrode, the EMF is probably developed by an oxidation-reduction reaction involving antimony and the

Table 5. Temperature coefficients for the glass and Sb-microelectrode in Tris and RB solutions.

		Temperature coefficient†			
		$\frac{dE}{dt}$ (mV/°C)	$\alpha \cdot \frac{dpH}{dt}$ (mV/°C)	$\frac{dE^*}{dt}$ (mV/°C)	$\frac{d\alpha}{dt}$ (-mV/pH/°C)
Glass elect.	Tris	1.05	1.63	-0.58	0.20
	RB	-0.43	0.15		
Sb-elect.	Tris	-0.06	1.63	-1.69	0.40
	RB	-1.18	0.15	-1.33	

The temperature coefficient of the electrode system is shown by the equation. The terms involved in the equation are given in the individual columns of the table. For explanation, see text.

$$\text{†Temperature coefficient} = \frac{dE}{dt} = \frac{dE^\circ}{dt} + pH \frac{d\alpha}{dt} + \alpha \frac{dpH}{dt} = \frac{dE^*}{dt} + \alpha \frac{dpH}{dt}.$$

Fig. 7. Reactions of antimony-antimony oxide electrode system.

thin layer of antimonous oxide (commonly antimony trioxide, Sb₂O₃) that is usually present on the surface of the metal. The whole reaction which will occur in the system is summarized in Fig. 7, illustrating that conditions of metal oxides have various influences on the behavior of the electrode. The main electrode reaction is indicated by thick lines. An additional reaction involving oxygen may also participate in this reaction. Simply, Sb₂O₃ is produced from 2Sb and 3/2 (O₂), by the reaction

This process itself does not directly generate electric current or potential, because there is no electron flow in this process. However, it certainly affects the rate of formation of Sb₂O₃ which is associated with a current generating system,

Therefore, some potential change, if any, would take place as a result of the interfering effect of reaction (6) on the main reaction (Eq. 7).

One can derive the change of EMF in the Sb-electrode, ΔE_{Sb} , as (see APPENDIX)

$$\Delta E_{\text{Sb}} = -59.2\nu(\Delta\text{pH}) + 14.8\nu r \Delta \log(P_{\text{O}_2}) \quad (\text{at } 25^\circ\text{C}). \quad (8)$$

(mV)

If the Sb-electrode behaved as a true oxygen electrode, then both ν and r would become unity and $dE/d \log(P_{\text{O}_2})$ would be 14.8 mV per tenfold change of P_{O_2} in mmHg, under conditions of constant pH (IVES, 1961). In our experiment, $dE/d \log(P_{\text{O}_2})$ was 11.7 ± 0.42 mV (at 25°C , $n=13$, mean $\pm$ SEM) (see Table 3) at the constant pH of 7.7 ± 0.1 . Our electrodes had a mean slope of -55.3 mV/pH (see Fig. 3), which indicated that ν was $0.93 (=55.3/59.2)$, and hence, r was $0.85 (=11.7/(14.8 \times 0.93))$. Thus, in view of various errors included, our result was essentially in agreement with the theoretical value and the data (14.7 mV at 25°C) of KAUKO and KNAPPSBERG (1939), but different from the data of MALNIC and his coworkers (1972) and PUSCHETT and ZURBACH (1974), who did not find a significant effect of P_{O_2} on the EMF of the Sb-microelectrode, and of QUEHENBERGER (1977), who reported a higher value of 58.5 mV per tenfold change of P_{O_2} . The reason for the discrepancy between our findings and data of these workers remains unclear. Even the predictions that the higher oxygen tension would form the higher antimony oxides (EL WAKKAD, 1950) and that the standard potential would be affected by oxygen tension (TOURKY and MOUSSA, 1948) are not yet clearly defined.

However, it can be stated from our data that the pH measurement in the range of 50 to 100 mmHg of P_{O_2} , ΔE is only ± 0.07 mV per 1 mmHg change of P_{O_2} . Since it corresponds to ± 0.0012 of ΔpH , the effect of P_{O_2} on the Sb-microelectrode is relatively small in the physiological range where no large change of P_{O_2} is produced.

Measurement of blood pH. As a biological application of the Sb-microelectrode, blood pH of bullfrog was determined at various room temperatures. pH

values tended to decrease with a rise of temperature with both Sb-micro and glass electrodes. This temperature effect was larger in the Sb-microelectrode than in the glass electrode, unless the correction for the change of temperature coefficient was made. By correction for the temperature effect by means of Eq. (2), the value of $\Delta\text{pH}_{\text{Sb-Glass}}$ was reduced from 0.044 ± 0.043 (SD) to 0.032 ± 0.043 (see Table 4).

Previously, YOSHIMURA (1936) reported that the pH value measured by means of the antimony electrode differed markedly from that determined by the hydrogen gas electrode or the glass electrode. His value for $\Delta\text{pH}_{\text{Sb-Glass}}$ ranged from -0.007 to -0.184 in goat plasma and $\Delta\text{pH}_{\text{Sb-H}_2}$ from 0.293 to 0.466 in horse plasma. Thus his conclusion was that the antimony electrode could not be applied to the determination of plasma pH. The reason for the discrepancy between our findings and his data is not clear, but would probably be related to the following facts: (1) Difference of electrode. His electrode was a plated and stick-type antimony electrode, whereas ours was a microelectrode with highly purified antimony metal (99.999%) mounted in a glass capillary, only the tip being made pH sensitive and having the advantages of suffering a lesser degree of stirring effect and minor heterogeneity of the metal surface. (2) Difference of calibration solution. Yoshimura used Mc Ilvaine buffer and Sørensen phosphate buffer for calibration, which have sizable and varied effects on the EMF of the Sb-electrode, whereas we used Tris buffer, which has the least effect (MATSUMURA *et al.*, 1980). (3) Difference of electrometry. We used electrode cables with highly insulating material (teflon), connected to an electrometer with high input impedance of more than $10^{14} \Omega$. Though Yoshimura used an excellent vacuum tube (Toshiba UX-54) whose grid current was of the order of 10^{-14} A, 40 years ago was not a favorable time for obtaining a better insulating material for the connecting cable and electronic apparatus.

In all likelihood, therefore, we may conclude that a more or less fair measurement of pH of biological fluid would be possible by the Sb-microelectrode in so far as the temperature effect is taken into account and calibration is properly done.

APPENDIX

Temperature coefficient

The temperature coefficient of the EMF of the pH calomel electrode system can be expressed as

$$\frac{dE}{dt} = \frac{dE^*}{dt} + \alpha \frac{dpH}{dt}. \quad (9)$$

By electrometry of the total electrode system, only the value for dE/dt can be measured. For the determination of individual components of the temperature coefficient, our approach was by means of the following steps.

In the first step, dE^*/dt can be assessed by comparing the theoretical and actually measured temperature coefficients of a well-characterized electrode system, such as a glass pH electrode with internal and external reference electrodes of calomel half-cells. In Tris buffer, for example, the theoretical temperature coefficient could be approximated (BATES, 1973), as

$$\begin{aligned}\frac{dpH}{dt} &\simeq -\frac{d \log K_w}{dt} + \frac{d \log K_b}{dt} \\ &\doteq -0.0330 + 0.0054 \\ &\doteq -0.0276 \text{ pH}/^\circ\text{C} \text{ (at } 25^\circ\text{C)},\end{aligned}\quad (10)$$

where K_w and K_b are the ionic product of water and the dissociation constant of organic base, Tris-(hydroxymethyl)-aminomethane HCl, respectively. Converting the unit of pH/ $^\circ\text{C}$ into mV/ $^\circ\text{C}$ and assuming α for the glass electrode to be -59.2 mV/pH, one can obtain the theoretical value for $\alpha \cdot dpH/dt$ as 1.63 mV/ $^\circ\text{C}$. The measured value for Tris buffer, dE/dt , was, on average, 1.05 mV/ $^\circ\text{C}$, as is shown in Table 2. From Eq. (9), dE^*/dt for the glass electrode is calculated to be -0.58 ($=1.05-1.63$) mV/ $^\circ\text{C}$. Thus, for the glass electrode system, one can use the empirical formula

$$\left(\frac{dE}{dt}\right)_{\text{GLASS}} = -0.58 + \alpha \cdot \frac{dpH}{dt}. \quad (11)$$

In the second step, the temperature coefficient which may be theoretical for Ringer bicarbonate solution (RB) is calculated from Eq. (11) for the glass electrode. As the measured value of dE/dt for RB was -0.43 mV/ $^\circ\text{C}$ (see Table 2), the value of $\alpha \cdot dpH/dt$ for RB is estimated to be 0.15 ($=-0.43-(-0.58)$) mV/ $^\circ\text{C}$.

Oxygen effect

In the presence of oxygen, reactions (6) and (7) proceed, generating an electron flow across the solid and liquid interfaces. If extra oxygen was added to main reaction (7), then the following electrochemical process might be expected:

where r is the molar ratio of participating gaseous oxygen with respect to the unit occurrence of the main redox reaction; n is the oxidation number of antimony metal, such as 3 (trioxide), 4 (tetroxide) and 5 (pentoxide); (s) and (l) indicate solid and liquid phase, respectively. In the above equation, if oxygen is not participating in the system, r is zero. Additionally, if n is 3, as is usually seen, then Eq. (12) is reduced to Eq. (7), which is independent of oxygen tension.

In diluted aqueous media, the EMF of this system would be practically a function of hydrogen ion concentration, $[\text{H}^+]$:

$$E_{\text{Sb}} = E_{\text{Sb}}^* + \frac{\nu RT}{F} \ln [\text{H}^+], \quad (13)$$

where ν is an empirical constant (dimensionless) which takes into account the non-ideal behavior of the Sb-microelectrode. For a more precise reaction, as described in Eq. (12), we have

$$E_{\text{Sb}} = \overline{E}_{\text{Sb}} - \frac{\nu RT}{F} \ln \frac{[\text{Sb}]^{(1-r)/n} [\text{H}_2\text{O}]^{(1/2)}}{[\text{H}^+][\text{Sb}_2\text{O}_n]^{(1-r/2n)} [\text{O}_2]^{(r/4)}}, \quad (14)$$

where $\overline{E}_{\text{Sb}}$ is the standard potential of the system, in which oxygen is involved. Since the partial molal volume of metal Sb is unity, the equation concerning the EMF (mV) of the Sb-microelectrode is written by the following expression:

$$E_{\text{Sb}} = \overline{E}_{\text{Sb}} - 0.1983\nu T \left[\text{pH} - \frac{r}{4} \log (\text{O}_2) - \frac{1-r}{2n} \log (\text{Sb}_2\text{O}_n) + \frac{1}{2} \log (\text{H}_2\text{O}) \right]. \quad (15)$$

(mV)

Further, Henry's gas law states

$$(\text{O}_2) = \beta_{\text{O}_2} (P_{\text{O}_2}), \quad (16)$$

where, β_{O_2} stands for the absorption coefficient of O_2 , and P_{O_2} denotes the partial pressure of oxygen in mmHg. Substituting Eq. (16) into Eq. (15), and assuming that the concentration of solid Sb_2O_n and liquid H_2O remains constant in the reaction, one can derive the change of EMF in the Sb-electrode, ΔE_{Sb} , as

$$\Delta E_{\text{Sb}} = -59.2\nu (\Delta \text{pH}) + 14.8\nu r \Delta \log (P_{\text{O}_2}) \quad (\text{at } 25^\circ\text{C}). \quad (8)$$

(mV)

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