

and occasional interruptions of service for recharging the storage cells, and the second is relatively complex and requires the manufacture or purchase of a number of special resistances.

The author feels that a description of the simple a-c relay developed in this laboratory will be useful to others requiring a sensitive relay for temperature con-

## ON THE GRAPHIC REPRESENTATION OF **IONIC EQUILIBRIA IN BLOOD SERUM**

DURING the session of 1915-16 I made a study of ionic equilibria in sea water of 2.3 millimols per liter alkaline reserve, and plotted the results on log-log paper.<sup>1</sup> Log  $[H^+]$  was measured on the x axis and log. CO, pressure (later reduced to mm of mercury) on the y axis. During the session of 1916–17 I plotted similar values for blood serums, but in this case the alkaline reserve (bicarbonate), titrated in a rotating hydrogen electrode vessel, varied from sample to sample, so distinguishing marks were used for each sample in marking the values on the log-log paper and it was found that the values of bicarbonate formed a logarithmic scale on an axis at  $45^{\circ}$  to the x and y axes.<sup>2</sup> Later I learned of the mathematical treatment of this subject by Hasselbalch<sup>3</sup> and applied the equation

$$[\mathbf{H}^+] = k_2 \frac{p \mathrm{CO}_2}{[\mathrm{BHCO}_3]}$$

to that point on each graph where  $pCO_{2} = [BHCO_{2}]$ and hence  $[H^+] = k_2$  and  $pH = pk_2$  (denoting log of reciprocal of  $k_2$ ). It was found that  $pk_2$  of sea water was 7.08 at 0°, 7.20 at 10°, 7.32 at 20° and 7.44 at 30° at the points where  $[BHCO_3] = pCO_2$ , but inspection of the graph showed that  $k_2$  varied slightly with  $CO_2$ 

<sup>3</sup> Biochem. Z., 78: 113, 1917.

trol or other purposes. The circuit is shown in the accompanying diagram. The relay consists of a "45" power amplifier vacuum tube, a Leach No. 1305 a-c relay (R), two resistors  $(G_1 \text{ and } G_2)$  and a transformer with a 110 volt primary (P), a 660 volt center tapped secondary (S) and a 2.5 volt center tapped filament supply winding (F) (Inca transformer, type C-31). TR is the mercury thermoregulator and H is the thermostat heating element. The only electrical supply required is 110 volts a-c. The power consumption (exclusive of H) is 35 to 40 watts. The resistors G<sub>1</sub> and G<sub>2</sub> have the values 2 and 7 megohms, respectively. The parts for the relay are commercially available and inexpensive.

A relay similar to the above has given nearly two years of trouble-free service, regulating the temperature of a covered water thermostat to  $\pm 0.002^{\circ}$  C. and another is controlling the temperature of an open stirred water bath at  $25^{\circ} \pm 0.01^{\circ}$  C.

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## SPECIAL ARTICLES

pressure. Whether this was due to partial change of BHCO<sub>3</sub> to B<sub>2</sub>CO<sub>3</sub> with fall of CO<sub>2</sub> pressure or due to experimental errors was not determined. In case of blood serums it was thought that errors in titrating [BHCO<sub>3</sub>] would be greater at lower values, and with the higher values  $pk_{2} = 7.5$  at 20°. From the data on sea water it seems evident that  $pk_2$  of blood serum would be at least 0.12 higher at 38° than at 20° and so a value of  $pk_2 = 7.62$  might be guessed at. Preliminary attempts at determination of  $k_{o}$  at 38° showed varying results and were interrupted by my entrance into military service, and after the war I constructed log-log-pH graph paper on three axes at angles of  $60^{\circ}$ with each other and posted it in the laboratory for the class in physiological chemistry. Since then many papers have appeared on ionic equilibria in blood and new values of the standard hydrogen electrode higher than those used by Sörensen have been used.

Hasselbalch and most later workers instead of titrating [BHCO<sub>3</sub>] of serum, added acid and pumped out the CO<sub>2</sub> and measured it and calculated [BHCO<sub>3</sub>] and, instead of using  $pCO_2$  in an equation, first calculated the CO<sub>2</sub> physically dissolved in the serum, calling it " $H_2CO_3$ ," using two constants k' and c where  $\mathbf{k'c} = \mathbf{k}_{a}$ .

$$[\mathrm{H}^+]_{\mathrm{s}} = k'c \, \frac{p\mathrm{CO}_2}{[\mathrm{BHCO}_3]_{\mathrm{s}}}.^4$$

where  $c = 0.0591 \alpha.5$ 

4 L. J. Henderson, "Blood," New Haven (1928) equations 6-7, p. 42.

<sup>&</sup>lt;sup>1</sup> Publication No. 251, Carnegie Institution of Washington, p. 36, Fig. 6, 1917. <sup>2</sup> Jour. Biol. Chem., 519: 522, Fig. 1, 1917.

Earlier workers used Bohr's value of  $\alpha = 0.541$  at  $38^{\circ}$  in which case  $c = 10^{-1.495}$ , whereas most recent workers have used Van Slyke, Sendroy, Hastings and Niel's<sup>6</sup> value of  $\alpha$  of 0.51 at 38°, in which case  $c = 10^{-1.92}$ . Hastings, Sendroy and Van Slyke<sup>7</sup> reviewing recent literature found pk' averaged 6.104, using Bohr's  $\alpha$ , and 6.13, using their own value of  $\alpha$ . In either case  $pk_2 = 7.625$ . If, however, the value given in their summary of pk' = 6.10 is used,  $pk_2$  becomes 7.62. Since the value of  $\alpha$  is not the same for all serums it seems of advantage to use  $k_2$  in place of k'c and plot quantities that can be directly determined: *i.e.*, pH,  $pCO_2$  and  $[BHCO_3]$ . Hence I have redrawn my log-log-pH paper with  $k_2 = k'c$  of Hastings, Sendroy and Van Slyke, and reproduce it here (Fig. 1).



Since at all points where  $pCO_2 = [BHCO_3]$ ,  $pH = pk_2$ , I have marked that value on the pH scale. In case any other value of  $pk_2$  is considered more correct it is only necessary to slide the numbers up or down the pH scale until the  $pk_2$  line corresponds to its new value.

On this graph paper it is easy to mark what takes place during a respiratory cycle or during acidosis or alkalosis, both compensated and uncompensated. In health and comparative rest the values of the blood fluctuate around the center of the chart, being in the arteries above and to the right of the center and in the veins below and to the left of the center. Under extreme conditions the values may go beyond the range of the graph. For example, in order to remove the compensating action of the respiratory center a cat was put under artificial respiration, and when this was markedly increased, the values for arterial blood moved off the graph upward and to the right, whereas the values for the veins remained nearer the center. When the maximum rate of the artificial respiration apparatus was reached an attempt was made to blow more CO, out of the blood by removing the surface layer from the base of the lungs with sandpaper and blowing a continuous stream of air through the lungs. The same result was obtained, the venous blood remaining near its normal value. The explanation of this was found in observing the output of the heart. When the respiratory center was put out of action the center or centers controlling the circulation (vasomotor and vagus centers?) regulated the blood and, although the arterial blood was very deficient in CO<sub>2</sub>, the blood moved so slowly through the capillaries that its normal  $CO_2$  content was restored.

Since the arterial blood is spread over 125 sq. m. of surface in contact with alveolar air in the lungs, it is safe to assume that the CO<sub>2</sub> partial pressure in the alveolar air is as close to that of arterial blood as could be determined in any ordinary apparatus. Although the partial pressure is not uniform in the different alveoli, the mixed alveolar air should be very close to the mixed arterial blood in CO<sub>2</sub> partial pressure and hence at  $38^{\circ}$  these three values of pH, CO<sub>2</sub> pressure and bicarbonate concentration may be determined in relation to the arterial blood taken from the living subject with precautions against loss of CO<sub>2</sub> in the sample. In venous blood, however, it seems to me that the CO<sub>2</sub> pressure is the most difficult to determine and it is better to determine pH and  $[BHCO_3]$  and find  $pCO_2$  on the graph.

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## SEXUAL PHASES IN PROSOBRANCH MOL-LUSKS OF THE GENUS CREPIDULA

PROTANDRY, proterogyny, true hermaphroditism and, occasionally, self-fertilization have long been known to occur in Gastropods. In this group the species of Crepidula are of special interest, however, because in C. plana, which is normally protandric, it has been thought that the association of the young animal with an older individual, particularly one in the female phase, is essential for the development, as well as for the maintenance, of the functional male phase.<sup>1</sup> In another species, C. fornicata, which is likewise protandric, the length of time which the animal spends in the male phase was believed to be correlated with its continued opportunity for insemination.<sup>2</sup>

<sup>1</sup> Harvey N. Gould, Jour. Exp. Zool., 23: 1-69; Jour. Exp. Zool., 23: 225-250, 1917.

<sup>&</sup>lt;sup>5</sup> Peters and Van Slyke, "Quant. Clinical Chem.," 1: 878, equation 11, 1931. 6 Jour. Biol. Chem., 78: 765, 1928.

<sup>&</sup>lt;sup>7</sup> Jour. Biol. Chem., 79: 183, 1928.