CCIII. A COMPARISON OF THE COLORIMETRIC AND ELECTROMETRIC METHODS FOR THE DETERMINATION OF THE p_H OF SERUM OR PLASMA.

BY RUSSEL JOHN FOSBINDER
AND JANETTA WRIGHT SCHOONOVER.

*From the Cancer Research Laboratories of the Graduate School of Medicine
of the University of Pennsylvania, Philadelphia, Pennsylvania.*

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THE colorimetric method, because of its simplicity and the rapidity with which determinations may be made, has been the object of many biochemical investigations. Levy, Rowntree and Marriott [1915] devised the dialysis method in which phenol red was added to the dialysate of the blood, and the resulting colour compared to a set of standards. Later, Dale and Evans [1920-21] improved this method by preventing the loss of CO_2 from the system. Cullen [1922] eliminated the error due to the Donnan effect in the dialysis method by a procedure employing diluted plasma, in which an empirical correction, determined by a comparison of the colorimetric results obtained at 20° with those obtained by the hydrogen electrode at 38° , allowed for protein, salt, dilution, and temperature effects. Employing the Gillespie principle, Hastings and Sendroy [1924] developed a system of bicolour standards which permitted the colorimetric determination of the p_H of plasma or of whole blood at 38° , obviating the use of the empirical Cullen C correction.

The accuracy of the colorimetric method.

The value of the Cullen C correction for human plasma is $0.22 p_H$, thus the calculation for the reaction of blood at 38° is:

$$[p_H]_{38^\circ} = [p_H]_{20^\circ} - 0.22.$$

Bennett [1926] apparently found that the value of C was not a constant for the same blood under various conditions. Hastings and Sendroy [1924], on the other hand, reported that the results of the colorimetric and electrometric methods on different species of blood agreed very well when the colorimetric determination was carried out at 38° . In addition to their own procedure, they employed the single colour standard method of Cullen, and the modification of Cullen's method devised by Hawkins [1923].

Austin, Stadie and Robinson [1925] compared the results of p_H determinations on dog, sheep, and human serum or plasma, determined gasometrically and electrometrically at 38° , with colorimetric results obtained at 38° and

at 20°. They concluded that the colorimetric reading of a serum or plasma, obtained by the technique of Hastings and Sendroy, could not be relied upon.

Myers and Muntwyler [1928], using the micro-colorimetric method of Myers, Schmitz and Booher [1923], state that "colorimetric and electrometric determinations of the p_H made on the same sample of blood agree within 0.02 p_H when both determinations are made at the same temperature." Furthermore, there seemed to be no apparent reason for the observed variations in the C correction. The results of 51 protein determinations which they carried out indicated that a change in protein concentration is not a factor. This is in agreement with Marrack and Smith [1924], who found that the Cullen correction did not change with abnormalities in plasma proteins.

Errera, Reding and Slosse [1929] have compared the results of plasma p_H determinations at 38°, obtained by the colorimetric method of Hastings and Sendroy, with those obtained by the use of the glass electrode. The results of 30 determinations on the plasma of normal and cancerous patients indicate that the colorimetric method is reliable.

These findings are further supported by those of Bayliss, Kerridge and Verney [1926], who found excellent agreement between results obtained by the colorimetric, hydrogen electrode and glass electrode methods.

THE PRESENT INVESTIGATION.

To test the reliability of the colorimetric method, it was decided to compare colorimetric readings made at 38° on plasma with p_H values obtained by the use of the glass electrode on the same material and for this purpose the apparatus described elsewhere by Fosbinder [1930] and by Fosbinder and Schoonover [1930] was used.

If plasma is diluted 20-fold, thereby reducing the partial pressure of the free CO_2 to approximately 1/20 of its original value, it is possible to carry out electrometric measurements on this material at 38° without loss of CO_2 by covering the surface of the liquid with paraffin oil. Errera, Reding and Slosse [1929] apparently found this procedure to be unsatisfactory. However, Hastings and Sendroy [1924] observed no appreciable shift in the p_H of bicarbonate buffers of physiological strength when they were diluted 5- to 20-fold with 0.154 *M* sodium chloride.

Procedure and results.

The p_H of plasma at 38° was determined by the colorimetric method of Hastings and Sendroy [1924]. The vacuum tube potentiometer developed by one of us was used to determine glass electrode potentials. Glass electrodes, of the form described by MacInnes and Dole [1929, 1930], and identical in composition, were employed.

The electrometric p_H values were determined with the use of the following cell

In order to diminish the liquid junction potential between the saturated potassium chloride and the diluted plasma, 0.15 *M* KCl containing phenol red was employed as the diluent. Adjustment of the potassium chloride indicator solution, which served as the plasma diluent in both the colorimetric and glass electrode methods, was accomplished by adding to it a solution of 0.01 *N* NaOH, containing in addition potassium chloride and phenol red in the same concentrations as those of the indicator solution. This procedure avoided the possibility of any error due to dilution. The maximum buffer value of the indicator solution was 0.10 *mM* of NaOH per unit p_H per litre, which is approximately 1/50 of the buffer value of the plasma. Fortunately, it was possible to substitute potassium chloride for sodium chloride, for Hastings and Sendroy [1929] found that the dissociation constants of phenol red in the two solutions are identical at 20°, therefore, we may use the sodium chloride value at 38°, 7.65, for potassium chloride.

The bicolour standards were compared with a recently prepared set of standards of known log *R* values. The results of this comparison are given in Table I.

Table I. *Log R and p_H values of phenol red bicolour standards.*

Apparent p_H values of indicator pairs	log <i>R</i>	Corrected p_H values of indicator pairs
7.15	-0.49	7.16
7.20	-0.45	7.20
7.25	-0.40	7.25
7.30	-0.35	7.30
7.35	-0.30	7.35
7.40	-0.25	7.40
7.45	-0.20	7.45
7.50	-0.15	7.50
7.55	-0.09	7.56
7.60	-0.05	7.60
7.65	0.00	7.65
7.70	+0.08	7.73

The procedure employed in determining the glass electrode p_H values was a comparison of the potentials observed with the diluted plasma with the potential of a *M*/15 phosphate buffer solution of known p_H . The p_H value of the buffer solution was determined daily both colorimetrically and electrometrically. The electrometric standard was 0.1 *N* HCl, which was assumed to have a p_H value of 1.08 at 38°. To calculate the p_H of the plasma the following equation was used

$$p_H (\text{plasma}) = p_H (\text{buffer}) + \frac{E - e}{0.0617},$$

where *E* = the potential observed with diluted plasma, and *e* = the potential observed with the buffer.

The technique employed in the determination of the p_H of the diluted plasma was as follows. 2 cc. of 0.15 *N* KCl indicator solution, adjusted to a p_H of approximately 7.4, were added to the electrode cell, and the glass electrode immersed in the solution. A layer of paraffin oil, approximately

7 mm. in depth, was then placed over the surface of the liquid, sealing the solution from the air. One-tenth of a cc. of plasma was then added to the diluent beneath the surface of the oil and the solution thoroughly stirred with a small glass rod. The plasma was introduced from a tuberculin syringe, so much plasma being drawn into the syringe that the colorimetric and the electro-metric readings could be made on the same sample. There was no evidence of CO_2 escaping from the diluted plasma, for the potential remained constant over a considerable period of time.

The results of 28 unselected determinations of normal and pathological plasmas are given in Table II.

Table II. p_H values of diluted plasma obtained by the colorimetric and glass electrode methods.

Description of plasma	p_{H38^e}	$[p_H]_{38^e}$	$[p_H]_{38^e} - p_{H38^e}$	$([p_H]_{38^e} - c_{p_H'}) - p_{H38^e}$
Normal	7.38	7.39	+0.01	0.00
	7.38	7.39	+0.01	0.00
	7.41	7.42	+0.01	0.00
	7.40	7.42	+0.02	+0.01
	7.34	7.35	+0.01	0.00
	7.37	7.37	0.00	-0.01
	7.39	7.39	0.00	-0.01
	7.41	7.39	-0.02	-0.03
	7.38	7.40	+0.02	+0.01
Normal plasma allowed to stand 24 hours	7.35	7.36	+0.01	0.00
	7.28	7.29	+0.01	0.00
	7.29	7.29	0.00	-0.01
	7.20	7.19	-0.01	-0.02
	7.28	7.29	+0.01	0.00
Cancerous	7.37	7.37	0.00	-0.01
	7.31	7.32	+0.01	0.00
	7.39	7.38	-0.01	-0.02
	7.38	7.37	-0.01	-0.02
	7.39	7.39	0.00	-0.01
	7.39	7.38	-0.01	-0.02
	7.39	7.39	0.00	-0.01
	7.41	7.42	+0.01	0.00
	7.42	7.42	0.00	-0.01
	7.35	7.36	+0.01	0.00
	7.40	7.38	-0.02	-0.03
	7.40	7.38	-0.02	-0.03
Cancerous? Goitre	7.36	7.35	-0.01	-0.02
	7.36	7.39	+0.03	+0.02
Average deviation	—	—	+0.002	-0.008
Maximum deviation	—	—	0.03	0.03

In column 5 is given the deviation between the two methods when the dissociation constant of phenol red is corrected to $\log R = 0$. [See Hastings and Sendroy 1929, p. 238.]

DISCUSSION.

The remarkably close agreement between the results of the two methods

$$[p_H]_{38^\circ} - p_{H38^\circ e} = +0.002 \text{ or } [p_H]_{38^\circ} (\text{corr.}) - p_{H38^\circ e} = -0.008$$

shows that there are no deviations which cannot be accounted for otherwise than by experimental error. Our observations on diluted plasma are in agreement with the results obtained on whole plasma by Hastings and Sendroy [1924], Myers and Muntwyler [1928], Errera, Reding and Slosse [1929], and others.

Most authors are in agreement that the normal p_H of the blood lies between 7.30 and 7.40. However, in the case of normal plasma, one would not expect to find so great a range of deviation. The average of 9 electrometric determinations made by us on the p_H of plasma of normal individuals is 7.38, with a deviation from the mean of $+0.005 p_H$. Cullen and Robinson [1923], in 27 determinations of the p_H of normal plasma, found a variation from 7.28 to 7.41. Nevertheless, it is significant that 21 of the 27 determinations lay between 7.35 and 7.40. Quoting Reding [1928], the average normal value obtained by the electrometric method was found by Michaelis to be 7.35; Cullen, 7.35; Van Slyke, 7.35; Vincent, 7.34. Normal values obtained by the colorimetric method are: Cullen and Hastings, 7.36; Bigwood, 7.36; Cullen and Robinson, 7.35. Corran and Lewis [1924], using the quinhydrone electrode, obtained an average value of 7.33 on the whole blood of nine normal patients at 37° . If we add to this value $0.06 p_H$, which is the protein error of the electrode, we find that the average value for normal blood is 7.39. Reding [1928], in the study of 25 normals, arrives at an average value of 7.36 with the use of the colorimetric method. Results obtained by other workers in our laboratory¹ on 15 normals yield a value of 7.37. From these studies it appears very probable that the average normal p_H of the blood lies between 7.35 and 7.38.

No attempt is made here to compare the p_H of the blood of cancer patients with that of the blood of normal subjects, since all of the samples of blood which we received were drawn from patients who had already received a considerable amount of treatment.

Our results are not influenced by the fluctuations of the acid-base balance during the day, as observed by Cullen and Earle [1929], since all our determinations were made upon blood which was drawn at approximately the same hour each day.

SUMMARY.

1. The colorimetric method of Hastings and Sendroy may be used with accuracy for the determination of the p_H of blood or plasma at 38° .
2. Determinations carried out at 38° on 28 samples of diluted plasma from

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cancerous and normal subjects reveal no discrepancy between the colorimetric and glass electrode methods.

3. The p_H of normal plasma or blood lies between 7.35 and 7.38.

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