CXLI. THE TECHNIQUE OF GLASS ELECTRODE MEASUREMENTS.

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IN the course of an investigation into the value of the glass electrode as a means of making continuous records of the hydrogen ion concentration of circulating blood, it was found, in the preliminary experiments in which estimations were made on single samples, that there were several sources of serious error. The difficulties encountered in devising a satisfactory apparatus for measuring glass cell potentials have been overcome, and a suitable form of thermionic valve electrometer has been evolved [Platt and Winfield, 1933]. The present paper is concerned with technical details which must be carefully considered before accurate results can be obtained consistently.

DESCRIPTION OF THE APPARATUS.

The glass cell system consists of an electrode vessel carrying in a waxed cork the glass membrane and a 0.1 *N* HCl-quinhydrone half-cell. The electrode vessel is provided with a tap *A* and is connected by a 3.5 *M* KCl bridge, as

Fig. 1.

shown in Fig. 1, to another 0.1 *N* HCl-quinhydrone half-cell. A reservoir containing 3.5 *M* KCl and fitted with a thermometer and a stopcock is so arranged that there are no differences in hydrostatic pressure throughout the system such as are likely to disturb the liquid-liquid junctions. The type of stopcock used in most of the experiments is shown in the figure, but a more convenient alternative

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arrangement (see inset Fig. 1) is a set of 3 three-way taps, the limbs at 120° with V-shaped bores. These are substituted for *B* and *D*, the third interrupting the bridge so that the liquid-liquid junction may be made at *D* and preserved undisturbed in subsequent manipulations at *B*.

The procedure adopted was as follows. With tap *C* closed, tap *B* in the position shown and *D* in the corresponding position, the clip on the rubber tubing at *E* was opened and the bore of *D* flushed with 0.1 *N* HCl-quinhydrone solution. Tap *C* was then opened, and the potassium chloride solution was washed through the bore of *D* as it was rotated in a clockwise direction for the formation of the liquid-liquid junction to the position shown in the diagram. The electrode vessel was filled through tap *A* from a Record syringe attached by narrow rubber tubing. The liquid-liquid junction was made in the same manner as at *D*, tap *C* being turned off when measurements were made. In this system it was found convenient, whilst making the junction at *B*, to maintain that at *D* by turning the latter through $\frac{1}{8}$ turn. The alteration of potential caused by this procedure was small—not greater than 0.5 mv.—and the original value was rapidly resumed.

The potential in the system was measured by the valve electrometer in conjunction with a small box type moving-coil galvanometer (Tinsley and Co.) and a slide-wire potentiometer (Cambridge Instrument Co.). Differences of 0.0001 volt were readily and rapidly detected using membranes of 20 to 200 megohms resistance. The electrometric system had a stable zero. The grid current flowing through the cell was of the order of 10^{-15} amp., giving a negligible fall of potential across the membrane. No appreciable current was taken from the cell, and the risk of polarisation of the membrane when the input circuit was out of balance was eliminated by means of a special switching device.

The glass cell system must be completely screened and the screen of this and the electrometer system earthed at a common point. The lead from the glass electrode was air-insulated as far as possible; for the rest, high grade heavily insulated flexible leads screened by flexible copper tubing were used. If the insulation of the cell is inadequate, the electrical leak may act as a shunt for the glass cell and lead to polarisation of the membrane [MacInnes and Belcher, 1931]. This is more liable to occur if the resistance of the membrane is high. The efficiency of screening and insulation was proved at intervals by determining the E.M.F. of a standard cell in series with a high resistance. A low reading was usually indicative of inadequate insulation, whilst defects in screening gave rise to irregular deflections of the galvanometer image when the standard cell and resistance were thrown into the input circuit.

DETAILS OF EXPERIMENTAL TECHNIQUE.

The selection, preparation and properties of the glass membrane.

The ability of a glass membrane to function as a perfect hydrogen electrode depends essentially on the chemical composition of the glass. There is general agreement that a clear soft soda-lime glass, free as far as possible from potash, alumina and borates, gives the best results [Hughes, 1928]. The stock used was supplied by the Corning Glass Co., New York (No. 015) and has the chemical composition— SiO_2 72 %, CaO 6 %, Na_2O 22 % by weight. According to MacInnes and Dole [1930], this glass has a low resistance and asymmetry potential and only a small error in alkaline solutions (0.1 *N* NaOH).

The membrane may be a thin bulb in a simple or modified form; a thin membrane (about 0.001 mm.) at the end of a glass tube, as in the MacInnes and

Dole [1929] type, or a capillary may be used [Youden and Dobrosky, 1931; Voegtlin and Kahler, 1932]. We have had considerable success with the simple bulb type which is easy to make and manipulate. The method followed is essentially that described by Voegtlin, de Eds and Kahler [1930], except that only a small portion of special glass is used. This is fused to a supporting tube of ordinary soft glass and is then drawn out to a capillary which is broken off about 1 cm. from the shoulder formed. The glass of the capillary is melted in a micro-burner into a centrally placed globule from which the bulb is blown. Prolonged manipulation in the flame should be avoided, as devitrification and annealing impair the properties of the glass for electrode purposes.

The supporting tube is not made of the glass 015, since, according to MacInnes and Dole [1930], 7×10^{-10} equivalent of base is given off from this glass per sq. mm. of surface in 24 hours. The composition of the glass used for the shank is usually such that its hydrogen electrode function is imperfect, so that it is desirable that there should be no appreciable current flow across it. Membranes of minimum thickness are therefore used in conjunction with relatively thick supporting tubes of low conductivity glass [MacInnes and Belcher, 1931]. Deviation currents, occurring with thick membranes and varying in amount according to the nature of the fluid on the two sides of the electrode and the area of the surfaces involved, are described by Kahler and de Eds [1931]. These authors suggest that a guard ring may be used for the electrode if the shank is short and wet. Reference has been made above to the danger of polarisation of the membrane due to leakage current, especially if thick membranes are in use. We have, however, had no trouble with the supporting tube when this is dried and painted with hot high melting-point paraffin wax.

A rough selection of the bulbs prepared by the foregoing method is made by filling them with water and taking those scarcely visible when inspected under water against a white background. Excluding occasional membranes containing minute holes due to rupture of air bubbles in the glass, those obtained by this method are found to be of suitable resistance and, with proper treatment, have low asymmetry potentials.

The value of the resistance of the membrane may be required, especially if the design of the potential measuring system is such that the cell resistance is a limiting factor. A method of measuring the D.C. resistance by applying only small potentials across the membrane may be used [MacInnes and Dole, 1930; Kahler and de Eds, 1931], or an approximate value may be obtained on a calibrated galvanometer when a known voltage (say 10 volts) is applied across the membrane when it is set up for taking a strain potential (see later). The value of the resistance of the membranes we prepared varied from 50 to 150 megohms and was generally higher than that usually obtained with the glass 015, since quite small bulbs (approximately 1 cm. diameter) were used. It is noteworthy that the values obtained by MacInnes and Belcher [1931] for the A.C. resistance of glass membranes are very much lower than, and roughly proportional to, the apparent D.C. resistance. This difference is, no doubt, largely due to a polarisation E.M.F. As a point of some practical importance it may be noted that the D.C. resistance of the glass membrane falls considerably with rise of temperature [Kahler and de Eds, 1931].

The glass membrane may be cleaned by steaming [Kerridge, 1925] or by soaking in dil. HCl followed by distilled water, preferably for several hours [Fosbinder and Schoonover, 1930]. The membrane should not be treated with strong alkalis or concentrated solutions of electrolytes. It should not be allowed to dry after preparation, since it depends for its hydrogen electrode function on

the water content of the glass phase [MacInnes and Belcher, 1931; Dole, 1931, 1932; Ssokolof and Passynsky, 1932]. We found that the membrane may be cleaned with alcohol and ether [Stadie *et al.*, 1931], though Dubois [1932] states that this procedure disturbs the stability of the cell he used. Contamination of the membrane with grease or paraffin should be sedulously avoided.

The terms zero correction, glass strain or asymmetry potential have been applied to the potential in the glass obtained when the solution is the same on both sides of the membrane. This entity is of importance, since, if large, it is likely to vary frequently and considerably. Even when using a suitable glass, large and variable asymmetry potentials may follow annealing, drying or chemical treatment. We have confirmed the findings of Greville and MacLagan [1931] that potentials may occur and persist when an unbalanced current flows in the input circuit of the electrometer. The glass potential has been shown to vary according to the p_{H} of the solution bathing the membrane [Kahler and de Eds, 1931].

Preparation of half-cells.

Different workers have used various combinations of calomel, silver-silver chloride and quinhydrone half-cells in conjunction with the glass electrode. We have used the following system:

Pt	0.1 N HCl- quinhydrone	Glass membrane	Unknown solution	3.5 M KCl	0.1 N HCl- quinhydrone	Pt
	p_{H_2}		A	a	b	
			p_{H_1}			

The platinum electrodes were made by fusing 2 to 3 in. of heavy gauge platinum wire to a piece of copper wire, then sealing the platinum into a piece of glass tubing of convenient size. It is easy to obtain a satisfactory seal if the hot electrode is covered with heated asbestos fibre and then allowed to cool slowly. The seal can be tested by heating the electrode in boiling water and allowing to cool, when the presence of a crack or of incomplete fusion is shown by the entrance of water into the glass tubing. The platinum was cleaned by heating up to 125° in chromic acid cleaning mixture, then slowly cooled, washed with tap-water, then with distilled water and kept in 0.1 N HCl till required [Morgan *et al.*, 1931]. 0.1 N HCl, kept at 38° was saturated with quinhydrone (B.D.H.) and the excess removed by filtration, nitrogen being bubbled through the solution at intervals. Biilmann and Jensen [1927] recommend 0.005 M quinhydrone solution (1.1 g. per 100 cc.), *i.e.* about one-third the amount required for saturation (3.5 g. per 100 cc. at 18°).

In comparison with calomel half-cells, the quinhydrone half-cells are easy to prepare and, using matched platinum electrodes, they can be reproduced to give potentials differing by less than 0.00001 volt [Biilmann and Jensen, 1927; Morgan *et al.*, 1931]. Compact forms, easily insulated, can be used. They do not exhibit the troublesome hysteresis phenomena with change in temperature found with calomel half-cells. Erratic values for quinhydrone half-cells may, however, be obtained at 38° if oxygen is not excluded. It is, moreover, convenient to have in the present system similarly constituted opposing half-cells; but, though these are matched initially, we find that a slow change in value takes place, possibly due to alkali from the glass in the smaller, or to oxidation in the larger, half-cell. If, however, the electrode is standardised at intervals against a buffer solution of known p_{H} , this change is not inconvenient. 0.1 N HCl is used in preference to a buffer solution, since Lammert and Morgan [1932] have shown

that quinhydrone electrode potentials are not nearly so accurately reproducible in solutions of other electrolytes as in 0.1 *N* HCl. It is an advantage too to have a solution of simple ionic composition in apposition to the membrane.

Liquid-liquid junction potentials.

An important consideration arising out of the use of 0.1 *N* HCl is that a significant junction potential is introduced in the cell chain at *b*. The values given for the potential at this junction vary, according to different authors, from 2 to 5 mv. Gross discrepancies are recorded in the junction potential, when such methods as saturated potassium chloride-agar bridges, narrow tubes or ground glass capped tubes are used in making the junctions [Maclagan, 1929]. There is a significant time change in the value of the junction potential which may vary considerably and inconsistently if these methods are adopted. Maclagan [1929] has shown that it is preferable to form the junction inside tubes not less than 3 mm. in diameter, thus conforming to the condition of cylindrical symmetry investigated by Guggenheim [1930]. Ferguson *et al.* [1932] claim to have reproduced in wide-bore taps a KCl-HCl junction to 0.1 mv., remaining constant over long periods to ± 0.04 mv. We have confirmed the finding of Maclagan, but find it inconvenient on account of difficulties with temperature control to use the method he recommends. The junctions were therefore made as described above in taps and tubes of internal diameter not less than 3 mm. In view of the time change of the junction potential at *b*, 5 to 10 mins. must be allowed for the attainment of a steady value, in order that accurate and reproducible results may be obtained. For the purposes of calculation it is assumed that the potential at *a* when the p_{H} of solution *A* is greater than 3 is always small and is the same within the limits of experimental error [Michaelis, 1926].

Standardisation of the cell system.

The discrepancies found in the values assigned to reference standards used in the measurement of p_{H} are such that, whilst small differences of p_{H} can be detected with considerable accuracy, absolute measurements cannot be established to less than 0.01 p_{H} [Clark, 1928]. The method we have used for standardisation of the glass electrode is based on the following considerations. If the membrane functions as a hydrogen electrode within the range of p_{H} covered in our experiments, then the potential difference (E_m) between the two sides of the membrane, when solutions of p_{H_1} and p_{H_2} are on either side, is given by

$$E_m = \frac{RT}{F} (p_{\text{H}_1} - p_{\text{H}_2}) \quad \dots\dots(1),$$

where R is the gas constant, T the absolute temperature, and F the faraday. The total E.M.F. (E) of the cell chain represented above is the algebraic sum of E_m , the glass potential (E_g), the liquid-liquid junction potentials at *a* and *b* and the difference, if any, between the potentials of the two half-cells. If the sum of the latter three factors be represented by e , then

$$E = E_m \pm e = \frac{RT}{F} (p_{\text{H}_1} - p_{\text{H}_2}) \pm e \quad \dots\dots(2),$$

p_{H_2} is constant and is taken as 1.08 [Clark, 1928]. p_{H_1} varies according to the solution in the electrode vessel.

$$\text{Put} \quad E_c = \frac{RT}{F} p_{H_2} \pm e \quad \dots\dots(3).$$

E_c is referred to as the cell constant; substituting in equation (2)

$$E = \frac{RT}{F} p_{H_1} - E_c \quad \dots\dots(4).$$

It is desirable to determine, firstly whether the membrane does in fact function according to the relationship between E_m and p_H expressed in equation (1) and, secondly, whether the other potential differences contributing to the total E.M.F. of the system remain constant. These conditions are fulfilled if identical values for the cell constant are obtained, using a series of phosphate buffer solutions of known p_H value. The buffer solutions were carefully prepared according to the method of Hastings and Sendroy [1924]. They were kept in the refrigerator in waxed bottles protected from contamination by CO_2 . We found that the differences of p_H as measured by the glass electrode agreed within the limits of experimental error with the values given by Hastings and Sendroy for the p_H of these buffers. When the value of E_c has been determined, the value, p_{H_x} , of an unknown solution introduced into the electrode vessel is easily found, for, if the potential of the system now is E_x , then

$$p_{H_x} = \frac{E_x + E_c}{\frac{RT}{F}} \quad \dots\dots(5).$$

The value of E_c is best determined by using buffer solutions of reaction and composition similar to those of the unknown solution. The cell system should be standardised carefully at frequent intervals, since we find, as Stadie *et al.* [1931] also have observed, that for various reasons E_c may slowly alter.

When 0.1 *N* HCl is put into the electrode vessel in place of solution A, then $E_m = 0$. The E.M.F. measured when the second quinhydrone half-cell is connected directly to this solution without the interposition of the KCl bridge is the glass potential E_g , assuming that the two half-cells balance out exactly. If now the KCl bridge is introduced, the behaviour of the liquid-liquid junction may be studied. When the junctions are made simultaneously and in a similar manner they should give equal and opposing potentials, the E.M.F. of the system being again a measure of the strain potential. The time change of the junction can be investigated by remaking one junction after the other has had time to attain a steady value. Junctions made as described above show by this method a change of less than 0.5 mv., which was usually complete within 5 minutes. Because of the doubt as to the absolute value of the 0.1 *N* HCl-3.5 *M* KCl junction, it is impracticable in making accurate measurements to compute the value of e and therefore to obtain a value for p_{H_x} from the formulae

$$E_m = E_x \pm e = \frac{RT}{F} (p_{H_x} - 1.08).$$

The system must therefore be standardised as described above.

The effect of temperature on glass electrode potential measurements.

As a temperature control of the glass cell system, various workers have used some form of air-bath; others, Fosbinder and Schonover [1930], Stadie *et al.* [1931] and Dubois [1932], have used some form of water-bath around part of the system. A copper water-jacketed air-bath, maintained at 38°, was used in our early experiments. Subsequently, on account of temperature fluctuations on opening the door of the thermostat, this was kept in a constant temperature

room at 35–36°. Appreciable temperature variations still occurred during manipulations, giving rise to unreliable potential measurements. Better results have been obtained by using a large completely screened cabinet (3 ft. × 4 ft. 6 in. × 6 ft.) built of zinc-covered plywood on a welded iron frame. The cabinet was electrically heated by screened elements controlled by a long tubular mercury-toluene regulator and a relay, the air being mixed by a fan driven by a motor fixed outside the cabinet. Operations were carried out inside the cabinet, *e.g.* the removal of blood from an arm thrust through a heat-insulated opening in the wall, or the cell system could be manipulated from the outside through two felted arm-holes. Suitable arrangements were made for lighting, transit, ventilation, *etc.* The electrical recording apparatus was set up outside the cabinet and was kept at room temperature. Apart from temporary disturbances due, for example, to entering the cabinet, it was found that the air temperature could be maintained at $38^\circ \pm 0.2^\circ$, owing, no doubt, to the large capacity and the presence of a complete metal lining. The temperature of the potassium chloride solution in the reservoir under these conditions was $37^\circ \pm 0.1^\circ$.

Solutions kept in the air-bath were always at a lower temperature than 38°. It was imperative therefore to avoid surfaces from which cooling might occur. Even when the closed glass cell system described above was used, we found that potential equilibrium was only slowly attained (15 to 20 mins.). The initial reading for the p_H of a phosphate buffer solution introduced into the electrode vessel from a stock kept in the air-bath in a closed vessel gave a value about 0.02 p_H more acid than that ultimately observed when the system had come to equilibrium, all manipulations being carried out inside the air-bath. Only when the solution before introduction was slightly warmer than 38° were steady potential readings obtained immediately. When the temperature was raised still further to, say 40°, an apparent acid change was observed.

Temperature measurements have been made of solutions inside the glass cell and the electrode vessel by means of small rapid reading thermometers graduated in 0.1° from 34 to 42°. In spite of a constant air-bath temperature of 38°, the fluid inside the glass cell in one of these experiments eventually remained steady at 36.8°. Fluids put into the electrode vessel from a stock at 40° gave an initial reading of the temperature of the fluid in the glass cell of 37.1°, falling to 36.8° in about 15 mins. Evidence was obtained that a fall in temperature occurred in the course of transference to the electrode vessel. The time taken for temperature equilibration was about that required for the attainment of potential equilibrium in previous experiments under comparable conditions. The apparent acid or alkaline change obtained when, from the E.M.F. measurements, values for the hydrogen ion concentration were calculated, assuming a uniform temperature of 37°, was therefore shown to be due to the temperature anomalies. The size of these changes for given solutions can be calculated by substitution in the formula $E = \frac{RT}{F} (p_{H_1} - p_{H_2})$. Suppose $p_{H_2} = 1.08$ and p_{H_1} of a phosphate buffer solution is 7; at 37° then, allowing for a fall of 0.029 p_H in the phosphate solution for 18° rise [Hastings and Sendroy, 1924],

$$E_{37^\circ} - E_{36^\circ} = +1.2 \text{ mv. } (\equiv 0.02 p_H).$$

Irregular temperature changes may follow fluctuations in the air-bath temperature. The components of the system are affected unevenly on account of differences in volume of the fluids concerned and the surface area of the containing vessels. The total E.M.F. is altered, since, in addition to the factor discussed above, the E.M.F. of the half-cells may differ by 0.00074 volt for

1° difference of temperature [Biilmann and Jensen, 1927]. The time lag of these changes in the case of both fall and subsequent rise was found to be considerable. The temperature and therefore the E.M.F. of the half-cell may be directly affected by the solution in the electrode vessel if the fluid immediately surrounding the platinum electrode is involved. Repeated washing of the membrane can be shown to produce potential variations in the same way. Dubois [1932], however, attributes the potential changes after washing to an electrical charge on the membrane caused by friction and dielectric absorption. The effect of temperature variations on liquid-liquid junction potentials is unlikely to be of practical significance in view of the results of Prideaux [1928]. Experiments have been made proving that the drifts of potential we described were not due to the time change of liquid-liquid junction potentials.

The foregoing considerations are of importance in relation to the so-called "acid change" in freshly shed blood described by Havard and Kerridge [1929]. Further details of this work on the measurement of the p_H of blood will be presented when a method now under investigation for making rapid and accurate measurements on small quantities of fluid is fully developed. It may be stated here without further comment that the results of Havard and Kerridge can be explained on the grounds of inadequate temperature control and that we have been unable to obtain any evidence of an "acid change" in blood of the type obtained in their experiments.

SUMMARY.

A detailed description is given of the design and construction of a glass electrode system.

Several possible sources of error are described and discussed, and the conditions necessary for obtaining consistent and accurate results have been investigated.

Careful temperature control is shown to be of considerable importance. Measurements made of the hydrogen ion concentration of buffer solutions may show apparent acid and alkaline changes due to temperature variations. Reference is made to the importance of these factors in the interpretation of the so-called "acid change" in blood.

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