CLXXXIII. THE HYDROGEN ION CONCENTRATION OF THE WHOLE BLOOD OF NORMAL MALES AND OF CANCER PATIENTS MEASURED BY MEANS OF THE QUINHYDRONE ELECTRODE[1].

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THE present paper contains an account of measurements of the hydrogen ion concentration (or the p_H) of whole blood by means of the quinhydrone electrode, an electrode introduced for the purpose of the measurements of hydrogen ion concentrations by Biilmann [1921]. Up to the present, all direct measurements of the p_H of blood have been carried out by means of either the indicator, or the hydrogen gas electrode methods. The indicator method is only applicable to blood plasma, and inaccuracies are liable to be encountered on account of the necessity of separating the plasma from the corpuscles. The hydrogen gas electrode method is cumbersome, both from the point of view of time, and also on account of the fact that elaborate precautions have to be adopted in order to prevent the loss of carbon dioxide.

The quinhydrone electrode, on the other hand, promised to be simpler, more rapid in action, and consequently convenient for the purpose in view, namely, a comparison of the p_H of the whole blood of normal and of cancer patients, respectively.

The quinhydrone electrode is essentially an oxidation-reduction electrode and is based on the equilibrium set up in aqueous solution between quinone and quinol, namely,

$$C_6H_4O_2H_2 \rightleftarrows C_6H_4O_2 + 2H^+ + 2\theta.$$

This equilibrium may be represented by the equation

$$\pi = \pi_0 + \frac{RT}{2F} \log \frac{[\text{Quinone}]}{[\text{Quinol}]} + \frac{RT}{F} \log H^+,$$

where π is the observed potential of such an electrode, and π_0 is that when the hydrogen ion concentration (or activity) is unity and the molecular ratio of

[1] This investigation was undertaken on behalf of the Liverpool Cancer Research Committee. Director: Professor W. Blair Bell of the University of Liverpool.

quinone and quinol is unity. The numerical value of π_0 is 0·9802 volt at 37°. In order to ensure that the molecular ratio of quinone and quinol is unity, the compound quinhydrone is employed. A solution of quinhydrone in water is widely dissociated to give equimolecular proportions of quinone and quinol.

Biilmann [1923] has shown that the values of the p_H of buffer solutions, measured by the quinhydrone electrode, agree with those measured by means of the hydrogen gas electrode, and this has been confirmed during the present work. Further it has been found during this investigation, that the quinhydrone electrode gives correct values for the p_H of solutions of lead salts. It has previously been shown by Denham and Allmand [1908] that the hydrogen gas electrode fails to function in solutions of lead acetate, owing to the reduction of bivalent lead ion to monovalent lead ion by hydrogen gas, with a corresponding decrease in the hydrogen pressure at the electrode. Thus in the case of lead salts, the quinhydrone electrode gives correct values for the p_H when the hydrogen gas electrode fails to function.

The Hydrogen ion Content of Blood.

In the measurement of the p_H of blood, the main cause of error lies in the fact that blood rapidly loses carbon dioxide, thereby impairing the efficiency of the bicarbonate system with the result that the blood becomes more alkaline. In view of this factor, rapid measurements were aimed at in the present investigation, the blood coming into contact with the air for the shortest possible time.

With reference to the use of quinhydrone, this substance itself might react with the blood (for example, interaction between quinone and haemoglobin to form methaemoglobin) and so cause erroneous results. It will be shown later, however, that such a reaction, should it occur, is comparatively slow.

The commonly accepted value for the p_H of blood, as measured by indicators (use of indicators is limited to the case of blood plasma only) and by the hydrogen gas electrode, is 7·4–7·5. Hasselbalch and Lundsgaard [1912], however, found by means of the hydrogen gas electrode that the p_H of defibrinated blood is about 7·4 and this value lies between those of the p_H of blood serum (7·8) and of red blood corpuscles (7·0). In the following work, the p_H of whole blood (not defibrinated blood) has been measured in every case.

Experimental.

For the purpose of the measurement of the p_H of blood, a small vessel of about 2 cc. capacity was employed. The vessel had a narrow side limb and could be completely filled with blood. In the first place it must be stated that when a *platinum* foil electrode was employed in conjunction with the quinhydrone, unreasonable values (such as $p_H = 13$) were obtained for the p_H of sheep's whole blood. Since the platinum foil functions other than as an

inert electrode (for example, as a catalyst in the oxidation of quinol), gold foil was substituted[1].

The E.M.F. of the following cell was measured at 37°:

Hg | Hg_2Cl_2 | KCl (N) | saturated KCl | blood saturated with quinhydrone | Au.

The value of the normal calomel electrode at the temperature of the experiment was taken as 0·5731 volt, whilst RT/F was 0·0615. The formula from which the results were calculated was the following:

$$p_H = \frac{0{\cdot}9802 - (0{\cdot}5731 + E)}{0{\cdot}0615},$$

E being the observed electromotive force of the above cell, its value being positive when the gold electrode was the positive pole of the cell.

Preliminary experiments, employing the gold-quinhydrone electrode, showed that values for the p_H of blood of the right order of magnitude could be obtained. It was concluded, therefore, that any reaction between the blood and the quinhydrone, in the presence of gold foil, was very slow. In these preliminary approximate experiments, however, the values, although of the correct order of magnitude, were not reproducible enough to render the method reliable as it stood.

The following technique for the measurement of the p_H of blood was eventually evolved. About 3 cc. of blood were collected rapidly from a vein (in the case of humans, of the arm) through a large hollow needle and shaken vigorously in a small vessel with a little quinhydrone for about ten seconds. Vigorous shaking was necessary, as the quinhydrone was not readily wetted by blood. The blood was then rapidly transferred to the electrode vessel and forced through the side limb by pressing down the rubber stopper containing the gold foil electrode. The blood, which was still warm and unclotted, attained the temperature of 37° in a few seconds and the reading was taken immediately. Alternately, time readings of the potential were taken and the readings were extrapolated to zero time. By the latter means, any variation due to slow chemical interaction could be detected. In general, however, the potential drift with time was so slow, that the reading obtained within a minute of the introduction of the quinhydrone into the blood could be taken as the correct one.

The above technique was followed in all of the subsequent work. The advantages are (*a*) a considerable saving of time as compared with other methods, (*b*) that the p_H of whole blood was obtained, since the blood was introduced into the electrode vessel before it had clotted. The absence of any sudden change in the E.M.F. during clotting indicated that the process of clotting did not affect the value of the p_H.

After each measurement, it was found essential to clean the gold electrode

[1] Whilst this work was in progress, a paper by Dixon and Quastel [1923] appeared in which it was stated that the use of gold was preferable to that of platinum in the measurement of reduction potentials, in this case of cystine-cysteine systems.

with chromic acid, followed by boiling in distilled water. Before a measurement, the electrode was tested by means of buffer solutions of known p_H.

Results.

(i) *Values of the p_H of whole human blood.* In the first place, normal human subjects were examined. From the results given in Table I, it will be seen that the average value at 37° is 7·33.

It may be mentioned that the average value obtained for human defibrinated blood at 37° by Schade, Neukirch and Halpert [1921] by means of the platinum-hydrogen electrode, employing a mixture of hydrogen and carbon dioxide, is 7·31.

Table I. *Values of the p_H of the whole blood of normal males at* 37°.

Exp.	p_H	Exp.	p_H
1	7·33	6	7·30
2	7·37	7	7·33
3	7·36	8	7·33
4	7·33	9	7·33
5	7·33		

Average value = 7·33.

(ii) *Values of the p_H of the blood of cancer patients.* In Table II are given the results of measurements of the p_H of the blood of cancer patients, together with details of any treatment received prior to the measurement of p_H.

It is evident from the results shown in Table II that there is no marked difference between the p_H value of the blood of cancer patients and that of normal human blood, the average values being 7·32 and 7·33 respectively. The extreme values obtained in the case of cancer patients, namely 7·19–7·45, lay further apart than those obtained for normal blood. The absence of any

Table II. *Values of the p_H of the blood of cancer patients at* 37°.

Case no.	Sex	Diagnosis	p_H	Previous treatment
5	Female	Carcin. breast	7·35	X-ray treatment
6	,,	Carcin. both breasts	7·39	X-ray treatment
7	,,	Carcin. cervix	7·30	Operation
8	,,	Carcin. stomach	7·37	None
10	,,	Carcin. thyroid	7·39	None
13	Male	Sarc. stomach	7·45	Operation
16	,,	Sarc. neck	7·35	X-ray treatment
17	Female	Carcin. cervix	7·21	Operation
18	,,	Carcin. colon	7·39	
19	,,	Carcin. rectum	7·20	
21	Male	Carcin. parotid	7·33	Radium treatment
22	Female	Recurrent carcin. of breast	7·37	Operation and X-ray treatment
23	,,	Carcin. rectum	7·31	None
24	,,	Carcin. breast	7·30	Operation (2 years previously) Radium (1 year previously)
25	,,	Recurrence after breast amputation	7·27	
26	Male	Sarcoma	7·20	Deep X-rays
27	Female	Carcin. uterus	7·19	Vaginal hysterectomy Three years' history of haemorrhage
		Average value	7·32	

appreciable difference in the p_H of the blood of normal and cancerous subjects is in agreement with results obtained by Woglom [1924], using the hydrogen gas electrode method of measurement. It may be stated generally that immediately following an operation, the p_H is lowered. This may be due either to the products of autolysis of damaged tissues, or to the effects of the enforced physical inactivity following an operation.

(iii) *The effect of intravenous injection of lead suspension (electrically prepared)*[1] *on the p_H of the blood of cancer patients (in connection with the action of lead on cancerous growths)* [cf. Blair Bell, 1922, 1924]. It was of importance to determine the effects of injections of lead suspension on the p_H of the blood. The concentration of the suspension (of which various amounts were given) was 0·1 % in respect of lead. The suspension itself was made slightly hypertonic, containing, in addition to lead and gelatin, 2 % sodium chloride, 0·05 % calcium chloride, and 0·05 % potassium chloride.

In Table III are summarised a group of results which suggest generally that treatment with lead suspension, injected intravenously, is accompanied by a fall in the p_H of the blood of cancer patients, that is, the blood becomes slightly more acid[2].

Table III. *The effect of lead injections on the p_H of the blood of cancer patients.*

Case	Date	p_H before treatment	Treatment Total amount Pb g.	Date	p_H after treatment
5	23. iv. 24	7·33	0·095	4. vii. 24	7·24
6	5. v. 24	7·39	0·01*	19. v. 24	7·24
7	14. v. 24	7·30	0·126	17. vi. 24	7·23
10	13. vi. 24	7·39	0·154	22. vii. 24	7·27
13	1. vii. 24	7·45	0·06	11. vii. 24	7·30
17	9. vii. 24	7·21	0·15	24. vii. 24	7·22
21	22. vii. 24	7·33	0·18	15. viii. 24	7·30
24	30. vii. 24	7·20	0·21	26. viii. 24	7·16
	Average value	7·32		Average value	7·24

* This patient underwent an operation immediately after the first measurement of p_H (*i.e.* before lead treatment had commenced).

Having obtained the general result that the effect of injection of lead is to lower the p_H of the blood, it was next necessary to determine whether such injections exerted a permanent depressing effect, or whether, between injections, the p_H tended to attain its original value. Measurements before and after injections carried out over a considerable period of time, in the case of five patients, showed that the p_H tends to rise to its original value. It was also found that the injection of a solution containing those substances, other

[1] This material is obtained by sparking lead electrodes under water containing calcium chloride and gelatin, as stabilising agents. By proper adjustment of conditions a suspension consisting of metallic lead together with hydroxide, and possibly a trace of carbonate, can be obtained in which the size of particles does not exceed 0·3 μ.

[2] It has been shown by La Barre [1924] that injections of electrargol cause a lowering of the p_H value of the blood plasma of guinea-pigs. This result is analogous to the above.

than the lead, which were present in the lead suspension (*i.e.* gelatin, sodium, potassium, and calcium chlorides) has practically no effect on the p_H.

SUMMARY.

1. The measurement of the p_H of whole blood by means of the quinhydrone electrode has been described. As platinum foil has been found to be unsatisfactory when employed as electrode metal in the case of blood, a gold foil electrode was used instead, with satisfactory results.

2. By this means it has been found that the average value for the p_H of normal human subjects is 7·33. It was also found that the p_H of the blood of cancer patients is identical, within the limits of error, with that of the blood of normal human subjects.

3. The effect of intravenous injection of a lead suspensoid, prepared by the Bredig sparking method, is to lower the p_H immediately following such injections; but the lowering is only temporary.

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