CLIV. ON THE MEASUREMENT OF CHLORINE ION POTENTIALS IN THE PRESENCE OF PROTEINS.

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CALOMEL and silver-silver chloride electrodes have been used extensively in the physical chemistry of the proteins for determining the concentration of chlorine ions in the presence of proteins [Bugarsky and Liebermann, 1898; Manabe and Matula, 1913; Oryng and Pauli, 1915; Pauli and Matula, 1917; Loeb, 1922; Northrop and Kunitz, 1924]. A few attempts have also been made to use these electrodes for chlorine determinations in biological fluids. Barkus [1924] reported some estimations of Cl' in gastric juice using Ag-AgCl electrodes; Neuhausen and Marshall [1922] used similar electrodes to find out whether all the chlorine in serum is free and diffusible; and Brown and Hill [1921] attempted to show the difference in chlorine concentration between oxygenated and reduced blood by the same means. On the other hand Warburg [1922] stated that such electrodes (concentration chains of the second order) in the presence of protein will not give potentials that conform with the Nernst equation, and that their use is therefore unsound; he argued this on purely theoretical grounds and brought no experimental evidence in support of it. In the following experiments an attempt was made to use the silver-silver chloride electrode for the determination of chlorine in human blood-plasma; it proved unsuccessful but, especially in view of Warburg's criticism, the observations seem worth recording.

APPARATUS AND TECHNIQUE.

The following chain was used:

The electrodes were made of pure silver wire, bent twice at their lower ends and covered over the bent parts with an electrolytically deposited layer of silver chloride; they were mounted on rubber corks fitted into glass electrode vessels with side tubes for making electrical contact with each other. In one vessel were placed about 4 cc. of a standard solution of pure 0.02 *M* KCl, in the other a similar quantity of the plasma or other solution under investigation; a direct liquid junction was effected by bringing the free ends of the side tubes

of the vessels into apposition, and slipping a short piece of rubber tubing over them. The potential difference between the two electrodes was then measured with a Cambridge and Paul direct-reading potentiometer, using a recently checked Weston cell; the result could be read to 0.0001 volt, which gave a deflection of 1 mm. on the galvanometer scale.

Before every experiment six electrodes were prepared by being cleaned and re-coated with fresh layers of silver and silver chloride. Individual variations between electrodes were then avoided by choosing two which gave no P.D. when immersed in the same KCl solution. These two were set up in the electrode vessels, which along with the standard cell were kept at a uniform temperature of $20^{\circ} \pm 0.02^{\circ}$, in an air thermostat of the kind described by Clark [1922]. Potentiometer readings were taken at varying intervals of time after the closure of the liquid junction.

OBSERVATIONS ON PURE KCl SOLUTIONS.

A preliminary series of observations was made on pure KCl solutions of known concentration, as a test of the reliability and accuracy of the apparatus and technique. The solutions were all approximately 0.1 *M*. The potentiometer readings sometimes showed fluctuations of a few tenths of a millivolt in the first 10 minutes after closure of the liquid junction, but after that in every case they remained steady to the nearest 0.0001 volt. They were observed at frequent intervals for periods varying from 25 to 140 minutes; breaking the junction and making a fresh one did not cause any change in the reading.

The logarithms of the concentrations of the solutions, and the E.M.F. obtained are given in Table I, and plotted in Fig. 1. The points lie on a straight line, as might be expected (over this narrow range of concentrations) from the equation for the E.M.F. of a concentration chain of this kind, viz.

$$E = \frac{2\eta RT}{nF} \ln \frac{c_1}{c_2} \frac{\gamma_1}{\gamma_2},$$

where E = E.M.F. in volts; η = transference number of cation = 0.496 for K; R = gas constant = 8.316; T = absolute temperature; n = valency; F = Faraday's constant; c_1 , c_2 = concentrations of anion; γ_1 , γ_2 = activity coefficients of anion.

From this straight line the E.M.F. corresponding to a Cl' concentration of 0.1 mol. per 1000 g. water can be read. It is 0.0370 volt. If this be substituted for E in the above equation, then since the ratio c_1/c_2 is known to be 0.10/0.02 and the values of the constants are known, γ_1/γ_2 can be calculated and is found to be 0.876. This agrees with the results of Noyes and McInnes [1920] who obtained the values $\gamma_1 = 0.745$ and $\gamma_2 = 0.849$ (by interpolation), giving a ratio of 0.878.

The preliminary series of observations therefore showed that the apparatus and technique were adequate; the E.M.F.'s obtained with known KCl solutions were steady, reproducible (as shown by the straight line in Fig. 1) and in agreement with standard observations.

OBSERVATIONS ON KCl SOLUTIONS CONTAINING PROTEIN.

The next step was to find out whether steady and reproducible E.M.F. could be obtained from solutions of known chlorine concentration, containing protein. For this purpose sheep or ox serum was dialysed in collodion tubes in a cool room against either 0.03 *M* sodium bicarbonate, or 0.03 *M* buffer solution of sodium acetate and acetic acid of p_H 5.5, until the dialysate gave no precipitate with silver nitrate; in one or two cases the protein solution was also tested for chloride by ashing and extraction with dilute nitric acid and found to be chlorine-free. The final concentration of the protein solution was determined by micro-Kjeldahl estimation of the nitrogen, and known amounts of pure KCl were then added by weight. Four control solutions were also prepared by dissolving KCl in the bicarbonate or acetate solutions used in the dialysis.

Observations of E.M.F. were made on 20 solutions in all; their composition and the final readings obtained are shown in Table I (Nos. 11 to 30).

Fig. 1. The relation of the E.M.F. to the logarithm of the concentration of KCl (Table I).

- + : Solutions 1 to 9, containing nothing but KCl in water.
- : Solutions 11 to 14, containing acetate or bicarbonate
- ▲ : Solutions 15 to 17, containing bicarbonate and 2.7 % protein.
- : Solutions 27 to 30, containing bicarbonate and 8.5 % protein.
- + : Solutions 20 to 22, containing acetate and 3 % protein.
- x : Solutions 18, 19, containing acetate and 7.8 % protein.
- : Solutions 23 to 26, containing acetate and 10 % protein.

The E.M.F. of the solutions containing salts but no protein, Nos. 11 to 14, usually fell a few tenths of a millivolt in the first 10 or 15 minutes after closure of the liquid junction, and thereafter remained satisfactorily steady.

In a solution containing protein, however, the E.M.F. was by no means steady. It was found that a slight mechanical disturbance, such as tapping the upper end of the electrode dipping into the protein solution, would regularly cause a sharp rise in the E.M.F.—a millivolt or so—followed by a gradual fall.

This difficulty was overcome by keeping the electrode in a state of continual vibration. The gong of an electric bell was removed and its clapper replaced by a flagellum of iron wire about 8 inches long. The brass connecting piece carried by the top end of the electrode wire was covered with insulating tape, and the end of the flagellum brought up against it. When the bell current was turned on, the "whipping" of the brass piece by the flagellum kept the upper and the lower ends of the electrode wire in a state of continuous agitation, which had the additional advantage of stirring the liquid in the electrode vessel.

Table I.

No.	Concentration of protein %	Salts present (other than KCl) 0.03 M	Log. mols. KCl per 100 g. water	E.M.F. volts
1	—	—	2.9943	0.0367
2	—	—	2.9381	0.0340
3	—	—	1.0585	0.0402
4	—	—	2.9912	0.0366
5	—	—	2.9558	0.0346
6	—	—	1.0134	0.0377
7	—	—	1.0339	0.0386
8	—	—	1.0445	0.0395
9	—	—	1.0735	0.0410
11	—	Sodium acetate and acetic acid	1.1101	0.0431
12	—	" "	1.1030	0.0426
13	—	" "	1.0186	0.0379
14	—	Sodium bicarbonate	1.0227	0.0382
15	2.7	" "	1.0112	0.0387
16	2.7	" "	1.0514	0.0408
17	2.7	" "	1.0899	0.0431
18	7.8	Sodium acetate and acetic acid	1.1339	0.0447
19	7.8	" "	1.0281	0.0391
20	3.0	" "	1.0441	0.0404
21	3.0	" "	2.9904	0.0376
22	3.0	" "	1.0956	0.0429
23	10.0	" "	1.0526	0.0413
24	10.0	" "	1.0894	0.0431
25	10.0	" "	2.9896	0.0382
26	10.0	" "	1.0188	0.0392
27	8.5	Sodium bicarbonate	1.0029	0.0389
28	8.5	" "	2.9388	0.0360
29	8.5	" "	1.0663	0.0427
30	8.5	" "	1.0326	0.0415

When this device was applied to the electrode in the protein solution, the E.M.F. readings showed a gradual fall of 1 to 2 millivolts in the first hour or so, after which they remained practically steady. Sometimes there was a slight drift of about 0.0001 volt per hour.

The whipping device was therefore used in all the observations on solutions 11 to 30. It was also used in the case of No. 9 in the first series, where it was found to make no difference whatever to the steadiness or to the absolute value of the reading in the case of a pure KCl solution.

The reproducibility of the readings obtained on the solutions containing protein is best assessed by plotting the E.M.F. against the logarithms of the known concentrations of chlorine (in mols. per 1000 g. water). This is done in Fig. 1, where the straight line is that given by the preliminary observations. It will be seen that the points for the four solutions (Nos. 11 to 14) containing

no protein practically coincide with this line, showing that the electrode is uninfluenced by the sodium acetate or bicarbonate present. On the other hand, the points for the solutions containing protein (Nos. 15 to 30) all lie above the line, by anything up to 0.0023 volt. This means that the presence of the protein alters the relationship of the E.M.F. to the concentration of chlorine. The discrepancy is not very great, but it seems to be irregular, for the deviation of the points in Fig. 1 from the straight line bears no relation either to the concentration of protein present or to the hydrogen ion concentration (bicarbonate or acetate buffer). So long as this discrepancy is unexplained or cannot be allowed for, the silver-silver chloride electrode, with this technique, cannot be used for the accurate estimation of chlorides in the presence of protein.

OBSERVATIONS ON OXALATED HUMAN PLASMA.

Haemoglobin-free plasma was obtained by centrifuging oxalated blood obtained from arm veins, and the potentials given by an electrode dipping into this plasma were observed with the technique already described. It was found impossible to get a satisfactorily steady reading. Nine attempts were made. Sometimes the reading showed an irregular drift; sometimes it showed an initial fall, a steady period, and then either a subsequent rise or a subsequent fall; in one case only it remained steady for a reasonable period (from the 97th to the 292nd minute). Moreover, if two sets of readings were made on different portions of the same specimen of plasma, at an interval of several hours or 1 day, they were found to be completely different. The unsteadiness of the readings, and this utter lack of reproducibility, are illustrated by the following figures:

(a) Fresh plasma		(b) Further quantity of same plasma 3 hours later; same electrode	
Time (minutes)	Reading (volts)	Time (minutes)	Reading (volts)
0	Liquid junction made	2	0.0501
3	0.0403	8	0.0525
10	0.0412	20	0.0530
24	0.0424	31	0.0515
37	0.0420	44	0.0500
50	0.0416	55	0.0482
63	0.0413	65	0.0469
99	0.0412	74	0.0462
136	0.0411	97	0.0452
154	0.0411	120	0.0447
		209	0.0440
		236	0.0438
		262	0.0436

When potentials were determined on successive days on the same plasma using a fresh electrode for each occasion an increase of 7 millivolts was observed on the second day. This would suggest that changes occur in the plasma with time which affect the activity of the chlorine ion. On the other hand, electrodes which had been immersed in plasma for 2 to 5½ hours gave, after washing, values of E in a pure solution of KCl which differed by 5 to 7 millivolts from those recorded before treatment with plasma. This is evidence that plasma has a definite "poisoning" effect on the electrode.

DISCUSSION.

The observations on pure KCl solutions. It is interesting to note that the E.M.F. in the case of No. 9 solution, in which the electrode was kept vibrating, is in conformity with those obtained in the other solutions where the electrode was still; this throws doubt on Brown and Hill's [1921] conclusion that in such measurements as these the liquid must be kept still.

The observations on KCl solutions containing protein gave E.M.F.'s which are all higher than was expected. The solutions appear to contain a greater concentration of Cl' than is known to be present (or else the activity coefficient of the ion is raised). Possible explanations of this are as follows.

1. There may be a disparity in the concentrations of ions between the solution inside the protein micellae and the solution surrounding them. Since the proteins are on the alkaline side of their isoelectric point, the concentration of chlorine outside the micellae (and therefore affecting the electrode) might be expected to be greater than that inside, and therefore greater than the average chlorine concentration of the whole solution. If this were the explanation, however, the apparent increase in chlorine concentration as judged from the E.M.F. should bear some relation to the concentration of protein present and to the hydrogen ion concentration; no such relation appears in the readings that were obtained.

2. The protein molecules or ions may be hydrated. Again, if this were the explanation, the apparent increase in chlorine concentration should be related to the protein concentration present. Moreover, it has been shown by Corran and Lewis [1922], in the case of sucrose, which is known to be hydrated in solution, that the activity of chlorine in the presence of sucrose is the same as in its absence, the concentrations being calculated in the total water present (free water plus water of hydration).

3. The behaviour of the electrode may be modified by the presence of protein. Oryng and Pauli [1915], using calomel electrodes, found a lower E.M.F. with salt-free dialysed serum-protein than with distilled water; in the chain they used this meant that serum-protein after being shaken with calomel appeared to have a higher concentration of chlorine than water shaken with calomel. In the light of other experiments they attributed this to the formation and ionisation of a mercury-protein complex, and in subsequent work they introduced a correction for this effect, assuming it to be proportional to the concentration of protein present. Warburg [1922] criticises this. He lays down two conditions theoretically necessary for the determination of Cl' electrometrically:

- (a) that the Hg or Ag ion concentration be determined solely by the activity of the Cl'; this is not the case if metal-protein complexes be formed;

- (b) that none of the other solutes be affected by the Hg or Ag ions.

He considers that in the presence of protein neither of these conditions is fulfilled, and the determination is impossible.

On the whole, the third of these suggested explanations seems to carry most weight. In the absence of further positive findings, the results that have been given provide an experimental justification for the criticism that Warburg made on purely theoretical grounds.

The observations on oxalated plasma show that in whole plasma there are more disturbing factors at work than simply the presence of protein, and the E.M.F. obtained are quite unreliable as a measure of the chlorine present. What constituent of plasma is responsible for the "poisoning" effect described was not determined, for in view of the unsatisfactory results obtained with the protein solutions it seems very unlikely that the electrometric method can be applied with success to the estimation of chlorine in plasma.

SUMMARY.

Chlorine ion potentials were measured by means of silver-silver chloride electrodes. The readings obtained in pure potassium chloride solutions were steady and reproducible. The readings obtained in potassium chloride solutions containing serum-protein were steady, but not accurately reproducible, and indicated a higher concentration of chlorine ions than was known to be present. The readings obtained in oxalated human plasma were not steady, and were not reproducible. Immersion in plasma "poisoned" the electrode. The technique as used is not applicable to the estimation of the chlorine ion in plasma.

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