XLV. THE ACCURACY OF THE DALE AND EVANS METHOD OF DETERMINING THE HYDROGEN ION CONCENTRATION OF BLOOD.

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THE method employed by Dale and Evans [1920] of finding the hydrogen ion concentration of blood is to place the blood in a small thin collodion tube and to suspend this in an isotonic saline solution. The whole is allowed to stand for about half-an-hour to allow equilibrium to be attained, and the hydrogen ion concentration of the outside solution is then determined by an indicator method. The hydrogen ion concentration of this solution is then taken as that of the blood. The hydrogen ion concentration determined by this method does not, however, according to Evans [1920] agree with the results obtained by the use of a hydrogen electrode placed in the blood, the hydrogen electrode giving, in his hands, results which were about $0.2\ p_H$ smaller than those obtained by the dialysis method. The diffusion method is supposed to be accurate to $0.02\ p_H$; at any rate the p_H of the dialysate can be determined to that degree of accuracy. Since the phosphate solutions used for comparison with the dialysate are standardised with a hydrogen electrode, it is quite clear that the p_H of the dialysate must be approximately correct, and hence the difference must be due, either to the presence of the collodion membrane or to some effect of the blood on the electrode.

Evans in his paper suggests a number of possible causes to account for the difference observed, but he does not consider the fact that even in equilibrium the concentration of the hydrogen ions may not be the same on both sides of the membrane. Such would be the case if a Donnan equilibrium were set up at the membrane. Considering the proteins of blood as providing ions incapable of diffusing through the collodion membrane we have the conditions necessary for the existence of a Donnan equilibrium, *i.e.* on one side of the membrane an indiffusible ion, along with diffusible ions, and on the other side diffusible ions only. Thus when equilibrium is set up there will not be an equal distribution of diffusible ions on the two sides of the membrane. Such a state has actually been demonstrated by Loeb [1921] in the case of

gelatin solution inside a collodion tube, the concentrations of the hydrogen ions being unequal on the two sides of the membrane.

If two liquids on opposite sides of a membrane be in equilibrium and the concentrations C_1 and C_2 of the diffusible ions be not the same on both sides, then there must be a potential difference across the membrane. This potential difference has been demonstrated by Loeb [1921] and whatever be the cause of the inequality of concentration it can be shown thermodynamically [Hill, 1923] that the potential difference E must be of the form

$$E = \frac{R \cdot T}{n \cdot F} \log \frac{C_1}{C_2}.$$

[n = valency of ion; F = electrical charge per equivalent.]

Hence by measuring the potential difference between the blood inside and the saline solution outside the ratio of the concentration of hydrogen ions in the blood to that in the outer solution can be determined. In the case of hydrogen ions at 18° the formula reduces to $E = 59 [p_H (\text{inside}) - p_H (\text{outside})]$ millivolts. It can be shown also that, in the case of a membrane potential, the side on which the concentration of hydrogen ions is the lower is the positive side.

In the presence of a considerable concentration of diffusible ions the difference of concentration occurring in a Donnan equilibrium must be small. In the case of blood therefore no considerable potential difference can be expected.

Method of measurement of membrane potential.

The potential difference across the membrane was measured by means of a potentiometer and two saturated KCl calomel electrodes. In many of the observations a high resistance galvanometer of extreme sensitivity was employed. The apparatus was the same as used in the actual determination of the hydrogen ion concentration by the Dale and Evans method. The small tube into which the saline was put was located in a wider tube, which was large enough to hold the whole of the apparatus when the vulcanite top had been placed on. The amount of saline in the small glass tube was arranged so that when the dialysing tube containing the blood was placed in it the saline came up to the top of the glass tube. When the whole was ready it was corked up and left for about three-quarters of an hour to ensure that equilibrium was attained. This is longer than the time generally allowed in hydrogen ion determinations. The outer tube was then filled with saturated KCl solution up to the lower rim of the vulcanite top. The cork of the dialysing tube was then removed, one saturated KCl calomel electrode placed in the blood and the other in the outer KCl solution. The calomel electrodes made contact through capillary points so as to avoid contaminating the blood with KCl. The potential difference was then measured between the two electrodes. Enough electrical contact was obtained round the edge of the vulcanite top, between

the dialysate and the KCl outside, for the readings to be made easily. The cell employed therefore was as follows:

Calomel electrode	Saturated KCl	Blood	Membrane	0.85 % saline	Saturated KCl	Calomel electrode
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Due allowance was made for any difference between the calomel electrodes employed by testing them together in the same solution. The results are given in the following table.

No. of experiment ...	1	2	3	4	5	6
P.D. (millivolts) }						
Blood positive }	2.44	2.90	2.31	0	-3.45	+1.15
p_H (inside) }						
$-p_H$ (outside) }	0.041	0.049	0.039	0	-0.058	0.020
$\frac{c_H \text{ inside}}{c_H \text{ outside}}$	0.91	0.83	0.91	1.0	1.14	0.95
No. of experiment ...	7	8	9	10	11	12
P.D. (millivolts) }						
Blood positive }	-5.3	-0.9	3.23	0.1	0.85	-0.5
p_H (inside) }						
$-p_H$ (outside) }	-0.090	-0.015	0.055	0.002	0.018	-0.009
$\frac{c_H \text{ inside}}{c_H \text{ outside}}$	1.22	1.035	0.88	0.99	0.96	1.02
No. of experiment ...	13	14	15	16	17	18
P.D. (millivolts) }						
Blood positive }	2.2	1.3	0.76	4.42	2.54	1.66
p_H (inside) }						
$-p_H$ (outside) }	0.037	0.022	0.013	0.075	0.043	0.028
$\frac{c_H \text{ inside}}{c_H \text{ outside}}$	0.92	0.95	0.97	0.86	0.91	0.94
No. of experiment ...	19	20	21	22		
P.D. (millivolts) }						
Blood positive }	0.45	0.66	0.53	0.75		
p_H (inside) }						
$-p_H$ (outside) }	0.008	0.011	0.009	0.013		
$\frac{c_H \text{ inside}}{c_H \text{ outside}}$	0.98	0.97	0.97	0.97		

In the above table, in spite of small individual variations, the ratio of c_H (inside) to c_H (outside) is consistently about unity. Its average value is 0.97, the average deviation from this value being only 0.06 (about 6 %), the probable error of the mean value only about 0.01 (about 1 %). A ratio of 0.97 between the true c_H of the blood and that of the dialysate corresponds to a p_H difference of only 0.013, which is outside the limits of accuracy of measurements by the dialysis method. The average deviation in the table corresponds to a p_H difference of 0.027 which is of the same size as the admitted error of the dialysis method. Hence, for practical purposes we may regard the method of Dale and Evans as giving correctly the hydrogen ion concentration of the blood as existing at the moment when the dialysis has been completed: for greater exactness 3 % may be subtracted from the c_H or 0.013 added to the p_H , to allow for the membrane effect.

If the membranes are used several times for determinations of the hydrogen ion concentration, they become stained and in such cases potential differences up to 11 millivolts, are obtained. It was found, however, that if 0.85 % saline

was placed on both sides of these membranes a potential difference was set up across them of the same order of size as obtained with blood on one side. Hence the stained membranes, for some unknown reason, introduce a large error into the hydrogen ion determinations. The membranes therefore were always tested before use by finding the membrane potential with saline on both sides. In most cases no potential difference was obtained but a small P.D. was found when the membranes had become slightly stained while testing for leaks. Thus for accurate determinations of the hydrogen ion concentration new membranes should be employed, or at any rate such as have been used before should be carefully tested before being used again.

SUMMARY.

In the Dale-Evans method of determining the hydrogen ion concentration of blood it was conceivable that an appreciable error might occur owing to a difference of concentration (due for example to a Donnan equilibrium) produced by the membrane itself. Such an effect would result in a measurable electrical potential difference. The actual P.D. has been measured and found to be small. On the average the hydrogen ion concentration in the blood is only 3 % less than in the dialysate, an amount smaller than the error of the method. For practical purposes, therefore, the c_H of the dialysate is identical with that of the blood as existing at the moment when dialysis is complete.

I wish to express my indebtedness to Dr R. E. Conway for the provision of the Dale-Evans apparatus, and for valuable suggestions.

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