

**THE RELATION OF OXYHÆMOGLOBIN TO THE CO<sub>2</sub>  
OF THE BLOOD<sup>1</sup>. BY J. M. H. CAMPBELL, M.B.,  
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THE relation of the blood proteins to the transport of the CO<sub>2</sub> by the blood is a problem that has been much debated. In the main there are two opposing views on this subject. According to one view the CO<sub>2</sub> is partly present as sodium bicarbonate, and partly united to the proteins by chemical combination or adsorption. According to the other view, the CO<sub>2</sub> is present altogether as alkaline bicarbonate, while the proteins act as acids, and thus compete with the CO<sub>2</sub> for the alkali, the amount of sodium bicarbonate depending on the concentration of the CO<sub>2</sub> according to the law of mass action.

The early history of the controversy<sup>(1-4)</sup> has been described fully by Loewy, who inclines to the first of these two views as representing the actual conditions in the body. However during recent years many workers<sup>(7, 8)</sup> have adopted the view that most of the CO<sub>2</sub> is present as bicarbonate, the proteins acting as acids. This is largely due to pioneer work of L. J. Henderson<sup>(5)</sup>. Hasselbalch<sup>(6)</sup> using improved methods introduced his well-known formula for the hydrogen ion concentration of the blood. This has been considered by Joffe and Poulton<sup>(14)</sup>.

The theory of amphoteric electrolytes established by James Walker<sup>(9)</sup>, follows directly upon the ionic theory and the law of mass

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action, and stands or falls with these two principles. According to the theory their behaviour depends on the hydrogen ion concentration, the substance acting as a base in an acid medium and acting as an acid when the hydrogen ion concentration is diminished. The isoelectric point when the substance is neutral, lies at an intermediate  $C_H$ . Michaelis and others(10) have determined the isoelectric points of hæmoglobin and various serum proteins, and in every case they are more acid than the normal reaction of the blood. Hence if these determinations are accepted these proteins must act as acids in the blood, and only act as bases when the  $C_H$  is much greater than ever occurs in the body.

There has been a tendency to regard the behaviour of plasma towards acids as identical with that of a solution of sodium bicarbonate. This has been strenuously opposed by Moore(11) as the result of his early work on the "reactivity" of the plasma.

The theory of direct combination of CO<sub>2</sub> with protein and especially with hæmoglobin, has received a fresh impetus from Buckmaster's work(12). He dialysed red blood corpuscles for varying lengths of time, and found that they took up more CO<sub>2</sub> than could be accounted for by simple solution in the mixture. The amount of combined CO<sub>2</sub> increased with the hæmoglobin content of the mixture, and with the tension of CO<sub>2</sub> to which the mixture was exposed. Buckmaster himself was doubtful whether this reaction played a great part in the body, because it was only at relatively high pressures of CO<sub>2</sub> that much CO<sub>2</sub> was actually taken up in combination. However Bayliss(13) in a very recent paper considers that carbon dioxide is carried by hæmoglobin. He also dialysed serum against sodium bicarbonate containing an excess of free CO<sub>2</sub> so as to ensure that there was no normal sodium bicarbonate present, and under these conditions, which resemble the conditions found in the body, he found that no CO<sub>2</sub> was chemically combined with the serum protein. He also states that the serum proteins do not act as acids; but we do not think his experiment (p. 176) is very conclusive, because actually less CO<sub>2</sub> was found in the serum and bicarbonate mixture than in the bicarbonate solution alone. We shall describe a somewhat similar experiment.

#### *Experiments with dialysed red cells.*

On the balance of evidence so far presented we inclined to the view that all the CO<sub>2</sub> is present as bicarbonate, the blood protein and particularly hæmoglobin playing the part of acids. It was important to

settle this question definitely, because Joffe and Poulton<sup>(14)</sup> have shown that Hasselbalch's formula can be applied to determine the  $C_H$  of blood by determining the  $CO_2$  content of the "true plasma" or "true serum" at a given  $CO_2$  pressure. Hasselbalch's formula is only correct if all the  $CO_2$  is present as bicarbonate. After we had carried out most of the experiments in this paper, we noticed a single uncontrolled experiment in one of Hasselbalch's papers<sup>(6)</sup> bearing on the same point. He used washed ox corpuscles dialysed for only three hours, and to this a solution of sodium bicarbonate was added, so that the solution was  $\cdot 025N$ . He then exposed the solution to three separate pressures of  $CO_2$  and estimated respectively the content of  $CO_2$ . At pressures below 90 mm. the solution contained less  $CO_2$  than corresponded to  $\cdot 025N$   $NaHCO_3$ , and the only possible explanation of this phenomenon is that the hæmoglobin acted as acid.

The importance of this question warrants a full investigation, and for this purpose we have used exclusively human blood. In some experiments blood from a case of myocardial degeneration was used. The patient showed some breathlessness but no cyanosis. In other experiments blood came from three normal individuals. The results of the different series agreed well together. The blood was defibrinated and centrifuged; the serum removed and the corpuscles washed with 0.9 p.c. sodium chloride solution. The corpuscles were dialysed against distilled water for 5-6 days. At the end of dialysis there was no perceptible cloud with silver nitrate and nitric acid. This method was very similar to that used by Buckmaster. In all the experiments the solution used contained 52 p.c. hæmoglobin, as tested by a specially standardised Haldane's hæmoglobinometer. This corresponded to 10.85 vols. of oxygen per 100 c.c.

Our first experiment (Table I) was undertaken to confirm Buckmaster's results. The solution of hæmoglobin in distilled water was exposed to various pressures of  $CO_2$  at  $38^\circ C.$ , and the total  $CO_2$  determined by Van Slyke's apparatus, as described by Joffe and Poulton. Joffe and Poulton found that in defibrinated blood the red blood corpuscles occupy about 50 p.c. of the total volume. Hence the hæmoglobin value of red corpuscles on Haldane's hæmoglobinometer scale is 200 p.c. We were using a 52 p.c. solution of hæmoglobin. Now the solubility of  $CO_2$  in red corpuscles at  $38^\circ C.$  is 0.45 (Bohr<sup>(15)</sup>), and in distilled water 0.555. The solubility in our solution falls somewhere between these two values; it was calculated as 0.527. The dissolved  $CO_2$  (Table I, col. 3) has been calculated from this coefficient. The  $CO_2$

actually combined with hæmoglobin is shown in col. 4. It varies from 15.8 c.c. p.c. at 74.9 mm. up to 39.6 c.c. at 805 mm. As a control of our method of analysis we have placed lower down in Table I four determinations of the amount of CO<sub>2</sub> taken up by distilled water at 38° C., and we have placed beside them for comparison the amounts calculated from the known solubility of CO<sub>2</sub> in water.

TABLE I. Vol. CO<sub>2</sub> in dialysed hæmoglobin solution at varying CO<sub>2</sub> pressures at 38° C.

CO <sub>2</sub> mm.	100 c.c. hæmoglobin solution contain		
	Total CO <sub>2</sub> c.c.	Dissolved CO <sub>2</sub> c.c.	Combined CO <sub>2</sub> c.c.
74.9	21.0	5.2	15.8
112	28.6	7.8	20.8
329	49.6	22.9	26.7
805	95.6	56.0	39.6

Vol. CO<sub>2</sub> in distilled water at 38° C.

CO <sub>2</sub> mm.	CO <sub>2</sub> found c.c.	CO <sub>2</sub> calculated from known solubility c.c.
79.2	8.7	5.8
217.6	21.8	16.0
415.2	27.8	30.3
570.6	43.2	41.7

Parsons(8) has explained Buckmaster's results by supposing that during dialysis the solution of corpuscles which originally contained NaHCO<sub>3</sub> lost CO<sub>2</sub>, so that some sodium hydrate combined with the hæmoglobin. He suggested that on saturating with CO<sub>2</sub> the hæmoglobin would give up its soda again and bicarbonate would be formed. This explanation will not apply to our experiments because the weight of ash found was 0.20 and 0.16 gms. p.c. respectively in the samples of hæmoglobin used. However calculation showed that the hæmoglobin solution contained 0.21 gm. of Fe<sub>2</sub>O<sub>3</sub> per 100 c.c. which corresponds pretty well with the actual values of the ash found.

Now we agree with Buckmaster that the CO<sub>2</sub> is actually combined with the hæmoglobin and also probably with the protein of the stroma of the corpuscles, but our explanation is that these solutions are so acid that hæmoglobin being an amphoteric electrolyte is acting as a base. A simple calculation will indicate the actual hydrogen ion concentration of a solution of CO<sub>2</sub> in water. The reaction is represented by  $H + HCO_3 \rightleftharpoons H_2CO_3$ . By the law of mass action  $(H)^2 = K (CO_2)$  where (H) and (CO<sub>2</sub>) are the molecular concentration of the hydrogen ions and dissolved CO<sub>2</sub> and K is the first dissociation constant of carbonic acid, which is  $4.15 \cdot 10^{-7}$  at 38° C. according to Milroy(16). Suppose we calculate (H) for a solution under a pressure of 40 mm. CO<sub>2</sub>, which is about

the pressure in the arterial blood. Then the  $\text{CO}_2$  dissolved is 29.2 c.c. per litre and the molecular concentration is  $\frac{29.2}{22400}$ , therefore

$$(\text{H}) = \sqrt{4.15 \cdot 10^{-7} \times .0013},$$

$$\therefore p_{\text{H}} = -4.62.$$

Solutions of  $\text{CO}_2$  at pressures employed by Buckmaster and ourselves will have a still higher  $C_{\text{H}}$  about 1000 times as much as blood. It is thus impossible to draw any conclusions as to the state of combination of  $\text{CO}_2$  in blood from these experiments.

The reaction of the blood is regulated by the presence of sodium bicarbonate, and so sodium bicarbonate was next added to the hæmoglobin solution of the previous experiment in such strength as to make a 0.041*N* solution. This particular strength was used because it approximately represented the total amount of alkali in the blood which according to Parsons(8) is available for combining with  $\text{CO}_2$ . Our control experiment (Table II) consisted in preparing a sodium bicarbonate solution of similar strength in distilled water and estimating its  $\text{CO}_2$  content at different pressures. The pressures varied from 1 mm. to 583 mm. The total  $\text{CO}_2$  varied from 90.3 to 129.7 c.c. per 100 c.c. of solution; but on subtracting the  $\text{CO}_2$  in solution the combined  $\text{CO}_2$  was the same at each pressure within the limits of experimental error, the mean value being 91.3 c.c. The fact that the combined  $\text{CO}_2$  of a pure sodium bicarbonate solution is independent of the pressure, if the latter is above a millimeter or two, is of course a well-known fact and Buckmaster(17) among others has published similar results.

TABLE II. Vol.  $\text{CO}_2$  in  $\text{NaHCO}_3$  solution at varying  $\text{CO}_2$  pressures.

$\text{CO}_2$ mm.	100 c.c. $\text{NaHCO}_3$ solution contain		
	Total $\text{CO}_2$ c.c.	Dissolved $\text{CO}_2$ c.c.	Combined $\text{CO}_2$ c.c.
1	90.3	0.1	90.2
15.6	92.0	1.1	90.9
32.5	93.8	2.4	91.4
33.7	91.6	2.5	89.1
62.8	98.2	4.6	93.6
148	103.8	10.8	93.0
221	111.5	16.1	95.4
368	118.0	26.9	91.1
583	129.7	42.6	87.1

Mean = 91.3

The value 91.3 vols. p.c. may be taken as representing the total available alkali in our solution of hæmoglobin and sodium bicarbonate. Table III gives our results for total and combined  $\text{CO}_2$  in this solution

at pressures varying from 4.8 mm. up to 596.4 mm. The results for the combined CO<sub>2</sub> are plotted out in Fig. 1. The interrupted line represents the CO<sub>2</sub> in the corresponding pure sodium bicarbonate solution.

TABLE III. Vol. CO<sub>2</sub> in solution of dialysed hæmoglobin and NaHCO<sub>3</sub> at varying CO<sub>2</sub> pressures.

CO <sub>2</sub> mm.	100 c.c. solution contain		
	Total CO <sub>2</sub> c.c.	Dissolved CO <sub>2</sub> c.c.	Combined CO <sub>2</sub> c.c.
4.8	52.6	0.3	52.3
4.9	52.4	0.3	52.1
11.0	58.3	0.8	57.5
21.9	64.8	1.5	63.3
40.7	75.7	2.8	72.9
43.6	79.5	3.0	76.5
50.7	79.0	3.5	75.5
52.5	73.9	3.7	70.2
78.1	79.5	5.4	74.1
85.5	85.8	6.0	79.8
90.0	83.4	6.3	77.1
110.5	100.0	7.7	92.3
119.8	94.6	8.3	86.3
151.4	104.0	10.5	93.5
221.9	114.5	15.4	99.1
226.7	113.6	15.8	97.8
310.4	123.5	21.6	101.9
338.0	130.5	23.5	107.0
435.3	140.3	30.3	110.0
596.4	153.7	41.5	112.2

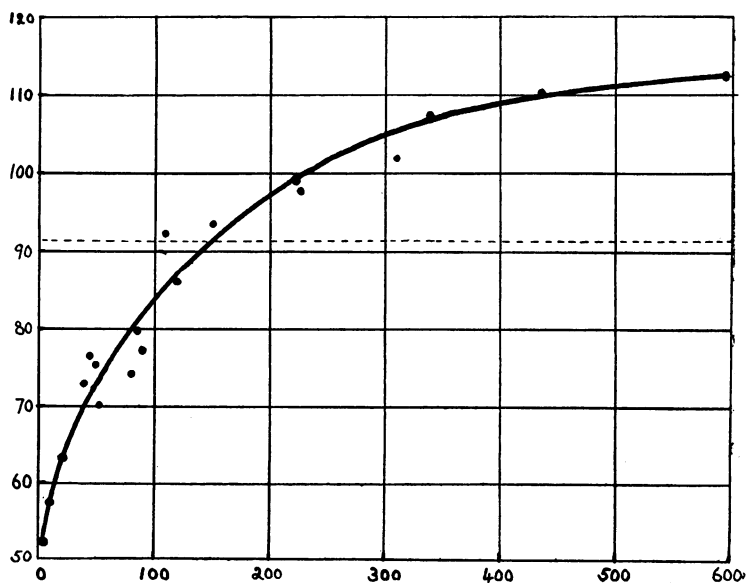


Fig. 1. Ordinate, percentage vol. CO<sub>2</sub>. Abscissa, CO<sub>2</sub> press. mm.

• NaHCO<sub>3</sub> + dialysed Hæmoglobin.  
 Interrupted curve, NaHCO<sub>3</sub> alone.

The effect of adding dialysed red blood corpuscles to sodium bicarbonate is to diminish the amount of combined  $\text{CO}_2$  below 150 mm. Thus at 4.85 mm. two independent results give a mean value of 52.2 vols. p.c. of  $\text{CO}_2$ , a reduction of 41.1 c.c. from the  $\text{CO}_2$  in the pure sodium bicarbonate solution. This effect gets less and less, until at 150 mm. the curve cuts the interrupted line. This is the isoelectric point of the proteins of the corpuscles, and at pressures below 150 mm. the corpuscle proteins act solely as acid and all the  $\text{CO}_2$  must be combined as sodium bicarbonate. At pressures above 150 mm. the solution takes up more  $\text{CO}_2$  than the pure sodium bicarbonate. Hence the extra  $\text{CO}_2$  must be combined directly with the protein. At 596.4 mm. for instance the combined  $\text{CO}_2$  is 112.2 c.c. p.c. Now of this 91.3 c.c. is combined as sodium bicarbonate and the difference 20.9 c.c. is combined with the protein. As already stated we have been dealing with all the protein of the corpuscles mixed together, and although the hæmoglobin probably has the greatest effect, we have no proof of this. However from the physiological point of view the experiments are satisfactory because we have used the mixture of proteins which occurs naturally in the corpuscles. It is to be noted that the curve is even throughout its course. There is no sudden alteration in curvature at the isoelectric point.

Before these results can be applied to the blood in the body, it is necessary to show which part of the curve corresponds to the physiological range as regards its  $C_H$ . Since below 150 mm. all the combined  $\text{CO}_2$  is present as bicarbonate it is perfectly legitimate to apply Hasselbalch's formula for calculating the  $C_H$ . These are the results:

$\text{CO}_2$ pressure mm.	Combined $\text{CO}_2$ c.c. p.c.	$p_H$
40	70.2	7.46
50	72.5	7.37
70	78.0	7.25
150	91.3	6.98

The normal  $p_H$  of blood is about 7.37 which corresponds to 50 mm. on the curve and this point lies well below the interrupted line, so that it is clear that hæmoglobin acts as an acid inside the physiological range of  $C_H$ .

The objection may be raised that our curve was much higher than the  $\text{CO}_2$  dissociation curve of blood, because at 40 mm. our solution contained 70.2 c.c. p.c.  $\text{CO}_2$ , whereas the blood of J. J. (see (14)) contained only 45 c.c. There are two reasons for the difference. Firstly, we used a comparatively dilute hæmoglobin solution; if the concentration of this had been increased the  $\text{CO}_2$  content would have been diminished.

Secondly, it will be shown later, that we were using too great a concentration of sodium bicarbonate. Hasselbalch's formula shows that the  $p_H$  increases with the combined CO<sub>2</sub>. If we had used less sodium bicarbonate the isoelectric point 6.98 would have been lower in Fig. 1, and with it all the points on the curve would have been shifted downwards. These objections do not of course invalidate our conclusions, which are based on the  $p_H$  of the solution and not on its CO<sub>2</sub> content. It is to be noted that Michaelis and Takahashi(18) found that the isoelectric point of hæmoglobin (separated from the protein of the stroma of the corpuscles) had a  $p_H$  of - 6.745 which is decidedly more acid than the value we have obtained for all the proteins of the corpuscles together.

*The combination of CO<sub>2</sub> in serum.*

We have made some similar observations on dialysed serum from the defibrinated blood of healthy men. The serum was dialysed against distilled water and the volume was found to have increased from 77 c.c. to 128 c.c. There was a thick sediment of globulin, and there was less than .02 gm. ash in 100 c.c. As with hæmoglobin we found that the solution of serum in distilled water combined directly with CO<sub>2</sub> but the amount of CO<sub>2</sub> combined was much less. In some of these experiments sodium chloride was first of all added to redissolve the globulin.

TABLE IV. Solution of dialysed serum and sodium bicarbonate.

CO <sub>2</sub> mm.	100 c.c. solution contain		
	Total CO <sub>2</sub> c.c.	Dissolved CO <sub>2</sub> c.c.	Combined CO <sub>2</sub> c.c.
11.8	85.3	0.7	84.6
27.7	86.1	1.9	84.3
47.0	88.7	3.3	85.4
114.0	99.1	7.9	91.3
188.0	102.3	13.0	89.3
290.5	112.9	20.2	92.7
436.0	119.6	30.2	89.4
706.0	145.6	49.1	96.5

The experiments with dialysed serum and sodium bicarbonate are given in Table IV. The sodium bicarbonate was the same strength as in the hæmoglobin experiments, (91.3 c.c. CO<sub>2</sub> p.c.). This strength of NaHCO<sub>3</sub> caused the globulin to go into solution. The coefficient of solubility was calculated as 0.528 taking the dilution of the serum protein due to the dialysis into account. Three determinations with a pressure less than 50 mm. gave values for the combined CO<sub>2</sub>, the highest of which was 85.4 c.c. p.c. This is 5.9 c.c. less than the CO<sub>2</sub> in the pure bicarbonate solution, so there is no doubt that the serum proteins act



as acids at these pressures, though their effect is nothing like so striking as the hæmoglobin. At 290.5 mm. the combined  $\text{CO}_2$  was 92.7 p.c. and at 706 it was 96.5 p.c., which means that at higher pressures the solution contains more  $\text{CO}_2$  than the pure  $\text{NaHCO}_3$  solution. Hence like hæmoglobin the serum proteins act as bases at these pressures. We obtained similar results using serum protein which had been precipitated, washed and dried by Hardy and Gardiner's method (19), and redissolved as far as possible in distilled water.

Although it is quite clear from our results that serum proteins like hæmoglobin behave as amphoteric electrolytes, many more determinations would be necessary to settle the exact isoelectric point, especially as the differences of  $\text{CO}_2$  involved are not so very much outside the experimental error of the method. We can only say that in our experiments it probably lies between 100 and 300 mm.  $\text{CO}_2$ . If 200 mm. is taken as the isoelectric point the calculated value for the  $p_{\text{H}}$  is 6.86. If 300 mm. is taken the  $p_{\text{H}}$  is 6.68. Michaelis(10) and his fellow workers again found by a different method much more acid values than this for the various serum proteins.

#### *Discussion of results.*

There would seem to be no doubt that both hæmoglobin and the serum proteins act solely as acids for a considerable distance on each side of the normal range of hydrogen ion concentration. Further the experiments with the hæmoglobin and bicarbonate solutions give data for calculating approximately the acid dissociation constant of oxyhæmoglobin. The reaction is represented thus:



from which  $\frac{(\text{Hb}) \times (\text{H})}{(\text{HbH})} = k$ ,  $k$  being the dissociation constant, the brackets indicating as usual the gramme molecular concentration of the various substances.

In the actual experiment a mixture of the free acid (oxyhæmoglobin) and its sodium salt is present. Under these circumstances (Hb) is the same as  $\alpha$  (Na.Hb) where  $\alpha$  is the degree of dissociation of the salt and (HbH) represents the concentration of free hæmoglobin, since the dissociation of the acid is now extremely small.

$$\frac{k}{\alpha} = (\text{H}) \times \frac{(\text{sodium salt})}{(\text{free acid})}.$$

Sodium salts of acids are nearly completely dissociated, but although this dissociation will be rather hindered by the presence of excess of

Na in the form of bicarbonate, still  $\alpha$  may be taken as unity as an approximation. The equation may be written thus:

$$p_K = p_H + \log \frac{(\text{sodium salt})}{(\text{free acid})} \dots\dots\dots (I),$$

$p_K$  being the exponential of the dissociation constant.

For calculating  $p_K$  a particular point on the curve in Fig. 1 is taken. For instance at 20 mm. the solution holds 63 c.c. p.c. CO<sub>2</sub>. This CO<sub>2</sub> is present as bicarbonate. Now the total sodium in the solution combines with 91.3 c.c. CO<sub>2</sub> p.c. Hence at 20 mm. 91.3 - 63 = 28.3 c.c. CO<sub>2</sub> is equivalent to the amount of sodium combined with the hæmoglobin.

The free acid will be represented by the difference between the total amount of sodium that the hæmoglobin can combine with when there is no free acid left, and the amount of sodium salt actually present at 20 mm., *i.e.* 28.3 c.c.

The total combining power of Hb for Na is obtained by a slight extra-polation of the curve downwards to 40 c.c. This point has been taken for a reason that will be explained later. In other words it is assumed that when by reduction of the CO<sub>2</sub> pressure the solution only holds 40 c.c. CO<sub>2</sub> combined as carbonate or bicarbonate, the hæmoglobin is completely saturated with sodium. Hence the total hæmoglobin combining power will be represented by 91.3 - 40 = 51.3 c.c. The free acid will be 51.3 - 28.3 = 23.0 c.c. Now the  $p_H$  of the solution at 20 mm. calculated from Hasselbalch's formula is -7.717. The equation (I) may be written:

$$p_K = -7.717 + \log \frac{28.3}{23} = -7.627.$$

This same calculation may be carried out at other points on the curve. Two more have been taken, *viz.* at 50 mm. when the combined CO<sub>2</sub> is 73 c.c. p.c., and 100 mm. when it is 83.5 c.c. In the first case  $p_K$  is found to be -7.631 and in the second -7.873. Of course all these three values should really be the same and there is certainly an extremely close agreement between the first two. The main uncertainty in the calculation is the amount of extra-polation that should be carried out. The actual point 40 was taken because by using this value more concordant results were obtained for  $p_K$  at different parts of the curve than by using any other value. The uncertainty is increased by the fact that below 2 mm. the CO<sub>2</sub> is present at any rate partly as carbonate, and so it is not correct to say that 91.3 - 40 quite represents Na combined with hæmoglobin as some of this Na will have combined with the bicarbonate to produce normal carbonate. Some results for  $p_K$  may be given

assuming that 50 c.c. (and not 40 c.c.) represents the point where all the hæmoglobin combining capacity for sodium has been satisfied. At 20 mm.  $p_K = -7.38$  and at 55 mm.  $p_K = -7.47$  and at 100 mm.  $p_K = -7.76$ .

It cannot be pretended that these calculations give more than a very rough idea of what the true dissociation constant is, but it probably is somewhere about  $10^{-7.7}$  or  $2 \times 10^{-8}$ . Unfortunately the data are not sufficient to calculate even approximately a value for the acid dissociation constant of the serum proteins.

Joffe and Poulton have given figures for the  $\text{CO}_2$  content of J. J.'s "true serum" and red blood corpuscles. They have also pointed out that since the hydrogen ion concentration of blood as ordinarily measured is that of the true serum it can be calculated by means of Hasselbalch's formula. The present paper by showing that the serum proteins act as acids within the physiological range justifies this procedure. Now that hæmoglobin has also been shown to act solely as an acid it is also quite justifiable to use the formula for calculating the  $\text{C}_H$  of the red corpuscles.

TABLE V. The  $\text{C}_H$  of serum and corpuscles in J. J.'s oxygenated and reduced defibrinated blood.

CO <sub>2</sub> mm.	O <sub>2</sub> serum		O <sub>2</sub> corpuscles		Differ- ence $\text{C}_H$ $10^{-8} \times$	Red serum		Red corpuscles		Differ- ence $\text{C}_H$ $10^{-8} \times$	Serum differ- ence $\text{C}_H$ $10^{-8} \times$	Cor- puscle differ- ence $\text{C}_H$ $10^{-8} \times$
	$p_H$	$\text{C}_H$ $10^{-8} \times$	$p_H$	$\text{C}_H$ $10^{-8} \times$		$p_H$	$\text{C}_H$ $10^{-8} \times$	$p_H$	$\text{C}_H$ $10^{-8} \times$			
10	7.71	1.95	7.52	3.0	1.05	7.74	1.8	7.67	2.1	0.3	0.15	0.9
20	7.51	3.1	7.41	3.9	0.8	7.55	2.8	7.50	3.2	0.4	0.3	0.7
30	7.40	4.0	7.31	4.9	0.9	7.43	3.7	7.40	4.0	0.3	0.3	0.9
40	7.32	4.8	7.26	5.5	0.7	7.35	4.5	7.32	4.8	0.3	0.3	0.7
55	7.23	5.9	7.18	6.6	0.7	7.26	5.5	7.24	5.8	0.3	0.4	0.8
70	7.16	6.9	7.12	7.6	0.7	7.19	6.5	7.18	6.6	0.1	0.4	1.0
90	7.08	8.3	7.05	8.9	0.6	7.12	7.6	7.13	7.4	-0.2	0.7	1.5

Table V gives the results of these calculations both for oxygenated and reduced blood. Values both for  $p_H$  and  $\text{C}_H$  are given. It will be seen that at all pressures up to 90 mm. the corpuscles are slightly more acid than the serum but the differences are greater in oxygenated than in reduced blood, since oxyhæmoglobin is evidently a stronger acid than reduced hæmoglobin. At 40 mm. for oxygenated blood the  $p_H$  of the true serum is 7.32 and for corpuscles 7.26. At 40 mm. for reduced blood the  $p_H$  of true serum is 7.35 and for corpuscles 7.32. The difference in  $\text{C}_H$  between serum and corpuscles becomes less as the  $\text{CO}_2$  pressure increases (cols. 6 and 11). In cols. 12 and 13 the difference between the oxygenated and reduced true serums are arranged and also between the oxygenated and reduced corpuscles. There is a bigger difference between the corpuscles than between the serums. It is true that we have only

proved that oxyhæmoglobin acts as an acid but there can be little doubt that reduced hæmoglobin acts similarly, so that we have felt it justifiable to calculate the  $C_H$  of the reduced corpuscles as well as of the oxygenated corpuscles.

In Milroy's experiments the red blood corpuscles were found to be extremely acid. Thus at 49 mm. CO<sub>2</sub> the  $p_H$  was 6.60 or about 0.7 less than the  $p_H$  of the serum. It is hardly conceivable that there should exist such a big difference as this in the circulating blood. Milroy's procedure was responsible at any rate for a large part of the difference. He centrifuged off the corpuscles, and then to the corpuscular deposit he added enough water to bring back the volume to that of the original blood. Blood contains approximately 50 p.c. of its volume as corpuscles, so that he diluted the corpuscular volume to about double its original volume. This would halve the concentration of sodium bicarbonate in the corpuscles which from Hasselbalch's formula would reduce the true  $p_H$  by 0.3. This at any rate accounts for part of the great increase of acidity that he found.

Our experiments with solutions of sodium bicarbonate added to hæmoglobin and serum proteins provide a useful schema of the condition of affairs in the blood. At low pressures of CO<sub>2</sub> the hydrogen ion concentration of the hæmoglobin solution is greater than that of the serum proteins. For example at 20 mm. CO<sub>2</sub> the  $p_H$  of the hæmoglobin bicarbonate solution is 7.72. At this pressure the serum bicarbonate solution which contained 84 vols. p.c. CO<sub>2</sub> has a calculated  $p_H$  of 7.83. As the CO<sub>2</sub> pressure increases the CO<sub>2</sub> content of the hæmoglobin bicarbonate solution increases much more rapidly than the serum bicarbonate solution, so that the difference between the  $p_H$ 's becomes less and less. Exactly the same thing is shown in Table V. At 20 mm. CO<sub>2</sub>, the difference between the  $p_H$ 's of serum and corpuscles of oxygenated blood is 1.0, at 90 mm. it is only 0.03.

As has already been pointed out<sup>(14)</sup> the condition in reality is much more complicated in blood, because there is an interchange of ions constantly occurring between corpuscles and plasma. The reason for this interchange taking place is clear, though the mechanism of its production is not clear. In the corpuscles the hæmoglobin is nearly four times as concentrated as in our solution and so the dissociation curve must be steeper. This means that raising the CO<sub>2</sub> pressure causes a greater increase in the CO<sub>2</sub> content of the corpuscles, so that the hydrogen ion concentration is more rapidly diminished. This excessive alteration in  $C_H$  is prevented by diffusion of acid from the serum.

It is clear from our experiments why all the  $\text{CO}_2$  can be expelled from blood by means of the blood pump. The hæmoglobin of the corpuscles is present in sufficient quantity to combine with all the available sodium of the blood, as the  $\text{CO}_2$  is gradually removed. In our experiment the concentration of hæmoglobin was less than in blood and the amount of available sodium was greater, so that there were still 40 c.c.  $\text{CO}_2$  combined with sodium when the  $\text{CO}_2$  pressure was reduced to zero. In blood the interchange of ions between plasma and corpuscles ensures that on reduction of pressure the residue of available sodium after the plasma proteins have been satisfied, receives acid from the corpuscles and so makes sodium available inside the corpuscles to combine with the hæmoglobin. In the separated serum the proteins are not sufficient to combine with all the available sodium, so that on reduction of pressure some  $\text{CO}_2$  is still left in combination with it.

The experiments with hæmoglobin and serum proteins also give an indication of the total amount of sodium bicarbonate in blood, or, expressing it more accurately, the total amount of sodium available for combining with  $\text{CO}_2$ . Parsons<sup>(8)</sup> has suggested that the sodium bicarbonate is 0.045*N* which corresponds to the total combined  $\text{CO}_2$  when blood is completely saturated with  $\text{CO}_2$ . This suggestion takes no account of the fact that while all the sodium of the blood will be present as sodium bicarbonate additional  $\text{CO}_2$  will be taken up in combination with the hæmoglobin and serum proteins when the hydrogen ion concentration is increased to this extent. On the other hand at the isoelectric point all the sodium is present as bicarbonate, and no  $\text{CO}_2$  is combined with protein.

It was pointed out that our experiments with the serum-bicarbonate solution showed that the most probable value for the  $p_{\text{H}}$  of the isoelectric point was 6.86. Now calculations show that this value corresponds to 180 mm. on the true serum curve of J. J.'s blood (cf. (14) Fig. 3). At this pressure the difference between curves B and C which gives the  $\text{CO}_2$  content in the serum of 100 c.c. blood, is 37.9 c.c. and this corresponds to the total available sodium in the serum, since this is the isoelectric point of the serum proteins. The  $\text{CO}_2$  in the corpuscles at this pressure will be partly present as bicarbonate and partly combined with hæmoglobin, since the reaction of the corpuscles at this pressure is more acid than the isoelectric point of the corpuscles, viz. 6.98.

Now the amount of  $\text{CO}_2$  in the corpuscles combined as bicarbonate will be the volume of  $\text{CO}_2$  in the corpuscles at the isoelectric point. Calculation by means of Hasselbalch's formula shows that when the

$p_H$  of the corpuscles is 6.98 the pressure is 117 mm. corresponding to 53.6 c.c. combined CO<sub>2</sub> in 100 c.c. corpuscles. The corpuscles in 100 c.c. blood at 117 mm. contain 29.9 c.c. CO<sub>2</sub> (cp. (14), curve C, Fig. 3), this represents the total available sodium in the corpuscles of 100 c.c. blood. The total available sodium in 100 c.c. of blood is the same as that in the serum and corpuscles, and is equivalent to  $37.9 + 29.9 = 67.8$  c.c. CO<sub>2</sub>, which means that the total sodium bicarbonate of blood is 0.03*N*.

There is admittedly some uncertainty in the calculation because our experiments with the serum-bicarbonate solution were not sufficient to settle accurately the isoelectric point of the serum proteins, and no allowance has been made for any diffusion of acid from corpuscles to plasma between the two isoelectric points. However we think that the value we have obtained (0.03*N*) is probably more nearly correct than that calculated by Parsons, viz. 0.045*N*, which is almost certainly a good deal too high.

#### CONCLUSIONS.

1. The proteins of blood combine directly with CO<sub>2</sub> when the reaction of the blood is very much more acid than ever occurs in the body.
2. Throughout the range of C<sub>H</sub> occurring in the body all the CO<sub>2</sub> is present as bicarbonate, and the blood proteins act as acids combining with sodium and competing for it with the CO<sub>2</sub>.
3. Experiments with dialysed red cells and sodium bicarbonate indicate that the isoelectric point of hæmoglobin has the value  $p_H = 6.98$ . The acid dissociation constant of hæmoglobin is  $2 \times 10^{-8}$ .
4. At all pressures of CO<sub>2</sub> the C<sub>H</sub> of the corpuscles is greater than the C<sub>H</sub> of the plasma. The difference becomes less as the pressure rises.
5. CO<sub>2</sub> is completely expelled from blood by the blood pump, because the blood proteins in their capacity as acids are present in sufficient concentration to combine with all the sodium of the blood.
6. The maximum possible quantity of sodium bicarbonate in blood (*i.e.* the total sodium available for combining with CO<sub>2</sub>) is 0.03*N*.

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