XLVIII. THE COMBINATIONS OF HAEMOGLOBIN WITH OXYGEN AND WITH CARBON MON-OXIDE. II.

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In the preceding paper Hill [1913] has expanded his formula

$$y/100 = \frac{Kx^n}{1 + Kx^n}$$

in which y = the percentage saturation of the haemoglobin with oxygen, x the oxygen pressure, K the equilibrium constant of the reaction and n the average number of molecules of haemoglobin in each aggregate. The formula was originally put forward [Hill, 1910] to cover the dissociation curves of haemoglobin in pure aqueous solution and in certain saline solutions. With the introduction of certain assumptions it may now be applied (1) to the affinity of blood for oxygen in the absence of carbonic acid, (2) to the affinity of blood for carbon monoxide in the absence of oxygen, (3) the partition of haemoglobin between oxygen and carbon monoxide in the presence of the two gases and either with or without acids such as CO_2 in the system. The validity of the theory depends not only upon the soundness of the reasoning on which it rests, but upon the accuracy with which it fits the vast number of experimental data by which it may be tested.

It may here be pointed out that the formula contains but two constants n and k : the experimental test therefore is a much more crucial one than in the case of the more adaptable formula of Douglas, Haldane and Haldane [1912], which contains three constants.

Since the formula was first published by Hill, I have devoted a great deal of attention to the accuracy with which it fits the facts. The correspondence is so striking that I propose to record it in the present paper.

I will treat of the evidence as regards

(1) the reaction $\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2$,

where the haemoglobin is dissolved in solutions of various salts,

(2) $\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2$ with reference to blood.

HAEMOGLOBIN IN SALINE SOLUTIONS.

In Hill's preliminary communication he tabulated the comparison of the curves calculated from the formula and the freehand curves drawn by Camis and myself [1910] for solutions of haemoglobin in water, 0.7% NaCl and 0.9% KCl.

The figures lost much of their force from want of a fuller presentation. It was not evident from a casual study of these data that all the curves they represented for which the value of n was more than unity were in essence S-shaped. It therefore seemed that there was an essential difference between these curves and those which were known to exist for blood, the latter being S-shaped curves.

Blood.

Blood offers a far more searching test than haemoglobin solution because the curves are much more spread out. There is no case on record in which a great number of determinations, say more than about a dozen, have been carried out on the blood of any one person at one time. Fortunately the blood of the persons who have been most thoroughly investigated seems the same at one time as at another¹; a single curve may be taken as representing the blood of one individual, for instance Douglas, Zuntz or myself, irrespective of the time at which the determinations were made.

Thirty-eight determinations of points on Douglas' normal dissociation curve at his existing alveolar CO₂ pressure of 40–41 mm. have been made. The series is especially valuable because the determinations have been made by somewhat different methods, by different persons, and on different scales. Rather more than half were made by me, and for all these the differential method of blood gas analysis was used, dilute ammonia unaided by saponin being employed in the blood gas bottles and in most cases 0.1 c.c. of blood being used for each analysis. Sixteen of the points shown were determined by Haldane and Douglas; they did not use the differential apparatus, but Brodie's adaptation of the old Barcroft-Haldane apparatus. They used 1 c.c. of blood for their analyses. Five determinations were made with dilute sodium carbonate as the fluid for taking the blood whilst the rest were made with ammonia. They used saponin in addition to the alkali. Moreover, Haldane and Douglas treated the gas in their tonometer somewhat differently from the way in which I did. Their method involved them in much greater corrections for the changes during analysis in gases held physically in solution. In spite of this their method was probably somewhat more accurate than mine.

¹ Barcroft [1911], Douglas, Haldane and Haldane [1912, p. 283].

In treating of the data I have adopted two methods. The first is to take all the data in order, to divide them into three groups as nearly as possible equal, to state (1) the difference between the observed and the calculated percentage saturation in the case of each observation, (2) the greatest difference in each direction and the mean difference in the case of each group and (3) the same in the case of the whole series. The following is the result:

Pressure obs.	% sat. obs.	Dev. from curve	Pressure obs.	% sat. obs.	Dev. from curve	Pressure obs.	% sat. obs.	Dev. from curve
9	6	+1	36	63	+0.5	59	84	-1
11	6	+3	36	67	+4.5	61	83	-3
17.5	26	+3	37	63	-1	61	84	-2
18	24	+1	38	65	-1	70	89	-0.5
20	27	-0.5	39	71	+3	77	92	+0.5
20	30	+2.5	39	67	-1	85	92	-1.5
23	38	+3	41	71	+1	85	94	+0.5
24	37	-1	42	75	+4	90	92	-2.5
26	39	-3	42	72	+1	95	99	+4
29	51	+2	49	77	-1.5	97	94	-1.5
30	50	-1	49	79	+0.5	96	96	+0.5
34	57	-2	53	78	-3	104	96	0
			59	81.5	-3.5	104	97	+1
			Greatest error			Mean		
Total of 38			+4.5			0.14 %		
			-3.5					

The second plan of treating the results is to divide according to the methods by which they were obtained. Taking the 16 results obtained by Haldane and Douglas, eight are above the line and eight below it. The greatest individual discrepancies are +4% and -3%. The mean of the sixteen is 0.19%. Of their sixteen results one differs from the calculated number by 4%, three by 3%, three by 1.5-2%, eight by 0.5-1% and one by under 0.5%: whilst of the whole series of thirty-eight, there is one of 4.5%, three of 3.5-4%, nine of 2.5-3%, six of 1.5-2%, eighteen of 0.5-1% and one of under 0.5%. The general run of the figures is so nearly the same in each of the two series, as to prove that the method of experiment does not affect the comparison at issue.

Nine points have been determined on the Zuntz blood at 40 mm. CO₂ pressure. I could not find that it differed from that of Douglas, it is therefore referable to the same curve. Of these nine, three fall on the curve (within 0.5% of it), four are below it and two are above it.

Oxygen pressure	0	12	30	37	37	43	61	79	97
Observed % saturation	0	4	50	61	61.5	72	86.5	92	95
Divergence from curve	0	-5	-1	-4	-3.5	0	+5	+1	0

Extreme divergence $\left\{ \begin{array}{l} +1 \\ -5 \end{array} \right.$

Mean divergence -1.3 %.

Fig. 1. Ordinate=percentage saturation with oxygen; abscissa=oxygen pressure.
 x Blood of Douglas. x Blood of Zuntz. + Blood of Haldane.

Referable to this curve also is the blood of Haldane which I have taken from the figure in the *Journal of Physiology*, vol. XLIV, page 283, as faithfully as the scale of the figure permits. The points are as follows:

Oxygen pressure	26	20.3	20.5	20.5	29	31	37	47	48	52.5	62	62	82	99	105
Observed % sat.	19	28.5	28	23	49	52	63	66	81	80	83	87	89.5	93	96
Div. from curve	0	-0.5	0	-5	0	+0.5	-1	+2	+4	+2	+1	0	+2.5	+1	+2.5

Extreme divergence $\left\{ \begin{array}{l} +4 \\ -5 \end{array} \right.$

Mean divergence 0.6 %.

The results of the comparisons between the curve drawn from the equation, and the actual determinations which I have cited may be summarised as follows. (1) Of the 63 determinations none differs by more than 5% from the calculated value, (2) the "average error" reckoning all departures from the calculated value as positive quantities is 1.6%, (3) the mean of all the errors reckoning those above the line as positive and those below it as negative is 0.02%.

It is known that the blood of different persons differs. I have compared the points which have been determined on my own blood with the curve, the constants of which are $n = 2.5$, $K = 0.000292$. Only about 20 points are available for the comparison.

Dividing them into two groups they are as follows:

Pressure of oxygen	0	1.5	7	13	22	28	36	36	37	38.5
% sat. observed	-1	2	4	12	37	55	69.5	71	68	72.5
Div. from calcd. value	-1	+1.5	0	-4	-2	0	0	+1.5	-2.5	+0.5

Extreme divergence $\left\{ \begin{array}{l} +1.5 \\ -4 \end{array} \right.$ Mean -0.6% .

Pressure of oxygen	38.5	43	50	50	50	50	57	65	65	65	89
% sat. observed	74.5	79.5	83.5	83.5	84.5	85	89	89	90	90.5	95
Div. from calcd. value	+2.5	+1	-0.5	-0.5	+0.5	+1	0	-3	-2	-1.5	0

Extreme divergence $\left\{ \begin{array}{l} +2.5 \\ -3 \end{array} \right.$ Mean -0.23% .

Taking the two series together the results may be summarised as follows:

- (1) no single determination differs by more than 4% from the calculated value,
- (2) the "average error," calculating all errors as positive, is 1.2% ,
- (3) the mean divergence, calculating points above the line as positive and those below as negative, is 0.36% .

Fig. 2. Zuntz¹ dissociation curve, 35 mm. CO_2 ; ordinate = percentage saturation with oxygen, abscissa = oxygen pressure in mm.

Of the cases of human blood which have been studied at all exhaustively by me, the least satisfactory from the present point of view is that of Zuntz at his existing alveolar CO_2 pressure of 34–35 mm. of CO_2 ; here, however,

¹ The data and pressure given on p. 62, *Journal of Physiol.* Vol. XLII, are slightly erroneous owing to a wrong correction for the zero of the gas burette.

the chief cause of discrepancy is a point done in duplicate (pressure 19 mm. percentage saturation 41 and 40%). The data are as follows. The curve here is very steep, and a slight error in the pressure measurement would account for it.

Pressure of oxygen	15.5	19	19	23	27	31	36	36	49	50	50	60	60.5	60.5	72
% sat. observed	21	40	40	50	58	63	75	68	83	86	83	85	92	88	90
Div. from calcd. value	-1	+6	+7	+4	+3	0	+4	-3	-1	+1	-2	-5	+2	-2	-3

Of these fifteen points one is on the line, seven above and seven below it, the average error counting all errors as positive is 3%, whilst the mean counting errors above the line, as positive, and those below it, as negative, is 0.66%.

So far as I know there are no other cases in which a sufficient number of points has been determined and these spread sufficiently uniformly over the curve to test the truth or otherwise of the theory.

At this point a few words may be said by way of comparison between the curve obtained from Hill's formula and that obtained from the formula of Douglas, Haldane and Haldane. Over a great portion of the curves obtained from the two formulae they agree so closely as to make an experimental test between the one and the other almost impossible.

At pressures below 20 mm. the two curves differ and it may be well to compare this portion of them with the observed points. This task is made easier because at very low oxygen pressures the difference between the blood of different persons is very slight and therefore the determinations—all too few—for human blood may be taken together for the purpose. This may be done the more freely by me because such error as there is in the application of results obtained from my blood to the curves of Douglas' blood which are those depicted, would favour the formula of Douglas, Haldane and Haldane.

Below 15 mm. there appear to be eleven determinations on human blood, of these the three lowest, at 0 and 1.5 mm. offer no test of the relative merits of the two curves, yet I have included them because they are all important to the argument as showing that the percentage saturations as observed start from the zero. Had there been some such error as incomplete laking of the blood or wrong correction for the gases in physical solution these points would not have fallen on the base line.

Below are the data of the eleven points.

Pressure of oxygen ...	...	0	0	1.5	6	7	8	9	11	12	12	13
Percentage saturation observed	0	-1	2	-1	4	+1	6	13	4	10	12	
Divergence from Haldane's curve	0	-1	+1	-6	-3	-7	-3.5	+1	-10	-4	-3.5	
Divergence from Hill's curve ...	0	-1	+2	-2.5	+1	-2.5	+1.5	+5.5	-5	+1	+1	

Averaging the points between 5 and 15 mm., (1) the extreme departures from Haldane's formula are +1 and -10 and from Hill's +5.5 and -5, (2) reckoning the "average error" (counting all the errors as positive) it is with regard to Haldane's formula 4.8% and with regard to Hill's 2.5%, (3) reckoning errors above the line as positive and below it as negative, the mean divergence from Haldane's curve would be 4.5% and from Hill's curve 1.2%. It seems clear from the figures and more clear from Fig. 3, that Hill's formula more closely represents the facts than does that of Haldane. Eight points is however a small number to take for such a purpose and while it is evident that the equation $y/100 = \frac{Kx^n}{1+Kx^n}$ has the better of the comparison, many more points would have to be determined before the argument could be regarded as settled on this count.

Fig. 3. Lower portion of dissociation curves of human blood. The upper curve is drawn from Douglas, Haldane and Haldane's formula, the lower curve from Hill's formula. Ordinate=percentage saturation with oxygen; abscissa=oxygen pressure in mm.

It may be taken as proved that either by accident or otherwise the curves for human blood at normal alveolar CO_2 pressure closely follow Hill's equation, which is founded upon a physical conception. The natural test is to see whether curves obtained under widely different conditions also agree with the formula.

The simplest way of varying the curves is to alter the concentration of the carbonic acid. In this way a whole series of curves may be produced as was shown by Bohr, Hasselbalch and Krogh [1904]. Poulton and I [1913] investigated the question of whether three curves of the series other than the normal curve at 40 mm. pressure could be represented by Hill's formula. The answer was striking for not only were the curves capable of expression by the general formula $y/100 = \frac{Kx^n}{1 + Kx^n}$, but over the whole series only one of the two constants (K) varied, n remaining at a constant value throughout. Hence it is possible from a graph relating the concentration of carbonic acid to the value of K to obtain the dissociation curves for concentrations of carbonic acid intermediate between those at which the determinations were actually made. As this has proved useful on many occasions I append the diagram.

Fig. 4. Ordinate = CO₂ pressure in mm. Abscissa = K .

It was shown by Orbeli and me [1911] that carbonic acid had no specific effect in changing the affinity of haemoglobin for oxygen, but that it shared this property with lactic acid: Mathison subsequently showed that other organic and inorganic acids had a like effect.

The following points were obtained by the addition of lactic acid (0.2%) to blood from which the carbonic acid had been as completely as possible removed by shaking.

Pressure of oxygen	10	20	20	30	30	40	50	50
Percentage saturation observed ...	11	39	40	64	68	82	87	86
Divergence from calculated results	+1	-1	0	0	+4	+2	+1	0
$n=2.5, K=0.000363$

Fig. 5. Equilibrium curves of CO haemoglobin drawn from Hill's formula. The points are the observations of Douglas, Haldane and Haldane. Ordinate=percentage of haemoglobin with CO; abscissa=CO pressure in mm.

In addition to this, curves might be cited in which both lactic and carbonic acid were added to blood, such curves for example as those given by me in the record of my work in Tenerife and by Orbeli and myself on the effects of low oxygen pressures on animals. The points on curves all follow the equation with the same degree of exactitude as those cited in this paper, but the points determined in each case were too few to make any detailed discussion of them worth while.

Most of what has already been said as regards oxyhaemoglobin curves might be repeated with regard to CO-haemoglobin. Fortunately a very complete series of data on this subject has recently been published by Douglas, Haldane and Haldane. In Fig. 5, these data are shown and along with them a system of curves drawn from Hill's formula. The scale on which the diagram is plotted is such that the centre point marked *A* of the curve corresponding to 40 mm. CO₂ would be on the same place in the figure as the centre point of the oxyhaemoglobin curve corresponding to the same CO₂ pressure. That being so it will be found that the whole figure is superposable upon the same figure for oxyhaemoglobin. Not only are the points then faithful to Hill's formula, but on a different scale the relation of *K* to the CO₂ pressure is the same in the case of oxy- as of CO-haemoglobin.

There is one more point to which I should draw attention, and which is involved in the fidelity of the observed points to the theoretical lines. Did the lines in Fig. 5 actually conform to Haldane's formula, there would have been the same slight discrepancy between the lines and the points at the bottom of the diagram in the case of CO-haemoglobin as in the case of oxyhaemoglobin and to just the same extent. When this difference reappears in another series such as the CO-series in which the points have been arrived at by wholly different methods (these were obtained by the method of carmine titration) the weight of evidence in favour of their having a real significance is greatly increased.

CONCLUSION.

1. The available data for the dissociation curves of blood agree very closely with the theoretical curves deduced from the following physical conceptions:

(a) That the reaction between haemoglobin and oxygen is a reversible chemical change $\text{Hb}_n + n\text{O}_2 \rightleftharpoons \text{Hb}_n\text{O}_m$.

(b) That *n* is the average number of molecules aggregated together, the value of *n* depending upon the nature and concentration of the electrolytes in the solution.

(c) That the effect of acids is to change the equilibrium constant of the reaction without sensibly altering the degree of aggregation of the molecules.

(d) That the above reaction does not involve the breakdown or reformation of the aggregates.

(e) That unsaturated oxides are unstable and break up into haemoglobin and saturated oxides.

2. The available data with regard to the reaction of CO and oxygen support an entirely similar conception of carboxyhaemoglobin.

3. So far as the curves deduced from the formula $y/100 = \frac{Kx^n}{1+Kx^n}$ (Hill) can be distinguished from those yielded by the formula

$$x = \frac{K_y [1+b(1-y)]}{(1-y)(1+ay)} \text{ (Douglas, Haldane and Haldane),}$$

the experimental evidence leans towards the former.

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