**The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves.** By A. V. HILL.

In a previous communication Barcroft and I gave evidence which seemed to us to prove conclusively that dialysed hæmoglobin consists simply of molecules containing each one atom of iron. The molecular weight is therefore $Hb = 16,660$. These experiments have not been published yet, but I shall assume the results.

Other observers (Reid, Roaf, Hüfner and Gansser) working on different solutions have obtained divergent results. The method used by all of them was the direct estimation of the osmotic pressure, by means of a membrane permeable to salts, but not to hæmoglobin. The method involves a relatively large error, because the quantity measured is small. It is doubtful however whether this can explain the discordant results.

Our work led me to believe that the divergence between the results of different observers was due to an aggregation of the hæmoglobin molecules by the salts present in the solution, a consequent lowering of the number of molecules, and an increase in the average molecular weight as observed by the osmotic pressure method. To test this hypothesis I have applied it to several of the dissociation curves obtained by Barcroft and Camis with hæmoglobin in solutions of various salts, and with hæmoglobin prepared by Bohr's method.

The equation for the reaction would be

$$Hb + O_2 \rightleftharpoons HbO_2,$$
$$Hb_n + nO_2 \rightleftharpoons Hb_nO_{2n},$$
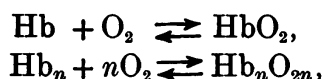
where $Hb_n$ represents the aggregate of $n$ molecules of Hb. I have supposed that in every solution there are many different sized aggregates, corresponding to many values of $n$.

If there were in the solution only Hb and $Hb_2$ the dissociation curve would be

$$y = \lambda \frac{K'x^2}{1 + K'x^2} + (100 - \lambda)\frac{Kx}{1 + Kx} \quad \ldots\ldots\ldots\ldots(A),$$

where $\lambda\,\%$ is as $Hb_2$, $(100 - \lambda)\%$ as Hb, $K'$ is the equilibrium constant of the reaction $Hb_2 + 2O_2 \rightleftharpoons Hb_2O_4$ and $K$ that of $Hb + O_2 \rightleftharpoons HbO_2$: $K$ has the value $\cdot125$ (Barcroft and Roberts).

Now in the figure is given the dissociation curve of Bohr's hæmoglobin. The points [⊙] are those given by Barcroft and Camis. The continuous curve is calculated by myself from the formula given above where

$$\lambda = 62, \quad K' = \cdot 0011, \quad K = \cdot 125.$$
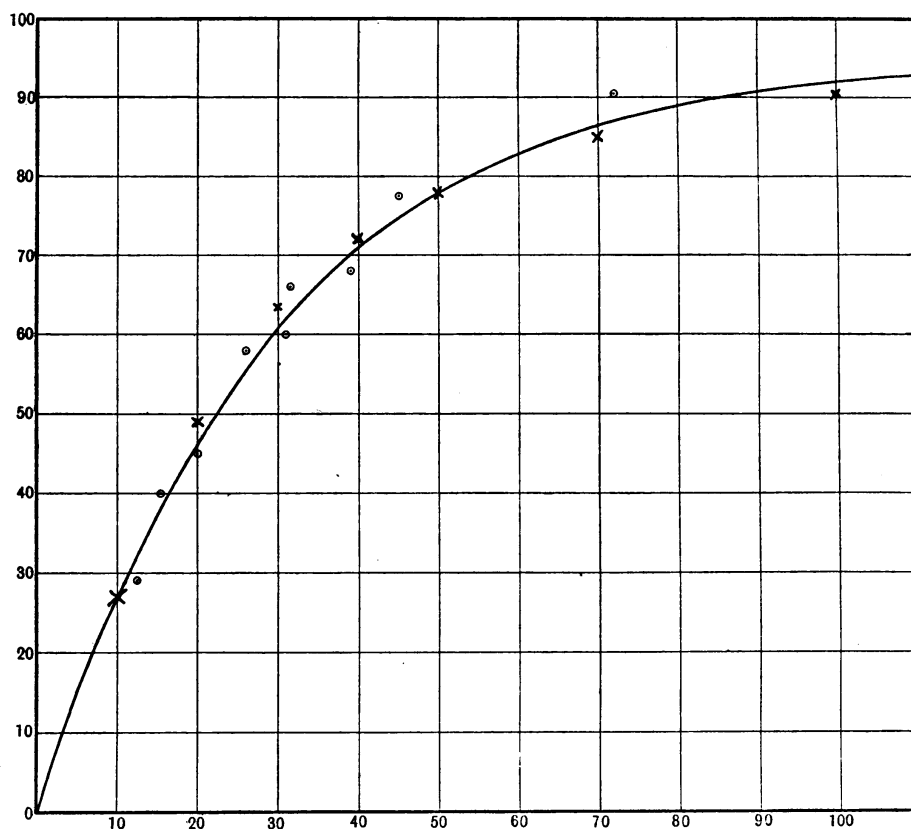


⊙ % saturations observed by Barcroft and Camis.
The continuous curve calculated from equation (A).
× % saturations calculated from equation (B) with $n = 1 \cdot 405$, $K = \cdot 01455$.

The curve fits the points remarkably well.

Again, I have taken Barcroft and Camis' curve for hæmoglobin in $Na_2HPO_4$. The calculated results are obtained from the formula (A) above where

$$\lambda = 84 \cdot 8, \quad K' = \cdot 0236, \quad K = \cdot 125.$$

| mm. $O_2$ tension | 10 | 15 | 20 | 25 | 30 | 40 | 100 |
|---|---|---|---|---|---|---|---|
| observed % saturation | 67·5 | 81 | 87 | 90·5 | 93 | 95 | 99·5 |
| calculated % saturation | 68·2 | 81·3 | 87·3 | 91 | 93 | 95·5 | 98·9 |

Now it is unlikely that in either of these cases there is only Hb and $Hb_2$: and as the calculation of the constants in these equations is very tedious I decided to try whether the equation

$$y = 100 \frac{Kx^n}{1 + Kx^n} \qquad \qquad (B)$$

would satisfy the observations.

My object was rather to see whether an equation *of this type* can satisfy all the observations, than to base any direct physical meaning on $n$ and $K$. As I show below all the experiments agree with this formula: and therefore there is no doubt that they will agree with the more general formula

$$y = \sum_{r=1}^{r=n} a_r \frac{K_r x^r}{1 + K_r x^r},$$

if the constants are chosen properly.

This represents the most general form of the dissociation curve on this hypothesis.

I. Barcroft and Camis' curve with $Na_2HPO_4$. (*Journ. of Phys.* xxxix. p. 125.) $n = 1\cdot670$, $K = \cdot0453$.

| mm. $O_2$ tension | 10 | 15 | 20 | 25 | 30 | 40 | 100 |
|---|---|---|---|---|---|---|---|
| observed % saturation | 67·5 | 81 | 87 | 90·5 | 93 | 95 | 99·5 |
| calculated | 68·2 | 81 | 87 | 90·7 | 93 | 95·5 | 99 |

II. Curve with $NaHCO_3$. (*Ibid.* p. 125.) $n = 1\cdot835$, $K = \cdot0155$.

| mm. $O_2$ tension | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 50 | 100 |
|---|---|---|---|---|---|---|---|---|---|
| observed % saturation | 52 | 69·5 | 79 | 85 | 89 | 91·5 | 93·5 | 96 | 99·5 |
| calculated | 51·5 | 69 | 79·2 | 85 | 89 | 91·4 | 93·2 | 95·2 | 98·8 |

III. Curve with KCl. (*Ibid.* p. 124.) $n = 2\cdot49$, $K = \cdot00204$.

| mm. $O_2$ tension | 10 | 15 | 20 | 25 | 30 | 35 | 50 | 100 |
|---|---|---|---|---|---|---|---|---|
| observed % saturation | 42·5 | 63 | 75·5 | 84·5 | 91 | 94 | 96 | 99 |
| calculated | 38·6 | 63·5 | 78 | 86 | 90·6 | 93·6 | 97·4 | 99·4 |

IV. Curve with NaCl. (*Ibid.* p. 122.) $n = 1\cdot778$, $K = \cdot0062$.

| mm. $O_2$ tension | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 50 | 60 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|
| observed % saturation | 27·5 | 41 | 60 | 69·5 | 75 | 79·5 | 83 | 85·5 | 91 | 98·5 |
| calculated | 28·5 | 45·8 | 58 | 67·2 | 74 | 78·8 | 82·7 | 87·7 | 90·8 | 96 |

V. Curve with Ringer's solution. (*Ibid.* p. 121.) $n = 2\cdot111$, $K = \cdot00427$.

| mm. $O_2$ tension | 10 | 15 | 20 | 25 | 30 | 40 | 50 | 100 |
|---|---|---|---|---|---|---|---|---|
| observed % saturation | 36 | 56 | 70 | 79 | 85 | 91 | 94 | 99 |
| calculated | 35·5 | 56·6 | 70·2 | 79·2 | 84·5 | 91·2 | 94·3 | 98·6 |

VI. Curve, 1 drop of $\overline{NH_4}_2CO_3$ to 3 c.c. of solution. (*Ibid.* p. 121.) $n=3\cdot19$, $K=\cdot000257$.

| mm. $O_2$ tension | 20 | 20·5 | 21 | — | 29·5 | 30·5 | — | 37 | — | 59·5 |
|---|---|---|---|---|---|---|---|---|---|---|
| observed $^0/_0$ saturation | 80 | 78·5 | 79 | — | 93 | 92·5 | — | 96 | — | 99 |
| calculated | 78 | 79·2 | 80·7 | — | 92·8 | 92·9 | — | 96·2 | — | 99·1 |

VII. Barcroft and Roberts' curve for dialysed hæmogloblin. (*Ibid.* p. 146.) $n=1$, $K=\cdot125$.

Thus the equation above satisfies all the observations given by Barcroft and Camis. This in itself is a gain, as instead of a curve we have now two constants $n$ and $K$, and the effect of any given treatment on the dissociation curve can be estimated as a numerical effect on $n$ and $K$.

Experiments are being done by Barcroft and myself using this method. The advantage of this method of analysing the results is that much labour is saved : *two* observations can give the *two* constants for any curve, and then the rest of the curve may be calculated.

There is also the theoretical interest that the observations can be made to fit curves of the type required by the hypothesis of an aggregation of the molecules of hæmoglobin to form larger molecules.

These dissociation curves seem therefore to be a useful method of inquiring into the nature and properties of colloidal solutions. The constants $n$ and $K$ can be calculated from six well chosen observations with practically no liability to error : and the effects of various external conditions on these two constants may very probably have a direct and important physical meaning. By the aid of the laws of chemical statics and dynamics this chemical reaction (with $O_2$) of colloidal matter may be made to give us some further insight into the nature of the laws that govern colloidal solutions.