

intendent's headquarters, elev. 3000 to 3300 ft., David Kaye 2, 3, in 1932 (CRB, UC). Copper River Region, trailing, 2.5 inches high, common in high tundra, West Fork of Eagle Creek, alt. 4500 ft., Wm. L. Pope 160, Aug. 22, 1902 (USN).

Kadiak Id.: Kadiak, L. J. Cole, July 1, 1899 (USN); Coville and Kearney 2312, July 20, 1899 (USN); C. V. Piper 4592, July 28, 1904 (CRB, USN). Kadiak Mountain, F. A. Walpole 1181 (pistillate type), July 30, 1900 (CRB, USN). Alaska Peninsula: Kukak Bay, Coville and Kearney 1537, July 1-5, 1899 (USN); Katmai Region, A. E. Miller, July 21, 1919 (USN).

Shumagin Ids.: Unga, F. A. Golder 40, Sept. 20, 1899 (USN). Unalaska: (U. S. Coast Survey Expedition to Alaska, 1867, under George Davidson), Dr. A. Kellogg 325 (PANS); Amakaak Id., at Unalaska, B. W. Evermann 156, Aug. 2, 1892 (USN).

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## THE OXYGEN EQUILIBRIUM OF HEMOGLOBIN AND ITS STRUCTURAL INTERPRETATION

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The work of the early investigators of blood (Bohr, Hasselbalch, Krogh, Barcroft, Haldane, Henderson) showed that the oxygen equilibrium of hemoglobin is affected by the hydrogen ion concentration of the solution, and, moreover, that it does not correspond to the simple equilibrium  $\text{Hb} + \text{O}_2 \rightleftharpoons \text{HbO}_2$  (in which the symbol Hb represents the portion of the hemoglobin molecule containing one heme and capable of combining with one oxygen molecule). After completing his osmotic pressure experiments showing that the hemoglobin molecule contains four hemes, Adair<sup>1</sup> suggested that the  $\text{Hb}_4$  molecule adds four oxygen molecules successively, with different equilibrium constants. He and Ferry and Green<sup>2</sup> found that their data on the oxygen equilibrium of hemoglobin solutions at constant pH can be represented by a four-constant equation corresponding to this conception, the values of the constants changing in a regular way with changing pH.

On the basis of certain postulates regarding the structure of the hemoglobin molecule, I have derived an equation involving only two constants which satisfactorily represents the data on the oxygen equilibrium at constant pH, and also an equation involving two constants which represents the change in the oxygen equilibrium with change in pH. The validity of these equations provides considerable support for a particular structure of the molecule.

The most accurate and extensive data on the oxygen equilibrium are those obtained by Ferry and Green<sup>2</sup> for horse hemoglobin in phosphate and borate buffers. Ferry and Green remarked that their curves of  $y$  (the ratio

$O_2$  absorbed/ $O_2$  content at saturation) versus  $p$  (the oxygen pressure) at different values of pH can be superimposed by making a change of scale for  $p$ . On doing this, the experimental points all fall near a smooth curve, as shown in figure 1. This curve can be reproduced roughly by the equation

$$y = \frac{Kp^n}{1 + Kp^n} \quad (1)$$

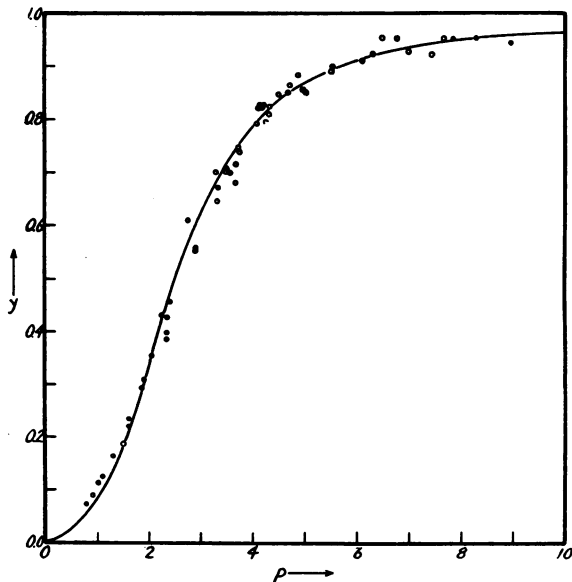


FIGURE 1

The experimental values of the fractional absorption of oxygen,  $y$ , plotted against the oxygen pressure  $p$  (the points obtained at various pH values being reduced to pH 8.30 by a change of scale in  $p$ ), compared with the theoretical curve of Equation 3, corresponding to the square structure of the hemoglobin molecule.

which corresponds to the equilibrium  $Hb_n + n O_2 \rightleftharpoons Hb_n(O_2)_n$ , the value of  $n$  being about 2.6. The curve can be accurately represented by Adair's four-constant equation

$$y = \frac{K_1p + 2K_2p^2 + 3K_3p^3 + 4K_4p^4}{4(1 + K_1p + K_2p^2 + K_3p^3 + K_4p^4)}, \quad (2)$$

and also by the two-constant Equation 3, which we shall now derive.

Let us make the following postulates regarding the structure of the hemoglobin molecule.

1. The hemoglobin molecule contains four hemes, each of which is

connected with  $k$  others, the connection being such as to permit the interaction of the hemes in pairs.

2. The four hemes are equivalent.

3. The interactions for connected pairs are equivalent.

We distinguish four cases, corresponding to  $k = 0, 1, 2$  and 3.

No interaction of hemes,  $k = 0$ . This leads to Equation 1 with  $n = 1$ , and is hence not compatible with experiment.

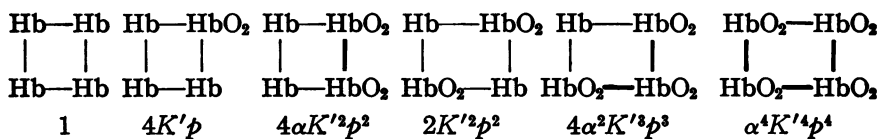
Interaction of the hemes in pairs  $\begin{pmatrix} \text{Hb} & \text{Hb} \\ | & | \\ \text{Hb} & \text{Hb} \end{pmatrix}$ ,  $k = 1$ . This leads to

an equation which can be approximated by Equation 1 with  $1 \leq n \leq 2$ , and is hence not compatible with experiment.

Hemes at the corners of a square, interactions along the sides of the square  $\begin{pmatrix} \text{Hb}-\text{Hb} \\ | & | \\ \text{Hb}-\text{Hb} \end{pmatrix}$ ,  $k = 2$ . This leads to Equation 3, in agreement with experiment.

Hemes at the corners of a tetrahedron, interactions along the edges of the tetrahedron,  $k = 3$ . This leads to Equation 4, in agreement with experiment; however, for other reasons the structure is less satisfactory than the square structure.

To derive Equation 3 on the basis of the square arrangement, we note that at the oxygen pressure  $p$  the relative amounts of the six molecular species present are the following:



The constants  $K'$  and  $\alpha$  have the following significance,  $RT \ln K'$  is the free energy change accompanying the addition of oxygen to heme, and  $RT \ln \alpha$  is the additional free energy stabilizing two interacting  $\text{HbO}_2$  groups; that is, the free energy is decreased by  $RT \ln \alpha$  for each interaction shown by a heavy bar. The factors 2 and 4 result from the symmetry numbers.

These relative amounts  $1:4K'p:(4\alpha + 2)K'^2p^2:4\alpha^2K'^3p^3:\alpha^4K'^4p^4$  of the substances  $\text{Hb}_4$ ,  $\text{Hb}_4\text{O}_2$ ,  $\text{Hb}_4(\text{O}_2)_2$ ,  $\text{Hb}_4(\text{O}_2)_3$  and  $\text{Hb}_4(\text{O}_2)_4$  lead directly to the following equation for  $y$ :

$$y = \frac{K'p + (2\alpha + 1)K'^2p^2 + 3\alpha^2K'^3p^3 + \alpha^4K'^4p^4}{1 + 4K'p + (4\alpha + 2)K'^2p^2 + 4\alpha^2K'^3p^3 + \alpha^4K'^4p^4} \quad (3)$$

The curve shown in figure 1 is calculated by means of this equation, with  $\alpha = 12$  and  $K' = 0.033$ . It is seen that the agreement with the experimental points is satisfactory. (The uniform deviation shown by the first

five points may be due to experimental error, or may be real, indicating that the theory is only approximate.)

The similar discussion of the tetrahedral configuration leads to the equation

$$y = \frac{K'p + 3\alpha K'^2 p^2 + 3\alpha^2 K'^3 p^3 + \alpha^3 K'^4 p^4}{1 + 4K'p + 6\alpha K'^2 p^2 + 4\alpha^2 K'^3 p^3 + \alpha^3 K'^4 p^4} \quad (4)$$

in which  $K'$  and  $\alpha$  have the same significance as before. This equation

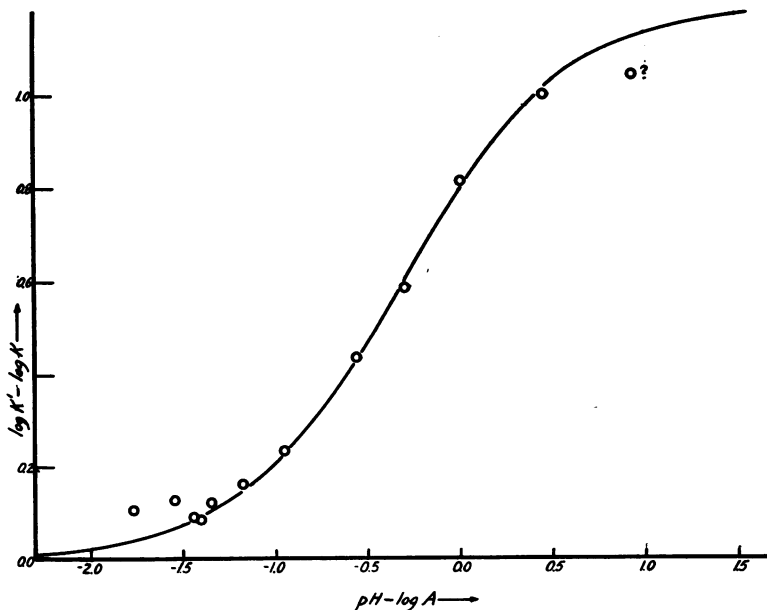


FIGURE 2

The dependence of the equilibrium constant  $K'$  of Equation 3 on pH. The circles are experimental values, and the curve is drawn according to Equation 6.

provides a curve almost indistinguishable from the one shown in the figure, the value of  $\alpha$  being about  $12^{2/3} = 5.3$ .

Although the tetrahedral configuration is thus compatible with the oxygen equilibrium data, we reject it in favor of the square configuration for the following reasons. The hemoglobin molecule is roughly spherical in shape, with a radius of about 29 Å, and it is very probable that the hemes are located on the surface of the molecule; if arranged tetrahedrally, they would be about 47 Å apart. It is very difficult to imagine a connection between two hemes this far apart which would lead to an interaction energy as large as  $RT \ln \alpha = 1000$  cal./mole. Moreover, the tetrahedral configuration would lead one to expect a three-fold axis of symmetry for

the hemes rather than the four-fold axis possessed by the porphyrin nucleus.

To account for the dependence of the oxygen equilibrium on the hydrogen ion concentration, we give quantitative expression to the familiar qualitative explanation that there is an interaction between the oxygen molecules of oxyhemoglobin and some acid groups. The fact that an equation of the form of 3 can be made to account for the data for various pH values by changing  $K'$  alone indicates strongly that each heme contains its own acid groups, the interaction of one heme with the acid groups of another being negligible.<sup>3</sup> Let us first assume that there is one acid group for each heme, with interaction constant  $\beta$  such that the free energy change on dissociation of  $\text{HHbO}_2$  to  $\text{H}^+$  and  $\text{HbO}_2^-$  is  $RT \ln \beta$  less than that of  $\text{HHb}$  to  $\text{H}^+$  and  $\text{Hb}^-$ , oxyhemoglobin being a stronger acid than hemoglobin. The relative amounts of the four molecular species (considering only one heme)  $\text{HHb}$ ,  $\text{Hb}^-$ ,  $\text{HHbO}_2$  and  $\text{HbO}_2^-$  are then  $1:A/[\text{H}^+]:K\beta:K\beta A/[\text{H}^+]$ , and the oxygen equilibrium formula becomes

$$y = \frac{K'p}{1 + K'p}$$

with

$$K' = K \frac{(1 + \beta A/[\text{H}^+])}{(1 + A/[\text{H}^+])}, \quad (5)$$

in which  $A$  is an acid-strength constant. It is found on similarly considering the 55 molecular species  $\text{H}_4\text{Hb}_4$ ,  $\text{H}_4\text{Hb}_4\text{O}_2$ , etc., corresponding to the square arrangement that the oxygen equilibrium is expressed by Equation 3, with  $K'$  dependent on the hydrogen ion concentration in the way given by Equation 5.

The dependence of  $K'$  on pH as found experimentally from the factors used in reducing the points of figure 1 to a single curve is shown<sup>4</sup> in figure 2. It is found that Equation 5 does not represent this dependence satisfactorily. If, however, we assume that there are two equivalent acid groups interacting with each heme, we derive the equation

$$K' = K \frac{(1 + \beta A/[\text{H}^+])^2}{(1 + A/[\text{H}^+])^2}, \quad (6)$$

which does agree satisfactorily with experiment,<sup>5</sup> as shown by the curve in the figure, calculated for  $\beta = 4$ ,  $-\log A = 7.94$  and  $K = 0.0035$ .

We conclude that the data on the oxygen equilibrium of hemoglobin indicate that the four hemes of the molecule are arranged at the corners of a square; each heme is connected with two others in such a way as to give rise to an interaction energy of  $RT \ln \alpha = 1500$  cal./mole for each pair of adjacent oxyhemes, and each heme contains two acid groups, the acid

group-oxyheme interaction energy being  $RT \ln \beta = 820$  cal./mole. The total oxyheme-oxyheme interaction energy is about 6000 cal./mole, and the total acid group-oxyheme interaction energy is about 6600 cal./mole.

The none too exact experiments on the carbon monoxide equilibrium and the carbon monoxide-oxygen balance of hemoglobin suggest that the interactions  $\alpha$  and  $\beta$  are effective for carboxyhemoglobin as well as for oxyhemoglobin. On the other hand, the results of Anson and Mirsky<sup>6</sup> on the carbon monoxide equilibrium of hemochromogen, which agree with Equation 1 with  $n = 1$ , show that there is no heme-heme interaction in the hemochromogen studied. It seems not improbable that the hemochromogens differ from hemoglobin mainly in that in the hemochromogens the hemes are independent and in hemoglobin four hemes form a conjugated system.

<sup>1</sup> G. S. Adair, *Proc. Roy. Soc.*, **A109**, 292 (1925); *Jour. Biol. Chem.*, **63**, 529 (1925).

<sup>2</sup> R. M. Ferry and A. A. Green, *Jour. Biol. Chem.*, **81**, 175 (1929).

<sup>3</sup> It is possible that the results of more accurate experiments would require this conclusion to be revised.

<sup>4</sup> The points for pH 5.8, 4.9 and 4.5 are not shown. These are obtained from only one or three measurements; they lie somewhat above the origin (0.1-0.2), which may indicate that some other interaction becomes effective in very acid solutions.

<sup>5</sup> We have neglected the mutual interaction of the two acid groups, the data not being sufficiently accurate and extensive to make a discussion of this interaction profitable.

<sup>6</sup> M. L. Anson and A. E. Mirsky, *Jour. Physiol.*, **60**, 50 (1925).

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## THE RATE AND EXTENT OF ANAEROBIC DECOMPOSITION OF SULFITE WASTE LIQUOR BY BACTERIA OF SEA BOTTOM MUD

### II. BACTERIOLOGICAL

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In Part One of this paper, to which the reader is referred,<sup>1</sup> the experimental set-up and procedure, chemical methods and results of chemical analyses were described in detail. Reviewing briefly, gallon bottles were filled about one-third full of marine mud and the rest of the bottle was filled with sea water, to which various amounts of the sulfite waste liquor (s.w.l.) had been added. The bottles were then incubated at 9°, 21° and 36°C.; the evolved gases were collected, measured and analyzed chemically. During the 310 day incubation period through which fermentation