THE OXYGEN EQUILIBRIUM OF THE HEMOGLOBIN OF THE EEL, ANGUILLA ROSTRATA

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(Received for publication, February 27, 1951)

Almost all vertebrates possess blood hemoglobins which have S-shaped oxygen equilibrium curves. Such S-shaped curves are an indication that each molecule contains several hemes, which interact in the sense that the oxygenation of one heme facilitates the oxygenation of the others. For the reaction of a single independent heme with oxygen, the oxygen equilibrium curve is a rectangular hyperbola. Hill's equation,

$$\frac{y}{100} = \frac{Kp^n}{1 + Kp^n},$$

represents fairly all such functions, at least between 10 and 90 per cent saturation. In an S-shaped curve n is greater than 1; in a hyperbolic curve n is 1.

In 1929 Kawamoto (1) presented data which he believed showed that the oxygen equilibrium curve of eel blood hemoglobin is hyperbolic. He stated that, "After trials, it was found that Hill's equation is satisfied only when the value of n . . . is made as small as unity." I have recalculated his data and find that they are best fitted when n is not 1.0, but 1.8.

In a single experiment, performed by methods described in the preceding paper (2), the oxygen equilibrium of eel hemoglobin was redetermined. The animal (*Anguilla rostrata*) was obtained from a commercial fish market, and was kept alive for about 2 weeks in an aquarium with fresh running water before use. In phosphate buffer (0.1 M $K_2HPO_4 - KH_2PO_4$) of pH 7.3 at 20°C., the oxygen pressure at half saturation is 12.5 mm. Hg and n is 1.8. These data are presented in the accompanying table on the following page.

Hill's equation can be rewritten in the logarithmic form, $\log \frac{y}{100 - y} = \log K + n \log p$. This is the equation of a straight line, with intercept log K and slope n. Kawamoto's and the new data are plotted in this form in Fig. 1. Kawamoto's measurements were made at several temperatures, and yield in

* I am greatly indebted to Professor George Wald for suggesting this problem. The experiments were supported in part by a grant to Professor Wald by the Medical Sciences Division of the Office of Naval Research.

Oxygen Equilibrium Function of Eel Hemoglobin

p is the oxygen pressure in mm. Hg. y is the percentage saturation with oxygen. 20°C.; pH 7.3.

p	y
mm. Hg	
0.66	1.82
1.33	1.82
1.99	1.82
3.32	12.7
4.65	16.4
6.65	23.6
10.6	42.2
13.3	53.7
16.6	63.6
19.9	69.0
26.6	79.1
36.5	86.9
46.5	90.0
158.0	98.2
760.0	100.0

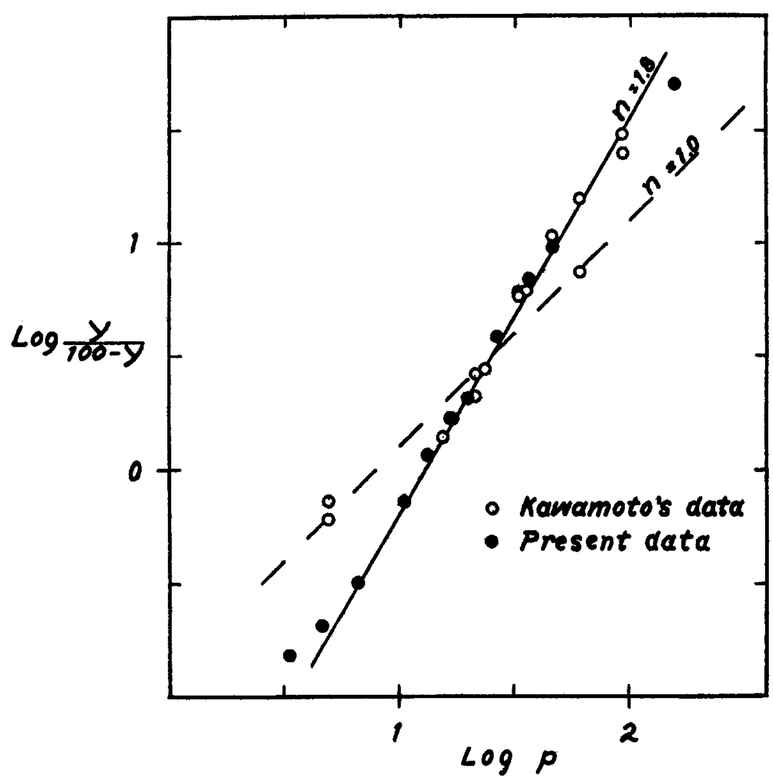

FIG. 1. The oxygen equilibrium of eel hemoglobin. y is the percentage oxygenation and p is the oxygen pressure in mm. Hg. Temperature 20°C. In this way of plotting the data, Hill's equation,

$$\frac{y}{100} = \frac{Kp^n}{1 + Kp^n},$$

yields a straight line of slope n. It is clear that the great majority of both Kawamoto's and our own data indicate n to be about 1.8. Only three of Kawamoto's points diverge significantly; these were obtained at 30°C. where some deterioration of hemoglobin may have resulted in deviations.

this plot a group of parallel lines. They have been moved along the log p axis so as to coincide in position.

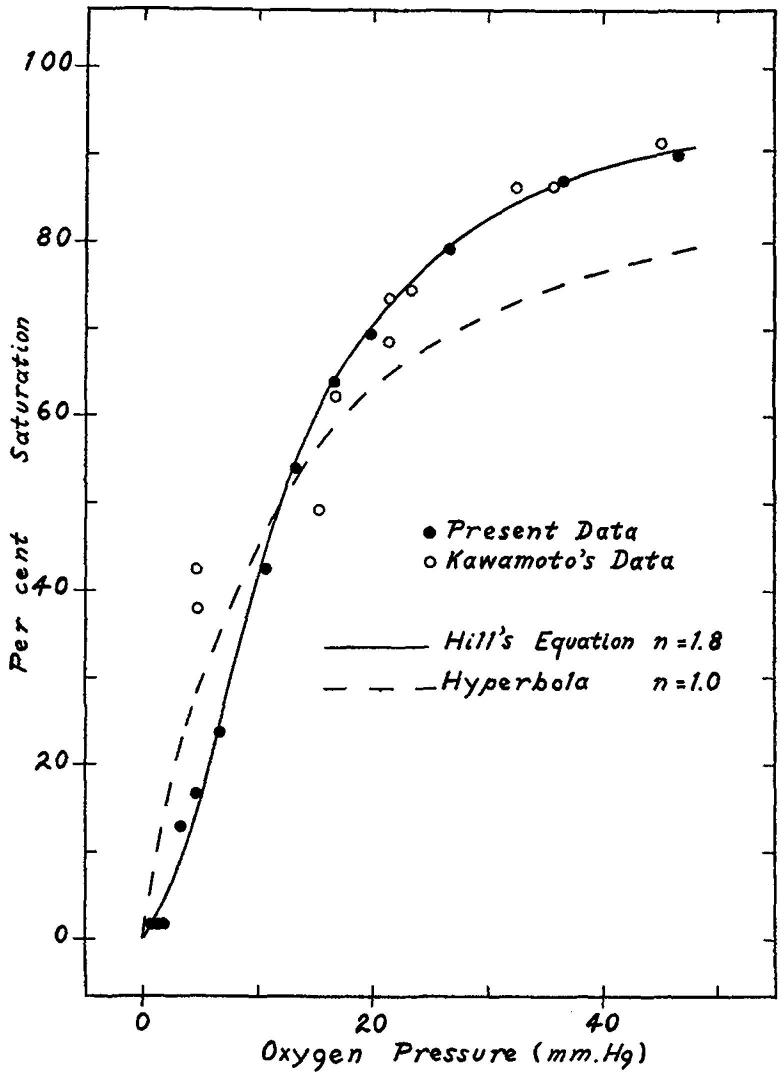

FIG. 2. The oxygen equilibrium of eel hemoglobin. Both sets of data follow Hill's equation when n is 1.8.

It is clear from Fig. 1 that Kawamoto's and the new data are in substantial agreement. The slope of the best line drawn through both sets of measurements shows that $n = 1.8$. Only three of Kawamoto's points fall appreciably off this line; these points all represent measurements made at 30°C., and possibly deviate because of some deterioration of the hemoglobin at this temperature.

In Fig. 2 all the data are replotted in the conventional way. The oxygen equilibrium function is clearly S-shaped. This leaves only one vertebrate, the lamprey, with a blood hemoglobin known to yield a hyperbolic oxygen equilibrium curve (3).

SUMMARY

Kawamoto had reported that eel hemoglobin has a hyperbolic oxygen equilibrium function, with n in the Hill equation equal to 1. On the basis of Kawamoto's data and with new measurements, it is shown that the equilibrium function is in fact S-shaped, as in most other vertebrates, and n in Hill's equation equals 1.8.

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