A Study of the Oxygen Equilibrium of Horse Hemoglobin

BY SHIRO TAKASHIMA[1]

The effect of ionic strength on the shape of oxygen saturation curve of hemoglobin was investigated. It was found that in a solution of high ionic strength, the fit of the Hill equation was satisfactory, but when the ionic strength of the solution was lowered, the saturation curve gradually deviated from the Hill equation, and simultaneously the oxygen partial pressure causing 50% saturation becomes progressively lower.

I. Introduction

Oxygen equilibrium of hemoglobin has already been the subject of repeated investigations. Most of the workers[2,3] have reported that the curve which is obtained by plotting the degree of saturation (y) against the logarithm of oxygen partial pressure (p) deviate more or less from the Hill equation: $y = KP^n/1 + KP^n$ (K and n being constants), according to which the y–log P curve must show a "regular" (or "symmetric") sigmoid flexure. Recently Wyman, et al.,[4] working with human hemoglobin freshly prepared from laked blood, obtained a curve with a typical sigmoid flexure, and they assumed that the "assymmetry" of the curves obtained by other workers might have been due to the unfreshness of the hemoglobin samples used in their experiments.

An older study made by Altschule and Hogness[5] has shown that both the dissociation constant (K) of oxyhemoglobin and the interaction constant (α) in the sense of Pauling vary with the ionic strength of the solution. It seems, therefore, possible that the discrepancy in the data reported by various investigators might have been due to the difference in the ionic strength of the solutions. The experimental results presented in this paper show that the form of the y–log P curve is largely dependent on the ionic strength of the solution, rather than on the freshness of hemoglobin sample as was suggested by Wyman, et al.

II. Experimental

Hemoglobin was prepared from horse blood cells by fractional crystallization with alcohol at about 0°. It was then recrystallized twice and washed several times with cold distilled water to remove the electrolytic impurities. The measurement of O_2 saturation was conducted immediately after dissolving the crystals in solution, each series of experiments being completed within a few hours. The optical density of the solution was measured at the wave length of 600–650 mμ. The pH and the ionic strength of the solution were varied by using phosphate or acetate buffers of different concentrations. The concentration of hemoglobin in the solution was about 1.0–1.5%.

Hemoglobin solution was first deoxygenated by alternative evacuation and filling with nitrogen until its optical density became constant. Then, CO_2-free air of known pressure was introduced, under evacuation, into the vessel until a steady value of the optical density was established. After each reading, the solution was deoxygenated and the reproducibility of the optical density of reduced hemoglobin was assured. The reproducibility of the optical density of

completely oxygenated hemoglobin was also tested at the beginning and end of each series of experiments. In some cases, higher values for 100% oxygenated hemoglobin were observed at the end than at the beginning of experiment, a fact which indicated the occurrence of gradual change in the nature of hemoglobin molecules during the course of the determination. In such cases the data of the whole series of measurements were discarded.

III. Results

The oxygen saturation curves obtained with solutions of various ionic strengths at pH 6.9 (phosphate buffer) and at 28° are reproduced in Fig. 1. As
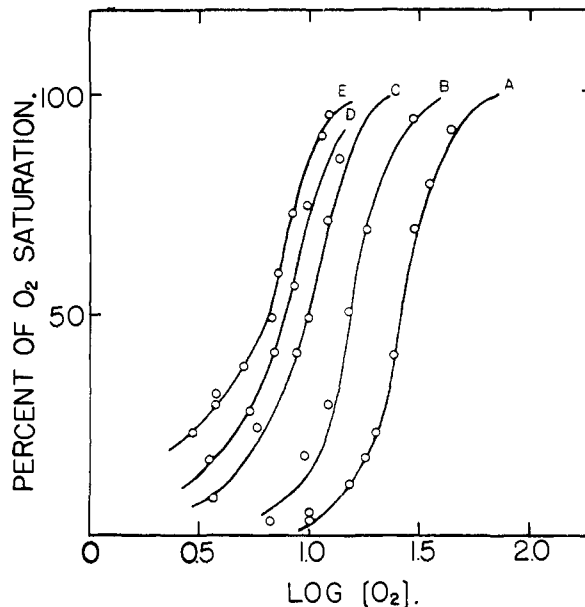
Fig. 1.—Degree of oxygenation of hemoglobin in function of O_2 partial pressure, as it changes with the ionic strength of the solution; pH 6.9 and temperature 28°. Concentration of phosphate buffer: A, $M/10$ (ionic strength, 0.3); B, $M/100$; C, $M/1000$; D, $M/10000$; E, unbuffered.

may be seen, both the form and the lateral position of the curves were markedly affected by the change of ionic strength. The oxygen partial pressure at which the degree of oxygenation was 50% became progressively lower with the decrease of ionic strength. In Fig. 2 the curves for the highest and lowest ionic strengths are superimposed in order to facilitate the comparison of their shapes. The curve obtained at higher ionic strength showed a fairly regular sigmoid flexure corresponding to the Hill equation, whereas the curve obtained at lower ionic strengths was decidedly "asymmetric," devi-

(1) School of Chemistry, University of Minnesota.
(2) W. H. Forbes and F. J. W. Roughton, J. Physiology, **71**, 229 (1931).
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(4) D. W. Allen, K. F. Guthe and J. Wyman, J. Biol. Chem., **187**, 393 (1950).
(5) A. M. Altschule and T. R. Hogness, ibid., **129**, 315 (1939).

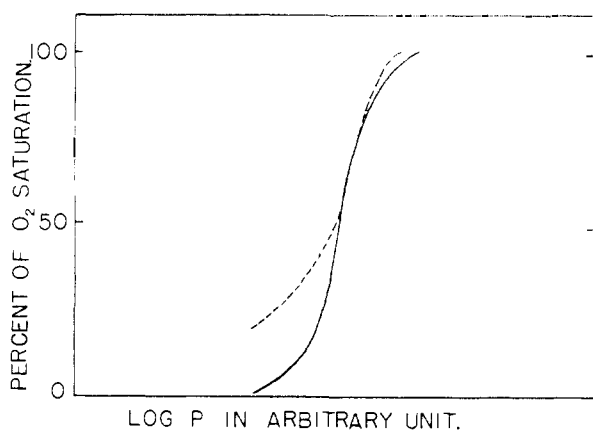ating from the Hill equation especially markedly at the lower portions of the curve.

Fig. 2.—Comparison of the forms of O_2-saturation curves obtained at higher and lower ionic strength (curves A and E in Fig. 1); pH 6.9 and temperature 28°.

To test whether the possible drop of pH accompanying the process of oxygenation had any influence upon the shape of the sigmoid curve, the oxygenation was measured at pH 6.0 (using acetate
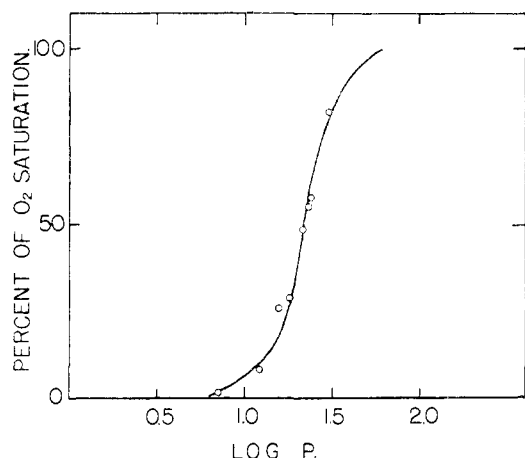

Fig. 3.—Degree of oxygenation of hemoglobin in function of O_2 partial pressure at pH 6.0 and at the ionic strength of 0.1 (acetate buffer); temperature 28°.

buffer) and at the ionic strength of 0.1. Comparison of the curve presented in Fig. 3 with that given in Fig. 1 (curve A; for pH 6.9 and ionic strength of 0.3) shows that the shape of the curve did not change at all at least in the pH range between 6.0 and 6.9.

Discussion

In the experiments reported in the present paper, there had always been intervals of several days between the extraction of hemoglobin from fresh horse blood and the performance of main experiment of the oxygenation measurement. The "symmetry" of the sigmoid flexure of the curve we have observed at higher ionic strength can, therefore, hardly be attributed to the freshness of the hemoglobin preparation. The regular sigmoid curve obtained at the ionic strength of 0.3 to 0.03 corresponds to the Hill equation with the n-value of about 3.0. This value seems to be irreconcilable with the well known formula of Pauling[6]

$$Y = \frac{KP + (2\alpha + 1)K^2P^2 + 3\alpha^2K^3P^3 + \alpha^4K^4P^6}{1 + KP + (4\alpha + 1)K^2P^2 + 4\alpha^2K^3P^3 + \alpha^4K^6P^6}$$

according to which the value of n cannot exceed 2.

Along the line of Pauling's idea, Altschule and Hogness had pointed out that the "interaction constant" (α) varies with the salt concentration in the hemoglobin solution. According to these investigations, α was 3 in a dialyzed solution and 12 in a buffered solution. If the equation $n = 2\sqrt{\alpha}/(1 + \sqrt{\alpha})$[7] is applicable, their data show that n was 1.3 in a dialyzed solution and 1.6 in a buffered solution. These values are roughly one-half of the value we have obtained for a solution of high ionic strength. Presumably, the hemoglobin molecule in the samples used in their experiment might have been split into two, since, as was pointed out by Wyman, the n-value should become smaller when splitting occurs in the hemoglobin molecule.

It is a pleasure to the author to thank Prof. Hiroshi Tamiya for his kind guidance in this research, and to Dr. R. Lumry of the University of Minnesota, for his many advices.

TOKYO, JAPAN

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