

# Oxygen Equilibrium of Hemoglobin J Cape Town

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**ABSTRACT** Polycythemia in carriers of hemoglobin J Cape Town or hemoglobin Chesapeake is thought to be produced by increased oxygen affinity of their blood. Both hemoglobins involve substitution of amino acid residue  $\alpha$  FG-4. Measurements reported here, of the oxygen equilibrium of purified hemoglobin J Cape Town, permit direct comparison of the two hemoglobins. J Cape Town exhibits lower oxygen affinity, and greater heme-heme interaction, than Chesapeake; both exhibit normal Bohr effects. Substitution of one polar amino acid residue for another of opposite charge (arginine  $\rightarrow$  glutamic acid) thus appears to create less disruption of the interface between  $\alpha$ - and  $\beta$ -chains than substitution of a nonpolar residue (arginine  $\rightarrow$  leucine).

portion of the blood was used for studies of ligand binding<sup>1</sup> and mobility in the ultracentrifuge.<sup>2</sup> Portions of the remainder were removed over the next 2 wk, maintaining sterility at all times. Red cells were washed three times, hemolysates were prepared with toluene and distilled water, and the hemoglobin components were separated by starch block electrophoresis at 4°C (5). After electrophoresis, the eluted hemoglobin solution was passed through a 4  $\times$  40 cm column of Sephadex G-25, which had been equilibrated with 0.1M phosphate buffer. Samples were adjusted to a concentration of 0.1 g/100 ml, and oxygen affinity was measured at 10°C, using a modification of the techniques of Allen, Guthe, and Wyman (6) and Riggs (7). Methemoglobin formation was monitored by measurement of optical density at 630 m $\mu$ ; the only samples (pH 6.8) which showed more than a very slight increase in OD were discarded.

## INTRODUCTION

Some, but not all carriers of hemoglobin J Cape Town (Hb J), ( $\alpha$ 92(FG-4) Glu) are polycythemic (1, 2). Oxygen affinity of a hemolysate containing 35% Hb J was increased, while the Bohr effect was normal, suggesting that polycythemia was produced by decreased delivery of oxygen to tissues (3). Heme-heme interaction in Hb J was significantly decreased, with an  $n$  value of 1.8. These findings were of great interest, for hemoglobin Chesapeake ( $\alpha$ 92 Leu), which also is produced by a mutation at FG-4, exhibits increased oxygen affinity and decreased heme-heme interaction ( $n = 1.3$ ) (4). We report here data on the oxygen equilibrium of purified Hb J, studied under the conditions used in previous studies of hemoglobin Chesapeake.

## METHOD

Blood was collected in Johannesburg in ACD (acid citrate dextrose) solution under sterile conditions. It was shipped in ice to Baltimore and arrived in excellent condition. A

## RESULTS

Oxygen affinity of Hb J was increased, and a normal Bohr effect was present (Fig. 1). Heme-heme interaction was decreased: the mean value of  $n$  from nine series of measurements, calculated by the method of least squares, was 2.22 (SD 0.20). Data for Hb J are compared with data for normal hemoglobin and Hb Chesapeake in Fig. 2: Hb Chesapeake has a lower p50 and a lower value of  $n$  than Hb J.

## DISCUSSION

Heme-heme interaction of purified Hb J was not decreased to the degree found by Lines and McIntosh in a hemolysate of whole blood (3). The difference between their results and ours may be due to graphical summation of the properties of two hemoglobins with different affinities for oxygen: the true dissociation curve must be sinusoidal, since Hb J plays a disproportionate role in oxygen binding at low oxygen tensions. Effects of the two hemoglobins might be resolved using a continuously recording oxygen electrode and linear coordinates (8); the small departure from linearity would not be noted when the logarithmic form of Hill's equation is utilized.

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<sup>1</sup> Nagel, R. L., Q. H. Gibson, and T. Jenkins. Ligand binding in hemoglobin J Capetown. Submitted for publication.

<sup>2</sup> Charache, S., R. Briehl, and T. Jenkins. Unpublished observations.

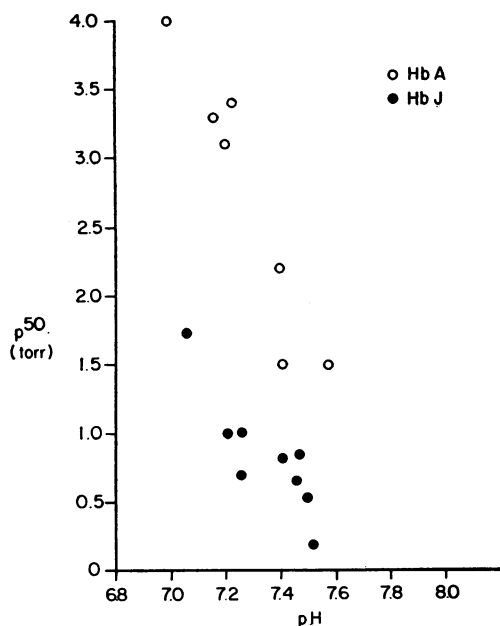


FIGURE 1 Bohr effect in hemoglobins A- and J-Cape Town. Each point represents the oxygen pressure required for half saturation of hemoglobin at 10°C in 0.1M PO<sub>4</sub> buffer at a given pH. Points were derived from lines plotted according to Hill's equation ( $\log S/1-S = n \log P_{O_2} - n \log p_{50}$ ), where S is per cent saturation, K is a constant, and  $n$  is related to the magnitude of heme-heme interaction. The oxygen affinity of Hb J is higher than that of Hb A, but the magnitude of the Bohr effect appears to be normal.

All abnormal hemoglobins with amino acid substitutions at the  $\alpha_1\beta_2$  interface between globin subunits have abnormal oxygen affinity, and Hb J is no exception

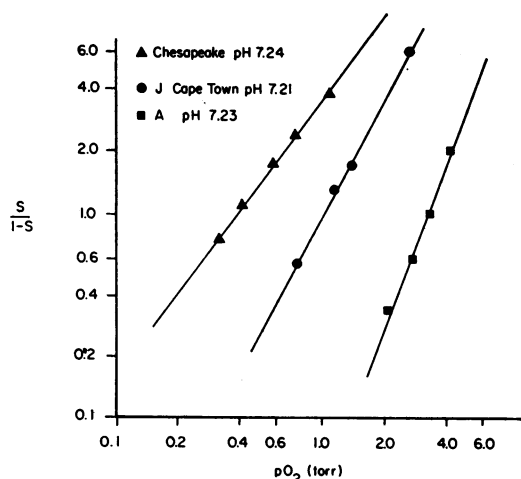


FIGURE 2 Oxygen affinity of hemoglobins Chesapeake, J-Cape Town, and A, plotted according to Hill's equation. Oxygen affinity of Hb J is less than that of Hb Chesapeake, and heme-heme interaction is greater (Chesapeake:  $n = 1.3$ ; J:  $n = 2.2$ ; A:  $n = 2.7$ ). Data for Hb Chesapeake are derived from Nagel, Gibson, and Charache (4).

[(9) and reference cited therein]. Analyses of X-ray diffraction data suggest that there are many differences between the structure of oxyhemoglobins A and Chesapeake (10). The oxygen equilibrium of Hb J is less abnormal than that of Hb Chesapeake, as are a number of physical properties: electron-spin resonance spectra (11), reactivity with ligands,<sup>1</sup> and sedimentation in the ultracentrifuge.<sup>2</sup> Substitution of a polar residue by another of opposite charge (arg → glu) thus appears to create less disruption of the  $\alpha_1\beta_2$  interface than does substitution by a nonpolar residue (arg → leu). Polycythemia produced by Hb J is less impressive than that produced by Hb Chesapeake, as might be expected from these findings (1, 2, 12).

#### ACKNOWLEDGMENTS

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