THE OXYGEN EQUILIBRIUM OF FETAL AND ADULT HUMAN HEMOGLOBIN

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Although the fetal and adult hemoglobins of many species, including man, are essentially similar in molecular weight and in spectroscopic characteristics, they exhibit marked differences in other chemical and physical properties (1-6). Therefore, they may be assumed to represent distinct chemical entities. The present study was undertaken to determine whether the chemical difference is expressed in variation of oxygen equilibria.

The oxygen equilibrium of hemoglobin is conveniently characterized in terms of Hill’s equation

\[ Y = \frac{K_p^n}{1 + K_p^n} \]

In this equation, \( Y \) is the fractional saturation of the hemoglobin with oxygen, \( K \) is a constant, and \( p \) is the oxygen pressure. The quantity \( n \) is a measure of the over-all interaction between the four hemes, and thus represents one fundamental characteristic of the oxygen equilibrium curve. Change in the value of \( n \) reflects increased or decreased interaction between the hemes, such as would be expressed by differences of the inflection and sigmoid character of the oxygen equilibrium curve.\(^1\) A second important characteristic of the curve is the oxygen affinity, conveniently defined by the reciprocal of the oxygen pressure at which the hemoglobin is half saturated (\( p_{0.5} \)).

Previous investigators have studied one or both of these aspects of fetal and adult human hemoglobins in whole blood (7-10) and as hemoglobin in solution (11-13). In the case of hemoglobin in whole blood or washed corpuscles, a greater affinity of the fetal than of the maternal specimens for oxygen has been indicated by most investigations, including a former one from this hospital (10). That study also indicated the value of \( n \) to be the same, within limits of error, for blood from the two sources. Changes in the serum salt concentrations were considered too small to account for

\(^1\) For a discussion of the significance of \( n \) as a measure of heme interactions see Wyman (6).
the shift in the fetal oxygen equilibrium curve. No significant difference was found between the oxygen affinity of blood from term and premature fetuses.

These results on whole blood stand in contrast to those obtained by other investigators working with hemoglobin from freshly laked cells (12, 13), from which it appears that the oxygen affinity of the fetal hemoglobin in solution is less than that of adult hemoglobin. Particular interest attaches to the work of McCarthy (11), who obtained oxygen equilibrium curves both from suspensions of corpuscles obtained from human maternal and fetal blood and from concentrated solutions of maternal and fetal hemoglobins. His solutions, dialyzed against \( \frac{m}{7} \) phosphate at pH 6.8, showed smaller differences between oxygen affinities of fetal and maternal hemoglobins than were found by previous workers with freshly laked cells, although the fetal hemoglobin solution still had a lower affinity than the maternal. The range of \( n \) found by McCarthy for maternal hemoglobin was 2.8 to 1.7 (average 2.3) and for fetal hemoglobin 2.8 to 1.5 (average 2.1). Similar low values of \( n \) (but within a narrower range) have been observed by Riggs after dialysis of human adult hemoglobin solutions (14). In view of the apparent inconsistency between the results on whole blood and hemoglobin solutions, oxygen equilibria of fetal and adult human hemoglobin from freshly laked cells have been reinvestigated.

**Methods**

Solutions of fetal hemoglobin were prepared from umbilical cord blood taken at birth. In most instances, as specified below, the maternal hemoglobin was from the simultaneously obtained blood of the mother. Samples from either source were centrifuged and the cells washed three or four times with 0.9 per cent NaCl solution, before being hemolyzed for 2 hours with an equal volume of distilled water and 0.4 volume of toluene. The hemolyzed mixture was centrifuged at high speed in a Servall angle centrifuge, the hemoglobin layer removed, and the process repeated until a clear solution was obtained. To this was added enough phosphate buffer to make the final phosphate concentration 0.4 m. The pH was measured with a glass electrode.

In Experiments 1 to 6 (Table I), the oxygen equilibrium was then determined. In Experiments 7 to 12 (Table II) maternal and fetal hemoglobin solutions prepared as above were treated to reduce differences in their dialyzable components before oxygen equilibria were measured. The two hemoglobin solutions from each of six deliveries were placed in Visking dialysis sacks and both dialyzed simultaneously against the same sample of 0.4 m phosphate buffer containing glucose at a concentration of 1.0 m. The glucose was added both to prevent dilution and to increase stability.
of the hemoglobin solution, as suggested by Pennell and Smith (15). Dialysis was continued for 8 to 16 hours at 4° with constant stirring of the outside solution. Because of the limited volume of that solution (50 cc. versus 5 cc. of each hemoglobin solution) this procedure was not expected to remove all dialyzeable components, but rather to render each pair of hemoglobin solutions alike in respect to such components.

The oxygen equilibrium measurements were carried out by a method previously described (16) involving a special tonometer into which known amounts of oxygen are injected after repeated evacuation to water vapor pressure and washing with nitrogen. Photometric analyses were made at a wave-length of approximately 710 mμ. All work was done at 20°.

Results

Table I summarizes the results obtained on fresh hemoglobin solutions, without dialysis. Fetal and maternal hemoglobins listed under the same experiment number in Column 1 represent blood samples taken simultaneously at one delivery, as in Experiments 1, 5, and 6. In Experiment 2, hemoglobin from a 31 week fetus was analyzed on two successive days; maternal blood was not obtained. Experiments 3 and 4 represent maternal blood only, taken at delivery. The concentrations of hemoglobin in the solutions, listed in Column 3, were determined from spectrophotometric differences in optical densities of the reduced and oxygenated sample. The values of n listed in Column 5 were obtained graphically from the slope of the curve of log Y/1 - Y versus log p between Y = 0.3 and Y = 0.7, where the curve is essentially straight. The oxygen affinity is expressed (Column 6) as the log of half saturation oxygen pressure. It should be noted that a change of log p1 of 0.01 corresponds to a change of 2 per cent in p1. Column 7 gives the value of log p1 reduced to an arbitrarily chosen reference pH of 7.20. In making this reduction it was assumed that, over the small pH range involved in these experiments, Δlog p1/ΔpH = -0.60, the figure graphically obtained from the present data and identical with the value obtained by Ferry and Green on horse hemoglobin for the same pH range (17).

Several observations may be made upon the data of Table I. The value of n is essentially the same for all hemoglobins, maternal and fetal. The oxygen affinity for all samples of maternal hemoglobin is the same, since the average value of log p1 reduced to pH 7.20 (Column 7) for these samples is 0.928 and only one figure differed from this by as much as 0.02, which corresponds to 4 per cent in p1 itself. The fetal hemoglobin affinities are equally uniform, since, including the values for the preterm fetus, the average value of log p1 (Column 7) is 0.988, with only one departure from this by as much as 0.018. On the other hand, the oxygen affinities of ma-
## Table I

### Oxygen Equilibrium of Undialyzed Hemoglobin

<table>
<thead>
<tr>
<th>Experiment No. and date of analysis</th>
<th>Source of hemoglobin</th>
<th>Concentration of hemoglobin in solution</th>
<th>pH</th>
<th>n</th>
<th>log p</th>
<th>Log p (pH 7.20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3) per cent</td>
<td>(4)</td>
<td>(5)</td>
<td>(6)</td>
<td>(7)</td>
</tr>
<tr>
<td>1</td>
<td>July 25, 1950</td>
<td>Term fetus</td>
<td>14.2</td>
<td>7.21</td>
<td>2.9</td>
<td>0.99</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; 26, 1950</td>
<td>Maternal</td>
<td>13.4</td>
<td>7.21</td>
<td>2.9</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>&quot; 30, 1950</td>
<td>31 wk. fetus</td>
<td>13.4</td>
<td>7.20</td>
<td>2.8</td>
<td>0.99</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; 31, 1950</td>
<td>31 &quot;</td>
<td>2.8</td>
<td>0.98</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Aug. 3, 1950</td>
<td>Maternal</td>
<td>14.0</td>
<td>7.20</td>
<td>3.0</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>8, 1950</td>
<td>&quot;</td>
<td>11.0</td>
<td>7.21</td>
<td>2.8</td>
<td>0.93</td>
</tr>
<tr>
<td>5</td>
<td>&quot; 29, 1950</td>
<td>Fetal</td>
<td>15.5</td>
<td>7.15</td>
<td>3.0</td>
<td>1.00</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; 30, 1950</td>
<td>Maternal</td>
<td>12.4</td>
<td>7.21</td>
<td>3.0</td>
<td>0.91</td>
</tr>
<tr>
<td>6</td>
<td>&quot; 7, 1951</td>
<td>Fetal</td>
<td>12.4</td>
<td>7.10</td>
<td>2.9</td>
<td>1.06</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; 8, 1951</td>
<td>Maternal</td>
<td>13.0</td>
<td>7.10</td>
<td>3.0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Fig. 1.** Oxygen equilibrium of maternal and fetal hemoglobin solutions from one mother and her infant at delivery. No dialysis. \( Y \) = fractional saturation of hemoglobin with oxygen; \( p \) = oxygen pressure.
ternal and fetal hemoglobins present a significant difference amounting to 0.06 in log $p_t$, or approximately 13 per cent in $p_t$. Fetal hemoglobin has the lesser oxygen affinity. This is illustrated by Fig. 1 which shows the original data for one pair of experiments made at pH 7.21. The direction of this difference between oxygen affinities of fetal and adult hemoglobin solutions is that observed by previous workers and is opposite to the findings on whole blood according to the work of Darling et al. (10) and of others (7–9).

The results obtained after the attempt to render fetal and adult hemoglobin solutions similar in dialyzable components are presented in Table II. The dialysis did not alter the value of $n$ for either the fetal or maternal hemoglobins. On the other hand, in sharp contrast to the results in Table I, no significant difference between oxygen affinities of maternal and fetal hemoglobins was found after dialysis. The mean value of log $p_t$ for maternal hemoglobin reduced to pH 7.20 (Column 7) is 1.013, only one point departing from this by as much as 0.017; that for fetal hemoglobin is also 1.013, the most discrepant point departing by 0.027. This identity of oxygen equilibria is illustrated in Fig. 2, which gives the data obtained in Experiments 8 and 10, in which all four hemoglobins were essentially at pH 7.20 so that no Bohr effect correction need be considered.

The foregoing experiments suggest that, although human fetal and adult hemoglobins presumably are chemically different, they are indistinguishable in regard to reaction with oxygen, provided that differences in dialyz-
able factors are removed. The dialyzable substance or substances responsible for the effect just discussed were not identified, but it has long been known that various ions (18) and other dialyzable substances (19) affect hemoglobin in such respects. A further possibility is suggested by the work of Riggs (14). He showed that increase in oxygen affinity demonstrable after dialysis of human hemoglobin solutions could be reversed by subsequent addition of glutathione, and thus may represent the loss of this dialyzable substance. No measurements of glutathione in maternal or fetal blood have been discovered in the literature.

**SUMMARY**

1. Fetal hemoglobin solutions were prepared by laking fresh red blood cells from one premature and three term human infants at birth. Adult hemoglobin solutions were similarly prepared from fresh red blood cells of six women at delivery. The oxygen affinities of the fetal hemoglobin solutions were consistently less than those of the maternal hemoglobin.
solutions by a difference of approximately 13 per cent in half saturation oxygen pressure ($p_{50}$). The calculated value of $n$, as an expression of inflection and sigmoid character of the oxygen equilibrium curve, was essentially similar for all ten hemoglobin samples.

2. The similarly prepared hemoglobin solutions from fetal and maternal blood obtained at delivery were dialyzed simultaneously against a common surrounding solution of restricted volume to equalize the concentrations of their diffusible components. Measurements made after this dialysis indicated approximately uniform oxygen affinities of the fetal and maternal hemoglobins from six term deliveries. The $n$ values remained unchanged and thus also uniform.

3. These findings suggest that reported differences in oxygen equilibria of human fetal and maternal hemoglobins may result from differences in the environment of the hemoglobin molecule rather than from intrinsic differences in the molecule itself.

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