Effect of Increased Maternal Hemoglobin Oxygen Affinity on Fetal Growth in the Rat

By Robert P. Hebbel, Elaine M. Berger, and John W. Eaton

A disparity between fetal and maternal hemoglobin oxygen affinities (i.e., $P_{50}^{\text{fetal}} < P_{50}^{\text{maternal}}$) is believed to be important in facilitating oxygen transfer from maternal to fetal circulation. However, human females with high oxygen affinity mutant hemoglobins ($P_{50}^{\text{maternal}} < P_{50}^{\text{fetal}}$) have successfully borne children, calling into question the importance of the usual maternal/fetal $P_{50}$ gradient. We have investigated the effect of artificially increased maternal hemoglobin oxygen affinity on fetal growth in the rat. Pregnant rats were exchanged transfused with homologous blood on the ninth day of gestation, the experimental group receiving donor blood having $P_{50}$ = 15.0 mm Hg achieved by prior incubation with sodium cyanate. This changed mean maternal $P_{50}$ from 41.1 to 24.6 mm Hg; the normal prenatal rat has $P_{50}$ = 24.7 mm Hg due to low erythrocyte 2,3-DPG content. Parameters reflecting the adequacy of fetal oxygenation were examined on the 20th and 21st days of gestation and at term (22 days). Fetuses from the experimental group were significantly smaller on the 21st day ($p < 0.001$) and at term ($p < 0.01$), and this was accompanied by placental hypertrophy. There was no significant difference in fetal weight on the 20th day of gestation unless a second exchange transfusion was performed to further lower maternal $P_{50}$. There was a trend towards erythrocytosis in the experimental group fetuses. We conclude that a narrowing of the $P_{50}$ difference between mother and fetus has adverse effects on fetal wellbeing in the rat.

The Human Fetus achieves normal growth and development in the relatively oxygen-poor uterine environment. Among the mechanisms that ensure adequate fetal oxygenation under this potentially adverse circumstance is the comparatively higher affinity of fetal hemoglobin (Hb) for oxygen (i.e., $P_{50}^{\text{fetal}} < P_{50}^{\text{maternal}}$), which facilitates the transfer of oxygen from maternal to fetal circulation. This is consistent with our previously stated argument that the high affinity of fetal Hb for oxygen is but one example of a general phenomenon: The most efficacious mechanism for dealing with hypoxic hypoxia is an increase in Hb oxygen affinity. Indeed, a higher fetal than maternal Hb oxygen affinity is seen almost universally among mammals. Hence, it is not surprising that there are anecdotal reports suggesting that low maternal $P_{50}$ does adversely affect human fetal survival. Both the ubiquity of high fetal Hb oxygen affinity and the suggested inimical influence of increased maternal Hb oxygen affinity imply that this relationship is not only beneficial, but also essential for the survival and normal development of the fetus.

Conversely, it has been noted that normal human gestation and parturition can still occur even when $P_{50}^{\text{maternal}}$ is less than $P_{50}^{\text{fetal}}$, as with maternal mutant hemoglobins having extraordinarily low $P_{50}$. Such observations suggest that while higher fetal Hb oxygen affinity may be helpful, it is not essential for fetal survival and development. Overall, the existing data from families with high oxygen affinity hemoglobins are insufficient to support any conclusions for humans.

There is a dearth of experimental data addressing this problem. Hence, we have examined the effect of artificially increased maternal Hb oxygen affinity on fetal growth in the rat. The results of our investigations in this mammalian model suggest that perturbation of maternal/fetal oxygen transport by lowering maternal $P_{50}$ does adversely affect fetal well being but is not incompatible with viviparity.

Materials and Methods

Experimental Design

Pregnant Sprague-Dawley rats (age > 80 days) were purchased with date of impregnation known. They were fed standard rat chow (Ralston Purina Company, St. Louis, Mo.) and allowed tap water ad libitum. On the ninth day of gestation, rats were exchanged transfused with either unaltered (control group) or carbamylated (experimental group) homologous rat blood. Fetuses were delivered by caesarean section on the 20th or 21st day of gestation, or spontaneous birth was allowed on the 22nd day of gestation. All surgical procedures utilized ether anesthesia.

This study was designed to control as much as possible the influence of variables other than those inherent in a shift of maternal $P_{50}$. The study of control and experimental animals was done concurrently, and animals were strictly randomized to the two groups. They did not differ significantly in terms of (1) maternal weight at the time of exchange transfusion or sacrifice, (2) weight gain during study, or (3) duration of pregnancy. Because the latter variable is particularly important due to the rapid fetal growth during the later stages of pregnancy, control animals were always sacrificed immediately before, but within 1 hr of, experimental animals; this would tend to minimize any differences in fetal weight between the two groups.

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Preparation of Donor Blood

Donor blood anticoagulated with ACD (Fenwal Laboratories, Deerfield, Illinois) was obtained from adult rats and pooled, after which erythrocytes were separated by centrifugation and suspended in 50 mM sodium cyanate made isotonic with NaCl and neutralized with 1N HCl. After a 60-min incubation at room temperature, the carbamylated erythrocytes were washed three times with isotonic NaCl, filtered through a CFI11 cellulose (Whatman Ltd., England) column, and resuspended to hematocrit (Hct) 40% in their original (pooled, ACD-anticoagulated) plasma. Donor blood treated in this manner had $P_{50} = 15.0 \text{ mm Hg}$ (at pH 7.40, 37°C, and 5.6% CO2). Control animals received blood handled identically except for the absence of sodium cyanate during incubation.

Exchange Transfusion

Recipient rats underwent cardiocentesis with a 23-gauge needle connected to a 3-way stopcock. Two milliliters of recipient blood were removed and replaced with 2 ml of donor blood, which had been warmed to 37°C. This cycle was repeated until one total volume (assumed to be 6% of body weight) had been infused, the total elapsed time being 4-6 min. This procedure was accompanied by a mortality rate of 30%, apparently due to stroke or pericardial tamponade. However, mortality was acute (always within 15 min of transfusion), and there was no apparent morbidity in survivors. There was no difference in the measured fetal parameters between our control group and unmanipulated animals.

Fetal Blood Collection

Fetuses with placenta attached were rinsed with isotonic NaCl and subjected to thoracotomy. The blood so obtained was drawn into capillary tubes previously flushed with heparin (10,000 U/ml). The umbilical cord was then severed at its midpoint, and the placenta and fetal carcass were weighed. Litters were examined for malformations, and uteri were examined for evidence of resorptions. Litters allowed to complete gestation were sacrificed within 2 hr of birth and are considered to be term fetuses.

On the first 4 fetuses removed from each litter, individual whole blood $P_{50}$ were determined by means of a Hem-O-Scan oxygen dissociation curve analyzer (Aminco, Division of Travon Laboratories, Silver Springs, Md). The resulting raw $P_{50}$ (determined at 37°C and 5.6% CO2) were corrected to pH 7.40 by simultaneously equilibrating the remainder of these blood samples against an identical gas mixture in a tonometer and determining their pH (Blood Gas Analyzer 213; Instrumentation Laboratory, Inc., Lexington, Mass.) at the same time as the 50% saturation point was reached on the Hem-O-Scan.

On all fetuses after the fourth, microhematocrit (Hct) and hemoglobin concentration (measured spectrophotometrically as cyanomerhemoglobin) were individually measured. Erythrocyte 2,3-diphosphoglyceric acid (2,3-DPG) and adenosine triphosphate (ATP) were determined on pooled fetal blood (2 pools/litter) by enzymatic methods. Reticulocyte counts were done on one pool/litter.

Maternal Parameters

Maternal $P_{50}$ was followed longitudinally by analyzing caudal blood as above. Periodically, maternal $P_{50}$ was determined simultaneously both by Hem-O-Scan and by standard mixing techniques to check the accuracy of the Hem-O-Scan determination. All such comparisons revealed the pH-corrected $P_{50}$ to be within 1.0 mm Hg of each other. Maternal Hb, Hct, and reticulocyte count were determined on blood obtained by cardiocentesis at the time of exchange transfusion and at sacrifice.

Statistical Methods

These studies employed 40 litters (22 control and 18 experimental) comprising a total of 450 fetuses. Litters were approximately evenly divided into those examined at 20, 21, and 22 (term) days. Because any differences between treatment groups would reflect variability between individual fetuses and between different litters in addition to that due to variability between treatment groups, data were examined by components of variance statistical analysis. Within each treatment group, the variance ratio ($F$) was used to examine fetal variance ($V_f$) versus litter variance ($V_l$). However, the addition of a third level of variance ($V_e$) that from treatment groups precluded the use of $F$. Hence, overall data analysis involved the determination of total variance ($V_t$) and the proportionate contributions to it by $V_f$, $V_l$, and $V_e$. Given the results of these two types of analyses (vide infra), data were also examined for statistical significance by Student's unpaired, two-tailed $t$ test.

RESULTS

Maternal Response to Exchange Transfusion

The effect of a single exchange transfusion on maternal hematologic variables is depicted in Table 1. The pregnant experimental group (middle row) manifested a significant shift in $P_{50}$ so that 24 hr after exchange transfusion $P_{50}^\text{experimental} = P_{50}^\text{control} + 1.2$, indicating an efficiency of exchange transfusion of about 60%. Subsequently, maternal $P_{50}$ increased somewhat in the experimental group, but at sacrifice (11 or 13 days after exchange transfusion), mean maternal $P_{50}$ was still 7 mm Hg less than that of control group animals. It should be noted that this tendency towards normalization of $P_{50}$ was not due to loss of cyanate from maternal erythrocytes, since this adduct binds irreversibly.

For comparison, hematologic data from the pregnant control group and from a group of nonpregnant experimental animals are also shown in Table 1. A number of differences are apparent. The hematologic variables from the pregnant control animals (top row) reveal those changes that are inherent in pregnancy: hemodilution (decrement in Hb and Hct) and expansion of RBC mass (mild reticulocytosis). There were no differences between these pregnant control animals and unmanipulated pregnant animals (data not shown). On the other hand, the nonpregnant experimental animals (bottom row) manifested only the effect of shifted $P_{50}$; expansion of RBC mass (brisk reticulocytosis) without the effects of hemodilution (hence, an increment in Hb and Hct). In contrast, the pregnant experimental group (middle row) shows evidence of influence both by pregnancy and shift of $P_{50}$. Hemodilution is apparent in that Hb and Hct progressively decrease. Expansion of RBC mass is evidenced by a higher reticulocyte count as compared to pregnant control animals. The data further suggest that this response to $P_{50}$ diminishes the apparent effect of hemodilution during pregnancy.
day 21 consisted of 21 litters with a total of 244 fetuses; experimental group consisted of 17 litters with 182 fetuses. They were approximately evenly divided for examination on day 20, day 21, and term. Statistical analysis of these data is presented in Table 3.

Effect on Fetal Growth

There was no significant effect of increased maternal Hb oxygen affinity on litter size (11.6 fetuses/litter for control and 10.7 fetuses/litter for experimental animals) nor in the apparent number of resorptions. Only one grossly malformed, nonviable fetus was found, and this was in an unmanipulated litter. The observed effect of lowered maternal P_{50} (by single exchange transfusion) on the fetus is depicted in Table 2. There was an approximately 10% difference between control and experimental groups in mean fetal weight on the 21st day of gestation, fetuses from the experimental group being smaller. This decrement in fetal weight was accompanied by a concomitant placental hypertrophy, as evidenced by an increment in mean placental weight and a decrement in relative fetal weight (as defined in Table 2). The mean weight of the fetoplacental unit was, nevertheless, lower for experimental litters.

In contrast, no effect of single exchange transfusion was apparent on fetuses examined on the 20th day of gestation (Table 2). However, an attempt was made to further perturb maternal oxygen transport by repeating the exchange transfusion process 24 hr after the initial exchange. Unfortunately, the (immediate) mortality of the repeated process was such that only one doubly-exchanged litter survived in each group to take examination on day 20. Sacrifice was on the 20th to 22nd day of gestation (11–13 days after exchange transfusion for nonpregnant animals). That the reticulocytosis of pregnant animals is less striking than that of nonpregnant animals probably reflects the inhibitory influence of estrogens during pregnancy.\textsuperscript{12,13}

Reflecting the presence of greater erythropoietic stimulus in shifted animals is the observation of a 50% greater maternal erythropoietin \textsuperscript{59}Fe uptake (measured on day 20; data not shown) in the pregnant experimental animals as compared to pregnant controls (2 animals in each group were given \textsuperscript{59}Fe by cardiocentesis 30 hr before sacrifice). That the reticulocytosis of pregnant animals is less striking than that of nonpregnant animals probably reflects the inhibitory influence of estrogens during pregnancy.\textsuperscript{12,13}

\begin{table}[h]
\centering
\caption{Effect of Exchange Transfusion on Parameters of Maternal Oxygen Transport*}
\begin{tabular}{llllllll}
& \multicolumn{2}{c}{Before Exchange} & \multicolumn{2}{c}{24 hr After Exchange} & \multicolumn{2}{c}{48–72 hr After Exchange} & \multicolumn{2}{c}{At Sacrifice} \\
& \multicolumn{1}{c}{P_{50} (mmHg)} & Hb (g/dl) & Hct (%) & Reticulocyte Count (%) \\
\hline
Pregnant control group & 40.5 & 24.6 & 25.4^* & 13.6 & 13.16^* & 39.0 & 36.6 & 2.7 & 4.7 \\
\textit{(n = 21)} & ±2.1 & ±2.4 & ±1.8 & ±1.7 & ±0.90 & ±1.00 & ±3.9 & ±3.5 & ±1.0 & ±1.0 \\

Pregnant experimental group & 41.1 & 26.4^† & 25.4^† & 33.1^† & 13.60^§ & 13.16^§ & 39.0§ & 36.6§ & 2.7§ & 4.7^§ \\
\textit{(n = 17)} & ±2.7 & ±2.3 & ±2.5 & ±1.7 & ±0.61 & ±0.65 & ±3.6 & ±1.3 & ±0.9 & ±1.8 \\

Nonpregnant experimental group & 39.6 & 24.8 & — & 35.7 & 14.67 & 16.87 & 42.3 & 46.7 & <1.5 & 13.3 \\
\textit{(n = 10)} & ±1.5 & ±2.7 & ±2.7 & ±0.99 & ±1.08 & ±2.8 & ±2.6 & ±3.4 & & \\

\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Effect of Increased Maternal Hemoglobin Oxygen Affinity on Fetal Growth*}
\begin{tabular}{lccc}
& \multicolumn{2}{c}{Control Group} & \multicolumn{2}{c}{Experimental Group} \\
& \textbf{Units} & \textbf{Mean ± SD} & \textbf{Range} & \textbf{Mean ± SD} & \textbf{Range} \\
\hline
\textbf{Day 20 of gestation} & & & & & & & & & & \\
Fetal weight & g & 2.141 ± 0.164 & 1.669–2.489 & 2.126 ± 0.163 & 1.748–2.466 & & & & & \\

\textbf{Day 21 of gestation} & & & & & & & & & & \\
Fetal weight & g & 3.985 ± 0.168 & 3.504–4.253 & 3.589 ± 0.160 & 3.349–3.862 & & & & & \\
Placental weight & g & 0.704 ± 0.108 & 0.503–1.010 & 0.850 ± 0.141 & 0.666–1.209 & & & & & \\
Weight of fetus plus placenta & g & 4.681 ± 0.223 & 4.106–5.231 & 4.433 ± 0.227 & 4.067–4.796 & & & & & \\
Relative fetal weight† & % & 85.0 ± 2.8 & 80.7–88.8 & 81.0 ± 2.5 & 73.5–83.6 & & & & & \\
At term & & & & & & & & & & \\
Fetal weight & g & 6.649 ± 0.359 & 6.085–7.329 & 6.223 ± 0.864 & 4.950–6.872 & & & & & \\
\hline
\end{tabular}
\end{table}

*Values are mean ± SD. Preexchange values were obtained immediately prior to exchange transfusion, which took place on the ninth day of gestation. Sacrifice was on the 20th to 22nd day of gestation (11–13 days after exchange transfusion for nonpregnant animals).

†Whole blood P_{50} at pH 7.40, 37°C, and 5.8% CO_{2}. For comparison, normal fetal P_{50} on days 20–21 is 24.7 ± 2.1 mm Hg.

§Differs significantly from pregnant control group (p at least <0.05 by Student’s t-test).

§§Differs significantly from nonpregnant experimental group (p at least <0.05).
be examined on the 20th day. Nevertheless, comparison of these two litters (each with 12 fetuses) revealed that the further shift of maternal P<sub>0</sub> (18.4 mm Hg 24 hr after the second exchange and 29.2 mm Hg at sacrifice) did result in a significant decrement (about 10%) in fetal weight and weight of fetoplacental unit on day 20 (p < 0.01 by t test).

**Statistical Analysis of Effect on Fetal Growth**

Analysis of the results in Table 2 for each study day by components of variance analysis revealed that within each treatment group, the variance ratio (F = V<sub>T</sub>/V<sub>F</sub>) was not significantly different from 1.0 (data not shown). When differences between treatment groups (V<sub>T</sub>) were examined (Table 3), it was evident that they made a negligible contribution to total variance (V<sub>T</sub>) on day 20. However, on days 21 and 22, a marked shift toward a significant contribution to V<sub>T</sub> by V<sub>F</sub> is apparent. These differences between treatment groups were significant on day 21 (p < 0.001) and at term (p < 0.01) (Table 3). On day 20, the groups differed significantly only in the case of the doubly exchanged animals.

**Possible Cyanate Effect**

Even though cyanate binds irreversibly to hemoglobin, the experimental method might still result in the administration of some trace amount of unreacted cyanate to pregnant animals. To evaluate this possibility, aliquots were taken from carbamylated blood prepared for transfusion and free cyanate was measured after conversion to carbamylcysteine. Such analysis revealed that no pregnant experimental group animal received more than 1 μM of unreacted cyanate, an amount we believe to be negligible. Nevertheless, evidence for physiologic effect on fetuses of such trace cyanate was searched for in two ways. First, carbamylated rat hemoglobin revealed alterations of the mobility of some Hb bands on isoelectric focusing; samples obtained from experimental group fetuses showed no cyanate effect on isoelectric focusing (the rat fetus has the same six hemoglobins as the adult). Second, no alteration of experimental group hemoglobin was discernible upon measurement of fetal P<sub>0</sub> (Table 4). Hence, we do not believe our results have been influenced by passage of unreacted cyanate to the fetus.

**Fetal Hematologic Parameters**

There was no significant difference between control and experimental groups in the measured fetal hematologic parameters (Table 4). However, in the experi-

### Table 3. Statistical Analysis of Fetal Growth Parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Day 20 of gestation</th>
<th>Day 21 of gestation</th>
<th>At term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
</tr>
<tr>
<td>Placental weight</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
</tr>
<tr>
<td>Weight of fetus plus placenta</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
</tr>
<tr>
<td>Relative fetal weight</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
<td>V&lt;sub&gt;F&lt;/sub&gt;</td>
<td>V&lt;sub&gt;T&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

*Proportionate contribution to total variance (V<sub>T</sub>) by variance between fetuses (V<sub>F</sub>) and between litters (V<sub>T</sub>) is represented by (V<sub>F</sub> + V<sub>T</sub>)/V<sub>T</sub>. Proportionate variance contributed by differences between treatment groups is indicated by V<sub>F</sub>/V<sub>T</sub>. For all rows in the table, variance ratio (F = V<sub>F</sub>/V<sub>T</sub>) did not differ significantly from 1.0 within each treatment group.

†Significance analysis by Student’s unpaired, two-tailed t test: NS, not significant.

### Table 4. Hematologic Parameters of Fetuses Exposed to Normal (Control Group) or Increased (Experimental Group)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Gestational Age (Days)</th>
<th>Hemoglobin (g/dL)</th>
<th>Hct (%)</th>
<th>2,3-DPG (μM/g Hb)</th>
<th>ATP (μM/g Hb)</th>
<th>P&lt;sub&gt;O&lt;/sub&gt; (mmHg)</th>
<th>Reticulocyte Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single exchange transfusion</td>
<td>Control</td>
<td>20 and 21</td>
<td>10.86 ± 1.34</td>
<td>43.1 ± 4.7</td>
<td>3.44 ± 0.53</td>
<td>12.77 ± 1.60</td>
<td>24.7 ± 2.1</td>
<td>95.8 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>20 and 21</td>
<td>11.31 ± 1.22</td>
<td>42.7 ± 4.2</td>
<td>3.21 ± 0.38</td>
<td>12.73 ± 0.34</td>
<td>25.7 ± 2.0</td>
<td>97.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>n - 244†</td>
<td>20 and 21</td>
<td>11.31 ± 1.22</td>
<td>42.7 ± 4.2</td>
<td>3.21 ± 0.38</td>
<td>12.73 ± 0.34</td>
<td>25.7 ± 2.0</td>
<td>97.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>n - 182</td>
<td>20 and 21</td>
<td>11.31 ± 1.22</td>
<td>42.7 ± 4.2</td>
<td>3.21 ± 0.38</td>
<td>12.73 ± 0.34</td>
<td>25.7 ± 2.0</td>
<td>97.3 ± 1.6</td>
</tr>
<tr>
<td>Double exchange transfusion</td>
<td>Control</td>
<td>20</td>
<td>9.82 ± 2.19</td>
<td>43.1 ± 3.5</td>
<td>3.96 ± 0.40</td>
<td>14.44 ± 0.07</td>
<td>21.9 ± 1.3</td>
<td>97.8</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>20</td>
<td>11.11 ± 0.39</td>
<td>49.1 ± 2.6</td>
<td>3.91 ± 0.02</td>
<td>13.43 ± 0.49</td>
<td>22.8 ± 1.5</td>
<td>98.0</td>
</tr>
<tr>
<td></td>
<td>n - 12</td>
<td>20</td>
<td>11.11 ± 0.39</td>
<td>49.1 ± 2.6</td>
<td>3.91 ± 0.02</td>
<td>13.43 ± 0.49</td>
<td>22.8 ± 1.5</td>
<td>98.0</td>
</tr>
</tbody>
</table>

*Values given as mean ± SD. The first maternal exchange transfusion took place on the ninth day of gestation. For doubly exchanged animals, a second exchange was done 24 hr after the first.

†n, Total number of fetuses, divided for these various measurements as described in Materials and Methods.
mental animals, a trend towards erythrocytosis was discernible.

Also evident in the fetal rats was a reversal of the normal adult 2,3-DPG:ATP ratio (DPG \( \gg \) ATP). These very low erythrocyte 2,3-DPG concentrations explain the high oxygen affinity of fetal Hb in situ.

**DISCUSSION**

**Underlying Assumptions**

Implicit in our interpretation of these data is the assumption that the effects we have observed are, in fact, due to a perturbation of maternal oxygen transport. Although we did not attempt to directly measure specific parameters of fetal oxygen acquisition, several lines of indirect evidence support this assumption.

The data indicate that the variety of compensatory mechanisms available to the P_{50}-shifted animals (reviewed by Longo et al.\(^1\)) were insufficient to obviate the effects of markedly shifted P_{50}. This contention is supported by the data in Table 2, as described above. Moreover, the increased erythroid iron uptake by shifted pregnant animals at sacrifice demonstrates continued hypoxic stimulation of erythropoietin-producing tissue and, hence, failure to achieve full compensation.

A second issue is whether or not the perturbed maternal oxygen transport was manifest as a deficit in oxygen delivery at the level of the placenta. The magnitude of the maternal P_{50} shift was such that it would be mathematically predicted to result in a large decrement in placental oxygen transfer rate and in umbilical blood oxygen tension.\(^1\) Interestingly, such predictions indicate that the anticipated effect on fetal oxygen acquisition would be approximately the same for a 30% decrement in maternal Hb concentration as for the shift in P_{50} that we induced. Thus, in support of our interpretation, we have found in related studies following this same experimental design (Hebbel et al., in preparation) that a 30% decrement in maternal Hb on day 9 of gestation results in a 10% decrement in day-21 fetal weight. Furthermore, as is the case in the present studies, this maternal anemia also failed to produce a significant effect on day-20 fetal weight. Thus, the same results are obtained using two quite different models of hypoxic perturbation of maternal oxygen delivery.

**Interpretation of Results**

These studies demonstrate that an increase in maternal Hb oxygen affinity has a small but significant adverse effect on the weight of 21-day and term rat fetuses. That this reflects a physiologically signifi-
blood oxygen tension by perhaps as much as 50%. Consequently, the relatively small effect observed here is a testimonial to the efficacy of various homeostatic mechanisms (including high fetal Hb oxygen affinity) in protecting the organism against the adverse effects of hypoxic hypoxia. This is quite similar to the apparent protection against altitude-induced hypoxic hypoxia conferred by high oxygen affinity hemoglobins in humans.

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