Regulation of Erythropoiesis

XXII. Erythropoietin Production in the Newborn Animal

By Alberto O. Carmena, Donald Howard and Frederick Stohlman, Jr.

Erythropoiesis in the newborn rat is characterized by the production of hypochromic macrocytes. This is in contrast to adult animals, in which microcytosis usually precedes hypochromia. During the week prior to birth, erythropoiesis is seen primarily in the liver, but shortly thereafter there is an explosive increase in myeloid red cell production so that by the fifth day of life approximately 60 per cent of the bone marrow nucleated cells are erythroid. During the early neonatal period, there is present in the bone marrow a primitive cell which frequently occurs in syncytia. A somewhat similar cell is seen in the liver during the hepatic phase of erythropoiesis; the hepatic syncytial cell presumably has the capacity to differentiate either into hematopoietic or hepatic cells. Whether this cell migrates from the liver to the bone marrow, or a similar cell develops in situ, has not as yet been established. As the animal matures, the number of these syncytial cells in the marrow diminish in frequency until in the adult animal, only an occasional cell of this type is seen and then it does not occur in syncytia. Coincident with the decrease in frequency of the syncytial cell is a decrease in the size of those red cells produced by the bone marrow, so that by the twenty-fifth to thirtieth day of life the indices are normocytic normochromic.

The occurrence of hypochromic macrocytes together with the presence of significant numbers of the syncytial cell led us to examine the possibility that regulation of erythropoiesis in the newborn animal differs from that of the adult. Starvation, which leads to a complete shutdown of erythropoiesis in the adult, does not importantly affect erythroid cellularity of the marrow or the peripheral reticulocyte count; there is also an unusual metaphase block seen in some litters starved prior to the tenth to twelfth day of life. Bilateral nephrectomy, which completely abolishes hematopoiesis in the adult, had relatively little effect on the erythroid compartment of the bone marrow in the newborn animal, indicating that red cell production in the newborn animal was independent of renally produced erythropoietin. Accordingly, it seemed...
Table 1.—Effect of Age on Erythropoietin Production in the Rat

<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>No. of Animals</th>
<th>Units of EP/ml.</th>
<th>Ambient</th>
<th>23,000 ft.</th>
<th>Ambient</th>
<th>23,000 ft.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0.76±0.07</td>
<td>2.5 ±0.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>25</td>
<td>0</td>
<td>4.6 ±0.8</td>
<td>4.1 ±0.8</td>
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</tr>
<tr>
<td>15</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>4.6 ±0.8</td>
<td>4.1 ±0.8</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>16</td>
<td>0</td>
<td>8.4±1.3</td>
<td>8.3 ±1.8</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>—</td>
<td>12</td>
<td>—</td>
<td>8.3 ±1.8</td>
<td>8.6 ±1.2</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>—</td>
<td>9</td>
<td>—</td>
<td>8.3 ±1.8</td>
<td>8.6 ±1.2</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>23</td>
<td>45</td>
<td>0</td>
<td>8.6 ±1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*No. of animals refers to the number of assay animals. Assays were carried out in hypertransfused mice.

It is important to measure the capacity of newborn animals to produce erythropoietin.

**Materials and Methods**

Sprague-Dawley rats were mated after 3 to 4 months of age. Fetuses were delivered spontaneously. Newborn animals of varying ages were exposed to a simulated altitude of 0.4 atmospheres in a decompression chamber for 18 hr. Litter-mate controls were withdrawn from the mother for a similar time period. Bilateral nephrectomies were done as previously described. Those nephrectomized animals to be exposed to hypoxia were placed in the chamber within 2 hr. of the operation. Blood was collected in heparin by cardiac puncture. Hematocrits were similar to those previously published so that significant dilution with heparin could be ruled out. The plasma was pooled and assayed in hypertransfused mice of the CF1 strain. Assay animals with hematocrits of less than 55 were discarded. Both iron incorporation and reticulocyte counts were used to estimate red cell production in the assay animals. A curve of erythropoietic activity was constructed using a house standard which had previously been standardized against both Standards A and B, and the erythropoietin concentration was expressed in units per milliliter plasma.

**Results**

After exposure to simulated altitudes of 23,000 ft. (0.4 atmospheres) for 18 hr., erythropoietic activity could be demonstrated in the plasma of 1 day old rats. The concentration of erythropoietin, 0.76 units/ml. of plasma, was substantially less than the level of activity of 8.6 units/ml. observed in adult rats after a similar exposure (Table 1). As the rats matured, there was a gradual increase in the amount of erythropoietic activity which could be detected in the plasma after exposure to hypoxia (Table 1). On the fifteenth day of life, 4.6 units/ml. were observed, and by the thirtieth day of life, we observed 8.6 units/ml. of plasma, approximating the levels of erythropoietin seen in adult animals exposed to this level of hypoxia.

In unexposed litter-mate controls, erythropoietin could not be detected. These control rats had been withdrawn from the mother for a period of 18 hr. prior to collecting plasma for the assay. It was considered that the absence of erythropoietin in control animals might have reflected a secondary effect of starvation on erythropoietin production. Accordingly, plasma from newborn

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Table 2.—Effect of Age on Erythropoietin Production in Nephrectomized Animals

<table>
<thead>
<tr>
<th>Age (Days)</th>
<th>No. of Animals†</th>
<th>Units of EP/ml. Ambient 23,000 ft.</th>
<th>Units of EP/ml. Ambient 23,000 ft.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>14</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>6</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Adult</td>
<td>20</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>

†No. of animals refers to the number of assay animals. Assays were carried out in hypertransfused mice.

animals which had not been subjected to this period of starvation were assayed for erythropoietin, but erythropoietic activity could not be demonstrated in their plasma.

As has been previously reported,6 nephrectomy in the adult rat appeared to abolish the production of erythropoietin in response to hypoxia. In 4 separate experiments, erythropoietin could not be detected in the plasma of nephrectomized adult animals after an 18 hr. exposure at 0.4 atmospheres (Table 2). In contrast, bilateral nephrectomy of the 5 day old rat reduced the amount of erythropoietin present in the plasma after exposure to hypoxia, but did not totally abolish production of the hormone (Fig. 1, Table 2). In three experiments in which a comparison was made between nephrectomized and intact animals after exposure to hypoxia, the values for nephrectomized animals were 1.2 units of erythropoietin per milliliter of plasma as compared to 2.5 units/ml. of plasma in the intact animal. Nephrectomy in the 15 day old animal reduced the erythropoietin levels from 4.6 units/ml. of plasma to 0.96 units/ml. of plasma. By the twenty-fifth day of life, nephrectomy abolished the production of erythropoietin in response to hypoxia.

DISCUSSION

The production of erythropoietin was examined during the neonatal phase of life of the rat and compared with that observed in the adult animals. Erythropoietin could not be demonstrated in the plasma of rats at ambient pressure during the neonatal period of life nor in the adult animals with the technics used. It is to be noted that the newborn rat is anemic with hematocrits of ∼ 30 per cent on the fifth day of life; the hematocrit remains at this level until the fifteenth to twentieth day of life after which it begins to rise and reaches values of 40 per cent by the thirtieth day of life.1 The erythroid activity is strikingly increased as judged by reticulocyte values of 89 per cent at 1 day, 45 per cent at 5 days, and 20 to 30 per cent between the tenth and twentieth days of life.1 Even in the face of the significant anemia present in these animals, erythropoietin could not be demonstrated in the plasma. In part, this may have been the result of increased utilization by an active bone marrow as has been described in adult animals.7 In our experience, however, in adult rats in the presence of hemolytic anemia and hematocrit values of this level, it has been possible to demonstrate erythropoietin in the plasma. These observations, then would suggest that bone marrow utilization of erythropoietin
Fig. 1.—Plasma erythropoietin concentration ± SE in animals of various ages after exposure to simulated altitude of 23,000 ft. Animals were nephrectomized 2 hr. prior to exposure. Erythropoietin could not be demonstrated in the plasma of nephrectomized adult animals (Table 2).

cannot entirely account for the failure to detect erythropoietin in the newborn animal at 5 days of life.

After exposure to hypoxia, erythropoietin was demonstrable in the plasma of neonatal animals. Erythropoietin in the plasma of these animals increased from levels of 0.76 units/ml of plasma in the 1 day old animals exposed to hypoxia to values on the thirtieth day of life of 8.4 units/ml. The latter was comparable to those values seen in adults exposed to a similar level of altitude for 18 hr. This age-dependent increase in the amount of erythropoietin produced in response to hypoxia is consistent with a gradual transition from a neonatal to an adult-type of regulation of erythropoiesis. The failure of bilateral nephrectomy to completely abolish the production of the erythropoietic-stimulating factor during the first 15 days of life indicates that erythropoietin production is in significant measure independent of the kidney. This may in part provide an explanation for the failure of bilateral nephrectomy to result in the suppression of erythropoiesis in the newborn rat. We are unable to draw any conclusions from the present experiments as to the extrarenal site of production of erythropoietic activity. In the adult, minor degrees of erythropoietin production have been reported in hypoxic nephrectomized animals, but we were unable to demonstrate erythropoietin after the relative-
ly acute exposures used in nephrectomized animals in these experiments. Rosse and Waldmann\(^9\) reported some increase in red cell production in para-
biotic rats in which the nephrectomized parabiont was exposed to hypoxia. The duration of exposure to hypoxia was longer than used in the experiments reported herein. It would appear, therefore, that extrarenal production of erythropoietin, while it occurs in adults, is of secondary importance. In man, it is well established that erythropoiesis can be supported in the absence of renally produced erythropoietin and, indeed, a response to hypoxia has been described.\(^10\)

We previously observed that exposure to 0.4 atmosphere for a period of 6 hr./day for 7 days failed to produce a demonstrable increase in red cell production in rats exposed prior to the sixteenth day of life.\(^11\) Perhaps a longer exposure to hypoxia, e.g., 18 hr. as used in these present studies, would have resulted in a modest increase in red cell production, although it is doubtful that the newborn rat could have survived repetitive exposures of this duration. It was considered that the inability of the newborn animal to respond to hypoxia in a fashion similar to that observed in the adult animal reflected either the inability to produce erythropoietin, a decreased sensitivity of the bone marrow cells to erythropoietin, or perhaps both. Garcia and Van Dyke\(^12\) have reported that the rat is able to respond to erythropoietin after the fifteenth day of life, but the response appeared to be suboptimal; data on the response at an earlier age is not available. The studies reported herein suggest that in part the failure to observe a more pronounced erythropoietic response to altitude in the younger animals related to a decreased capacity to produce erythropoietin. Studies in progress on the effect of erythropoietin in hyper-
transfused animals during the first 10 days of life should shed light on the ability of the bone marrow to respond to erythropoietin at that age.

The progressive increase in the capacity of the newborn animal to produce erythropoietin is in concert with our previous suggestion that during the hepatic phase of fetal life, red cell production is controlled differently than in the adult animal and that during late fetal and early neonatal life, as splenic and myeloid erythropoiesis evolve, there is a gradual transition from the fetal to the adult-type of regulation of red cell production.\(^1,5,4\) Whether there is an analog to erythropoietin responsible for regulation of red cell production in the fetus is unknown. It is of interest that the transition from fetal to adult-type regulation and the concomitant increasing capacity for erythropoietin production is temporally correlated with the decrease in the numbers of syncytial cells which are present in the fetal liver and during early myeloid erythropoiesis. It might be suggested that the syncytial cell serves as a fetal “stem cell” and is gradually replaced by a more differentiated erythropoietin-
sensitive cell. Syncytial cells have been described in the human fetus during hepatic and early myeloid red cell production.\(^13\) These events in man, however, occur during intrauterine life, and myeloid red cell production is well established during the last trimester of pregnancy. The rat at birth is quite immature and perhaps analogous to the human fetus during the fifth and sixth months of gestation. In animals such as the guinea pig, in which the
degree of maturity at birth is similar to that seen in human beings, syncytial cells are no longer seen in the bone marrow of the newborn animal.\textsuperscript{1,4,5} There also appears to be an adult-type of regulation of red cell production as judged by response to erythroid perturbation such as starvation.\textsuperscript{4} It is tempting to suggest, therefore, that the transition to adult erythropoiesis and erythropoietin production which we have observed in late fetal and early neonatal life in the rat occur during the last trimester of intrauterine life in those species, such as man, in which the newborn is mature.

**Summary**

Erythropoietin production was examined in the newborn rat. Even though there was significant anemia present during the early neonatal phase of life in the rat, erythropoietin could not be demonstrated in the plasma. Exposure to simulated levels of altitude of 23,000 feet for a period of 18 hr. resulted in the production of demonstrable levels of erythropoietin in the plasma. The amount of erythropoietic activity which could be demonstrated in the plasma of hypoxic animals increased with age from values of 0.76 units/ml in the 1 day old animal to normal adult values of approximately 8 units/ml by the thirtieth day of life. Bilateral nephrectomy prior to exposure to hypoxia in the 5 day old and 15 day old rat reduced the amount of erythropoietin to values of 50 per cent and 25 per cent of those observed in the intact hypoxic animal. By the thirtieth day of life, erythropoietin could no longer be demonstrated in anephric animals exposed to hypoxia.

It is concluded that there is a gradual increase in the capacity of the rat to produce erythropoietin in response to an hypoxic stimulus, and that in significant measure during the early neonatal phase, erythropoietin production is accomplished by extrarenal sources.

**SUMMARIO IN INTERLINGUA**

Le production de erythropoietina esseva exanime in rattos neonate. Ben que grados significative de anemia esseva constatate durante le prime parte del phase neonate in le vita del ratto, nulle erythropoietina poteva esser demonstrate in le plasma. Le exposition a simulate nivellos de altitude de 23,000 pedes durante un periodo de 18 horas resultava in le production de demonstrabile concentrationes de erythropoietina in le plasma. Le quantitate de activitate erythropoietic que poteva esser demonstrate in le plasma de animales hypoxic accresceva in le curso de lor maturation ab un valor de 0.76 unitates per ml al etate de 1 die ad approximativemente un valor de 8 unitates per ml (le valor adulte normal) al etate de trenta dies. Nephrectomia bilateral ante le exposition a hypoxia in animales de 5 e de 15 dies de etate reduceva le quantitate de erythropoietina per 50 e 75 pro cento de lo que esseva caracteristic del intacte animales hypoxic. Al etate de trenta dies, il esseva non plus possibile demonstrar erythropoietina in animales anephric post exposition a hypoxia.

Es concludite que le ratto ha un augmento gradual in le capacitate a producer erythropoietina in responsa a un stimulo hypoxic e que durante le prime parte del phase neonate le production de erythropoietina es effectuate—a mesura significative—in sitos extrarenal.

**ADDENDUM**

Since submission of this manuscript, Naets and Wittek have reported the presence of erythropoietin in the plasma of an anephric patient. See Blood 31: 249, 1968.
REFERENCES


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