Prevention of Neonatal Anemia in the Rat by the Pituitary Erythropoietic Factor

By A. N. Contopoulos, D. C. Van Dyke, S. Ellis, M. E. Simpson, J. H. Lawrence and H. M. Evans

Anemia develops immediately after birth in many animals as well as in man. The neonatal anemia displays itself by decrease in red cell count, hemoglobin, and hematocrit. Iron storage in the body does not prevent the development of this anemia. At birth the hematologic values are higher than in the adult, but they decline rapidly to reach a low point within days or weeks following birth, depending on the species. Several explanations have been offered explaining this change in the blood picture. The initial high values, according to Goldloom and Gotlieb result from the unsaturated oxygen level in fetal blood. When, at birth, the oxygen tension rises, the increased number of red cells is not needed and the cells are rapidly destroyed. Mollison suggested that the anemia results from a more rapid rate of destruction of erythrocytes in the newborn than in the adult. Atrophy of the erythropoietic tissue in the liver has also been considered as responsible for this physiologic anemia. Wintrobe attributed this anemia to a "relative deficiency of antipernicious-anemia principle."

Evidence has recently been presented by this group of investigators in support of the concept that the pituitary contains a specific erythropoietic factor. This principle is capable of repairing the post-hypophysectomy anemia in the rat, and of inducing polycythemia in the normal, hypophysectomized, or adrenalectomized rat. Since there is evidence that some pituitary hormones are not produced or released in the fetal and neonatal period, insufficiency of the pituitary erythropoietic factor may result in anemia at this critical period when the offspring is suddenly deprived of maternal hormones.

It is the primary object of this study to investigate the effect of the anterior pituitary erythropoietic factor on neonatal anemia. However, the assumption has been made that the anterior pituitary is effective in stimulation of erythropoiesis only through mediation of the hormones of its target organs, therefore the effects of these hormones on neonatal anemia have also been investigated. Further, as high protein diets have been considered salutary in post-hypophysectomy anemia, and as iron is involved in hemoglobin production, these substances also were tested, alone and in combination with hormonal factors.

Materials and Methods

Rats of the Long-Evans strain were used in these experiments. Litters were chosen consisting predominantly, or wholly, of males, as males only were injected. Each experimental group was so constituted that it contained 6 (or more) animals from as many different litters. The newborn rats were marked or identified by their color and coat pattern. The
mothers were maintained on a diet satisfactory for lactation. The injections were started
the 4th day of life and were continued daily for 14 consecutive days, or until the animals
were 18 days of age, at which time the neonatal anemia is known to be most severe. The
groups of suckling rats were removed from the cages only for the short periods required
for injection. After each injection the experimental group was placed with a different
lactating female in order to equalize the milk supply. With proper care the young were not
rejected because of this procedure.

The following substances, in addition to the anterior pituitary erythropoietic factor,
were tested for their capacity to prevent the development of the anemia of the newborn
rats: testosterone propionate; thyroxin; bovine plasma albumin; a combination of testos-
terone propionate; thyroxin; bovine plasma albumin; a combination of testosterone pro-
pionate, thyroxin and bovine plasma albumin; whole adrenal cortical extract; and iron
gluconate. The doses in which these substances were administered will be found in table 2.

Groups of controls, uninjected or injected with sterile physiologic saline, were maintained
parallel with each series of experiments.

At autopsy the endocrine organs were weighed in order to detect evidence of the physio-
logic activity of the hormones administered.

At the end of the injection period blood volumes were determined by the Fe59 labeled
red cell dilution method. The labeled cells were obtained from a Long-Evans donor rat
previously injected with Fe59. The experimental rats were injected through the jugular
vein with 0.1 ml. of donor blood containing approximately 0.02 μc Fe59. After allowing 6
minutes for the blood to mix, a sample of blood was drawn from the aorta into a heparinized
syringe. A known volume of this blood was pipetted into a vial and its activity counted
directly in a scintillation counter. The total blood volume was calculated from the frac-
tion of the injected activity recovered in this sample. The total blood volume thus calcul-
ated, multiplied by the hematocrit (determined in Wintrobe tubes), gave the total circu-
lating red cell volume. The total circulating red cell volume was then divided by the body
weight of the animal, and the results are presented in terms of ml. of red blood cells per
100 Gm. body weight.

The hemoglobin concentration was determined by the method of Turner and the results
are presented in Gm. per 100 ml. The total blood volume multiplied by the hemoglobin con-
centration gave the total circulating hemoglobin. The total circulating hemoglobin was
then divided by the body weight of the animal and the results are presented in terms of
hemoglobin Gm. per 100 Gm. body weight.

RESULTS AND DISCUSSION

The anemia which begins to develop in the rat immediately after birth be-
comes progressively more severe, and by the eighteenth day the values for hemo-
globin, hematocrit and red cell volume reach the lowest level. The anemia dis-
appears by the thirty-fifth day, at which time the hematologic values attain the
normal adult level. The hematologic values during this period are summarized
in table 1.

Table 2 shows that all substances used to prevent the development of neo-
natal anemia were ineffectual, with the exception of the pituitary erythropoietic
factor. Testosterone propionate did not affect the hematologic values; the dose
used resulted in a tenfold increase in the seminal vesicle weight and a decrease
in the adrenal weight (table 3). Thyroxin, administered in doses known to be
adequate for normal growth and skeletal differentiation in the hypophysec-
tomized rat, was without effect on erythropoiesis. Combinations of testosterone
propionate and thyroxin were also ineffective. Combinations of testosterone pro-

* Diet 1 consists of 67.5 per cent wheat, 15 per cent casein, 7.5 per cent skim milk powder,
6.75 per cent hydrogenated vegetable oil, 1.0 per cent fish oil, 0.75 per cent NaCl, 1.5 per
cent CaCO3, KI added (analysis 1 μg iodine per g diet).
† Upjohn Adrenal Cortex Extract, Alcohol 10%. The Upjohn Co., Kalamazoo, Michigan.
TABLE 1.—Development and Recovery from Neonatal Anemia in the Male Rat

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>No. of rats</th>
<th>Body weight (Gm.)</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Hemoglobin/100 Gm. body weight (Gm.)</th>
<th>Hematocrit (%)</th>
<th>Red cell volume/l00 Gm. body weight (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7</td>
<td>6 ± 0.5†</td>
<td>12.5 ± 0.6</td>
<td>0.64 ± 0.03</td>
<td>38.5 ± 0.6</td>
<td>2.50 ± 0.06</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>20 ± 2.0</td>
<td>9.5 ± 0.3</td>
<td>0.59 ± 0.03</td>
<td>35.5 ± 0.6</td>
<td>2.12 ± 0.02</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>36 ± 2.0</td>
<td>7.5 ± 0.2</td>
<td>0.49 ± 0.04</td>
<td>29.5 ± 0.5</td>
<td>1.72 ± 0.05</td>
</tr>
<tr>
<td>18</td>
<td>27</td>
<td>38 ± 1.0</td>
<td>6.9 ± 0.5</td>
<td>0.44 ± 0.03</td>
<td>25.6 ± 0.6</td>
<td>1.58 ± 0.05</td>
</tr>
<tr>
<td>23</td>
<td>8</td>
<td>52 ± 3.0</td>
<td>8.2 ± 0.6</td>
<td>0.50 ± 0.02</td>
<td>30.7 ± 0.2</td>
<td>1.95 ± 0.06</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
<td>72 ± 4.0</td>
<td>10.5 ± 0.08</td>
<td>0.58 ± 0.04</td>
<td>40.0 ± 0.6</td>
<td>2.30 ± 0.05</td>
</tr>
<tr>
<td>35</td>
<td>7</td>
<td>95 ± 5.0</td>
<td>12.3 ± 0.6</td>
<td>0.68 ± 0.02</td>
<td>43.2 ± 0.8</td>
<td>2.31 ± 0.03</td>
</tr>
</tbody>
</table>

* Standard Error = \( \sqrt{\frac{\sum d^2}{n(n - 1)}} \)

TABLE 2.—Effectiveness of Erythropoietically Active Substances in Prevention of Neonatal Anemia

<table>
<thead>
<tr>
<th>Treatment* substance</th>
<th>Daily dose (mg.)</th>
<th>No. of rats</th>
<th>Body weight (Gm./100 ml.)</th>
<th>Hemoglobin (Gm.)</th>
<th>Hemoglobin/100 Gm. body weight (Gm.)</th>
<th>Hematocrit (%)</th>
<th>Red cell volume/l00 Gm. body weight (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.0</td>
<td>27</td>
<td>38.0 ± 1.0</td>
<td>6.0 ± 0.2</td>
<td>0.44 ± 0.01</td>
<td>25.6 ± 0.6</td>
<td>1.59 ± 0.04</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>1.0</td>
<td>10</td>
<td>39.5 ± 1.2†</td>
<td>7.2 ± 0.2</td>
<td>0.49 ± 0.01</td>
<td>24.1 ± 0.6</td>
<td>1.63 ± 0.03</td>
</tr>
<tr>
<td>Thyroxin</td>
<td>0.0025</td>
<td>5</td>
<td>39.0 ± 1.0</td>
<td>6.8 ± 0.1</td>
<td>0.45 ± 0.01</td>
<td>24.2 ± 0.3</td>
<td>1.60 ± 0.03</td>
</tr>
<tr>
<td>Bovine plasma albumin</td>
<td>0.5</td>
<td>7</td>
<td>42.5 ± 1.3</td>
<td>6.8 ± 0.2</td>
<td>0.44 ± 0.01</td>
<td>25.6 ± 0.6</td>
<td>1.61 ± 0.04</td>
</tr>
<tr>
<td>Bovine plasma albumin</td>
<td>4.0</td>
<td>6</td>
<td>43.5 ± 1.0</td>
<td>6.8 ± 0.2</td>
<td>0.46 ± 0.02</td>
<td>26.2 ± 0.8</td>
<td>1.70 ± 0.03</td>
</tr>
<tr>
<td>Combination T. P. 1 mg. with albumin</td>
<td>0.5</td>
<td>9</td>
<td>39.5 ± 0.8</td>
<td>6.5 ± 0.2</td>
<td>0.43 ± 0.01</td>
<td>26.2 ± 0.8</td>
<td>1.67 ± 0.04</td>
</tr>
<tr>
<td>Thyroxin 2.5 µg. with albumin</td>
<td>4.0</td>
<td>6</td>
<td>44.5 ± 1.0</td>
<td>6.5 ± 0.2</td>
<td>0.43 ± 0.01</td>
<td>27.1 ± 0.9</td>
<td>1.70 ± 0.02</td>
</tr>
<tr>
<td>Adrenal cortical extract</td>
<td>5 RU</td>
<td>5</td>
<td>38.0 ± 0.9</td>
<td>6.7 ± 0.1</td>
<td>0.43 ± 0.01</td>
<td>26.0 ± 0.7</td>
<td>1.61 ± 0.04</td>
</tr>
<tr>
<td>Iron gluconate</td>
<td>0.2</td>
<td>5</td>
<td>38.5 ± 0.8</td>
<td>6.9 ± 0.1</td>
<td>0.46 ± 0.01</td>
<td>24.5 ± 0.4</td>
<td>1.60 ± 0.02</td>
</tr>
<tr>
<td>Pituitary fraction</td>
<td>0.1</td>
<td>16</td>
<td>37.5 ± 1.0</td>
<td>7.8 ± 0.2</td>
<td>0.51 ± 0.02</td>
<td>35.1 ± 0.8</td>
<td>2.26 ± 0.03</td>
</tr>
</tbody>
</table>

* All substances injected IP except testosterone propionate which was given subcutaneously.
† Standard Error = \( \sqrt{\frac{\sum d^2}{n(n - 1)}} \)

pionate and thyroxin with a high protein diet have been reported to repair post-hypophysectomy anemia; such combinations were tested but were found to be without effect on erythropoiesis. As the diet of the newborn could not be changed protein in the form of bovine plasma albumin was injected. (The serum protein was also tested by itself for erythropoietic activity and no change in hemoglobin, hematocrit and red cell volume resulted.) The effect of whole adrenal extract was tested at doses five times that necessary for maintenance of adrenalectomized rats. No changes in the hematological values were present in injected animals after administration of the adrenal cortical extract. Administration of high doses of iron gluconate was ineffective.
TABLE 3.—Weights of Pituitary Target Organs in Experimental Groups

<table>
<thead>
<tr>
<th>Injected with</th>
<th>No. of animals</th>
<th>Adrenals (mg.)</th>
<th>Thyroids (mg.)</th>
<th>Seminal vesicles (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>27</td>
<td>10.3 ± 0.4*</td>
<td>4.3 ± 0.3</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>10</td>
<td>6.4 ± 0.5</td>
<td>2.8 ± 0.1</td>
<td>57.0 ± 3.0</td>
</tr>
<tr>
<td>Thyroxin</td>
<td>5</td>
<td>10.5 ± 0.6</td>
<td>2.5 ± 0.1</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>Bovine plasma albumin</td>
<td>7</td>
<td>9.8 ± 0.6</td>
<td>3.5 ± 0.1</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>Testosterone propionate, thyroxin, and bovine plasma albumin</td>
<td>9</td>
<td>6.6 ± 0.7</td>
<td>2.5 ± 0.1</td>
<td>55.0 ± 0.2</td>
</tr>
<tr>
<td>Adrenal cortical extract</td>
<td>5</td>
<td>10.5 ± 0.6</td>
<td>4.9 ± 0.5</td>
<td>7.0 ± 0.6</td>
</tr>
<tr>
<td>Pituitary fraction</td>
<td>16</td>
<td>10.6 ± 0.6</td>
<td>3.7 ± 0.2</td>
<td>5.9 ± 0.5</td>
</tr>
</tbody>
</table>

* Standard Error = \( \sqrt{\frac{\sum d^2}{n(n-1)}} \)

Only those pituitary fractions of proved erythropoietic activity in repair of the post-hypophysectomy anemia were tested in the newborn rat. The preparation of such active extracts has been described recently.11

These pituitary preparations prevented the neonatal anemia; no decrease in hematocrit, hemoglobin or red cell volume occurred. The hematological values of the injected animals exceeded those of their controls as follows: 48 per cent in red cell volume, 38 per cent in hematocrit and 16 per cent in hemoglobin per 100 Gm. of body weight (table 2). These increases are statistically significant at the 1 per cent level.

No evidence of the presence of known pituitary hormones in these pituitary preparations was obtained from the weight or histology of the pituitary target organs of the young rats (table 3). It could be argued, however, that these animals are not as sensitive test animals for the pituitary target hormones as are hypophysectomized rats. Tested in hypophysectomized rats the pituitary extracts were found to contain only adrenocorticotropic hormone in appreciable amounts, and were free of thyrotropic hormone, interstitial cell stimulating hormone, follicle stimulating hormone, and growth hormone at the level used in this experiment. However, evidence has been presented that the adrenals are not necessary for the stimulation of the erythropoietic tissues in the rat.11

The results reported here show that the erythropoietic tissues in the newborn rat respond to an erythropoietic factor present in the pituitary. It must therefore be assumed that this substance is either not produced or not released in adequate amounts from the neonatal pituitary.

**SUMMARY**

Of all the substances tested for the prevention of the neonatal anemia in the rat, only the pituitary preparations, erythropoietically active in the repair of post-hypophysectomy anemia, were able to prevent the development of neonatal anemia of the newborn rat.

**SUMMARIO IN INTERLINGUA**

Inter omne le substantias probate como preventivos de anemia neonatal in rattos, solo le preparatos pituitari—active como agentes erythropoietic in le
reaparation de anemia post-hypophysectomy—esseva capace a prevenir le disveloppamento de anemia in rattos neonate.

REFERENCES

Prevention of Neonatal Anemia in the Rat by the Pituitary Erythropoietic Factor

A. N. CONTPOULOS, D. C. VAN DYKE, S. ELLIS, M. E. SIMPSON, J. H. LAWRENCE and H. M. EVANS