Effect of Prolactin on Erythropoiesis in the Mouse

By J. H. Jefson and L. Lowenstein

The effect of various steroid and protein hormones on erythropoiesis has been demonstrated in the past. Gonadectomy in male rats results in reduced erythrocyte counts, and injection of testosterone is well known to increase the red-cell count in normal rats, dogs and monkeys and in castrated rats. Injection of gonadotrophins into normal animals is said to have slight effect on erythropoiesis except through stimulating production of testosterone. In 1942, Vollmer et al. administered a prolactin preparation, and other hormones to groups of hypophysectomized rats. Injection of prolactin extract resulted in average increase of red-cell counts of 25 per cent and of hemoglobin of 16 per cent over the pre-injection values in three animals. Bone marrow sections appeared to have increased numbers of erythrogenic elements. The extent of repair, however, was not so great as that seen in the thyroxine treated groups and the prolactin preparations available at that time were not homogeneous, although the prolactin treated animals showed no change in the histology or the weight of their adrenals, testes or thyroids.

Recently, Fisher and Crook found that administration of adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), adrenocortical extract, hydrocortisone, corticosterone, 11-dehydrocorticosterone, 3,5,3'-tri-iodothyronine, or angiotensin enhanced Fe incorporation into the erythrocytes of hypophysectomized rats, whereas growth hormone and testosterone, up to 2 mg., had no significant effect in these animals.

In 1958, Bond found that the total blood volume and red-cell mass were increased significantly in lactating rats as compared with values in the non-pregnant and non-lactating female rats.

We have employed the polycythemic mouse assay method to determine if certain hormones altered erythropoiesis in the presence of suppressed erythropoietin production and the resulting suppressed erythropoietic state. In the hypophysectomized rat previously used as an assay animal, normal endocrinological homeostasis is no longer present and it is questionable whether a specific erythropoietic effect of a given substance can be determined. In the polycythemic mouse assay, normal endocrinological status is maintained and a specific effect on erythropoiesis can be observed following administration of test materials.
The hormones investigated in these experiments included testosterone, ovine prolactin, bovine growth hormone, and progesterone. The effect on erythropoiesis of injections of pooled plasma from lactating mice and from pregnant mice was also studied in the polycythemic mouse. In addition, the effect of long-term administration of prolactin and testosterone on erythropoiesis was studied in the normal and orchidectomized mouse.

**MATERIAL AND METHODS**

*Effect of Test Materials Upon Fe$^{59}$ Incorporation Into Erythrocytes of the Polycythemic Mouse*

Male Swiss mice weighing 20–25 Gm., and inbred in this laboratory, were made polycythemic by exposure to 10 per cent O$_2$ environment for 21 days. Each animal received 1 mg. of Imferon$^{15}$ at the beginning of the hypoxic exposure. At the end of three weeks, the hematocrits of the animals ranged from 67–72 per cent. On the fourth and fifth days following removal from the hypoxic environment, each group of five animals received a subcutaneous injection of the test material. Fifty-six hours later, 0.5 µc. Fe$^{59}$ Cl$_3$ in 0.1 ml. physiologic saline was injected into the tail vein. Seventy-two hours later, 0.5 ml. of blood was withdrawn by cardiac puncture for counting in a scintillation counter and for duplicate microhematocrits. Fe$^{59}$ standards were prepared at the time of injection.

Fe$^{59}$ RBC uptake (in the polycythemic mouse) was calculated on the basis of an expected blood volume equal to 7.5 per cent of the body weight$^{16}$ by the formula:

$$\text{Per cent Fe}^{59}\text{ uptake} = \frac{\text{CPM}_\text{standard injected}}{\text{CPM}*/\text{ml. bld.} \times 0.075 \times \text{body weight (Gm.)} \times 100}$$

*Effect of Test Materials on Red Cell Volume (RCV) in Normal and in Orchidectomized Mice*

Normal male mice were divided into groups of five. Several groups, each of five mice, were orchidectomized 2 weeks before the beginning of the injection of test materials. The test materials were injected subcutaneously 6 times weekly until a total of 25 injections had been received. At the end of this period, the total circulating red cell volume (RCV) was determined with Cr$^{51}$-tagged red cells by a modified method of Gray and Sterling.$^{17}$

Homologous red cells were obtained from donor mice by withdrawing blood via cardiac puncture into a syringe containing acid-citrate dextrose solution (ACD). Contents of the syringe were centrifuged at 2000 rpm., the supernatant was removed and stored in the cold. Sodium bichromate, 0.5 µc., of high specific activity (614 mc./mg.) was then added per 1 ml. of packed cells. This suspension was incubated at room temperature for 30 minutes, then centrifuged in the cold, the supernatant removed, and the cells gently washed twice with isotonic saline. After further centrifugation at 2000 rpm. and removal of supernatant, the cells were resuspended in approximately the original volume of saved chilled plasma-ACD solution. A sample of 0.2 ml. of the mixed suspension was diluted in 10 ml. of distilled water for use as a standard. Each animal was anesthetized, and 0.2 ml. of Cr$^{51}$ labelled homologous red-cell suspension was injected into the tail vein. Fifteen minutes later, blood was aspirated via cardiac puncture; 0.5 ml. was counted in a well-type scintillation counter and the blood volume was calculated. Heparinized micro-hematocrits were determined in duplicate. The per cent reticulocytes per 1000 erythrocytes was counted in air dried cover slip preparations made after mixing equal parts of blood and Brilliant Cresyl Blue.

*Ovine prolactin* (Merck): Intact and orchidectomized polycythemic mice received sub-

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*CPM = Counts per minute above background.*

†See footnote, page 728.
cutaneous injections of 0.2 ml. of 0.85 per cent saline containing 125 µg., 250 µg., 500 µg., or 1 mg. of a highly purified preparation of prolactin on 2 consecutive days. Normal and orchidectomized normal mice received 125, 250, or 500 µg. in 0.1 ml. of 0.85 per cent saline daily, subcutaneously 6 times a week, over a period of 1 month, to a total of 25 injections. The prolactin preparation was prepared by a modification of Reissfeld technic.18

**Testosterone** (Schering Research H-4): Fifty µg. in 0.1 ml. of sesame oil was injected subcutaneously into unoperated and orchidectomized polycythemic mice on 2 consecutive days. Fifty µg. of this preparation was also injected daily, 6 times a week, over a period of 1 month, into intact and orchidectomized mice, to a total of 25 injections.

**Bovine growth hormone** (Horner, 1958, Lot No. GE/5): Two hundred µg. in 0.2 ml. of 0.85 per cent saline was given subcutaneously on 2 consecutive days to polycythemic mice. This substance was prepared by the method of Li, C. H. et al.19

**Analytical grade cobaltous chloride hexahydrate** (Fisher Scientific No. 705558): 0.2 ml. of 0.85 per cent saline containing 1 µM. of CoCl₂-6H₂O was given subcutaneously, on each of 2 consecutive days, to polycythemic mice.

**Pregnant and lactating mouse plasma:** On the fifth day postpartum, lactating mouse plasma was collected by centrifugation of blood taken in heparinized syringes, pooled and frozen until use. One ml. of this plasma was administered subcutaneously to polycythemic mice on each of 2 consecutive days. Pregnant mouse plasma was collected between 16-19 days of pregnancy, pooled and frozen until use. One ml. was given subcutaneously, on each of 2 consecutive days to the polycythemic mouse.

**Progesterone** (Upjohn No. 8405): Two hundred µg. in 0.2 cc. of sesame oil was administered subcutaneously on each of 2 consecutive days to polycythemic mice.

**RESULTS**

**Effect of Hormones and Cobalt upon Erythrocyte Fe⁵⁹ Incorporation**

Table 1 shows the RBC Fe⁵⁹ incorporation following injection of saline, ovine prolactin, testosterone, progesterone and the pooled plasmas of both pregnant and lactating mice. RBC Fe⁵⁹ incorporation in the groups of intact polycythemic mice receiving 500 µg. and 1000 µg. prolactin, lactating mouse plasma, pregnant mouse plasma, CoCl₂-6H₂O, and testosterone was significantly greater than the saline-injected controls (p = < .05-< .01). Prolactin, at 250 µg. dose, was only statistically significant at the 10 per cent level, and the 125 µg. dose produced no significant response. Progesterone produced no significant change of RBC Fe⁵⁹ incorporation in the polycythemic mouse. There was a slight, but not statistically significant increase of RBC Fe⁵⁹ incorporation following injection of bovine growth hormone (0.1 < p < 0.05).

Eight intact polycythemic mice receiving 1 ml. of nulliparous female plasma on 2 consecutive days showed incorporation of Fe⁵⁹ into erythrocytes of 0.67 ± .07. Thus, normal female mouse plasma was shown to contain no erythropoietic stimulating activity.

In orchidectomized polycythemic mice, prolactin doses of 125 to 500 µg. did not significantly increase RBC Fe⁵⁹ incorporation, although a small but consistent elevation was produced by the 125 to 250 µg. dose range (fig. 1). Again, injection of testosterone (50 µg. x 2) was followed by a significant in-

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*We wish to thank Dr. S. Solomon for supplying us with the testosterone and progesterone; Dr. L. Mitchell of F. W. Horner Co. for the bovine growth hormone, and the Merck Research Institute, Westpoint, Pa., and Rahway, N. J., for the ovine prolactin.*
Table 1.—Effect of Administration of Hormones and Plasma from Pregnant and Lactating Mice on Per Cent Fe	extsuperscript{59} RBC Incorporation in Polycythemic Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Daily Dose on 2 Consecutive Days</th>
<th>% Fe	extsuperscript{59} Incorporation (Mean ± S.E.)*</th>
<th>Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unoperated Polycythemic Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5</td>
<td>—</td>
<td>0.43 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Bovine G.H.</td>
<td>5</td>
<td>200 μg.</td>
<td>2.81 ± 1.03</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>5</td>
<td>125 μg.</td>
<td>0.81 ± 0.48</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>5</td>
<td>250 μg.</td>
<td>2.13 ± 0.8</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>8</td>
<td>500 μg.</td>
<td>3.91 ± 1.46</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>5</td>
<td>1000 μg.</td>
<td>3.68 ± 1.13</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Progesterone</td>
<td>3</td>
<td>200 μg.</td>
<td>1.72 ± 0.95</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Testosterone</td>
<td>4</td>
<td>50 μg.</td>
<td>2.46 ± 0.43</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>CoCl	extsubscript{2} 6H	extsubscript{2}O</td>
<td>5</td>
<td>1 μM.</td>
<td>4.45 ± 1.37</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Lact. mouse pl.</td>
<td>4</td>
<td>1 ml.</td>
<td>6.71 ± 0.54</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Preg. plasma</td>
<td>4</td>
<td>1 ml.</td>
<td>13.95 ± 5.48</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>(16-19 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal female mouse plasma</td>
<td>8</td>
<td>1 ml.</td>
<td>0.67 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Orchidectomized Polycythemic Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>5</td>
<td>125 μg.</td>
<td>1.55 ± 0.87</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>5</td>
<td>250 μg.</td>
<td>1.32 ± 0.67</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Ovine prolactin</td>
<td>4</td>
<td>500 μg.</td>
<td>0.85 ± 0.32</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Testosterone</td>
<td>5</td>
<td>50 μg.</td>
<td>4.60 ± 1.0</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Saline</td>
<td>4</td>
<td></td>
<td>0.42 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

*S.E. = \sqrt{\frac{\sum d^2}{n(n-1)}}

†Level of significance of per cent Fe	extsuperscript{59} RBC incorporation as compared to control.

crease of erythrocyte Fe	extsuperscript{59} incorporation (P < .05), and this increase was greater in the orchidectomized mouse than in the normal mouse.

Effect of Repeated Injections of Prolactin and Testosterone on the Red Cell Volume (RCV) in the Intact and Orchidectomized Male Mouse

The effect of prolactin administration on the RCV is summarized in table 2 and figure 2. Following orchidectomy, the control red-cell volume did not significantly decrease in comparison with the unoperated, uninjected control. Maximum effect was obtained with 125 μg. and 250 μg. per day, associated with an increase in the reticulocyte count (fig. 3). The per cent of control for 125 μgm. dose was 164.9 per cent for the normal and 165.2 per cent for the orchidectomized mouse. After repeated injections of 250 μg. of prolactin, the RCV was 155 per cent and 141 per cent above the control, in intact and orchidectomized mice, while at the 500 μg. dose level the per cent of control was 132.6 per cent and 132.7 per cent respectively. Thus, there was no significant difference between orchidectomized and unoperated mice.

All of these values were significantly greater than those of the uninjected control animals. The RCV and the reticulocyte counts of animals receiving 50
μg. of testosterone was also raised significantly above control levels. The increase of RCV was greater in orchidectomized than in intact mice.

The spleens of all mice were examined at autopsy and showed no gross pathology or evidence of splenomegaly to suggest sequestration of erythrocytes.

DISCUSSION

The effect of highly purified preparations of prolactin on erythropoiesis in the normal and polycythemic mouse has not been reported previously. When polycythemic mice were injected with 500 μg. and 1000 μg. of prolactin, a statistically significant increase of Fe59 RBC incorporation over that of the control was observed. No such increase occurred when polycythemic mice were orchidectomized and then treated with prolactin on 2 consecutive days.

Treatment of normal and orchidectomized male mice with prolactin over a 1-month period resulted in an increase of the red cell volume, which was maximal after a daily dose of 125 μg. of the material, but was not significantly different from the 250 μg. dose. The repeated administration of 500 μg. of prolactin resulted in a significant increase of red cell mass, but was somewhat lower than that of the smaller doses.

The reticulocyte counts of animals receiving prolactin were also elevated, as were the hematocrit values, which were maximal at the 250 μg. dose level. The discrepancy between hematocrit and RCV can be explained by a greater increase of plasma volume after the 125 μg. dose than after the 250 μg. dose of prolactin (table 2). Since the spleens were grossly normal in size and ap-
pearance, and the control values fell within the normal limits of red cell mass of 2.67–2.99 ml/100 g. in the mouse (20,21), splenic sequestration of Cr\textsuperscript{51}-tagged red cells seems unlikely.

Administration of testosterone produced an increase of Fe\textsuperscript{59} RBC incorporation following short-term injection. The increase of RCV following long-term injection of testosterone was greater in the orchidectomized mice than in the intact mice. Therefore, it would seem that the erythropoietic stimulating effect of exogenous testosterone is greater in the orchidectomized than in the intact mouse.

The repeated injection of 125 \( \mu \)g. of prolactin over a period of 1 month in orchidectomized mice produced an equivalent increase of RCV to that which followed similar injections of 50\( \mu \)g. of testosterone, indicating that prolactin stimulated erythropoiesis in these animals in the absence of the testes and probably did not act through stimulation of increased testosterone production. However, in animals receiving prolactin for a short period of time, it appeared that the testes may have played some role in the increased erythropoietic responsiveness of the intact animal.

Lactating mouse plasma and plasma from mice in the last trimester of pregnancy produced greater RBC Fe\textsuperscript{59} incorporation in polycythemic non-pregnant mice than prolactin, testosterone, or cobaltous chloride in the doses given. Pregnant rat plasma has been shown to be active in stimulating erythropoiesis.\textsuperscript{22} Whether the erythropoietic stimulatory effects of plasma from lactating and pregnant mice are due to gonadotrophins or to other hormones is unknown. The RCA is seen to increase towards the end of pregnancy in the human\textsuperscript{23,24} and in the rat,\textsuperscript{25} hence stimulation of erythropoiesis by plasma from pregnant animals is not surprising. Furthermore, the greater effect of lactating and pregnant mouse plasma on erythropoiesis in the mouse than that produced by testosterone and prolactin may be due to species specificity of the active plasma component, although the magnitude of the dose of testosterone and prolactin may have been responsible.

Review of the literature reveals that a number of endocrinologic changes occur during pregnancy which may well vary from species to species; hence all the observations reported need not necessarily apply to the mouse. However, some general scheme can be formulated as to the events occurring in the mouse.

The gonadotrophins increase in the circulation from the fourteenth day of gestation to parturition.\textsuperscript{27} The progesterone titre of the blood of pregnant mice is high during the first half of gestation, decreases by the tenth day, after which a progressive rise of estrogens until parturition has been reported.\textsuperscript{28} The various metabolic effects of estrogens during pregnancy are complex and their role in relation to erythropoiesis by mediation through other hormones is unknown. Estrogens have been observed to reverse the stimulating effect of erythropoietin on erythropoiesis and to inhibit the incorporation of Fe\textsuperscript{59} into erythrocytes in rats.\textsuperscript{29} Estrogens, however, are known to increase pituitary prolactin in the circulating blood of rabbits\textsuperscript{30,31} and prolactin has been found to be present in significant amounts in pregnant mice.\textsuperscript{32}
Table 2.—Effect of Prolactin and Testosterone on the RCV in Normal and Orchidectomized Male Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Weight (Gm.)</th>
<th>Dose µg./d. (25 injections)</th>
<th>Het. % (Mean ± S.E.)*</th>
<th>RCV ml./100 Gm. (Mean ± S.E.)</th>
<th>RCM % of Control</th>
<th>TBV ml./100 Gm. (Mean ± S.E.)</th>
<th>Retic. % (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninj.</td>
<td>4</td>
<td>26.8</td>
<td>—</td>
<td>41.7 ± 1.6</td>
<td>2.82 ± 25</td>
<td>164.9</td>
<td>6.79 ± .36</td>
<td>2.38 ± .29</td>
</tr>
<tr>
<td>Prolactin</td>
<td>4</td>
<td>30.3</td>
<td>125</td>
<td>47.3 ± .17</td>
<td>4.65 ± .38</td>
<td></td>
<td>9.54 ± 1.21</td>
<td>4.90 ± .33</td>
</tr>
<tr>
<td>Prolactin</td>
<td>4</td>
<td>26.8</td>
<td>250</td>
<td>53 ± 1.8</td>
<td>4.37 ± .21</td>
<td></td>
<td>155.0</td>
<td>8.44 ± .66</td>
</tr>
<tr>
<td>Prolactin</td>
<td>5</td>
<td>26.8</td>
<td>500</td>
<td>51 ± .2</td>
<td>3.74 ± .16</td>
<td></td>
<td>132.6</td>
<td>7.39 ± .45</td>
</tr>
<tr>
<td>Testosterone</td>
<td>4</td>
<td>30.0</td>
<td>50</td>
<td>45.4 ± 1.14</td>
<td>3.55 ± .25</td>
<td></td>
<td>125.9</td>
<td>7.81 ± .42</td>
</tr>
<tr>
<td><strong>Orchidectomy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninj.</td>
<td>5</td>
<td>32.7</td>
<td>—</td>
<td>41 ± .5</td>
<td>2.69 ± .04</td>
<td></td>
<td>6.56 ± .11</td>
<td>2.64 ± .24</td>
</tr>
<tr>
<td>Prolactin</td>
<td>4</td>
<td>28.3</td>
<td>125</td>
<td>48 ± .41</td>
<td>4.66 ± .37</td>
<td></td>
<td>165.2</td>
<td>9.73 ± .83</td>
</tr>
<tr>
<td>Prolactin</td>
<td>3</td>
<td>31.4</td>
<td>250</td>
<td>52 ± 2.1</td>
<td>3.79 ± .42</td>
<td></td>
<td>140.9</td>
<td>6.51 ± .8</td>
</tr>
<tr>
<td>Prolactin</td>
<td>6</td>
<td>29.1</td>
<td>500</td>
<td>49 ± .61</td>
<td>3.57 ± .28</td>
<td></td>
<td>132.7</td>
<td>7.35 ± .62</td>
</tr>
<tr>
<td>Testosterone</td>
<td>5</td>
<td>33.1</td>
<td>50</td>
<td>45.2 ± 1.39</td>
<td>4.64 ± .38</td>
<td></td>
<td>164.5</td>
<td>10.30 ± .83</td>
</tr>
</tbody>
</table>

*S.E. = \sqrt{\frac{\sum d^2}{n(n-1)}}
It is reported that adrenocortical activity in the rat decreases from the fifteenth to the twenty-first day of gestation and that pregnancy and estrogens induce important alterations in adrenal steroid metabolism, increasing corticosteroid binding protein (transcortin) levels in the blood and transcortin-binding of corticosterone or cortisol, but not increasing non-protein bound cortisol.

It is unlikely that the pregnant plasma erythropoietic stimulating factor is derived from estrogens themselves. Furthermore, progesterone was shown to have no significant effect on incorporation of Fe into erythrocytes (table 1). The low adrenocortical activity found in the rat during the third trimester and the altered adrenal steroid metabolism observed would appear to eliminate adrenal hormones as the active component. The presence of gonadotrophins, including prolactin, and of erythropoietin remain possible causes of the observed increased erythropoietic activity. It has been suggested that "gonadotrophins" have an erythropoietic effect through the stimulation of testosterone. However, this seems unlikely in the pregnant animal and the stimulating effect of prolactin on erythropoiesis in orchidectomized mice, at least when prolactin is given over a prolonged period of time, would appear to rule out testosterone as a mediator for this effect. Prolactin is gonadotropic in the rat and mouse and not in other mammals. A previous report on the effect of prolactin administration on erythrocyte counts, hemoglobin and bone marrow of hypophysectomized rats showed a slight repair of erythropoiesis. However, replacement of any one of several hormones alone or in combination may restore the red cell mass to normal after the development of the anemia which follows pituitary ablation. The prolactin extracts available at
that time, however, probably contained some impurities not present in our preparation, including ACTH.12

The effect of a highly purified preparation of prolactin on erythropoiesis in the sensitive polycythemic mouse and its ability to increase red cell mass above the normal in the intact and orchidectomized mouse in the presence of a normal endocrinologic state would suggest a significant sensitivity of the erythropoietic elements to prolactin.

Both prolactin and erythropoietin have been found to increase the red cell mass. Erythropoietin may be physiologically increased during pregnancy, secondary to an increased erythropoietin requirement during the late stages of pregnancy.23-26 Furthermore, there may be transplacental passage of fetal erythropoietin into the maternal circulation or it is possible that erythropoietin is produced by the placenta.28 Thus erythropoietin or prolactin, alone or in combination, may be responsible for the increased activity of pregnant mouse plasma. Prolactin could act by stimulating the production of erythropoietin. Also, the possible role of the recently described placental lactogenic-like substance,38 may require consideration in considering the erythropoietic stimulating effect of pregnant mouse plasma. There also remains the possibility of such hormones as thyroxine, acting in combination with prolactin and erythropoietin to promote increased erythropoiesis. Furthermore, only 2 ml. of pregnant mouse plasma were required to obtain a substantial increase of incorporation of Fe59 into erythrocytes. This quantity of plasma may not contain enough prolactin alone to stimulate erythropoiesis and, therefore, act in conjunction with other hormones.

During and after parturition, adrenocortical function increases in rats38.
and mice, and has been found to be increased during lactation in humans. The increased erythropoietic activity of lactating mouse plasma, therefore, may be due to one or a combination of three factors: (1) adrenocortical steroids which are known to stimulate erythropoiesis, (2) prolactin, which has been shown, in this paper, to increase the RCV, or (3) erythropoietin itself, the primary source of which has been shown to be the kidney. Elevation of erythropoietin may occur following blood loss at parturition. However, we found the 24 hour postpartum hematocrit to be 38.0 per cent ± 1.06 (normal female = 40.4 per cent ± 1.46). This implies that parturitional blood loss is probably not great enough to stimulate erythropoiesis. The postpartum bone marrow is known to be active. In the absence of severe blood loss with a normal hematocrit, an active bone marrow would be expected to utilize available plasma erythropoietin. Stohlman found a decreased erythropoietin titre as the bone marrow activity increased following an hypoxic stimulus in rats. The "lactation polycythemia" observed by Bond, suggests that an active erythropoietic stimulating factor is present during the lactation period. The erythropoietic stimulating effect of prolactin and the need for further evaluation of its role as the cause of erythropoietic stimulating activity of lactating mouse plasma, has been discussed in a preliminary report from this laboratory. Whether the ability of prolactin to stimulate erythropoiesis is by a direct action on the bone marrow, or whether it acts through the adrenal gland or through the kidney with a secondary rise of erythropoietin has yet to be evaluated.

**Summary**

The effect of prolactin, growth hormone, progesterone, testosterone, lactating mouse plasma (fifth postpartum day), pregnant mouse plasma (sixteenth to nineteenth day of pregnancy) on erythropoiesis in the polycythemic mouse has been studied and compared. Prolactin was found to stimulate erythropoiesis in the intact mouse. Also, pregnant mouse plasma and lactating mouse plasma had an erythropoietic stimulating effect. Prolactin produced an increase in the red cell mass when administered over a prolonged period to normal and orchidectomized mice. This suggests that the testes are not necessary for the action of prolactin. It is suggested that prolactin could function as an erythropoietic stimulatory component in pregnant and lactating mouse plasma.

**Summario in Interlingua**

Esseva studiate comparativemente le effectos exercite super le erythropoiese in muses polycythemic per prolactina, hormon de crescentia, progesterona, testosterona, plasma de muses lactante (quinte die post parto), e plasma de muses pregnante (dece-sexte a dece-none die del pregnantia). Esseva trovate que prolactina stimula le erythropoiese in muses intacte. Plasma de muses pregnante e plasma de muses lactante etiam habeva un effecto stimulatori super le erythropoiese. Prolactina produceva un augmento in le massa del erythrocytos quando illo esseva administrate durante prolongate periodos de tempore a muses
intacte o orchidectomisate. Isto suggere que le testes non es necessari pro le action de prolactina. Se presenta le possibilitate que probactina es le componente de plasma ab muses pregnante e lactante be quab stimula le erythropoiese.

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Joanne H. Jepson, M.D., C.M., Hematology Fellow, Division of Hematology, Department of Medicine, Royal Victoria Hospital, McGill University, Montreal, Quebec.

Louis Lowenstein, M.D., Hematologist-in-Charge, Division of Hematology, Department of Medicine, Royal Victoria Hospital, Associate Professor of Medicine, McGill University, Montreal, Quebec.
Effect of Prolactin on Erythropoiesis in the Mouse

J. H. JEPSON and L. LOWENSTEIN