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Erythopoiesis During Pregnancy and Lactation in the Mouse. II. Role of Erythropoietin.* (30971)

JOANNE JEPSON AND LOUIS LOWENSTEIN (Introduced by J. S. L. Browne)
Department of Medicine, Division of Hematology, Royal Victoria Hospital and McGill University Clinic, Montreal, Canada

An increase of total blood volume and red cell volume in lactating rats has been demonstrated by Bond(1). During lactation in mice, plasma volume and red cell volume increased(2) and their plasma had increased erythropoietic activity(3). While prolactin has been found to stimulate erythropoiesis in polycythemic mice(3), in postpartum nonlactating mice(2), and in orchidectomized male mice(3), other hormones active during lactation have not shown an erythropoietic effect(3,4).

Since erythropoietin is a known potent erythropoietic stimulant, the relation of this hormone to the erythropoietic effect of prolactin was investigated to determine if prolactin acted directly on the bone marrow or through stimulation of ESF production.

Materials and methods. To determine how rapidly erythropoiesis was suppressed following exposure to hyperoxia, the incorporation of Fe59 into erythrocytes was determined in normal female mice exposed to 60% O2-40% N2 environment for 1, 2 and 5 days. Twenty-four hours prior to their removal from this environment, 0.5 μc of Fe59 C13 was injected into the tail vein and immediately upon removal, blood was obtained by cardiac puncture. The 24-hour incorporation of Fe59 into erythrocytes of these groups of mice was compared to that of normal unexposed female mice.

Since hyperoxia abolishes the secretion of erythropoietin(5), groups of normal female mice and of postpartum lactating mice, 0-12 hours from birth were placed with their newborn in a 60% O2-40% N2 environment for periods of 5, 10 and 15 days. Two similar groups of mice were simultaneously exposed to hyperoxia and injected daily with 250 μg of prolactin† for 15 days. Upon removal from the hyperoxia, the RCV was determined as described previously(2) in all mice and compared with the values obtained in normal females, in 15-day lactating mice in normal atmosphere, in normal female mice injected daily with 250 μg of prolactin for 15 days, and in normal mice and nonlactating postpartum mice exposed to 10% O2 environment for 15 days.

To determine whether hyperoxia would influence the plasma erythropoietic activity of lactating mice, plasma was collected as previously described(3) on the 1st, 5th and 10th postpartum days from lactating mice exposed from the time of parturition to hyperoxia. For comparison, plasma was collected from normal female mice exposed to *hypoxia* (10% O2-90% N2) for 24, 48 and 72 hours. The erythropoietic activity of these plasmas was

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then determined in hypoxia induced polycythemic mice by injecting them with 1 ml of plasma on 2 consecutive days and then measuring the incorporation of Fe59 into their erythrocytes at 72 hours as previously described(3).

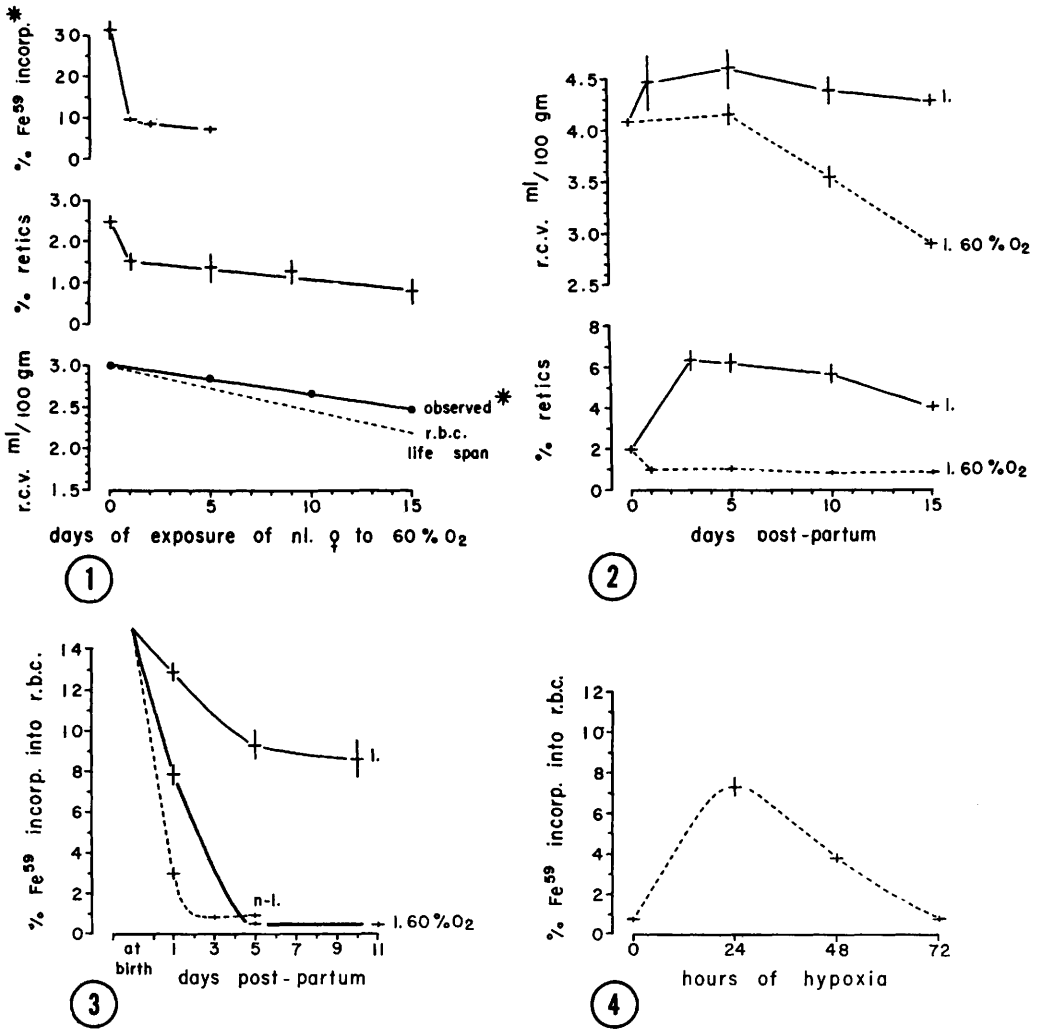


FIG. 1. Effect on erythropoiesis of normal female mice of exposure for 1-15 days to hyperoxia (60% O_2). (Top) Incorporation of Fe59 into erythrocytes at 24 hr. (Middle) % reticulocytes. (Bottom) Red cell volume (r.c.v.), ml/100 g body weight. * ●——● That observed during exposure to hyperoxia; ------- r.b.c. life span calculated on basis of red cell life span of 50-55 days, assuming complete suppression of erythropoiesis. Each group was composed of a minimum of 5 mice; vertical bar is S.E. of mean.

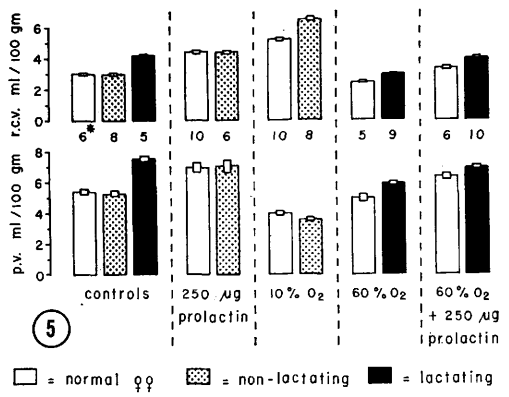
FIG. 2. Comparison of r.c.v. (ml/100 g) and % reticulocytes of hyperoxic exposed (60% O_2) and unexposed lactating mice (l) at various intervals postpartum. Each group composed of a minimum of 5 mice. Vertical bar is S.E. of mean.

FIG. 3. Comparison of erythropoietic activity of plasma collected from lactating (l), non-lactating (n-l), and lactating mice exposed to hyperoxia (l—60% O_2), as measured by incorporation of Fe59 into erythrocytes, at 72 hr, of polycythemic mice. Each group composed of a minimum of 5 mice. Vertical bar is S.E. of mean.

FIG. 4. Erythropoietic activity of plasma collected from normal female mice exposed to 24, 48 and 72 hr of hypoxia (10% O_2) as measured in polycythemic mice, by 72 hr incorporation of Fe59 into their erythrocytes. Each group composed of a minimum of 5 mice. Vertical bar is S.E. of mean.

Results. It is evident from the top curve in Fig. 1 that ESF production was suppressed by hyperoxia in normal female mice. Incorporation of Fe59 into their erythrocytes was only 25% of that of normal mice and the major reduction occurred within 24 hours after their exposure to hyperoxia. There was a sharp drop of reticulocytes within 24 hours followed by a more gradual decrease over the next 14 days and the red cell volume (RCV) decreased gradually over the 15 days of exposure to hyperoxia (Fig. 1). If erythropoiesis were completely suppressed by the hyperoxia, the expected decrease of RCV over the 15-day period of the experiment, calculated on the basis of the red cell life span of 50-55 days(6) would be as plotted in Fig. 1. It shows a similar decline to that of the observed decrease of RCV. Since the Fe59 incorporation data suggested that erythropoiesis was only 75% suppressed, it would be expected that the observed erythrocyte mass should be slightly higher than this calculated value (Fig. 1).

Exposure of postpartum lactating mice to hyperoxia did not impair lactation and the newborn had a normal weight gain. Reticulocytes decreased within 24 hours, as in the hyperoxic exposed normal female mice, and remained so throughout hyperoxic exposure, in contrast to a significant increase occurring in similar animals not exposed to hyperoxia (Fig. 2). After 5 days of hyperoxia, the mean RCV of these lactating mice was equivalent to that at parturition, but was significantly less than that of the unexposed 5-day lactating mice (Fig. 2). The mean RCV of these mice gradually returned to nonlactating levels between the 5th and 15th day of exposure, whereas the erythropoietic activity of their plasma disappeared by the 5th day (Fig. 3). Although incorporation of Fe59 into erythrocytes of normal mice decreased rapidly within 24 hours of exposure to hyperoxia (Fig. 1), plasma of lactating mice exposed to hyperoxia for 24 hours (Fig. 3) still retained considerable activity (7.83% Fe59 incorporation) at this time, although it subsequently decreased to very low values. This value was intermediate between that of plasma collected 24 hours after parturition from nonlactating



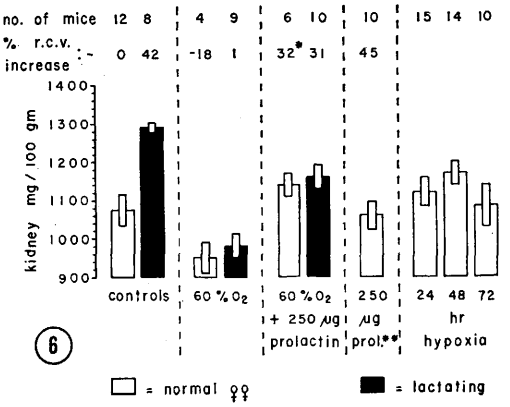


FIG. 5. Comparison of r.c.v. and p.v. (ml/100 g) of normal female mice, of nonlactating 15-day postpartum mice and of 15-day lactating mice treated for 15 days as shown in Figure. Vertical bar represents S.E. of mean. *Number below each vertical bar indicates number of mice in each group.

FIG. 6. Comparison of mean kidney weight (mg/100 g) of lactating and normal female mice treated as shown. % r.c.v.: % increase of r.c.v. over that of normal females. Duration of exposure or treatment, 15 days. Vertical bar is S.E. of mean. * % increase of r.c.v. over that of hyperoxic exposed mice. ** Ovine Prolactin (Merck).

mice (3%) and from lactating mice (12.88%) not exposed to hyperoxia. When normal mice were exposed to *hypoxia,* their plasma attained peak erythropoietic activity (7.28%) in 24 hours (Fig. 4).

Fig. 5 portrays the red cell volumes (RCV) and plasma volumes (PV) per 100 g body weight of the various groups. As values for both RCV and PV were practically identical in the normal control female mice and in the nonlactating mice at 15 days postpartum, both groups are referred to as the controls,

with which all other values are compared unless otherwise stated. Values for both RCV and PV showed essentially the same increase in normal female mice and in nonlactating mice receiving 250 μg of prolactin daily for 15 days and also in the 15-day postpartum lactating mice, maintained in normal atmosphere, *i.e.*, there was a 42-45% increase of RCV and a 30% increase of PV in these mice compared with that of the controls. When secretion of endogenous erythropoietin was stimulated by exposing normal female and nonlactating mice to 10% O2 for 15 days, their RCVs increased by 86% and 108% respectively. However, in contrast to the increase after prolactin administration and during lactation, the plasma volumes of these hypoxic mice decreased sharply, although not sufficiently to compensate completely for the increase of RCVs.

When both normal female and lactating mice were exposed to hyperoxia for 15 days, their RCVs decreased significantly from their previous levels. No significant change occurred in the PV of normal mice in contrast to the slight decrease of PV in the lactating mice. When, in addition to hyperoxic exposure, 250 μg of prolactin was administered daily for 15 days to normal female and lactating mice, both the RCV and PV increased significantly. The RCV of these normal and lactating treated groups was 32% and 31% respectively, above the hyperoxic controls. These values were somewhat less than that which resulted from prolactin administration to mice maintained in normal atmosphere.

Kidney weights are shown in Fig. 6. It is evident that kidney weights of lactating mice were increased over those of normal mice. Following 15 days of exposure to hyperoxia, the mean kidney weights of both normal and lactating mice decreased significantly below those of normal mice. When normal and lactating mice were concurrently injected with prolactin and exposed to hyperoxia, the mean weights increased to greater than those of the control group but were still less than those of lactating mice 15 days postpartum. There was no increase in normal female mice injected with prolactin, and not exposed to hyperoxia. The mean kidney weights increased in mice exposed to 24 and 48 hours of *hypoxia,* but had returned to normal after such exposure for 72 hours.

Discussion. Although prolactin stimulates erythropoiesis in polycythemic mice(3) and maintains the increased RCV observed at parturition in nonlactating postpartum mice(2), its relationship to erythropoietin secretion is unknown. Twenty-four hours of hyperoxia suppressed erythropoietin secretion in normal mice with subsequent decrease of Fe59 incorporation into erythrocytes and reticulocyte output. Despite exposure of lactating mice to 24 hours of hyperoxia, their plasma still contained considerable erythropoietic activity, suggesting that either other erythropoietic factors were present or erythropoietin had not completely disappeared. The rapid disappearance of erythropoietic activity from the plasma of 24-hour nonlactating mice suggests that the major part of the erythropoietic factor(s) present in the plasma of lactating mice must be secreted in the early postpartum. The erythropoietic activity of plasma collected from mice exposed to *hypoxia* for 24 hours was erythropoietically active but by the end of 72 hours of exposure to *hypoxia,* the plasma erythropoietic activity had disappeared, probably due to utilization of erythropoietin by the bone marrow(7,8). In comparison, lactating plasma remained erythropoietically active despite increased erythropoiesis.

Unlike lactating mice maintained at normal atmosphere, the RCV and postpartum reticulocyte count of lactating mice exposed to hyperoxia failed to rise early postpartum and plasma erythropoietic activity was lost by the 5th postpartum day. However, the RCV of these mice did not decrease rapidly within 24 hours of birth as was observed in nonlactating mice(2). These data suggest that the plasma of lactating mice contained erythropoietic factors which were not present in nonlactating postpartum mice. Since erythropoietin has an estimated half-life of 3-5 hours (9), one would expect that the hyperoxic environment would have eliminated the erythropoietic activity in the plasma of lactating mice by 24 hours. The cause of the continued elevation of plasma erythropoietic activity in

lactating mice exposed to 60% O2 is unknown. One may speculate that it may be due to an increase of erythropoietin binding protein with concomitant increased erythropoietin-plasma protein linkage and reduction of its degradation or to the presence of other erythropoietic substances, but experimental data are not available to support these suggestions.

The bone marrow of mice exposed to hyperoxia is extremely sensitive to erythropoietic stimulants(4) and all mice exposed to hyperoxia and injected with prolactin had a significant increase of RCV. Whether this erythropoietic response was the result of direct stimulation of the bone marrow by prolactin or if the prolactin stimulated the secretion of erythropoietin cannot be determined. The main site of erythropoietin production is the kidney and injection of prolactin into humans increases the glomerular filtration rate and renal plasma flow(10). The observed increase in mean kidney weights of lactating mice, of mice concurrently exposed to hyperoxia and injected with prolactin, and of mice exposed to hypoxia alone, suggests these stimuli act on the kidney and perhaps lead to increased secretion of erythropoietin. Preliminary experiments with bone marrow cultures show no direct effect of prolactin on total uptake or incorporation of tritiated thymidine, uridine or leucine (unpublished). This further supports the hypothesis that prolactin stimulates erythropoiesis by stimulating the production of erythropoietin.

It is evident that plasma volume is increased in lactation and following administration of prolactin. Normal female mice, nonlactating postpartum mice and those groups of mice exposed to hyperoxia, restored their PVs to lactational levels following prolactin injection, despite the absence of lactation. These findings suggest that the increased PV during lactation is due to the increased secretion of prolactin. The PV in normal mice, however, is not increased following exposure either to hypoxia, which specifically stimulates erythropoietin secretion, or after exposure to hyperoxia, which suppresses erythropoietin secretion. These data indicate that prolactin has an effect, either direct or indirect, on PV, independent of the action of erythropoietin.

Summary. Suppression of erythropoietin secretion in lactating and normal female mice by 60% oxygen decreased their RCV and reticulocyte output and abolished plasma erythropoietic activity. In normal mice and in nonlactating postpartum mice, administration of prolactin resulted in an increase of RCV and of PV to values equivalent to those of 15-day postpartum lactating mice. Administration of prolactin to lactating mice and to normal female mice concurrently exposed to hyperoxia prevented the decrease of RCV and of PV which resulted from exposure to hyperoxia alone. The data indicate that in mice, the prolactin preparation used acted as an erythropoietic stimulant and caused a plasma hypervolemia which was not related to the action of erythropoietin.

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