Canad. Med. Ass. J.
May 4, 1968, vol. 98

Endocrine Control of Maternal and Fetal Erythropoiesis

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FOR many years pregnancy was thought to be associated with anemia. However, it has been clearly demonstrated that with adequate diet, iron and vitamin supplementation, erythropoiesis is, in fact, accelerated during pregnancy. Reticulocytosis, bone marrow hyperplasia, an increased red cell mass, increased plasma iron turnover rates, utilization of ^{59}Fe by erythrocytes and increased erythropoietic activity in the plasma have been demonstrated in rodents[1-5] as well as in the human.[6-11] The decrease in peripheral hematological constituents is caused by expansion of the plasma volume which occurs between the third and fourth month of gestation[8] in the human and at mid-gestation in rodents.[4]

In view of increased erythropoietic activity during pregnancy, one might expect increased titres of erythropoietin in the plasma and urine of these patients. However, the erythropoietic content of urine collected from humans at various stages of gestation and specifically extracted for erythropoietin is minimal during the first half of gestation, and reaches a peak of activity between the 20th and 28th week. Thereafter, the erythropoietic content of urine extracts decreases to levels similar to those found in early gestation.[9] The increased erythropoietic activity found in plasma during late gestation,[9-11] despite the low amount of erythropoietin excreted in the urine at this time, is an interesting observation which may be related to several factors including altered metabolism and excretion of erythropoietin, increased tubular reabsorption or increased binding of this hormone to a plasma protein carrier. The situation would be analogous to the binding of thyroxin and corticosteroids to their protein carriers which takes place to an increased degree during pregnancy and which is associated with increased concentration of these transport proteins.

Probably a more important point to be considered is the presence of factors other than erythropoietin in the plasma of pregnant subjects which may augment the effect of the latter when such plasma is tested in the polycythemic mouse assay.[4] It is of interest that retroplacental blood collected from humans contained more erythropoietic activity than did peripheral blood.[12] This finding could be related to concentration at this site of factors which are of fetal or placental origin. Diffusion of these factors into the increased total blood volume would lead to dilution and the consequent decrease of observed activity. Separation of retroplacental plasma into components of different molecular weight on Sephadex G-100 reduced the sum of the erythropoietic activity of the components when compared to that of whole plasma. The activity was located in two fractions, one of which was related to a fraction which generally contains erythropoietin, and the other, of smaller molecular weight, immunologically identified as placental lactogen. The effect of whole plasma was much greater than that of the two fractions added together, indicating that a synergistic effect occurred when whole plasma was tested.[13] Placental lactogen, a protein hormone produced by syntrophoblastic cells, is found in maternal plasma as early as the sixth week and is present in high concentration from the second trimester until term.[14, 15] The concentration of this hormone increases when expansion of plasma volume first becomes evident in the human and when reticulocytosis and increased excretion of erythropoietin are at their maximum.[9]

One of the roles of placental lactogen during pregnancy appears to be to augment the action of erythropoietin on erythropoiesis. Alone, this hormone produced a small erythropoietic response in polycythemic mice,[16, 17] similar in magnitude to that produced by testosterone. This response was thought to be due to enhancement of small titres of endogenous erythropoietin in test animals by HPL,[4] since its action could be abolished by pretreating polycythemic mice with antisera to erythropoietin. In addition, its erythropoietic effect in hypophysectomized mice, which is greater than that in intact animals,[4] could be abolished when these mice were transfused to hematocrit levels of 75%.[18] When human placental lactogen was given to polycythemic mice in combination with erythropoietin, a synergistic response occurred, demonstrating the ability of this hormone to enhance the effect of erythropoietin. The experiments

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Supported by Grant MBT-1664 from the Medical Research Council of Canada.

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indicated that endogenous erythropoietin was required for placental lactogen to be effective.

The plasma volume of experimental animals was also increased following the injection of human placental lactogen,[4] but the mechanism of its action was not elucidated. Increased secretion and urinary excretion[19, 20] of aldosterone and high plasma levels of placental lactogen[14] and renin[21] are present during pregnancy. Since administration of human placental lactogen stimulates the excretion of aldosterone[22] and increased aldosterone production appears to be associated with an increased plasma volume, the hydremia of pregnancy could be either a direct effect of placental lactogen or an indirect effect through its action on aldosterone secretion.

Observations from experiments with animals demonstrate further the complex endocrine interrelationships which affect erythropoiesis during pregnancy. Erythropoietic plasma factors in pregnant mice are mobilized between implantation of the blastocyst and the attainment of endocrinological competence by the placenta around the tenth day of gestation. At this midgestational period reticulocyte counts are increased, the serum iron is decreased, and the erythropoietic content of plasma is slightly elevated. In the latter half of pregnancy the red cell mass increases and is associated with increased erythropoietic activity of the plasma.[2-4] With induction of pseudopregnancy in mice, a condition in which some of the endocrine changes of true pregnancy are simulated in the absence of syntrophoblastic tissue, no change of hematological parameters is found;[23] this suggests that trophoblastic tissue supplies or stimulates the production of factors which contribute to the control of erythropoiesis and to the accompanying increase of plasma volume which occurs both in humans[8] and rodents.[4]

The control of erythropoiesis during pregnancy is also complicated by increased titres of estrogens and perhaps other hormones. Estrogens have been shown to inhibit the utilization of erythropoietin by the stem cell, and large doses of erythropoietin are required to overcome the inhibition.[24, 25] The estrogenic inhibitory effect is partially prevented by progesterone compounds.[23] Pharmacological doses of estrogens appear to impair production of erythropoietin.[26] Estrogens also abolish the effect of human placental lactogen, but with the addition of progesterone to this combination, the erythropoietic effect of placental lactogen is preserved.[27] In pseudopregnant mice, where progesterone, estrogens and perhaps prolactin are present in increased concentrations, utilization of iron by erythrocytes is not impaired.[23] When the results of these experiments are combined, they demonstrate the complex interrelationship of various hormones which influence erythropoiesis during pregnancy, and it becomes apparent that a properly balanced ratio of hormones is required to maintain erythropoiesis. It is of interest that prolactin, a hormone biologically similar to human placental lactogen, also enhances, synergistically, the effect of endogenous erythropoietin[28] and is thought to influence erythropoiesis during lactation.[29, 30]

FETAL ERYTHROPOIESIS

Whether fetal erythropoiesis is initiated and maintained by a humoral regulator is not entirely clear. In mice, fetal erythropoiesis appears to be initiated in the absence of maternal erythropoietin,[31] indicating that the fetus either does not require erythropoietin or manufactures its own. Since de-embryonated chick blastodiscs form erythroblasts and synthesize hemoglobin in the apparent absence of a humoral regulator,[32] the initial differentiation of primitive mesenchymal cells into erythroid precursors may be entirely independent of an adult humoral factor. On the other hand, although chick blastodiscs incubated with erythropoietin respond by an increased synthesis of hemoglobin,[33] incubation at an early stage of the mouse yolk sac with erythropoietin produces no increase of heme synthesis.[34] These differences may reflect species variation in the ontogeny of erythropoietin control, since further studies have shown that when erythropoiesis is active in the liver during a later period of development of the fetal mouse, liver cells, obtained around the 14th day of gestation, are responsive to erythropoietin in an *in vitro* system, but the effect diminishes with the progression of fetal development[34] as the number of erythroid cells in the liver decreases with transference of erythropoiesis to the bone marrow. It is of interest that the mouse yolk sac, which produces only fetal hemoglobin,[35] is unresponsive to erythropoietin, while the fetal liver erythroid cell, producing adult hemoglobin,[36] is responsive to this hormone.

Other differences in embryonic erythroid tissue are also noted. Red cell carbonic anhydrase is not present in either the chick or mouse yolk sac and appears only in red cells arising from the bone marrow. At birth, the mouse cell contains only 10% of the red cell carbonic anhydrase of the adult and reaches a plateau level seven weeks after birth.[37] In man, the findings are similar and parallel those in the mouse in regard to their time relationships.[38]

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In neonatal rats, erythropoiesis *in vivo* was unresponsive to erythropoietin.[39] This failure to respond may indicate that other factors are required in the postnatal period to provide an optimal environment for the effective action of erythropoietin.

To date, the rather scanty experimental data appear to indicate that early embryonic erythropoiesis is independent of the adult humoral factor, erythropoietin. During the later stages of development the fetus appears to regulate erythropoiesis independently by production of its own hormone.

Observations in rodents are difficult to extrapolate to the human fetus which, at least during the later stages of development, produces erythropoietin as evidenced by detectable erythropoietic activity in the plasma of cord blood obtained from full-term infants.[40-44] It was evident as early as the 31st week of gestation,[44] when erythropoiesis is decreasing in the liver and increasing in the bone marrow. Hemoglobin concentration is increased early during fetal life and at birth,[45, 46] and erythropoiesis is accelerated as indicated by reticulocyte counts, erythroid hyperplasia of the bone marrow and increased turnover of iron. However, the number of erythroid precursors decreases in the early neonatal period[47] following the rapid increase of arterial oxygen saturation,[48] which immediately relieves the hypoxemia present at birth.[45, 48]

The decrease of erythropoiesis in the early weeks of life has been attributed by some authors to a failure of the bone marrow; others suggest that it represents an adjustment to an environment which is richer in oxygen and is associated perhaps with more effectual oxygen dissociation of fetal hemoglobin in the infant, and with the relatively increased red cell mass. The state of affairs is analogous to the descent of humans from high altitudes to a normal atmospheric pressure,[49, 50] known to be accompanied by suppression of production of erythropoietin and consequently the rate of erythropoiesis. The same changes are seen when polycythemic mice which were chronically exposed to reduced atmospheric pressure are returned to normal ambient pressure.[51, 52] This concept of reduced erythropoiesis due to increased oxygenation is also confirmed by the rapid disappearance of erythropoietin from the cord blood of normal infants early in the neonatal period, most likely owing to suppression of endogenous erythropoietin. Conversely, erythropoietic activity in the cord blood and amniotic fluid of severely anemic newborn infants and in the cord blood of infants who are hypoxic, secondary to congenital heart disease, remains increased during the first few weeks of life.[53] The latter group of hypoxic infants also maintain their red cell mass while normal infants show a drop of hemoglobin to levels below normal. These observations suggest that the human fetus is not only capable of producing erythropoietin as early as the 31st week of gestation, but is also capable of responding to it. The depression of erythropoiesis in the postnatal period would therefore be due primarily to improved oxygenation at birth. However, in the early months of infancy other factors may come into play which contribute to the eventual decrease in peripheral blood parameters.[47, 54, 55]

The physiology of initiation and control of erythropoiesis in the human fetus during early stages of development is a field which will provide fertile ground for experimentation in the future.

PLACENTAL TRANSFER OF ERYTHROPOIETIN

Transfer of fetal erythropoietin across the placenta to the mother may occur in the human;[12, 56, 57] this may or may not occur in the mouse.[28, 31] Hypertransfusion of pregnant mice and maintenance of an increased red cell mass throughout the gestational period appear to prevent an increase of reticulocytes in the mother. However, reticulocytes in pregnant mice are already depressed in late pregnancy and are further decreased by exposure to high oxygen environments. Despite this observation, some plasma erythropoietic activity persists and is not abolished by incubation with antisera to erythropoietin, indicating either that the erythropoietin is bound to a protein carrier which protects its antigenic sites or that other factors are present in this plasma which augment the effect of endogenous erythropoietin.[4]

In the human, reticulocyte counts are higher in the blood of mothers of severely anemic fetuses than in the blood of mothers of nonanemic fetuses.[56, 57] However, the degree of fetal anemia and the cord blood erythropoietin titre can not be correlated with maternal reticulocyte counts, and the erythropoietin content of the plasma of mothers of anemic infants is not significantly increased over that of mothers of normal infants.[57] Failure to demonstrate differences of plasma erythropoietin does not exclude placental transfer of fetal erythropoietin, since it may be greatly diluted by the expanded blood volume of the mother. It is not known whether transfer of maternal erythropoietin to the fetus occurs in the human.

Summary Increased erythropoiesis during pregnancy is predominantly controlled by erythropoietin. Such factors as placental lactogen may enhance the effect of endogenous erythropoietin. Factors such as estrogens may tend to inhibit the effect of erythropoietin on the bone marrow but complex physiological mechanisms are set in motion to balance the hormones, and the ultimate result is the maintenance of increased erythropoiesis. It is unlikely that increased erythropoiesis in very early pregnancy in the rodent is due to the placenta acting as a large arteriovenous fistula, causing decreased arterial oxygen saturation and the consequent increase of erythropoietin production. This possibility is not excluded in the human and may be a contributing factor, especially in the later stages of pregnancy. The expansion of plasma volume during pregnancy appears related to such factors as placental lactogen, perhaps through the stimulation of aldosterone secretion. Control of fetal erythropoiesis is even more complex and appears to be independent of maternal control. In the early stages of differentiation of the erythron, it may be entirely independent of any humoral control and considered to be an essentially wild type of growth from the primitive mesenchymal cell which is not subjected to adult regulatory mechanisms. In late fetal life, at least in the human, an erythropoietin-like hormone is produced and the fetal erythron is responsive to this humoral regulatory mechanism.

Résumé C'est l'érythropoïétine qui est le facteur principal en cause dans l'augmentation de l'érythropoïèse durant la grossesse. Certains facteurs, comme le lactogène placentaire, peuvent accroître l'effet de l'érythropoïétine endogène. Par contre, les estrogènes peuvent contribuer à inhiber l'activité de l'érythropoïétine dans la moëlle osseuse, mais des mécanismes physiologiques complexes sont mis en branle pour équilibrer le jeu des hormones, et ceci se traduit finalement par le maintien d'une érythropoïèse augmentée. Chez le rongeur, l'érythropoïèse est augmentée tout au début de la grossesse, mais il est fort improbable que ce phénomène soit attribuable au placenta qui agirait à l'instar d'une vaste fistule artérioveineuse, laquelle, en réduisant la saturation artérielle en oxygène, augmente la production d'érythropoïétine. Cette possibilité n'est cependant pas à exclure chez l'homme et peut constituer un facteur contributoire à l'hyperérythropoïèse, spécialement durant les derniers stades de la grossesse. L'expansion du volume plasmatique qu'on observe durant la grossesse est fonction de la présence de certains facteurs, comme le lactogène placentaire, peut-être par l'intermédiaire d'une stimulation de la sécrétion d'aldostérone. La régulation de l'érythropoïèse fétale est encore plus compliquée mais elle semble indépendante d'une régulation par l'organisme maternel. Aux premières phases de la différenciation de l'érythron, elle peut être entièrement indépendante de toute influence humorale et doit être considérée comme un type de tumeur échappant essentiellement aux règles normales et provenant du mésenchyme primitif, lequel ne dépend d'aucun mécanisme régulateur comme chez l'adulte. A la fin de la vie fétale, du moins chez l'être humain, une hormone apparentée à l'érythropoïétine apparaît et l'érythron fétal réagit à ce mécanisme régulateur humoral.

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