# Erythropoiesis Stimulating Agents: Clinical Pathology Effects in Rats Secondary to Extreme Erythrocytosis

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## ERYTHROPOIESIS

Erythropoiesis is the proliferation and progressive differentiation of hematopoietic stem cells into mature erythrocytes, and is primarily regulated by cellular oxygen levels.[1] Many cytokines, transcription factors and growth factors govern erythropoiesis. Erythropoietin is the principle hormone regulator of erythropoiesis, and is a glycoprotein hormone produced by renal peritubular interstitial fibroblasts in response to hypoxia.[1] Hypoxia upregulates erythropoietin gene expression by allowing accumulation of hypoxia inducible factor-1 (HIF-1), which binds to HIF response elements of the erythropoietin gene.[1] Erythropoietin stimulates colony-forming unit-erythroid cells to differentiate into rubriblasts. Continual proliferation, differentiation, synthesis of hemoglobin and iron accumulation of erythroid precursor cells leads to the formation of mature erythrocytes. Interleukin-3, stem cell factor, granulocyte-macrophage colony stimulating factor, among other cytokines, growth factors and transcription factors, also regulate erythropoiesis.[1]

**Stages of Erythrocyte Maturation**

Hematopoietic stem cells → → Colony forming units—erythroid → Rubriblast → Prorubricyte → Rubricyte → Metarubricyte → Reticulocyte → Erythrocyte

## ERYTHROPOIESIS STIMULATING AGENTS

Erythropoiesis stimulating agents (ESAs) are synthetic compounds that directly or indirectly mimic the actions of endogenous erythropoietin, and have been developed to treat anemia caused by chronic renal failure, chemotherapy and other etiologies. Several compounds are currently marketed for these purposes, including Procrit®, Epogen® and Aranesp®, although continual development of novel ESAs is currently underway to improve the safety, potency and half-life of these compounds. While many of the genes, growth factors and transcription factors could serve as potential targets to stimulate or enhance erythropoiesis, the two mechanisms which are most commonly utilized are:

**Recombinant erythropoietin administration**. Erythropoietin is highly conserved among species,[1] and exogenous administration of erythropoietin can result in stimulation of erythropoiesis in a variety of species.

**Hypoxia-inducible factor (HIF-1) prolyl hydroxylase inhibition**. These compounds act to increase erythropoietin levels by inhibiting the degradation and de-stabilization of the HIF-1 transcription complex.

## PRECLINICAL SAFETY STUDIES IN RATS

Normocythemic Sprague Dawley rats are commonly used in the preclinical safety assessment of novel ESAs. In preclinical safety studies with various ESAs, including recombinant erythropoietin and HIF-1 prolyl hydroxylase inhibitors, administration of the ESA to normocythemic rats results in the following expected pharmacologic effects:

**Increases in reticulocyte counts with resultant erythrocytosis**
These changes can be marked. Increases in erythrocyte mean cell volume (MCV) and decreased erythrocyte mean corpuscular hemoglobin concentration (MCHC) are also noted, and are associated with reticulocytosis. Reticulocytes are larger, and have a lower hemoglobin concentration than mature erythrocytes. Microscopic changes to erythrocytes associated with accelerated erythropoiesis may also be noted on blood smear evaluation, including increased polychromasia and the presence of Howell Jolly bodies. Hemoglobin crystals may also be noted, and can occasionally be abundant.

**Splenic extramedullary hematopoiesis and bone marrow erythroid hyperplasia**
Extramedullary hematopoiesis is primarily noted in the spleen, but can be noted in other tissues, including the liver.

## CLINICAL PATHOLOGY EFFECTS SECONDARY TO ERYTHROCYTOSIS

Effects on clinical pathology data secondary to erythrocytosis are commonly noted, particularly at higher dose levels and erythrocyte counts, and may include:

**Prolongations in prothrombin time and activated partial thromboplastin time (APTT)**
Most coagulation tests require citrated plasma with a 1:9 anticoagulant to blood ratio. The citrate concentration can have significant effects on coagulation times, and over-citrated samples may have reduced coagulation activity and prolonged coagulation times.[2] With erythrocytosis, plasma may become over-citrated due to displacement of plasma by erythrocytes. However, it should be noted that mortality and thrombotic events have been associated with ESA administration in preclinical species,[3] so careful evaluation of all correlative study data is critical.

**Increases in serum total bilirubin concentration**
Bilirubin is produced from the degradation of heme from senescent erythrocytes and heme-containing proteins by macrophages of the spleen, liver and bone marrow. Bilirubin is removed from circulation by the liver and kidneys.[4] Increased serum bilirubin concentration results when the rate of bilirubin production exceeds the rate of bilirubin clearance. With erythrocytosis, increased serum total bilirubin concentration likely results from increased erythrocyte degradation. It is unclear if erythrocyte lifespan is altered with erythrocytosis. Increases in serum bilirubin concentration typically lack microscopic correlates on histopathologic examination. *In vitro* hemolysis may be noted with increased frequency in animals with erythrocytosis, and may contribute to increased bilirubin concentrations, as well.

**Decreases in serum glucose concentration**
Glucose is the major energy source for mature erythrocytes in most species.[5] Glycolysis continues in blood cells *in vitro*, and lowers blood glucose. Blood glucose is expected to decrease by 5% to 10% per hour if blood cells remain in contact with plasma or serum. However, erythrocytosis will accelerate this process.[6] Animals with low serum glucose related to erythrocytosis typically do not display clinical signs of hypoglycemia.

**Increases in aspartate aminotransferase activity**
Aspartate aminotransferase (AST) is a cytoplasmic and mitochondrial enzyme that is found in hepatocytes, skeletal and cardiac myocytes and erythrocytes.[7] Because AST is present in erythrocytes, accelerated red cell turnover may increase AST activity. Increases in AST activity are typically minimal, and lack microscopic correlates on histopathologic examination.

**Decreased serum and plasma yields**
Plasma and serum yields are often reduced with erythrocytosis, and assays should be prioritized in the event of insufficient sample volume.

**Approximate Clinical Pathology Changes in Rats Secondary to Erythrocytosis Following ESA Administration**

| Representative Erythrocyte and Reticulocyte Increase Above Baseline or Controls | | | | |
|---|---|---|---|---|
| **Erythrocytes** | +40% | +50% | +60% | +80% |
| **Reticulocytes** | +50 to 100% | +100 to 150% | +200 to 350% | +200 to 400% |
| **Effects Secondary to Erythrocytosis** | | | | |
| **APTT** | +20 to 40% | +30 to 60% | +40 to 65% | +50 to 100% |
| **PT** | 0% or negligible | 0% or negligible | +10 to 15% | +15 to 20% |
| **Bilirubin** | +60 to 100% | +60 to 200% | +120 to 200% | +200 to 300% |
| **Glucose** | -30 to 50% | -40 to 70% | -50 to 70% | -50 to 70% |
| **AST** | +20 to 30% | +20 to 30% | +50 to 70% | +50 to 70% |

ESA: Erythroid stimulating agent; APTT: Activated partial thromboplastin time; PT: prothrombin time AST: Aspartate aminotransferase activity

## CONCLUSIONS

Erythropoiesis-Stimulating Agents (ESAs) are important tools utilized by physicians in the treatment of anemia resulting from chronic kidney failure, chemotherapy, certain treatments for Human Immunodeficiency Virus (HIV), and also to reduce the number of blood transfusions during and after certain major surgeries.[8] Continued development of novel ESAs is currently ongoing to improve the safety, efficacy, and administration of these compounds. Administration of an ESA to normocythemic rats results in erythrocytosis, which can be marked, particularly at higher dose levels or with repeated administration. Clinical pathology effects secondary to erythrocytosis are commonly noted, and include prolongations in coagulation times, increased serum bilirubin concentration, decreased serum glucose concentration, and increased AST activity. Identification of these secondary effects as a result of erythrocytosis and not as off-target compound-related effects are important in the accurate safety assessment and continued development of these compounds.

## REFERENCES

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