ERYTHROPOIESIS IN VITRO

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ABSTRACT

Various culture systems are now available to study erythropoiesis in vitro. These include suspension and cloning culture techniques. Suspension culture techniques are frequently used for studying biochemical changes in erythroid cells, while cloning culture techniques are suited for investigating the nature of erythroid stem cells. Some aspects of human erythropoiesis will be clarified by employing new methods such as the Marbrook diffusion chamber technique.

INTRODUCTION

Erythropoiesis has been studied using a number of in vivo and in vitro techniques. These studies have clarified many aspects of erythropoiesis—among them, the existence of erythropoietin as the major humoral regulator of erythropoiesis, and concepts surrounding erythroid stem cell proliferation and maturation.^{1,2} Certain biochemical changes in target cells induced by erythropoietin have been found^{3,4} but the site and mode of action of this hormone are poorly understood.⁵ Also, relatively little is known of the factors controlling pluripotent stem cell differentiation. The introduction of reliable in vitro techniques for assessing erythropoiesis has led to changes in our concepts of the pathogenesis of certain human erythropoietic disorders.

The purpose of this communication is to review the use of in vitro culture systems in the study of erythropoiesis and to discuss some insights obtained from recent studies.

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ESTABLISHED IN VIVO TECHNIQUES

1. *ERC (erythropoietin-responsive cell)*

The ERC can be defined as the cell which precedes the earliest recognizable erythroblast but differentiates into the erythroid cell line.¹ This concept was introduced by Gurney *et al.* in 1962 when they reported a method to study erythroid stem cell kinetics.⁶ In this method, irradiated mice were used as test animals. The mice were previously made polycythemic by hypertransfusion in order to suppress endogenous erythropoiesis. At varying times after irradiation, erythropoietin was injected into test animals. Forty-eight hours after the injection of erythropoietin, ⁵⁹Fe was administered intravenously. Seventy-two hours after the injection of ⁵⁹Fe, the animal were sacrificed and the erythropoietic response was assessed by the percentage of ⁵⁹Fe incorporated into newly formed red blood cells.

In general, suppression of erythropoiesis by hypertransfusion is sufficiently complete that no erythroblasts are seen in the spleen of the normal recipient mouse.⁷ After an injection of erythropoietin, pronormoblasts and normoblasts appear in the spleen within 24 and 72 hours respectively. A similar phenomenon is observed when the spleen of the polycythemic mouse is exposed to erythropoietin in vitro.⁸

Mice rendered polycythemic by hypertransfusion or extreme hypoxia are sensitive to exogenous erythropoietin and have little active erythropoiesis.^{9,10} The bioassay of erythropoietin or erythropoietic activity of test materials is conveniently performed with these animals.¹

2. *CFU-S (colony-forming unit, spleen)*

In 1961 Till and McCulloch described an in vivo colony-forming technique in which macroscopically visible spleen colonies were observed when a lethally irradiated mouse was injected with isologous bone marrow cells.¹¹ Mixed colonies of erythroid, granulocytic, and megakaryocytic cells were found in the spleen and the clonal origin of the colonies was demonstrated later by Becker, *et al.*¹² The spleen colony assay may be used to quantify the pluripotent stem cell in murine hematopoietic tissues.

HISTORICAL PERSPECTIVE OF IN VITRO CULTURE METHODS

In 1910, Carrel and Burrows first reported the culture of bone marrow, utilizing fibrin clots.¹³ In 1937 a simple method of suspension culture was described by Osgood and Brownlee in which medium supplemented with human

cord serum was used.¹⁴ In 1958 Matoth and co-workers reported a culture method in which bone marrow cells were suspended in plasma clots and the medium containing test material was added to the clots in culture tubes.¹⁵ The erythro-stimulatory effect of sera from anoxic patients on human marrow was shown by counting the number of early and intermediate normoblasts. In 1959 Reisner developed an organ culture system for bone marrow explants.¹⁶ A glass cylinder, sealed at one end by a cover glass, was used as a culture vessel. When human bone marrow was cultured on a tantalum gauze in this glass well, the continuous outflow of hematopoietic cells into the surrounding medium was observed microscopically over five months. Erythroblasts were seen during the first month. In the same year, Berman and Powsner reported a method of suspension culture in a roller tube.¹⁷

From the beginning of the 1960's, biochemical studies on erythroid cells started with the availability of crude erythropoietin preparations.¹⁸⁻²³

Long-term hemoglobin synthesis in vitro has been reported by Kraus,²⁴ Hall and Motulsky²⁵ in culture systems maintained at a low temperature (27°C), then by Gabuzda and Silver,²⁶ and recently by Wood²⁷ in cultures containing added erythropoietin.

In 1971, colony formation by erythroid cells in vitro was demonstrated by Stephenson *et al.* in a plasma clot culture system using mouse fetal liver.²⁸ The cell which gave rise to an erythroid colony in vitro was designated "CFU-E" to discriminate it from granulocytic and monocytic colony-forming units in vitro (CFU-C). In 1974, Iscove *et al.* reported that erythroid colony formation was also observed in culture containing methylcellulose as a supporting matrix.²⁹ Both techniques need added erythropoietin to allow the growth of erythroid colonies in vitro.^{28,29}

Liquid culture systems which have been developed in recent years by Wood³⁰ and Golde *et al.*³¹ will be dealt with in detail in this article, as well as other culture systems.

METHODOLOGY

The in vitro culture methods which have been developed recently can be classified simply into two types: suspension culture systems and in vitro cloning techniques.

a. Suspension Culture Systems

1. *Measurement of heme synthesis in vitro*^{19,32}

In 1963, Krantz, Gallien-Lartigue, and Goldwasser reported a culture method

that demonstrated an increased rate of heme synthesis in rat bone marrow cells incubated with erythropoietin.¹⁹

This short-term suspension culture (for up to 72 hours) is performed in Petri dishes where the cells are suspended in NCTC-109 medium supplemented with autologous or human serum (and, later, fetal calf serum) with or without erythropoietin. Four hours before the termination of culture, ⁵⁹Fe, preincubated with autologous serum to bind it to transferrin, is added to the plates. After the pulse with ⁵⁹Fe, the cells are lysed and hydrochloric acid is added to dissociate heme from the globin. Heme is extracted into cyclohexanone or other solvent and the radioactivity of ⁵⁹Fe incorporated into heme is counted in a gamma counter. The result is expressed as the rate of increased heme synthesis compared with control cultures.

This system is probably the prototype of all the other culture methods to study biochemical aspects of differentiating erythroid cells in the presence of erythropoietin.

2. *Measurement of hemoglobin synthesis in vitro*

Although the radioactivity of heme determined by the method of Krantz *et al.*¹⁹ is considered to be equivalent to that of hemoglobin,^{5,33} heme and globin synthesis in erythroid cells can be studied separately, utilizing other isotope tracers such as radioactive glycine, Δ -aminolevulinic acid (Δ ALA), leucine, and valine. The incorporation of these tracers into heme or globin can be analyzed precisely by the extraction of heme and by electrophoretic or chromatographic separation of hemoglobins and globin chains.

3. *Measurement of the uptake of radioactive iron, glucosamine, uridine, and thymidine*

The radioactivity of these tracers incorporated into intracellular nonheme iron,²³ the stroma,³⁴ RNA,²⁰ and DNA^{21,22} of erythroid cells, respectively, is measured after utilizing appropriate techniques for precipitation and extraction. ³H-thymidine incorporation is often expressed as the percentage of labelled erythroid cells on autoradiography.³⁵

4. *Other suspension culture techniques*

1. Wood described a method of human bone marrow culture in Leighton tubes containing medium supplemented with 50% AB serum and added erythropoietin.³⁰ The medium is changed every 48 hours and erythropoietin was added to the new medium. In this culture system, normoblasts at all stages of maturation are observed even after 10 days in culture. ³H-thymidine is used for autoradiography.

2. Golde *et al.* described a culture method that supports human erythro-

poiesis in vitro for periods up to two weeks.³¹ The Marbrook diffusion chamber employed in this method consists of a glass tube closed at one end by a dialysis membrane mounted in an Erlenmeyer flask. A cell suspension (3×10^6 cells in 1 ml of medium) is incubated in the glass tube with added erythropoietin. The flask is filled with 50 ml of McCoy's 5A medium supplemented with 20% fetal calf serum. The medium is not changed during culture. Proliferation and maturation of nucleated erythroblasts are assessed by trypan blue viability determinations, differential counts, and ^3H -thymidine labelling indices.

b. In Vitro Cloning Techniques

1. CFU-E (*colony-forming unit, erythroid*)

1. Plasma clot technique^{28,36,37}

One ml of cell suspension is added to a Petri dish containing 0.1 ml of citrated bovine plasma and the mixture is allowed to clot. The medium contains added erythropoietin and other additional requirements. Cultures are incubated at 37°C in humidified 5% CO₂ in air, usually for 2 days (murine cells) or 7-9 days (human cells). After incubation, the plasma clots are removed from the culture dishes and spread out on a glass slide for staining and counting. Colonies of eight or more cells showing a positive hemoglobin reaction are scored.

In 1974, McLeod *et al.* reported an improved plasma clot system with the use of disposable microtiter plates.³⁶ The required amount of the medium per well is one-tenth of that in the original method.

2. Methylcellulose culture techniques²⁹

Murine or human hematopoietic cells are mixed thoroughly in a tube containing erythropoietin and α medium supplemented with 0.8% methylcellulose, 30% fetal calf serum, and 1% bovine serum albumin. A total volume of 1 ml is placed in a Petri dish (35 mm). Erythroid colonies containing eight or more cells are counted directly under an inverted microscope after the same periods of incubation as mentioned above for a plasma clot culture. Erythroid colonies are easily distinguished from granulocytic colonies in cultures of murine cells, but in the case of human cells the discrimination of colonies may be difficult. Therefore, if necessary, the adherent cells are removed before plating. When desired, individual colonies may be removed, then fixed and stained on a glass slide.

2. BFU-E (*erythropoietic burst-forming unit*)

Recently, Axelrad *et al.* observed, using the modified plasma clot culture system, the formation of "bursts" in cultures of mouse bone marrow exposed to high concentrations of erythropoietin for four days.³⁸ These consisted of a large

number of erythroid cells which appeared after six days of culture and reached a plateau at 7–9 days. The cell which gave rise to a “burst” was named BFU-E.

On the other hand, using the methylcellulose culture system, Iscove and Sieber reported that the addition of a large amount of purified erythropoietin in a single dose also resulted in the formation of macroscopic erythroid colonies by the tenth day in culture of mouse bone marrow.³⁰

c. Significance of CFU-E and BFU-E

It has been suggested that there are several stages of differentiation between CFU-S and CFU-E^{40,41} and the relationship of these two components is not as close as that of CFU-S and CFU-C,⁴² but rather CFU-E may be the same population as ERC in vivo.⁴¹ Hypertransfusion decreases the number of CFU-E but not BFU-E.³⁸ BFU-E has a slower sedimentation velocity than CFU-E.³⁸

These findings suggest that BFU-E may be a precursor of CFU-E in vivo, although further studies will be needed to characterize its properties.

It has been reported that the addition of sulfhydryl compounds increases plating efficiency markedly in a methylcellulose culture of mouse bone marrow.³⁹ However, the effect seems to depend on whether the cells require these compounds or not.⁴³

INSIGHTS GAINED FROM IN VITRO STUDIES

1. *Normal erythropoiesis*

It is now obvious that erythropoietin is the primary regulator of erythropoiesis in higher organisms.¹ The production of this hormone is stimulated under conditions that create tissue hypoxia and is suppressed under opposite conditions. Whether the hormone exists in renal tissue in an inactive form binding to a lipid inhibitor or exists as erythrogenin which catalyzes the conversion of a plasma globulin precursor into erythropoietin is controversial.^{1,44}

Many other hormones and substances have been known to modulate erythropoiesis in vivo.^{1,45,46} Among them, androgens have been investigated intensively because of their therapeutic value in the treatment of certain human anemias.

Androgens and certain 5 β -H steroids are known to exert an effect on erythropoiesis in two different ways: stimulation of erythropoietin production and a direct effect on hematopoietic tissue.^{47–54}

Stimulatory effects of androgens on erythropoiesis have been reported utilizing in vitro culture techniques. Reisner observed that testosterone stimulated erythropoiesis using an organ culture technique.⁵⁵ Stimulation of DNA synthesis by testosterone was demonstrated in suspension cultures of human bone marrow

using ^3H -thymidine.⁵⁶ Increased rates of heme^{49,53} and globin⁵³ synthesis by 5β -H steroids were shown utilizing the method of Krantz *et al.* and a similar suspension culture method. Increased synthesis of an RNA which had a similar sedimentation coefficient to globin messenger RNA was reported in cultures of human fetal liver cells incubated with testosterone for five hours, followed by a one-hour pulse with radioactive uridine.⁵⁷ These reports imply that 5β -H steroids actually modulate the proliferation or maturation of erythroid cells in vivo.

While the number of CFU-E has been reported to increase under the influence of androgens,⁵⁸⁻⁶⁰ an effect of some steroids with 5β configuration on CFU-S was also suggested by Byron using the ^3H -thymidine suicide technique.⁶¹ His data suggested that these steroids may trigger CFU-S into cycle or shorten cell cycle times. This idea has been supported by other investigators. Moriyama and Fisher reported that the stimulatory effect of testosterone administered to rabbits was suppressed by busulfan which is known to block the inflow of CFU-S into ERC.⁵⁰ Singer and Adamson recently reported that etiocholanolone-stimulated CFU-E had little sensitivity to a suicidal dose of ^3H -thymidine while erythropoietin- and fluoxymesterone-stimulated CFU-E were very sensitive.⁶² This finding suggests that different steroids may act on different stem cell subpopulations.

Prostaglandins and cyclic nucleotides have been known to stimulate erythropoiesis in vivo.⁶³⁻⁶⁹ The effect is probably due to stimulation of erythropoietin production or some erythropoietin-dependent processes because nephrectomy⁶⁹ or the injection of anti-erythropoietin^{63,66,67,69} blocks the effects of these substances. It has been reported that prostaglandins augment the effect of erythropoietin in vitro by increasing the rate of heme synthesis and glucosamine incorporation into the stroma.⁶⁴ Cyclic AMP and dibutylic cyclic AMP (dbcAMP) have been reported to increase the rate of ΔALA synthesis in rat bone marrow cells.⁷⁰ Heme synthesis has also been shown to be stimulated by dbcAMP in cultures of human bone marrow.⁷¹ These data suggest that the stimulation of erythropoiesis in vivo by these substances may be in part due to direct effects on hematopoietic tissue.

2. Erythropoietin action

Erythropoietin is a glycoprotein hormone with a molecular weight of about 46,000.³ Erythropoietin acts on erythropoietin-responsive cells (ERC) to induce proliferation and differentiation along the erythroid cell line, but it also acts on early and possibly even late erythroblasts to accelerate their maturation.^{5,72} An effect of the hormone on the microvascular system of hematopoietic tissue has also been suggested by studies using in vivo microscopic techniques.⁷³

Biochemical changes induced by erythropoietin in ERC have been investigated using in vitro culture systems.³ The earliest event is a synthesis of a

type of RNA (150S) restricted to the nucleus. Following increased synthesis of DNA, about six hours (10 hours in cells from plethoric animals) after erythropoietin is added to culture, an increase in hemoglobin synthesis becomes evident. These findings have been derived mainly from cultures of rat bone marrow³ but fetal liver of rodents has also been used frequently in this area of study since fetal liver contains relatively high percentages of erythroid precursors capable of responding to erythropoietin *in vitro*.⁷⁴ In cultures of mouse fetal liver, it was reported that addition of erythropoietin caused an increase in DNA synthesis within 20 minutes and in RNA and hemoglobin synthesis in two hours.²² Terada *et al.* reported that there was a 6- to 10-fold increase in globin messenger RNA activity by 10 hours after erythropoietin was added to cultures of mouse fetal liver.⁷⁵

Since many hormones are known to act via specific receptors,⁷⁶ it is most likely that erythropoietin requires receptors to initiate its action. Surface protein receptors to this hormone were postulated by Chang *et al.*⁷⁷ Erythropoietin action may not be dependent on the cyclic AMP system.⁷⁸ However, it was reported recently that erythropoietin raised intracellular concentrations of cyclic GMP in a time- and dose-dependent manner, while cyclic AMP content did not change in cultures of rat fetal liver.⁷⁹

An active cytoplasmic fraction of a protein nature capable of stimulating RNA synthesis in marrow cell nuclei was recently extracted from erythropoietin-treated rat marrow cells by Chang and Goldwasser.⁸⁰

3. Regulation of hemoglobin synthesis

Hemoglobin synthesis is the primary function of differentiating erythroid cells. Both proliferation and maturation of erythroid cells are triggered by erythropoietin, but there is no clear evidence that this hormone increases the rate of hemoglobin synthesis per erythroid cell.^{1,4} Recently, Glass *et al.* reported that erythropoietin stimulated hemoglobin synthesis of murine erythroid cells which were fractionated by velocity sedimentation, at all stages of maturation.⁷²

The "hemoglobin switch" that is seen in certain sheep and goats under erythropoietic stress has been studied in recent years by using new *in vitro* culture techniques.⁸¹⁻⁸³ Utilizing the modified method of Krantz *et al.*, Adamson and Stamatoyannopoulos showed direct evidence that erythropoietin mediates the hemoglobin A→C switch of sheep.⁸¹ They observed that hemoglobin C was synthesized preferentially in the bone marrow culture of sheep with hemoglobin A, stimulated with erythropoietin in a dose-dependent fashion and this effect was blocked by anti-erythropoietin. Barker *et al.* reported that in short-term suspension culture in the presence of erythropoietin, goat bone marrow began

to synthesize β^c chain after 48 to 72 hours when β^A chain synthesis was falling off.⁸² Barker and co-workers also cultured goat bone marrow in the plasma clot system of McLeod *et al.*⁸³ They observed that higher concentrations of erythropoietin were needed to induce β^c globin synthesis as compared with the amounts of the hormone required to grow erythroid colonies.

The culture method of Wood has been used to study hemoglobin synthesis in vitro.^{27,84} It was shown by Shchory and Weatherall that α , β , and λ chain synthesis of human fetal liver were maintained for 12 days with a constant ratio of $\alpha/\beta + \lambda$ up to eight days.⁸⁴ Wood reported that adult bone marrow continued to synthesize hemoglobin A for up to 12 days in the presence of erythropoietin while hemoglobin F synthesis in vitro by adult marrow was not demonstrated.²⁷

The precise mechanisms controlling differential gene expression in erythroid cells will be investigated further utilizing in vitro culture techniques.

4. Human erythropoietic disorders

1. Pure red cell aplasia

The autoimmune nature of this disease has been suggested by many investigators.⁸⁵⁻⁸⁸ By utilizing the method of Krantz *et al.*, an inhibitor of heme synthesis has been found in the plasma of patients with pure red cell aplasia.^{86,88} An anti-erythropoietin-like substance reported by Jepsen and Lowenstein in the plasma of a patient with panmyelopathy⁸⁹ may be found in some cases of pure red cell aplasia as Krantz indicated.⁹⁰ In fact, a serum IgG antibody to circulating erythropoietin was demonstrated recently by Peschle *et al.* in one patient with this disease.⁹¹

2. Aplastic anemia

This disease has been postulated to be due to an abnormality of the pluripotent stem cell or an altered microenvironment surrounding hemopoietic cells because aplastic anemia is characterized by pancytopenia of varying degrees of severity.⁹² The defects of genetically anemic W/W^v and S1/S1^d mice exist in the stem cell and the microenvironment respectively,¹ although in man there is no clear evidence for a microenvironmental defect as a cause of aplastic anemia.

By utilizing the culture system of Krantz *et al.*, two types of response were observed in patients with bone marrow failure.⁹³ Some marrows responded normally with an increased rate of heme synthesis while other marrows did not respond at all. However, recovery of responsiveness was observed in the non-responsive group when the patients improved clinically. Subnormal responses to erythropoietin were also reported in patients with aplastic anemia utilizing the same culture system.⁹⁴ No erythroid colonies were found to grow in plasma clot cultures of bone marrow from six patients with congenital and acquired aplastic

anemia.⁹⁵

These observations do not explain the nature of this disease because no heme synthesis or colony formation could occur with either a markedly decreased number of erythroid progenitor cells or a complete block of their responsiveness to the hormone.

In patients with Diamond-Blackfan syndrome it was reported that erythroid colonies grew even when the bone marrows had less than 1% identifiable erythroblasts although the numbers of CFU-E were below normal.⁹⁶ CFU-E were normal in patients treated with prednisone. The authors suggested that decreased responsiveness of erythroid progenitor cells to erythropoietin can be modified by corticosteroids but clinically the response to corticosteroids is known to vary in this disease.⁹⁷ Therefore, in some cases another explanation may be required. In connection with this, it was demonstrated by Golde *et al.* that corticosteroids augmented the action of erythropoietin and increased the number of CFU-E in cultures of mouse fetal liver and adult bone marrow of both mouse and man.⁹⁸

3. Other anemias

The anemia of chronic renal failure is caused by many factors such as hemolysis, bleeding tendency, and impaired production of erythropoietin.⁹⁹ An inhibitor of heme synthesis was found in the plasma of uremic rabbits by using the method of Krantz *et al.*¹⁰⁰ The increased number of CFU-E in the bone marrow of uremic rabbits and normal response of the CFU-E to erythropoietin *in vitro* were reported by Moriyama and Fisher.¹⁰¹ These findings may have bearing on the treatment of uremic patients. The suppression of erythropoiesis in these patients is partially ameliorated by androgens. Perhaps a technique can be developed to remove the plasma inhibitor. It was reported that testosterone enanthate increased erythrocyte 2,3-diphosphoglycerate in patients with chronic renal failure on hemodialysis.¹⁰² This effect results in enhancement of oxygen supply to the tissues by shifting the oxygen dissociation curve to the right. The pathogenesis of anemias associated with neoplasmas, inflammation, and other chronic diseases may be clarified by *in vitro* studies.

4. Polycythemia vera

In contrast to secondary polycythemia, augmented erythropoiesis in polycythemia vera is not associated with increased plasma or urinary erythropoietin levels.¹⁰³ Although the autonomous nature of this disorder has been postulated,^{104,105} it has been known that erythropoiesis in polycythemia vera is still under a humoral control *in vivo*.^{106,107}

Using a short-term suspension culture technique, Krantz reported that bone marrows of patients with polycythemia vera responded poorly to erythropoietin

while a normal response in terms of heme synthesis was seen in patients in remission after busulfan therapy.¹⁰⁸ This finding in vitro has been supported by other investigators.^{106,109} However, it has been demonstrated recently by Golde and Cline that polycythemic marrows responded to added erythropoietin in cultures maintained for up to 18 days in diffusion chambers.^{110,111} It was also shown that without added erythropoietin, erythropoiesis was more marked and prolonged as compared with normal marrows. The discrepancy in the results gained from these two different types of culture system may be explained by the difference in incubation time. In the method which Krantz employed, the cultures were harvested within 72 hours.¹⁰⁸ On the other hand, when polycythemic marrows were cultured in the in vitro diffusion chamber system, the stimulatory effect by erythropoietin was not obvious until after three days.^{110,111}

Prchal and Axelrad cultured polycythemic marrows in a plasma clot system in which some erythroid colonies formed without added erythropoietin.¹¹² However the majority of CFU-E were shown to be dependent on erythropoietin. Zanjani *et al.* demonstrated that the "endogenous" colony formation was completely blocked by anti-erythropoietin.¹¹³ This finding suggests at least that all the CFU-E of polycythemic marrows are dependent on erythropoietin.

Myelostimulatory factors in the plasma of patients with polycythemia vera also have been investigated.¹¹⁴

Recently, strong evidence was presented by Adamson *et al.* that polycythemia vera is a clonal stem cell disorder similar to chronic myelocytic leukemia.¹¹⁵ Only the A-type isoenzyme of glucose-6-phosphate dehydrogenase was found in red cells, granulocytes, and platelets of two female patients with polycythemia vera who had A and B type isoenzyme in fibroblasts and lymphocytes.

The nature of erythroleukemia and erythroblastic crisis of chronic myelocytic leukemia may be clarified by in vitro studies. Clinical observations suggest that erythropoiesis of the former may be dependent on erythropoietin^{116,117} and that of the latter may not.¹¹⁸

SUMMARY AND CONCLUSIONS

The culture methods and insights gained from in vitro studies have been reviewed in regard to erythropoiesis.

Biochemical changes of differentiating erythroid cells have been found by employing short-term suspension culture system. Proliferative aspects of erythroid progenitor cells can be studied most readily by using in vitro cloning techniques. Some suspension culture systems which have been developed in recent years appear to be suited for studying both proliferative and maturational aspects

of erythropoiesis.

The results obtained from in vitro studies should be cautiously interpreted because of the limitations of the culture system used.

It is hoped that in vitro culture techniques will be used more widely and frequently and that insights gained from these studies will shed light on clinical problems of human erythropoietic disorders.

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