Humoral Regulation of Erythropoiesis VII. Shortened Survival of Erythrocytes Produced by Erythropoietine or Severe Anemia. (26784)

FREDERICK STOHLMAN, JR. (Introduced by George Brecher)
National Institute of Arthritis and Metabolic Diseases, Nat. Inst. of Health, Public Health Service, U. S. Department of H.E.W., Bethesda, Md.

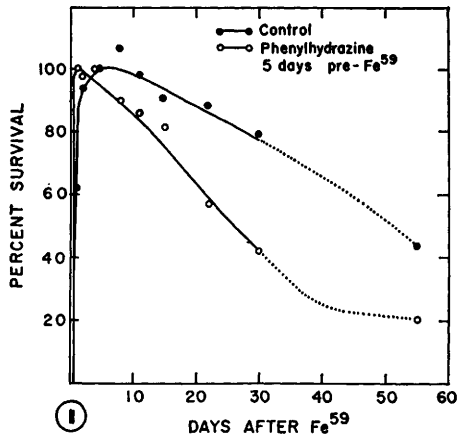
After severe blood loss or destruction of the entire red cell mass with phenylhydrazine, the hemoglobin in the rat will return to pretreatment levels within 10-15 days. Thereafter there is continued production at about normal levels as estimated by reticulocyte counts and iron incorporation studies. At this time in the phenylhydrazine treated rat there are no cells older than 15 days so that there is no senescent loss; senescent loss must also be strikingly reduced in the bled animals. Polycythemia, however, does not ensue. These observations suggest that the mean life span of red cells formed in response to severe anemia is shortened. A shortened life span of red cells produced in response to acute blood loss has been reported by Berlin and Lotz(1).

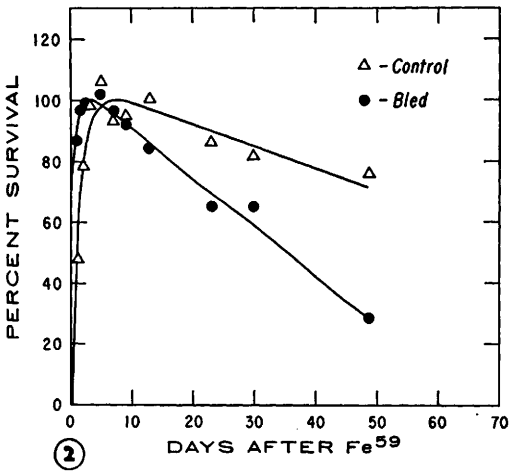
Erythropoietine is believed to be the stimulus for increased red cell production in both bled and phenylhydrazine treated animals. It therefore seemed likely that cells produced in response to erythropoietine would also have a shortened red cell survival. Data to document this hypothesis are presented here.

Materials and methods. Female Sprague-Dawley rats were used throughout; test animals weighed 135-160 g at beginning of the experiment; donor rats weighed 200 g or more. Phenylhydrazine was given in doses of 4 mg/100 g on days 0, 1 and 3. In the experiments on acute blood loss, rats were bled by cardiac puncture 1.5% of body weight on days 0 and 1. The animals receiving erythropoietine were transfused with packed red cells obtained from heparinized normal blood 4 days prior to the first injection of erythropoietine; each animal was given a volume of red cells equivalent to 1.65% of body weight. In the first experiment 5 subcutaneous injections of 0.5 cc of erythropoietine containing ~ 10 C.S.(2) units were given at 12 hour intervals. In a second experiment 0.5 cc of erythropoietine containing ~ 10 C.S. units was given subcutaneously on 3 occasions 24 hours apart. Erythropoietine was obtained by alcoholic extraction of urine from a patient with congenital hypoplastic anemia.

Measurement of survival. Plasma bound Fe^{59} was injected intravenously as previously described(3); 3 hours and at daily intervals thereafter 5 mg of non-radioactive iron (Imferon) was given intramuscularly to minimize reutilization of Fe^{59}. Fifty treated and 50 control animals were randomized for each experiment. Five animals were used to determine each point. Twenty minutes prior to exsanguination washed Cr^{51} tagged normal cells were injected intravenously for blood volume determination(3). Fe^{59} and Cr^{51} counts were separated with a single channel spectrometer. Total red cell Fe^{59} activity was determined(3) and expressed as the per cent of injected dose. These values were plotted against time and per cent survival was determined using the peak Fe^{59} incorporation value as the 100% value. Red cell counts were done electronically(4); hematocrits were measured by Strumia's method(5) and reticulocytes were counted by Brecher's method(6).

Results. 1. Phenylhydrazine. Radioiron was given 2 days after the last injection of phenylhydrazine. At that time the reticulocyte count was ~ 75%, the peak reticulocytosis of 90-100% occurring on the 6th-7th day. The hematocrit was ~ 20, with a mean corpuscular volume (MCV) of 100 μ^3 (normal ~ 55 μ^3). The survival of the cells tagged at this time was clearly shortened (Fig. 1). In a second experiment, Fe^{59} was injected on the 10th day when the hematocrit was ~ 40 and the reticulocytes ~ 3.0%. Degree of shortening of red cell survival was less than when cells were labeled on the 5th day; for example on the 20th day after Fe^{59} there was ~ 80% survival compared with





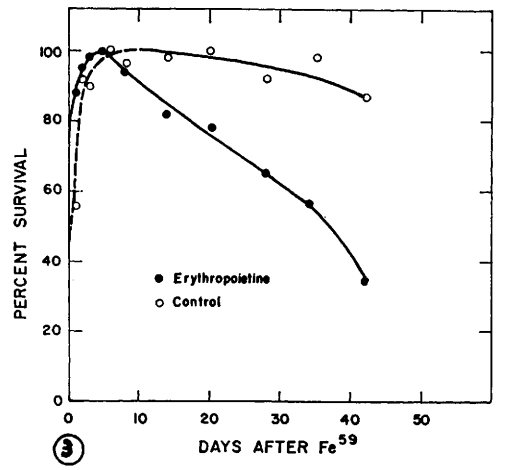


FIG. 1. Survival of red cells produced in response to phenylhydrazine induced anemia. Radioiron was given 5 days after first inj. of phenylhydrazine.

FIG. 2. Survival of red cells produced in response to acute bleeding.

~ 60% survival in the group given Fe^{59} on the 5th day after phenylhydrazine and ~ 90% in the control group.

2. Bled Animals. The animals were injected with Fe^{59} 48 hours after the initial bleeding. At this time the hematocrit was ~ 25, the MCV ~ 61 μ^3 and reticulocytes ~ 10%. The following day the reticulocytes were ~ 14%; peak reticulocytosis of ~ 21% was reached on the 6th day after bleeding. The period of survival of the red cells produced in response to this severe blood loss was also shortened (Fig. 2).

3. Erythropoietine Treated Group. At time of injection of erythropoietine the hematocrit of recipient animals was ~ 55 (normal 40-44) and reticulocytes 0.1-0.2% (normal 1-3%). Five injections of erythropoietine produced a reticulocytosis of 10-12% on the 4th day, an increase in hematocrit to ~ 60 and in the MCV to ~ 61 μ^3. After erythropoietine was stopped, reticulocyte values returned to 0.1-0.2%. Thereafter the hematocrit and blood volume slowly decreased until they returned to normal at ~ the 28th day, when the reticulocyte count was normal. Radioiron was given 12 hours after the last injection of erythropoietine. The survival of cells tagged at this time was significantly shortened (Fig. 3). In another experiment 3 injections of erythropoietine were given at 24 hour intervals and Fe^{59} was given 24 hours after the last injection of erythropoietine. There was a reticulocytosis of ~ 4% associated with a borderline shortening of red cell life span.

Discussion. There was a shortened life span of red cells formed in response to severe anemia, whether due to phenylhydrazine or blood loss. A similar effect was demonstrated with erythropoietine, which is known to be in part responsible for the increased red cell production in severe anemia. The use of hypertransfused animals, which reduced normal production to a minimum, permitted us to study primarily the life span of cells produced in response to erythropoietine. Values for the control animals were comparable to

FIG. 3. Survival of red cells produced in response to erythropoietine.

those observed by Berlin and Lotz with C^{14} glycine(1) and Harriss and Belcher with Fe^{55} followed by Imferon(7). There was a variable degree of random destruction between experiments in control groups but the curves for the experimental groups shown in Fig. 1-3 were well outside the normal range observed in these and 5 other experiments in our laboratory.

Van Dyke and Berlin(8) have previously published data which led them to conclude that cells produced in response to erythropoietine had a normal life span. The apparent discrepancy between their conclusions and ours might be explained by a dose effect. However, their data are not inconsistent with ours. They induced polycythemia by administration of erythropoietine over a 10 day period, hematocrits rising from 40 to 55. Midway in the period of treatment, C^{14} labeled glycine was given, and *specific activity of heme* determined thereafter. Specific activity reached a maximum by the 10th day and thereafter remained constant for the next several weeks. This led them to conclude that the life span of the cells was normal. In fact after erythropoietine was discontinued, red cell production should have stopped due to the induced polycythemia. At this point the labeled cells produced in response to erythropoietine would have been 0-10 days old. Senescent loss of older cells formed prior to administration of erythropoietine should have continued. Due to the polycythemia-induced suppression of erythropoiesis, these cells would not be replaced. If the survival of labeled cells was normal, the loss of cells with *unlabeled heme* without replacement would result in a constant increase in the proportion of labeled cells and hence an increase in the *specific activity* of heme. When erythropoiesis is suppressed the specific activity of heme can remain constant only when loss of labeled cells is equal to loss of unlabeled cells. This must have occurred in the studies reported by Van Dyke and Berlin and implies a shortening of the life span of cells formed in response to erythropoietine.

Erythropoietine acts primarily through differentiation of stem cells into the erythroid compartment(9,10). After large doses of erythroypoietine, the magnitude and rapidity of the response suggest that the differentiated cells either skip divisions or that generation times are shortened(11). In either event the result is the release of a "younger" reticulocyte which is much larger than the normal reticulocyte(12,13). In the phenylhydrazine treated animals, for example, MCV was 100 μ^3 in contrast to the normal of 55 μ^3. In the bled and erythropoietine treated groups the shift in MCV was less marked but normal cells were still present in these animals. To shift the MCV from 55 μ^3 to 61 μ^3 by introducing a population of macrocvytes amounting to 10% of the total population requires that these macrocytes have an average MCV approaching 100 μ^3. Size distribution studies bear out the increased size of these newly formed cells(12). It may be that these macrocytes have a shortened cell survival due to their size and shape much as do spherocytes in hereditary spherocytosis. The rapid rate of formation of cells may also lead to other defects which affect life span.

It is not possible to state whether production of the large cells is a dose-dependent phenomenon. The dose relationship is being investigated. If erythropoietine invariably results in production of large cells, this strongly supports our argument that erythropoiesis is not under a single control. Though data presented on the effect of high doses of erythropoietine do not offer conclusive proof they are in agreement with the hypothesis that red cell production is regulated by more than one mechanism, erythropoietine being a panic mechanism(14).

Summary. Red cells formed in response to erythropoietine or severe anemia have a substantially shortened life span. The possibility is proposed that this is related to the macrocytic character of the cells. The results are in agreement with the hypothesis that erythropoietine is not the sole regulator of red cell production.

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Received May 29, 1961. P.S.E.B.M., 1961, v107.

Reticulocyte Size and Erythropoietic Stimulation. (26785)

GEORGE BRECHER AND FREDERICK STOHLMAN, JR.

National Institute of Arthritis and Metabolic Diseases, Nat. Inst. of Health, Public Health Service, U. S. Department of H.E.W., Bethesda, Md.

It is generally accepted that reticulocytes are larger than mature red cells(1). The manner in which the oversized reticulocyte matures into a red cell of normal size has not been defined. Recently, Stohlman found that red cells produced during recovery from severe phenylhydrazine anemia have a markedly shortened life span(2). The mean corpuscular volume of the reticulocytes so produced was nearly twice that of normal mature red cells. The results suggested that these macrocytic cells are replaced by cells of more normal size. This thesis was confirmed in the present studies by measuring the distribution of cell sizes; the results indicate that a correlation exists between rapidity of red cell regeneration and size of reticulocytes.

Methods. Groups of female Sprague-Dawley rats weighing 140-160 g were injected s.c. with either 0.1 or 0.3 ml of a 2% solution of phenylhydrazine hydrochloride (PHH) in distilled water on days 0, 1, and 3 of the experiment. A third group served as controls. In one experiment, one group of animals was injected with 0.3 ml PHH on days 0, 1, and 3, then given 650 r whole body irradiation on day 4. Rats were exsanguinated by cardiac puncture at various intervals following last injection.

Mean corpuscular volumes (MCV) were determined from hematocrits measured by a micro method and red cell counts measured with the Coulter Counter(3). Red cell size distribution curves were obtained with a Coulter Counter Model B supplied with an automatic graphout. The Model B is a modification of the basic Coulter instrument which generates impulses proportional to cell volume as individual red cells pass through a 50 micra aperture. An electronic gating device of the Model B* permits discrimination of impulses of different magnitude, corresponding to different cell volumes. The graphout automatically records their frequency distribution. In the resulting graphs, the ordinate represents relative frequencies. The linear scale of the abscissa corresponds to the amplitude of the electronic impulses. The proportionality factor between amplitude of impulses and cell volume can be determined by appropriate calibration and units on the abscissa converted to μ^3. This has been done in Fig. 1. The peaks or modal values of distribution curves are, as a rule,

* Available from Coulter Electronics, Inc., Chicago, Ill.