Responsiveness of Hematopoietic Tissue to Erythropoietin in Relation to the Time of Administration and Duration of Action of the Hormone

By John C. Schooley

The cells of the blood and bone marrow are maintained in a steady state in spite of the fact that most of these cells are not self-maintaining populations. An enormous proliferative capacity is required to maintain this steady state. It has been postulated that there exists a self-maintaining population of stem cells in the bone marrow. The stem cell or stem cells differentiate and subsequently, through proliferation, give rise to the various blood cells.

Although the identities of the stem cells are unknown, various investigators have proposed kinetic models which can explain and predict some characteristics of the stem cell population. Osgood and Lajtha and co-workers have published thorough discussions of their theoretical models of stem cell kinetics.

The responsiveness after mild irradiation of the hypertransfused mouse to a standard dose of the hormone erythropoietin has been utilized by Gurney, Lajtha and Oliver as an experimental system to test their theoretical model. Various aspects of this experimental system for investigating stem cell kinetics have been discussed by Gurney. He points out that since no reliable simple method for quantitating the erythropoietic response exists, and since the challenge of the stem cells by erythropoietin cannot be instantaneous, the usefulness of their experimental model is seriously limited. The first of these limitations is primarily the time and effort required for more precise measurements. The second limitation, however, is more serious and is due to the fact that erythropoietin has a prolonged biologic half-life, and that an effective concentration of erythropoietin must exist. Thus, in studies of the stem cell population utilizing the hypertransfused mouse, it is likely that the stimulating dose of exogenous erythropoietin generally used acts during the entire test period, whereas conditions within the stem cell population and the developing erythroid population are changing as a result of the initial action of erythropoietin.

The recent development of an immune serum which can neutralize the biologic activity of erythropoietin provides a means of limiting the availability of the stimulating dose of erythropoietin in the intact animal. In the present experiments, changes in the responsiveness of the stem cell population of the hypertransfused mouse, after erythropoietin stimulation, have been measured.

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Supported in part by the U. S. Atomic Energy Commission and in part by Cancer Research Funds of the University of California.

Submitted June 29, 1964; accepted for publication Sept. 18, 1964.

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Methods

Female C3H mice weighing about 23 Gm. were hypertransfused with 2 daily intraperitoneal injections of 1 ml. of packed red blood cells obtained from isogenic donors. The donor red blood cells were washed 3 times with saline, and the buffy coat was removed after each wash. Normally, erythropoietin* was injected intravenously into the hypertransfused mice on the fifth day after the last transfusion. Fifty-six hours after the erythropoietin injection, 0.5 μc. of Fe59 as iron citrate (specific activity approximately 10 μc./μg.) was injected intraperitoneally, and 72 hours later a sample of blood was taken by cardiac puncture. In all experiments, the interval between the injection of Fe59 and the sampling of the blood of the assay animals was 72 hours, but the time of injection of erythropoietin and immune serum relative to the time of Fe59 injection was varied. Therefore, the time of these injections is always given relative to the time before or after the Fe59 injection. Thus, in 1 experiment groups of hypertransfused mice received injections of erythropoietin 56, 32, and 8 hours before and 16 hours after the injection of Fe59, i.e., the normal time (56 hours before), and 1, 2, and 3 days later than normal. In another experiment, groups of hypertransfused mice were injected intravenously with 0.25, 0.5, 1.0, 2.0, or 4.5 cobalt units of sheep plasma erythropoietin 56 hours before the Fe59 injection, and at various times later enough immune serum (0.2 ml.) to neutralize the biologic activity of the largest dose of erythropoietin was injected intravenously into each mouse. Blood was collected from the mice of each group 72 hours after the Fe59 injection, regardless of the time of immune serum injection. In another experiment, 0.5 cobalt unit of sheep plasma erythropoietin was injected intravenously 56 hours before the Fe59, and another similar injection of erythropoietin was made at various intervals after the first erythropoietin injection. In some cases, immune serum was injected 6 hours after the second erythropoietin injection. The radioactivity in 0.5 ml. of whole blood was measured in the assay mice, and the per cent of the injected Fe59 in the total red blood volume was calculated. It was assumed that the blood volume of the hypertransfused mice was 7 per cent of the total body weight. Values from any animal which lost weight or whose hematocrit was less than 55 per cent at the time of sampling were discarded. Each individual group in each experiment consisted of 6 to 10 mice. The standard error of the mean is indicated for each value.

Immune serum capable of neutralizing the biologic activity of sheep plasma erythropoietin was obtained from rabbits immunized with human urinary erythropoietin. The schedule of immunizations and characterization of the immune serum have been described previously.11

Results

The effects of varying the interval between the injection of erythropoietin and the injection of Fe59 were measured. If 0.5 cobalt unit of erythropoietin was given 56 or 32 hours before the injection of Fe59 (the normal time and 1 day later than normal), the uptake of Fe59 into the calculated blood volume 72 hours after the radioiron administration was 3.29 ± 0.31 and 3.16 ± 0.4 per cent, respectively. However, if the erythropoietin injection was given only 8 hours before the Fe59 injection (2 days later than normal), the Fe59 uptake was decreased to 0.41 ± 0.08 per cent. The Fe59 uptake observed when the stimulating dose of erythropoietin was given 16 hours after the Fe59 injection was 0.09 ± 0.01 per cent which is indistinguishable from the values found in saline-injected controls. These results indicate that very little Fe59 is taken

*Sheep plasma erythropoietin, A1-0336, No. 103194A obtained from the Hematology Study Section of the National Institutes of Health.
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Fig. 1.—The effect of a divided dose of erythropoietin relative to the effect of the same total amount of erythropoietin given as a single dose. Unit response is defined as that observed following a single intravenous dose of 1.0 cobalt unit of erythropoietin. The curve represents the response, relative to this unit response, when the dose was divided into two 0.5 cobalt unit doses given at times separated by the intervals (in hours) indicated on the abscissa.

up by cells during the early development of the wave of erythropoiesis. After the wave of erythropoiesis has progressed for 56 hours, a significant uptake of Fe¹⁹⁹ is observed, but this uptake is not significantly different from that observed when the wave has progressed for 32 hours. The Fe¹⁹⁹ uptake found in the assay animal 72 hours after the injection of Fe¹⁹⁹ is the result of a complex series of events such as the stage of the wave of erythropoiesis at the time of Fe¹⁹⁹ injection, the hemoglobin synthesizing ability of the individual erythroid cells present at this time, the rate of disappearance of the injected Fe¹⁹⁹, the rate of release of the newly formed red cells into the peripheral blood, etc.

The effect of varying the interval between the injection of the two identical doses of 0.5 cobalt unit of erythropoietin on the erythropoietic response is shown in figure 1. The first injection of erythropoietin was given 56 hours before the injection of Fe¹⁹⁹, and the second dose of erythropoietin was given at various times after the first injection. The Fe¹⁹⁹ uptake was determined 72 hours after the injection of Fe¹⁹⁹. The 72-hour uptakes are plotted as a function of the interval between the first and second erythropoietin injections. When the interval between the first and second dose of erythropoietin was 72 and 96 hours, the second dose of erythropoietin was given 16 and 40 hours after the injection of Fe¹⁹⁹. The values for the Fe¹⁹⁹ uptake are given relative to the Fe¹⁹⁹ uptake observed when no interval existed between the first and second erythropoietin injection, i.e., a total of 1 cobalt unit was injected in one dose. If 0.5 cobalt unit of erythropoietin is given at the usual time (56 hours before
Fe$^{59}$) and again 24 hours later, the erythropoietic response is about 2.5 times greater than that observed when 1.0 cobalt unit of erythropoietin is injected 56 hours before Fe$^{59}$ or about 5 times the response found with only 0.5 cobalt unit of erythropoietin. The magnitude of the erythropoietic response observed when the total dose of erythropoietin is fractionated into two doses 24 hours apart is not only greater than that observed when the dose is not fractionated but is greater than the sum of the responses found when each dose is given singly at these times. The increased erythropoietic response observed when the total dose of erythropoietin is divided into two injections is evident even when the interval between the two injections is only 6 hours. It has been stressed previously that the Fe$^{59}$ uptake observed in the assay animals is the resultant of a complex series of reactions occurring in the erythroid population before and during the time Fe$^{59}$ is available. Obviously the series of events becomes even more complex following multiple injections of erythropoietin, since the waves of erythropoiesis produced by each injection of erythropoietin will be at different stages of development when the Fe$^{59}$ is injected. Presumably, this accounts for the observation that when the interval between the two fractionated doses is increased to 48 or 72 hours, the erythropoietic response is not greater than that observed when the dose is not fractionated. However, the responses in this case do tend to be greater than the sum of the individual responses observed when 0.5 cobalt unit of erythropoietin is injected at these times without fractionation. Gurney et al. did, however, observe that a given dose of erythropoietin was about twice as effective when administered in two partial injections separated by 48 hours. The discrepancy between their findings and the present experiments is probably related to the fact that their dose of erythropoietin was greater than that used in the present experiments and their erythropoietin was injected subcutaneously rather than intravenously. These differences in dosage and route of injection suggest that an effective level of erythropoietin was maintained in their experimental animals for a longer interval of time following each erythropoietin injection than in the experiments reported here.

The above results suggest that the responsiveness of hematopoietic tissue to erythropoietin is increased after the initial exposure to erythropoietin. This possibility was further investigated by allowing the second dose of erythropoietin to act for the limited time of 6 hours. The results of these experiments are shown in figure 2. The times of injection of the doses of erythropoietin and antibody relative to the time of injection of Fe$^{59}$ are shown to the left of this figure. The uptake of Fe$^{59}$ into the calculated blood volume 72 hours later is indicated on the right. The amount of erythropoietin initially injected is indicated by the height of the vertical line. The decreasing amount of erythropoietin following injection is plotted for convenience as if the T$^1/2$ was 12 hours in these hypertransfused mice. The first experiments shown in this figure simply re-emphasize the results already mentioned and show that fractionating the total dose of 1 cobalt unit into two injections 24 hours apart of 0.5 cobalt unit gives a much greater response (experiment 4) than 1 cobalt unit (experiment 3) or the sum of the responses observed when a total of 0.5 cobalt unit of
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Fig. 2.—A diagrammatic representation of a series of experiments where the time of injection of erythropoietin and antibody against erythropoietin is varied. The actual times of injection are indicated on the abscissa to the left. The amount of erythropoietin injected is indicated by the initial height of the shaded areas. The magnitude of the erythropoietic response is indicated for each experiment by the bars to the right.
erythropoietin is injected either 56 or 32 hours before Fe\textsuperscript{59} (experiment 1 and 2). Experiments 3, 6, 7, and 9 of figure 2 indicate that if 1.0 or 0.5 cobalt unit of erythropoietin is injected either 56 or 32 hours before the injection of Fe\textsuperscript{59} and allowed to act on the marrow for only 6 hours, measurable erythropoietic responses occur, but they are not different from one another. If 1.0 cobalt unit of erythropoietin is injected 56 hours before the Fe\textsuperscript{59} injection and allowed to act 30 hours before the injection of immune serum, the erythropoietic response is about the same as that observed when no immune serum is injected (experiments 3 and 8). If, however, 0.5 cobalt unit is injected 56 hours and 32 hours before the Fe\textsuperscript{59} and immune serum injected so that the first dose of erythropoietin acts for 30 hours and the second dose acts for 6 hours, the erythropoietic response is significantly greater than that observed when 1 or 0.5 cobalt unit is injected without fractionation and allowed to act only 30 hours or during the entire assay (compare experiments 1, 3, and 10). But the response is not as great as that found when the fractionated dose acts during the entire assay period (experiment 4). The erythropoietic response given when 1 cobalt unit acts on the hypertransfused mouse marrow for 30 hours is shown in experiment 8. This is about two times the response seen when 0.5 cobalt unit of erythropoietin acts during the same interval. The response observed in experiment 10 with fractionated doses of 0.5 cobalt units each is much greater than the sum of the responses seen even with the larger dose of 1.0 cobalt unit in experiments 8 and 9. When 0.5 cobalt unit of erythropoietin acts for 30 hours (the period from 56 to 26 hours before the injection of Fe\textsuperscript{59}), the Fe\textsuperscript{59} uptake is \(2.8 \pm 0.27\) per cent, and when this same dose acts for 6 hours during the period 32 to 24 hours before the Fe\textsuperscript{59} injection, the Fe\textsuperscript{59} uptake is \(0.42 \pm 0.07\) per cent. The sum of these two values is \(3.2 \pm 0.28\) per cent which is considerably less than the \(10.6 \pm 0.45\) per cent observed when 0.5 cobalt unit is injected into the same animal 56 and 32 hours before the Fe\textsuperscript{59} injection and allowed to act until the twenty-sixth hour before Fe\textsuperscript{59} injection. Thus, the second injection of 0.5 cobalt unit of erythropoietin stimulated the marrow to give a response during the short interval of 6 hours of \(10.6-3.2\) or about 7 per cent. This is almost eighteen times the response observed when 0.5 cobalt unit acts on the marrow during the same time interval in animals which have not received an earlier injection of erythropoietin. Similar results are shown in the last four experiments of figure 2. In these experiments 0.5 cobalt unit was injected 56 hours before the Fe\textsuperscript{59} followed by another similar injection 6 hours later. The erythropoietic response is shown in experiment 11. Notice that the response is about double that observed when both injections of 0.5 cobalt units are injected at the same time (experiment 3). If the action of both injections of erythropoietin is limited to 12 hours by the injection of immune serum 6 hours after the second injection of 0.5 cobalt unit of erythropoietin, a significant erythropoietic response results. This response is much greater than that observed when 0.5 (experiment 13) or 1.0 cobalt unit (experiment 14) acts on the marrow for 12 hours. Thus, even at this early time after the first injection of erythropoietin, the data indicate that a second injection stimulates the marrow to a greater extent than when the marrow has not been previously exposed to exogenous erythropoietin.
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Fig. 3.—Erythropoietic response observed following the intravenous injection of different doses of erythropoietin. The horizontal lines represent the responses observed when the indicated doses of erythropoietin are allowed to act for the entire assay period. The curves represent the responses observed when the same respective doses act for the limited time indicated on the abscissa.

The above experiments were repeated using divided doses of 0.25 units of erythropoietin given with an interval of 24 hours between the first and second injection. When 0.5 unit of erythropoietin was given in two divided doses of 0.25 unit 56 and 32 hours before the Fe59 injection, the Fe59 uptake in the calculated blood volume of the mice 72 hours after the Fe59 injection was 10.4 ± 1.38 per cent, but when the same dose was given undivided 56 hours before the Fe59, the response was only 3.29 ± 0.31 per cent; i.e., the response following the divided doses was about 3 times greater. If the divided doses were allowed to act for only 30 hours (56 to 26 hours before the Fe59 injection) by the injection of immune serum 6 hours after the second injection of erythropoietin, the response was 6.23 ± 1.06 per cent.

The responsiveness of the stem cell population to different doses of erythropoietin acting during limited periods of time was investigated. The results are shown in figure 3. The different doses of erythropoietin were injected 56 hours before the injection of Fe59, and antibody was injected at various times after the injection of erythropoietin. The interval between the times of erythropoietin and antibody injection is indicated on the abscissa. The uptake of radiolabeled iron into the calculated blood volume 72 hours after the injection of radioiron is indicated on the ordinate. The erythropoietic response observed following the injection of 0.25, 0.5, 1.0, 2.0, and 4.0 cobalt units of erythropoietin when antibody was not injected is indicated by the appropriate horizontal lines. The erythropoietic response found when antibody
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was injected at various times following erythropoietin is indicated at each time for each dose of erythropoietin. These responses are connected with appropriate lines corresponding to the dose of erythropoietin injected. The Fe$^{59}$ uptakes of 0.25 and 4.0 cobalt units of erythropoietin following intravenous injection are about 2 per cent and 16 per cent, respectively, with the other doses having intermediate values. These responses are submaximal; larger doses of erythropoietin do give larger Fe$^{59}$ uptakes.

The injection of antibody 24 hours after the injection of 4.0 or 2.0 cobalt units of erythropoietin significantly reduced the subsequent development of the wave of erythropoiesis as measured by the decreased Fe$^{59}$ uptakes. Similar injections of antibody at this same time had little if any effect on the erythropoietic response seen with doses of erythropoietin of 1.0 cobalt unit or less. At 48 hours, the injection of antibody had little effect on the erythropoietic responses given by the injection of 4.0 and 2.0 cobalt units of erythropoietin, i.e., the responses were not significantly different than those found when antibody was not injected. Presumably the depression in the erythropoietic response following the injection of antibody is the result of the removal by neutralization of circulating erythropoietin which has not acted on the receptive cells of the bone marrow. Large numbers of immature erythroid cells are present in the marrow at the time of the antibody injection, and these cells must mature in order to give the erythropoietic response eventually seen. This indicates that the antibody has no effect on these maturing erythroid cells, and therefore that erythropoietin is not necessary for the maturation of erythroid cells.

Injection of antibody 6 hours after the injection of the different doses of erythropoietin markedly reduced the various erythropoietic responses; however, the responses found are small but measurable. They are not significantly different from one another even though there is a sixteenfold difference between erythropoietin doses. This finding suggests that during the first 6 hours following erythropoietin injection there is a limited number of receptive cells in the bone marrow, and all these cells are triggered to differentiate by even the smallest erythropoietin dose. When the doses are allowed to act for longer intervals, the erythropoietic responses given by each dose become more and more different. By the twenty-fourth hour all the doses except 0.25 and 0.5 cobalt units are significantly different from one another, and by the forty-eighth hour the responses given by these small doses are also significantly different.

When 4 cobalt units of erythropoietin are allowed to act for 24 hours, the erythropoietic response of the mouse is much greater than four times the response seen when this dose is allowed to act only 6 hours; i.e., the response seen after 24 hours exposure of the marrow to erythropoietin is about 30 times greater than that observed after a 6-hour exposure. During the time interval between 18 and 24 hours, the erythropoietic response increased from $3.84 \pm 0.25$ per cent to $9.30 \pm 0.82$ per cent, an increase of about 5.5 per cent. This increase is about 1.5 times greater than the erythropoietic response seen during the entire first 18 hours. This increase occurs in spite of the fact
that the exogenous erythropoietin is disappearing from the assay animal. In nonhypertransfused animals, the T½ for this disappearance has been reported to be about 1 to 3 hours.12,13 These present findings suggest that in the hypertransfused mouse the T½ may be much longer. Regardless of how much of the injected erythropoietin has disappeared by the eighteenth hour, this data indicates that the marrow is much more sensitive to erythropoietin stimulation during the eighteenth to twenty-fourth hour following erythropoietin injection than during the entire first 18 hours. The magnitude of this increased sensitivity depends, of course, on the actual T½ for the disappearance of exogenous erythropoietin in hypertransfused animals.

**Discussion**

Erythropoiesis, measured either by the incorporation of radioiron or by the presence of identifiable erythroid cells in the hematopoietic tissues, virtually ceases in the mouse 4 or 5 days after the production of an increased red cell volume by transfusion.14 The abolishment of erythropoiesis is probably due to the absence of erythropoietin production.15 The presence of an inhibitor of erythropoiesis in the plasma of polycythemic animals has been claimed;16 however, we have been unable to confirm this finding (Schooley and Garcia, unpublished observations).

The injection of exogenous erythropoietin into a polycythemic mouse gives rise to a predictable orderly wave of erythropoiesis. In the spleen17 and bone marrow,10 this wave of erythropoiesis is characterized by the appearance of a peak percentage of proerythroblasts 1 day after erythropoietin injection which is followed on the second day by a peak percentage of basophilic erythroblasts. On the third day a peak percentage of reticulocytes occurs in the peripheral blood. By the fifth day after a single injection of erythropoietin the hematopoietic tissue is again devoid of identifiable erythroid cells. Two methods have been utilized to quantitate the erythropoietic response; the uptake of Fe59 in the blood volume of the hypertransfused mouse measured 128 hours after the injection of erythropoietin and 72 hours after the injection of Fe59; and/or the percentage of reticulocytes is measured in the peripheral blood on the third day after erythropoietin injection. Measurements of either of these parameters give graded responses for doses of erythropoietin ranging from about 0.25 to 6.0 cobalt units. The magnitude of the response for any particular dose of erythropoietin depends somewhat on the route of injection as well as the vehicle of injection; i.e., subcutaneous injections give larger responses than intravenous injections, and subcutaneous injections in serum give larger responses than similar injections in saline (Garcia and Schooley, unpublished observations). It has been demonstrated that there is a smaller erythropoietic response to a single submaximal dose of erythropoietin than there is to the same amount given in divided doses.18

This fact has been utilized in one assay for erythropoietin.9 A great deal of data indicates that erythropoietin regulates erythropoiesis primarily by regulating the differentiation of stem cells into the erythroid population.1,2 Some arguments, however, have been advanced which suggest
that erythropoietin may, in addition, have an effect on nucleated erythroid cells.\textsuperscript{19,26} Schooley and Garcia\textsuperscript{10} have presented evidence consistent with experiments presented here that erythropoietin is not necessary for the maturation of erythroid cells. Thus, the injection of antibody capable of neutralizing erythropoietin into polycythemic mice which have received erythropoietin does not prevent the development of the wave of erythropoiesis. The magnitude of the erythropoietic response depends on the dose of erythropoietin injected and the time of antibody injection. Eventually, with the doses used in the current experiments, a time occurs when the injection of antibody has no effect on the magnitude of the erythropoietic response even though the hematopoietic tissue contains large numbers of nucleated erythroid cells at the time of the antibody injection. The finding that injections of large amounts of erythropoietin into young 14-day-old rats, whose marrows contain large numbers of nucleated erythroid cells, does not further stimulate erythropoiesis\textsuperscript{20} also suggests that the injected erythropoietin has little effect on nucleated erythroid cells.

Jacobson et al.\textsuperscript{21} demonstrated in an elegant experiment that when rat bone marrow cells are injected into lethally irradiated polycythemic mice, the leukocytes of the chimera are rat type, but rat erythrocytes are not observed. Rat red cells were produced in such animals when erythropoiesis was stimulated. They suggested that the stem cells specific for rat red cells either remained dormant in the mouse until erythropoiesis was stimulated or that the stem cells of the rat are pluripotent. It is also possible that the specific stem cell for rat red cells was actively dividing in such animals but simply died when not stimulated to differentiate into an erythroid cell. More recent autoradiographic observations indicate that the stem cell, which differentiates into erythroid cells, is continuously proliferating in the polycythemic mouse in spite of the fact that differentiation into erythroid cells does not occur.\textsuperscript{22,23} The fate of these proliferating stem cells, if not triggered to differentiate into erythroid cells, cannot be resolved until the question of the pluripotential nature of the stem cell is settled.

Following the intravenous injection of erythropoietin into the polycythemic mouse, dispersion of the hormone must occur rapidly, and stem cells in various stages of their proliferative cycle must presumably encounter the hormone. When different doses of erythropoietin are injected and allowed to act in the polycythemic mouse for the limited period of 6 hours, the erythropoietic response is stimulated to the same extent even though the dose varies sixteenfold. This suggests that only a small number of the stem cells stimulated by erythropoietin during these 6 hours are receptive to the differentiative action of the hormone. Which of the various stages in the proliferative cycle are receptive to the action of the hormone is an open question. Preliminary autoradiographic observations indicate that when H\textsuperscript{3}-thymidine and erythropoietin are injected into polycythemic mice at the same time, the nucleated erythroid cells present in the marrow 2 days later are rarely labeled; whereas, when the H\textsuperscript{3}-thymidine is injected at increasing intervals after the erythropoietin injection, increasing percentages of the nucleated
erythroid cells are labeled. This suggests that the stem cells stimulated by the erythropoietin do not pass into the DNA synthetic phase of interphase (the S phase) either just before or after stimulation. Erslev has shown in the rabbit that when mitotic division was arrested by colchicine during a 20-hour period of anoxia, the onset of the reticulocyte response, though delayed 1 or 2 days, was not decreased. In similar unpublished experiments, I have found that the injection of colcemid (1 mg./Kg. body weight) into polycythemic mice simultaneously with exogenous erythropoietin has little effect on the development of the wave of erythropoiesis. However, the injection of the same dose of colcemid 24 hours after the erythropoietin injection almost completely abolished the erythropoietic response. This suggests that the stem cell is receptive to the action of erythropoietin sometime after metaphase and before the commencement of DNA synthesis. Lajtha et al. recently proposed a model of stem cell kinetics based on the concept that the stem cells are what he terms a Type IIb population. He defines this population as those cells which are capable of growth; at any single time only a random proportion of the cells are in this state of growth. The state of growth is defined as a cell cycle (G1-S-G2 periods) followed by cell division. The length of this cell cycle is constant. The cells not in cell cycle are in a state of dormancy or what Lajtha terms “G0.” He proposes that G0 follows mitosis and is of an indeterminate length, and further, that differentiative actions on stem cells can occur only when the stem cells are in G0, i.e., the cells are receptive only during G0.

The fact that different doses of erythropoietin acting on the marrow for the limited period of 6 hours stimulate erythropoiesis to the same extent suggests that, in terms of Lajtha’s model, all the cells in G0 have been stimulated to differentiate. When, however, these same doses of erythropoietin are allowed to work longer periods of time, significant differences in the responses obtained for each individual dose of erythropoietin are observed. The increased erythropoietic response observed when 4.0 cobalt units act for an additional 6-hour interval, i.e., the dose acts for 24 hours instead of 18 hours, compared to the erythropoietic response observed when the same dose acts for the first 6-hour interval after injection, suggests that a continual recruitment of receptive stem cells occurs after the first exposure of the marrow to erythropoietin. The results obtained when divided doses of erythropoietin are injected also suggest that increased numbers of receptive cells are found in the marrow after the first exposure of the hypertransfused mouse to erythropoietin. This conclusion is also supported by the experiments in which the second dose of erythropoietin was allowed to act on the marrow for the limited period of 6 hours. Thus, it appears that very soon after the removal of receptive cells from the stem cell population by the action of erythropoietin the stem cell population detects the loss and recruits even larger numbers of receptive cells. How the stem cell population detects this loss and directs the recruitment of more receptive cells is an intriguing problem for future work. The conclusion that a recruitment of stem cells occurs assumes that the increased erythropoietic responses observed in these cases...
are not the result of an action of erythropoietin on the differentiated but not yet identifiable erythroid stem cell, i.e., does not cause an increased number of divisions of cells which have already been committed towards the erythroid line of development. This assumption appears to be valid considering the effects of erythropoietin on the polycythemic mouse during the first 12 hours after the initial injection of erythropoietin, since the marrows of the erythropoietin injected and control mice cannot be distinguished cytologically from one another. However, at later times it cannot be assumed, on the basis of these experiments alone, that erythropoietin has no effect on early proerythroblasts. With these reservations, the results suggest that if most of the stem cell population is in a receptive state in the polycythemic mouse, i.e., in the state of G₀, the recruitment of even larger percentages of cells into G₀ from cells in cell cycle would be difficult to accomplish within 6 hours considering the time required for a proliferative cell cycle. In terms of Lajtha’s model, it would appear necessary to postulate that if a large percentage of the stem cell population is in G₀, these cells are receptive to the actions of differentiative agents only during limited times while in the state of G₀. Thus, the increased numbers of receptive cells in the present experimental situations could arise from divisions of stem cells, as well as an increase in the number of cells in a receptive stage of G₀.

Recently, Till, McCulloch and Siminovitch²⁴ have advanced a stochastic model of stem cell kinetics, similar to Osgood’s model,⁴ based on their experiments on the production of spleen colonies following the transplantation of hematopoietic tissue into lethally irradiated mice. They indicate that stem cells have a probability of either dividing and producing two stem cells or differentiating and leaving the population, i.e., a birth or a death process. They suggest that these probabilities are controlled; either increased numbers of stem cells would occur if the probability of differentiation was decreased, or increased numbers of differentiated cells would result by decreasing stem cell division. They conclude, however, that erythropoietin is not involved in regulating the “death” process, but is involved in determining the path of differentiation which the differentiated cell, produced as a result of the death process, will take.²⁵ This conclusion, they claim, is necessary to explain the fact that the number of colonies formed by a cell suspension is not decreased in the presence of erythropoietin. Such a decrease would be expected in their model if the colony-forming cell is a stem cell. One could instead postulate that the differentiating effect of erythropoietin on stem cells is simply decreased by an increase in the probability of a stem cell division. The increased probability for stem cell division might be regulated by the same mechanisms which bring about the recruitment of stem cells or erythropoietin sensitive cells.

**Summary**

Following the injection of erythropoietin either in a single large dose or in multiple doses, a change in the responsiveness of the hematopoietic tissue occurs. The fact that different doses of erythropoietin stimulate erythropoiesis to the same extent when the action of the hormone is limited to 6 hours by
the injection of antibody suggests that the stem cells are receptive to the action of erythropoietin only at some limited time in their individual life cycle. It is suggested that this period is sometime after metaphase and before the commencement of DNA synthesis in the interphase state of individual stem cells. It is further suggested that the increased responsiveness of the hematopoietic tissue to erythropoietin following injection is due to recruitment of stem cells into this receptive state. This recruitment may be due to both the division of stem cells and the movement of cells through cell cycle into the receptive state. The results are discussed in relation to two recent models of stem cell kinetics.

**Acknowledgments**

The author wishes to express his appreciation for the valuable technical assistance of Mrs. Linda N. Cantor and Mrs. Virginia W. Havens.

**References**

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