Studies on the Mechanism of the Increased Dose-Response of Erythropoietin After Stimulation with Erythropoietin

By Jan Fogh

A number of authors have observed that the response to erythropoietin (ESF) in the erythremic mouse assay is substantially enhanced when the ESF dose is administered in fractions. Fogh demonstrated that the response to 1.0 IRP units of ESF, determined by Fe incorporation into RBC or by the number of reticulocytes in the blood, is increased one to four days after a previous ESF stimulation. It was suggested that this increase in dose response is the explanation for the increased effect of the fractionation, and that the increased dose response is caused by an increase in number of ESF-responding cells provoked by the previous ESF injection.

The nature of the cell that responds to ESF has not been elucidated. It has been definitely proven that at least one ESF-responding cell must be a primitive cell preceding the earliest recognizable cell in the red cell series. Some authors suggest that in addition to the primitive progenitor, nucleated red cells within the erythron are responsive to ESF. If ESF exerts a stimulatory effect on erythroblasts, and such an effect leads to an increase in the net production of red cells, the explanation of the increased dose response after ESF stimulation would be rather trivial. The spleen and bone marrow of erythremic mice, which have not previously been stimulated with ESF, contain no nucleated red cells. Consequently the response to the first injection of ESF is mainly the result of interaction of ESF with primitive ESF-responding cells. (This must especially be the case if the ESF dose is small and administered i.v. or i.p., so that the hormone, which has a T% in the blood of a few hours, is eliminated before any nucleated red cells have been developed.) From one to four or five days after the first injection of ESF, however, nucleated red cells will be available, and an additional effect on these cells of a second ESF injection could be the cause of the increased dose response of ESF after ESF stimulation.

An alternative explanation could be that the increased dose response is caused by an increase in number of primitive ESF-responding cells per se. The term "primitive ESF-responding cells (P-ERC)" will be used with reference to the primitive cells which, when exposed to ESF, respond instantaneously and contribute to an increase in red cell production.

The purpose of the present work was to determine the significance of nucleated red cells for the increased dose response to ESF after previous ESF stimulation.

From the Department of Nuclear Medicine, Rigshospitalet, Copenhagen, Denmark, and Argonne Cancer Research Hospital, University of Chicago, Chicago, Ill. The Argonne Cancer Research Hospital is operated by the University of Chicago for the United States Atomic Energy Commission.

First received May 12, 1969; accepted for publication December 22, 1969.

Supported in part by a Public Health Service International Postdoctoral Research Fellowship No. F05 TW 1202-02.
INCREASED DOSE-RESPONSE OF ERYTHROPOIETIN

Studies by Keighley and Lowy, and Reissmann and Ito have shown that a blockade of the ESF-induced formation of nucleated red cells in the erythremic mouse can be achieved by injection of a small dose of Actinomycin D (Act-D) simultaneously with the ESF dose. Reissmann and Ito demonstrated that the drug completely suppresses erythropoiesis of normal mice due to disappearance of erythroid marrow cells, without affecting the production of other blood cells. After the injection of Act-D was discontinued, the red cells production was reestablished in two days. They concluded that Act-D inhibits the differentiation of "stem cells" into the red cell series, and that the drug causes little or no reduction of the number of "stem cells" available for differentiation.

The ESF-induced formation of nucleated red cells can also be inhibited by using erythremic mice shortly after they have been exposed to irradiation. A severe impairment of the response to ESF following irradiation with 150 rad has been reported by Alexanian et al., Gurney, and others, and mice exposed to 850 R, with one femur shielded have been found to be completely refractory to ESF for a period of four days, after which a recovery gradually takes place.

These two methods of temporarily blocking nucleated red cell formation were used in the present experiments in order to test if an increased dose response to ESF can be elicited under experimental conditions where no nucleated red cells are present. ESF was injected into erythremic mice at a time when the blockade was effective. At a later time, when the ability to produce red cells had recovered, a second dose of ESF was given, and the response to this dose was compared with the response to an identical dose given without previous injection of ESF.

MATERIAL AND METHODS

Erythremic CF No. 1 female mice were used in all experiments. In experiments in which Act-D was given, the mice were rendered erythremic by exposure to carbon monoxide hypoxia for 16 days. In experiments in which animals were irradiated, the erythremia had been induced by exposure to intermittent low atmospheric pressure for 21 days. When the latter method was used, it was necessary to give the animals a transfusion of 0.5-mi. packed homologous red blood cells on the fourth day after termination of the hypoxia in order to maintain high hematocrit values throughout the observation time. Total body irradiation was administered on the sixth day after cessation of the hypoxic period by a 250 Kv. Maxitron unit with a filter of 0.25 mm. Cu + 1.00 mm. Al, a target distance of 83.8 cm. and a dose rate of 60 R. per minute. The mice were placed in perforated lusteroid tubes on a lucite turntable and rotated 33 rpm during the exposure. Human urinary and sheep plasma erythropoietin containing 0.5-2.0 IRP-units per mg. of protein were kindly supplied by Dr. E. Goldwasser. Solutions of Actinomycin D with a concentration of 10 μg./ml. saline were made and kept in sealed vaccine bottles in the dark at -20°C until immediately before use. Injections of ESF and Act-D were not started earlier than the sixth day after termination of the hypoxia since the endogenous erythropoiesis has not ceased completely until this time, and since the responsiveness to ESF does not stabilize until five to six days after previous stimulation with ESF. All injections were administered i.v. except in experiments with irradiated mice to which ESF was given i.p.

*Department of Biochemistry and Argonne Cancer Research Hospital, University of Chicago.
†Merck, Sharp & Dohme.
JAN FOGH

1.0 μCi. 59Fe as ferric citrate* was given i.v. at the time indicated in the figures. Blood was collected from the orbital cavity 72 hours later, and the incorporation of 59Fe into the red blood cells was determined after the radioactivity had been measured in a well-type scintillation crystal. Brilliant cresyl blue was employed for supravital staining of reticulocytes; 4000 to 6000 RBC were examined per smear. Results from the mice whose final hematocrit was less than 55 per cent were discarded.

EXPERIMENTS AND RESULTS

1. Dose of ESF, Mode of Administration and Times of Injections and Sampling of Blood of the Assay

For reasons given in the discussion it was decided to use only 0.5 IRP-units of ESF administered i.v. as standard dose in the following experiments.

Since the planned experiments would deal with responses that would exceed the response to a single i.v. injection of 0.5 IRP-units of ESF, it was necessary to establish the schedule by which maximum recording of the response to this dose would be achieved. Morphological studies of the hematopoietic tissue of erythremic mice following a single injection of ESF have indicated a maximum transit time from pronormoblast to release of reticulocyte into the peripheral blood of 72 to 96 hours. Systematic studies on the 59Fe-incorporation as a function of time following the injection of 59Fe have also shown that a final and maximum level of the curve is not reached until three days after the administration of 59Fe to ESF-stimulated erythremic mice. An interval of 72 hours between the injection of 59Fe and blood sampling was consequently used in all the experiments. In order to determine the optimal time for administration of 59Fe in relation to injection of ESF groups of erythremic mice who were injected i.v. with 0.5 IRP-units of ESF, 59Fe was administered at different intervals later to different groups, and 72-hour 59Fe incorporation was measured. The upper curve in Fig. 1 shows that maximum value of 59Fe incorporation is obtained when the interval between injection of ESF and injection of 59Fe is 48 hours.

2. The Effect of Act-D on the Response to ESF in Erythremic Mice

Preliminary experiments (not shown) had demonstrated that the duration of the inhibitory effect of Act-D on the red cell production is shorter for smaller doses of Act-D, and shorter if the drug is administered i.v. rather than i.p.. It was also found that a minimum dose of 2.15 μGm. Act-D is needed in order to block the response to 0.5 IRP-units of ESF, when Act-D and ESF are injected simultaneously i.v. to erythremic mice.

The lower curve in Fig. 1 shows the results from experiments in which groups of erythremic mice received simultaneous i.v. injections of 0.5 IRP-units of ESF and 2.15 μGm. of Act-D. 59Fe was given at different intervals later to different groups, and 72-hour 59Fe incorporation into RBC was determined. It is seen that the injection of Act-D completely suppresses the response to ESF. This was also confirmed by histological examination of the spleen and bone marrow which contained virtually no erythroid cells. The mice were checked up to four days after the combined injections, and a delayed response to ESF,

*Amersham, England. Specific activity 12–18 mCi./mg. of iron.
Fig. 1.—Seventy-two-hour incorporation of $^{59}$Fe into RBC of erythremic mice following 0.5 IRP-units of ESF (●), or 0.5 IRP-units of ESF +2.15 μGm. Act-D (□). Injections of ESF and ESF + Act-D given i.v. on sixth day after termination of hypoxic period. $^{59}$Fe given i.v. on different days later. (Mean ± standard deviation of mean) (number of animals per group in parentheses).

which has been observed following simultaneous injections of vincristine and ESF, is not seen following simultaneous injections of Act-D and ESF.

The duration of the inhibitory effect on erythropoiesis of 2.15 μGm. Act-D was studied by injecting Act-D into groups of erythremic mice along with or at different intervals before injections of 0.5 IRP-units of ESF. All groups re-
Fig. 2.—Seventy-two-hour incorporation of $^{59}$Fe into RBC of erythremic mice. All mice received 0.5 IRP-units of ESF i.v. on eighth day after termination of hypoxia and $^{59}$Fe 48 hours later. 2.15 $\mu$Gm. Act-D given i.v. 0, one, two days before ESF to different groups of mice (M $\pm$ S.E.M.) (number of animals in parentheses).

received $^{59}$Fe 48 hours after administration of ESF, and 72-hour incorporation into the red blood cells was measured. The results, which are shown in Fig. 2, demonstrate that the inhibitory effect of 2.15 $\mu$Gm. Act-D on the response to 0.5 IRP-units of ESF has practically ceased in two days. The difference in $^{59}$Fe incorporation of mice injected with Act-D two days before ESF and of mice that received only ESF is not significant ($P > 0.20$).

3. The Response to 0.5 IRP-units of ESF Two Days After a Previous ESF Injection

The response to 0.5 IRP-units of ESF injected two days after a previous ESF stimulation in erythremic mice was studied by the method described in an earlier publication. The experimental scheme and results are shown in Fig. 3. A, B, C and S represent groups of erythremic mice. The mice in group A received a single injection of 0.5 IRP-units of ESF on the sixth day after termination of the hypoxia. Group B received two injections of 0.5 IRP-units, one on the sixth and one on the eighth day. Group C received a single injection of 0.5 IRP-units on the eighth day. The mice in group S were controls injected with saline. $^{59}$Fe was given to all groups on the 10th day, i.e., 48 hours after the second ESF injection in group B and the ESF injection in group C.
The incorporation of $^{59}$Fe into the peripheral blood was measured 72 hours later.

Although the mice in group A had been stimulated with ESF, the $^{59}$Fe incorporation, which was recorded in the group, was not significantly greater than the value observed in the saline injected mice ($P > 0.20$). The explanation for this is obviously that $^{59}$Fe was administered to group A four days after the injection of ESF, i.e., at a time when most of the ESF-induced erythroid cells had matured, had completed their synthesis of hemoglobin, and had entered the peripheral blood.

The mice in group C had been stimulated with an identical ESF dose, but since $^{59}$Fe in this group was given 48 hours after the ESF injection, i.e., at a time when most of the ESF-induced erythroid cells were within the hemopoietic tissue, the $^{59}$Fe incorporation of group C was much greater, and the value, as mentioned above, represents the maximum recordable response to 0.5 IRP-units of ESF when the dose is given i.v. as a single injection.

The mice in group B that received two injections each of 0.5 IRP-units of
ESF, i.e., a combination of the injections used in groups A and B, showed a $^{59}$Fe incorporation that substantially exceeded the response predicted if the two injections had acted independently. The contribution of the first of the two ESF injections to the $^{59}$Fe incorporation in group B must be equivalent to the results obtained in group A. The contribution of the second ESF dose to the total $^{59}$Fe incorporation in group B consequently can be expressed as (B-A). This calculated value was found to be significantly greater than the maximum recordable $^{59}$Fe incorporation of a single injection of 0.5 IRP-units of ESF (group C), ($P < 0.0025$).

The number of reticulocytes appearing in the peripheral blood of groups A, B and C are shown in Fig. 4 Left. The peak values were observed three days after injection of ESF in group A and C, and no or very few reticulocytes were found on the fifth day after the ESF injection. The curve of group B had two peaks—one on the third day after the first ESF injection, and one on the third day after the second injection of ESF. Because of the similarity of the timing of the two peaks and the peaks observed when the two ESF injections were given separately, it is reasonable to assume that the two peaks in group B represent the response to the first and the second ESF injection respectively. The calculated difference (B-A), indicated by the dotted line, can be considered as the discrete response to the second ESF injection in group B. The peak, as well as the area of this constructed curve, is considerably greater than the peak and area of the curve of group C.

The results of the reticulocyte counts, consequently, were in agreement with the $^{59}$Fe incorporations, and the data of the experiments show that the erythropoietic response to 0.5 IRP-units of ESF is increased two days after a previous stimulation with ESF.

4. The Response to 0.5 IRP-units of ESF Two Days after Simultaneous Injections of ESF and Act-D

Three groups of erythremic mice A', B' and C' received injections of 0.5 IRP-units of ESF at the same time and with the same interval as in the experiments shown in Fig. 3 and Fig. 4 Left, and a control group S was injected with saline. In order to suppress the production of erythroid cells induced by the ESF dose given on the sixth day to groups A' and B', these groups received simultaneous i.v. injections of 2.15 µGm. Act-D. The inhibitory effect of this Act-D dose on the response to 0.5 IRP-units of ESF has been described and illustrated above (Item 2 and Figs. 1 and 2). An injection of Act-D was also given to the mice in group C'.

The $^{59}$Fe incorporation in group A' did not differ from that of the saline injected controls ($P > 0.35$).

In group C' the injection of Act-D had little or no effect on the response to the ESF dose that was injected two days later. Although the mean value of the $^{59}$Fe incorporation of group C' was somewhat less than the mean value of group C (Fig. 3), the results from the two groups were not significantly different ($P > 0.20$).

Even if the first of the two injections of ESF in group B' gave no erythropoie-
Fig. 4 Left.—Reticulocyte response following scheme of injection used in Fig. 3. 4 Right.—Reticulocyte response following scheme of injection used in Fig. 5.
Fig. 5.—Scheme of injections and doses of ESF identical with Fig. 3 except for additional injections of Act-D to Groups A', B', and C' on sixth day after termination of hypoxia. Mean ± S.E.M. of 72-hour 59Fe incorporation shown for each group. Response (A') does not differ from (S). Difference between (B' - A') and (C') significant (P < 0.025).

5. The Effect of 400 R. Total Body Irradiation on the Response to ESF in Mice

Erythremic mice were exposed to 400-R. total body irradiation on the sixth day after termination of the hypoxic period. 1.0 IRP-units of ESF was injected i.p. to groups of mice on different days after the exposure. 59Fe was given i.v. 48 hours after ESF, and the incorporation into the red cells was measured 72...
hours later. The results in Fig. 6 show that the ability to respond to the ESF dose is completely abolished for three to four days after irradiation, and serial sections of bone marrow following injection of ESF revealed no, or very few, erythroblasts during this period. On the fifth and sixth day the responsiveness started to recover.

The incorporation of $^{59}$Fe was much lower on the days immediately after irradiation than the values usually obtained in nonirradiated erythremic controls, and the mean ± SEM of $^{59}$Fe incorporation of 23 erythremic irradiated and saline-injected mice was found to be 0.03 ± 0.02 per cent. This is about a sevenfold reduction as compared to the values from nonirradiated saline-injected erythremic mice (0.21 ± 0.03 per cent). The reason for this discrepancy is not known. Histologic studies of bone marrow, spleen, and peripheral blood, however, showed no signs of effective erythropoiesis in irradiated erythremic mice until the $^{59}$Fe incorporation had risen above 0.3 per cent, and this value was consequently considered as reliable baseline for the erythropoietic function in irradiated as well as in nonirradiated erythremic mice.

Instead of giving the priming ESF dose in a single injection, as in the experiment illustrated in Fig. 6, the dose was administered in four fractions of 0.25 units and given at 12-hour intervals beginning immediately after the irradiation. Figure 7 shows that, whether $^{59}$Fe was injected 1½ days (group D) or 3½ days (group A") after the last of the four ESF injections, no response was obtained. The four injections, however, must have changed the pattern of erythropoietic recovery, since a definite response to 1.0 IRP-units of ESF was obtained 1½ days later or on the third day after irradiation of the mice (group B"). Injection of 1.0 IRP-units of ESF on the third day to a group which had received no previous ESF stimulation gave no response (group C") and even 2.0 IRP-units failed to give any response at that time (group E). The experiment demonstrates that ESF enhances the responsiveness to ESF of irradiated erythremic mice.
Fig. 7.—Responsiveness to ESF of erythremic mice following total body irradiation with 400 R. A", B" and C" show enhanced responsiveness to 1.0 IRP-units of ESF. Discrete response to last ESF injection in Group B", calculated as (B" - A") significantly greater than response of (C"), (P < 0.005). D. E and S served as controls. For details see text.

**DISCUSSION**

It is generally assumed that the response to ESF is the result of interaction of ESF with some target cells. The dose response of most ESF assays is linear within a wide dose range and has an upper plateau for high doses when plotted as log dose vs linear response. This is in agreement with a "hit and target type of action," and indicates that only a limited number of cells is available for ESF during the time of the assay. Keighley et al.\(^{2}\) have shown that the responsiveness to ESF does not decrease in mice or rats, even when massive doses of ESF have been given for long periods of time. This, and the observation that the dose response in the erythremic mouse assay increases significantly if the time during which the cells are challenged with ESF is extended, i.e., by subcutaneous rather than by intravenous injection,\(^{21}\) or by fractionated administration of the dose,\(^{4}\) indicates that at any time only a limited number of hemopoietic cells is responding to ESF, and that a recruitment of ESF-responding cells (ERC) takes place following the initial interaction of ESF with the cells already present.

Information about the recruitment phenomenon may be obtained by comparing the dose response to ESF of erythremic mice shortly after a previous stimulation with ESF with that of previously nonstimulated animals. This
method assumes, however, that the response to the challenging ESF injection is determined mainly by the number of ERC present at the time of injection. In experiments in which the neutralizing effect of antibody to ESF was used, Schooley\textsuperscript{21} demonstrated that ESF in doses ranging from 0.25–4.00 IRP-units gave the same response when allowed to act for only six hours. Consequently, the recruitment must play a very important role in the ordinary ESF-assay even if only one ESF injection is given. Gurney\textsuperscript{15} has pointed out that an exact determination of the number of ERC present at a given time would require an instantaneous challenge with ESF. This is not possible since ESF is eliminated exponentially from plasma with a T\% of some hours,\textsuperscript{8–10} and the response even to a single injection will accordingly reflect the number of ERC present at the time of injection as well as the number of ERC which have been recruited during the time when an effective concentration of ESF was present. The contribution of the recruitment to the final erythropoietic response will depend on the dose and on the mode of administration of ESF. The larger the dose of ESF administered, the longer high levels of ESF will be present in the plasma, and the challenge time of the erythropoietic stimulus will be longer if the ESF dose is given subcutaneously rather than intravenously or intraperitoneally. Consequently if the purpose of an experiment is to measure the number of ERC actually present, only small doses of ESF should be given i.v. or i.p., and in the present experiments 1.0 IRP-units or less were used.

A fixed rate of recruitment would be adequate to explain the enhancing effect of fractionated administration of ESF. Our earlier experiments,\textsuperscript{5} however, have demonstrated that the dose response to ESF, and therefore the number of cells which actually respond to ESF, is increased one to four days after a previous stimulation with ESF. A number of authors,\textsuperscript{6,7,22,23} suggest that ESF, in addition to an effect upon undifferentiated primitive precursors, has an effect upon differentiated erythroid precursors. It was therefore likely that the increase in the number of ERC one to four days after a stimulation with ESF simply is a reflection of the induction by the prior ESF stimulation of such nucleated red cells into the erythron. The present experiments, however, have demonstrated that an increase in dose response of ESF can be obtained under conditions where no nucleated red cells are available for ESF to act upon. If, therefore, ESF has an effect upon such cells, this effect is not involved or is at least not important for the increase in dose response. The explanation for the observed increase in response to ESF of group B' over group C', Fig. 5, must be an increase in number of primitive ESF-responding cells (P-ERC) which have been triggered into the erythron.

The experiments demonstrate, in other words, that not only does a recruitment of P-ERC occur following the initial interaction of ESF with the cells available, but that there is an overshoot resulting in an increase in the number of P-ERC over the number present in a nonstimulated animal.

By comparing Fig. 3 with Fig. 5 it can be seen that the mean value of $^{59}\text{Fe}$ incorporation of group B, in which nucleated red cells were present at the time of the second ESF injection, was somewhat higher than the mean value
of group B’, in which no nucleated red cells were present when the second ESF dose was given. A similar nonsignificant difference was found between the responses of group C and C’, in neither of which were nucleated red cells present at the time of injection of ESF. The difference between (B) and (B’) therefore, is probably caused by a slight inhibition of the second ESF dose in group B’ by the Act-D dose that was given two days before, rather than being caused by a stimulatory effect of the second ESF dose in group B upon nucleated red cells. The study thus indicates that if ESF has an effect upon nucleated red cells, such an effect does not lead to an increase in the net production of red cells. Whether ESF has an effect upon the transit time of erythroid cells through the marrow by causing “skipped divisions”,24 or upon the release of reticulocytes from marrow,25 cannot be elucidated on the basis of the present experiments, since such effects per se would not increase the net production of red cells.

It is noteworthy that in most of the studies that have indicated an effect of ESF upon nucleated red cells, the $^{59}$Fe incorporation into RBC has been measured 18 to 24 hours after administration of the tracer. Systematic investigation of the $^{59}$Fe incorporation curve in ESF-stimulated erythremic mice have, as mentioned earlier, showed that a maximum and final level of radioactivity in the blood is usually not obtained until three days after the injection of $^{59}$Fe,4 which is in agreement with a maximum transit time in the marrow for the cells of about 72 hours.17 Eighteen or 24 hours after the injection of $^{59}$Fe, therefore, only a fraction of the labeled cells in the erythropoietic wave has been released from the marrow, and since the value of the $^{59}$Fe incorporation at any time is an integrated expression for the labeled cells that have entered the blood, the 18- or 24-hour $^{59}$Fe incorporation does not necessarily reflect the net number of red cells produced by ESF. The results obtained 18 or 24 hours after administration of $^{59}$Fe will, furthermore, be very sensitive to changes in the marrow transit time, just as the results of studies in which the number of reticulocytes or the distribution of $^{59}$Fe between bone marrow, spleen and blood has been determined 24 or 48 hours after injection of ESF.23 It is possible that the apparent discrepancy between the results of authors who claim an effect of ESF upon nucleated red cells, and the results of those who can find no support for such an effect, is caused by differences in timing employed in the assays by the different authors.

Reissmann and Somarapoompichit26 have reported that ESF enhances repopulation of the erythroid marrow in mice treated with 4-Fluorouracil, an inhibitor of DNA-synthesis which kills proliferating cells and completely eradicates erythropoiesis in normal mice. Their observation is in agreement with the results of the present study. The authors considered a delayed action of ESF on the surviving P-ERC or an action on their precursors as possible explanations. A delayed response to ESF, however, was not observed in the present experiment for at least four days after simultaneous injection of ESF and Act-D (Fig. 1).

In agreement with the results shown in Fig. 7 are the reports by Stohlman,27 who demonstrated an enhancing effect of ESF on the recovery of erythropoiesis.
after irradiation, and by Hajadukovic and Szirmai and Naidu and Reddi, who found that severe bleeding or injection of ESF after irradiation stimulate the erythropoietic recovery.

The apparent discrepancy between the findings of Weisman et al. that the responsiveness to ESF after X-ray recovers at the same rate whether the animals are rendered erythremic before or after the irradiation, and our observation that ESF enhances the recovery could be due to difference in concentration of ESF in the two experiments. In Weisman's experiment the postirradiation stimulation was due to endogenous ESF presumably in low concentration. In the present study (Fig. 7) 0.25 IRP-units of ESF were injected each 12 hours for two days after the irradiation, challenge which in nonirradiated erythremic mice gives a substantial response.

Based on the observation of an overshoot in number of P-ERC after stimulation with ESF, it is reasonable to conclude that the recruitment of these cells is subjected to an active regulation. The same conclusion has earlier been reached by Gurney and Fried who suggested that a humoral factor is involved in the regulation, since the P-ERC within the lead-shielded leg of a mouse increased in number when similar cells in the rest of the body were damaged by irradiation. The present study indicates that this humoral factor could be ESF, but some questions will have to be answered, before any conclusion can be made.

Is Recruitment of P-ERC stimulated Directly or Indirectly by ESF?

Lajtha et al. have proposed that the size of a stem cell pool is controlled by a feedback mechanism by which a message is given to the remaining cells when cells are removed from the pool, causing an increased proliferation until the size of the pool is regained. This mechanism leads eventually to a temporary overshoot in pool size, as demonstrated in computer testing of Lajtha's kinetic model of hemopoietic stem cells, and in experiments in which the recovery of responsiveness to a small dose of ESF after irradiation was studied. Gurney et al. and Hanna have shown that P-ERC are dividing cells and since they also have the ability to differentiate they can be considered as stem cells. Although the results shown in Figs. 4 and 5 indicate that ESF has a direct effect on the recruitment, it could be that, even if no differentiation into pronormoblasts occur when ESF and Act-D are injected simultaneously, a number of P-ERC may be affected in such a way that they can no longer be considered as stem cells, and the feedback mechanism therefore comes into effect, which leads to an increase in pool size.

The enhancing effect of ESF upon the recovery of erythropoiesis after X-ray demonstrated in Fig 7, on the other hand seems to favor the possibility that ESF exerts a direct effect on the recruitment of P-ERC. The impaired response to ESF in irradiated animals is assumed to be due to damage of hemopoietic stem cells, and the recovery of the responsiveness in the days after the irradiation, to a gradual increase in the number of P-ERC as the results of recruitment. Since the number of competent P-ERC in irradiated mice already must be severely reduced, it is reasonable to postulate that the
encompassing effect of ESF upon the recovery is due, not to an additional removal of P-ERC, but to a direct effect of ESF upon the recruitment.

Is Recruitment Exclusively Controlled by ESF, or are Other Factors Involved?

A number of observations indicate that the recruitment of P-ERC can take place without the presence of ESF. The data shown in Fig. 6 demonstrate that the responsiveness to ESF recovers spontaneously in erythremic irradiated mice, and this has been observed by many others. The assumption in these experiments has been that the production of ESF, which ceases in erythremic mice, remains completely repressed after irradiation. However, it is not unlikely that the normal mechanism by which the production of ESF is regulated is put out of function for a period of time after irradiation. Schooley found an increased concentration of ESF in plasma from nonerythremic mice only one day after irradiation at a time when there was hardly any decrease in the hematocrit value, and we have recently demonstrated a slight but significant increase in ESF concentration in serum from erythremic mice two days after total body x irradiation with 400 rad (unpublished data). The stimulatory effect of ESF upon the recruitment of P-ERC seems well documented. Further studies will be needed in order to elucidate the mechanism by which ESF exerts this effect and the mechanism of the recruitment itself.

SUMMARY

The responsiveness to ESF of the erythremic mouse is increased one to four days after a previous stimulation with ESF. The formation of nucleated red cells by the priming ESF stimulation was suppressed either by simultaneous injections of ESF and Actinomycin D, or by giving the priming ESF challenge to erythremic mice shortly after they had been exposed to 400 R. total body irradiation with X-rays. By these techniques it was demonstrated that the presence of nucleated red cells within the erythron is not important for the observed increase in dose response of ESF after ESF stimulation. It was concluded that the recruitment of primitive erythropoietin-responding cells is enhanced by ESF.

ACKNOWLEDGMENTS

I wish to thank Mrs. Annelise Persson, Miss Evelyn Gaston, Mrs. Edna Marks and Mr. James Bland for technical assistance, and Dr. Leon Jacobson for letting me use his laboratory.

REFERENCES

4. Fogh, Jan: A sensitive erythropoietin


30. Weisman, M., Martinson, D., Fried,


Studies on the Mechanism of the Increased Dose-Response of Erythropoietin After Stimulation with Erythropoietin

JAN FOGH