PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 36–38 (1980)

The Effect of Plethora on Erythropoietin Levels[1,2] (40718)

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Several mouse bioassay methods are used to assay for erythropoietin (Ep). The common denominator of these bioassays is the suppression of erythropoiesis by plethora in the test animal. The commonly employed techniques used to produce plethora are hypoxia (1), carbon monoxide (2), and transfusion (3). The first two methods are effective in producing a state of plethora by stimulating endogenous Ep production (4). Plethora presumably by the increased number of red cells supplies increased amounts of O_2 to the tissues, suppressing Ep levels and hence diminishing red cell production. The third method of suppressing erythropoiesis in experimental animals is hypertransfusion. This procedure is associated with a near absence of recognizable erythroid precursors from the bone marrow 5 days after transfusion (4). The latter method is a more physiologic means of suppressing erythropoiesis in that one does not create a period of intense erythroid activity prior to the suppression of erythropoiesis (5). Irrespective of the method employed to produce plethora in the assay animals it is clear that erythroid activity is virtually absent from the bone marrow at the time the unknown substances which are being tested for Ep activity are injected into the plethoric animal.

It has been presumed that the aforementioned techniques have suppressed endogenous Ep production in the test animal. Since most Ep bioassays cannot detect less than 50 mU/ml plasma one could not prove by bioassay the presence of lower than normal levels of plasma Ep. The development of a radioimmunoassay (RIA) for Ep permits one to measure Ep levels in normal mice. In the present study we measured Ep levels in mice rendered plethoric by hypertransfusion with homologous red blood cells.

Materials and methods. Animals. Donors. Donor blood was obtained from exbreeder CF_1 mice (Charles River Breeding Laboratories, Wilmington, Mass.). They were bled by cardiac puncture using heparinized syringes. The blood was pooled, centrifuged for 30 min at 2000 rpm, then the plasma was removed. The remaining packed red cells were washed three times in normal saline and the final hematocrit was adjusted to 70%.

Recipients. CF_1 virgin female mice, 12 to 16 weeks old and weighing 25 to 30 g were used. They were housed in the laboratory for at least 2 weeks prior to use; they were fed water containing tetracycline and neomycin (0.5 g/liter of each) diluted with sterile water in order to suppress the bacterial flora in the gastrointestinal tract. One milliliter of blood (hct 70%) was injected intraperitoneally on 2 consecutive days (Days 0 and 1). They were sacrificed daily for the following 8 days. The group sacrificed on Day 1 received only one ip injection of blood (hct 70%). The mice were bled by direct cardiac puncture into heparinized syringes. Hematocrits were done on each sample using the microhematocrit technique. The individual samples were centrifuged and the plasma was separated and stored at −20°C for 1 month.

[1] Supported in part by the National Heart, Lung and Blood Institute (HL 22650 and HL 22469) and the U.S. Department of Energy under Contracts EY-C-02-0016 and W-7405-ENG-48.

[2] The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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0037-9727/80/010036-03$01.00/0

Erythropoietin assay. Ep was measured on individual plasma samples using an Ep RIA technique, details of which have been described previously (6). Pure human Ep was used for iodination (kindly supplied by Dr. Eugene Goldwasser, Department of Biochemistry, University of Chicago). It was obtained by extraction from urine of severely anemic humans and had a specific activity of 70,400 units/mg protein. The standard reference Ep preparation consisted of the second IRP of human urine (obtained from the National Institute for Medical Research, London, England). The Ep antiserum was produced in rabbits immunized with human urinary Ep. It has previously been shown that this Ep RIA crossreacts with a variety of animal Ep, including mouse Ep (6). The minimum level of detectability of this assay is approximately 4.0 mU/ml which is equivalent to an absolute amount of 0.4 mU/ml. The linear portion of the curve covers a range from approximately 4.0 to 100 mU/ml. Repeated determinations on the same samples of serum vary with an intra assay standard deviation of 8.8% and an inter assay standard deviation of 12.6%.

Results. The hematocrits of the hypertransfused mice increased on Day 1 to 60.3 ± 2.9% from the pretransfusion value of 43.9 ± 0.4%. They were stable between approximately 65 and 71% over the ensuing 8 days (Fig. 1a). The Ep levels decreased from the normal range of 11.2 ± 0.9 to 4.3 ± 1.1 mU/ml on the third day post-transfusion. The post-transfusion Ep levels were significantly different from the pretransfusion Ep levels in all experimental groups ($P < 0.05$ on Days 1, 6, and 7 and $P < 0.01$ on all Days 2–5 and 8). The combined Ep values from Days 3 to 8 were significantly different from control with a P value of < 0.001 (Fig. 1b).

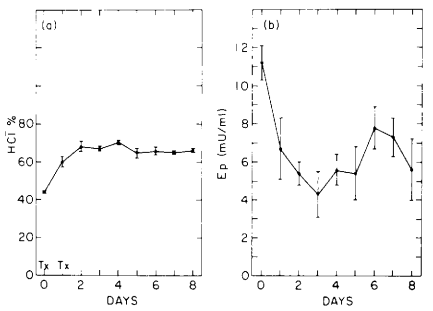

FIG. 1. Hct (a) and Ep (b) levels after hypertransfusion. The symbols indicate the mean ± standard error of the mean of values from nine mice in the control and four mice in each experimental group.

Discussion. Transfusion-induced plethora is followed by a 50% reduction in endogenous Ep levels in mice which presumably is a causative factor in the marked reduction in the recognizable erythroid compartment in the bone marrow (4).

It is of interest that Ep levels were not abolished by increasing the hematocrit 1.75 times. This degree of plethora has been reported to be associated with an increase in the viscosity of blood (7) and could be responsible for producing some degree of tissue hypoxia at the site and/or sites of Ep production, thereby accounting for the Ep levels that are observed. Kilbridge *et al.* (8) demonstrated that transfusion of hypoxic mice with either oxyhemoglobin or methemoglobin red blood cells suppressed Ep production in mice if the hematocrit was greater than 60%. Methemoglobin red cells were not as effective as oxyhemoglobin red cells in suppressing Ep levels if the hematocrits were less than 60%. Factors related to the increased hematocrit and not improvement of oxygen transport appeared to be responsible for the suppression of Ep levels observed in their studies.

Several investigators have reported that there are erythropoietic inhibitors in the plasma of animals and man with experimentally induced polycythemia (7–12); others have failed to confirm this finding (13). We have not excluded this alternative explanation in the present study.

Summary. The mechanism by which plethora suppresses erythropoiesis in experimental animals has been assumed to be related to a decrease in erythropoietin (Ep) levels. Prior to the advent of a radioimmunoassay (RIA) for Ep this theory could not be confirmed because of the insensitivity of the bioassay for Ep. In the present study hypertransfusion induced plethora was associated with approximately a 50% reduction in plasma Ep levels in CF_1 mice.

The technical assistance of Ms. Greta Duke and Mr. David Wei is gratefully acknowledged.

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Received May 7, 1979. P.S.E.B.M. 1980, Vol. 163.