Response of Friend Virus-Infected Bone Marrow Cells to Erythropoietin in vitro

The effect of erythropoietin (ESF) on erythropoiesis in vitro has recently received considerable attention. Investigators working with murine spleen cells in tissue culture[1,2] found that cells from plethoric (hypertransfused) mice respond to ESF by increased Fe^{59} incorporation in a manner similar to cells from normal mice. However, other studies on human bone marrow[3,4] indicate that ESF has little or no effect on cells from patients with polycythemia vera while in normal marrow cells Fe^{59} incorporation into heme is greatly increased.

Investigations in this laboratory on a variant of Friend virus which rapidly induces hypervolemic polycythemia in susceptible mice[5] have recently shown that this virus-induced erythropoiesis is ESF-independent[6]. It was therefore of interest to determine whether bone marrow cells from polycythemic mice would respond to ESF in vitro.

Cells were obtained from 7- to 8-weeks-old male Ha/ICR Swiss mice by perfusing their femora with a needle and syringe. The cells were suspended in RPMI No. 1640 medium supplemented with 20% fetal calf serum, 10% mouse plasma, penicillin and streptomycin. ESF (step 1 lyophilized sheep plasma from Connaught Laboratories, Toronto) was dissolved in medium to a concentration of 5 U/ml. Cells (10^6/ml) in a final volume of 2.8 ml were placed in 35×10 mm petri dishes, 0.2 ml of diluted ESF was added (final concentration 0.33 U/ml) and the cultures were incubated at 37 °C in 5% CO_2. (0.2 ml of medium only was added to control cultures.)

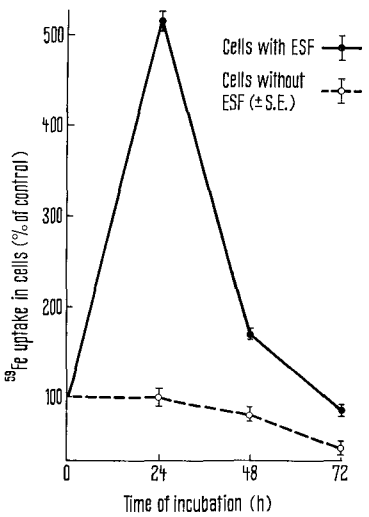

Fig. 1. Comparison of Fe^{59} uptake in normal marrow cells with and without erythropoietin (ESF) (0.33 U/ml).

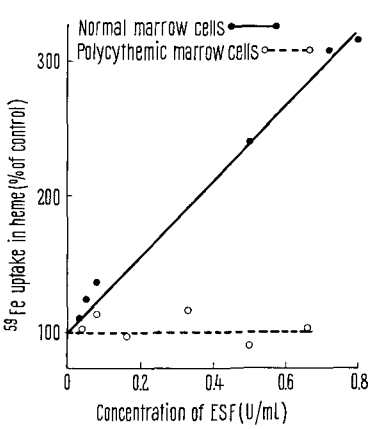

Fig. 2. Dose-response curves for normal and polycythemic bone marrow cells after 24 h incubation with erythropoietin (ESF).

At 24 h intervals 1 μc of $Fe^{59}Cl_3$ (Abbott Laboratories) was added to 3 of the cultures which were then reincubated for an additional 4 h. The cells from these cultures (and cells from 3 control cultures) were then transferred to centrifuge tubes, sedimented, washed twice with normal saline, and lysed by adding cold, acidified (pH 2) distilled water, according to TEALE[7]. The amount of Fe^{59} contained in the aqueous and heme fractions was determined with a well-type scintillation counter.

The results of a preliminary experiment (Figure 1) show that after 24 h normal marrow cells incubated with ESF took up 5 times as much Fe^{59} as cells without ESF. Thereafter, the Fe^{59} incorporation declined, even in ESF-treated cells. Therefore, a 24 h incubation period was selected for succeeding experiments.

To determine the relative sensitivity of marrow cells from normal versus virus-infected polycythemic mice, varying amounts of ESF were incubated with both types of cultures for 24 h. The dose-response curve for normal marrow (Figure 2) was significantly greater than that for polycythemic marrow cells ($P < 0.001$), which showed no significant response to ESF even at a concentration of 0.6 U/ml.

MIRAND[8] has suggested that in murine virus-induced polycythemia, the site of erythropoiesis gradually shifts from the bone marrow to the spleen. Preliminary experiments with bone marrow and spleen cells in the system described here indicate that this shift may occur as early as the fourth day after i.v. infection of mice with a high virus dose[9].

Zusammenfassung. Erythropoietin stimuliert in vitro die Aufnahme von Fe^{59} in normale Knochenmarkzellen der Maus, nicht aber in Knochenmarkzellen polycythämischer Tiere.

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9 This investigation was supported in part by research grants No. GY-4077 from the National Science Foundation, Nos. CA-08847 and CA-07745 from the National Institutes of Health, Public Health Service, and the John A. Hartford Foundation, Inc.