Erythropoietic Stimulating Factor (ESF) as a Stimulant of Cell Growth *in vitro*.* (28997)

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(Introduced by Perry Morgan)
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The existence of an erythropoietic stimulating factor or factors (ESF) or erythropoietin has been established beyond reasonable doubt(1,2,3,4,5). Evidence(6,7,8) was advanced that the stimulatory effect of ESF on erythropoiesis was due to increase in rate of differentiation of primative stem cells into the early population of cells. Recently Leaders *et al*(9) found that erythropoietin extracts or procedures which increase endogenous ESF production stimulated growth of Novikoff hepatoma in rats. They proposed that ESF might be a nonspecific growth factor. The present study is concerned with the ability of ESF to stimulate growth of cultures of cells *in vitro*.

Materials and methods. Two cell lines were used. One line of cells was cultured in this laboratory from the bone marrow of a human monocytic leukemia patient (No. 16 bone marrow) and has been in continuous culture for 3 years. The other line of cells, human synovial membrane (McCoy), was obtained from Dr. Charles Pomerat, Pasadena Foundation for Medical Research, Pasadena, Calif., and has been in continuous culture in this laboratory for 5 years.

Medium. Cells were cultured in 199 medium(10) with 10% horse or human serum. At times of drug administration the concentration of serum was reduced to 5%.

Techniques. 50,000 or 100,000 cells were suspended in 0.5 or 1.0 ml of medium in 16 × 150 mm pyrex culture tubes. Four tubes of controls and the same number of tubes for each treatment were counted at 2-day intervals for up to 13 days. All cultures were incubated at 37°C.

Counting. To suspend cells for counting, the medium was decanted and replaced with 0.2% trypsin (0.5 ml). When the cells were suspended, 0.01% crystal violet in 0.1 M citric acid (0.5 ml) was added. Cells were counted by the hemocytometer method(11).

Drug treatment. Erythropoietin (ESF) was obtained from the Armour Pharmaceutical Co. through the U. S. Public Health Service. This ESF was prepared from sheep plasma by the method described by White *et al* (5). One unit of ESF is equivalent to 5 μM of cobalt chloride using the fasted rat assay (12). ESF was dissolved in sterile distilled water to make a stock solution of 200 units/ml and was prepared freshly for each experiment by dilution with medium to the proper concentration. Cells were treated with ESF at the following times: 1) initially when tubes were first prepared, 2) when cell population returned to starting level of 100,000, 3) three days following return of cell population to starting level, 4) six days following return of cell population to starting level. At time of ESF administration, medium was decanted and replaced with fresh medium only or with medium containing "active" or "inactive" ESF. The "inactive" ESF did not produce an erythropoietic response *in vivo* (12) following "storage inactivation." ESF was used in concentrations between 0.0125 and 2.0 units/ml.

Results. Determination of cell culture characteristics. Experiments were performed to determine the growth characteristics of the 2 cell lines. After cultures of cell lines were started, a decrease in the number of cells was observed. The data recorded in Table I for the McCoy cells is typical for both cell lines. If approximately 100,000 cells were cultured in 1 ml of medium, recovery to the

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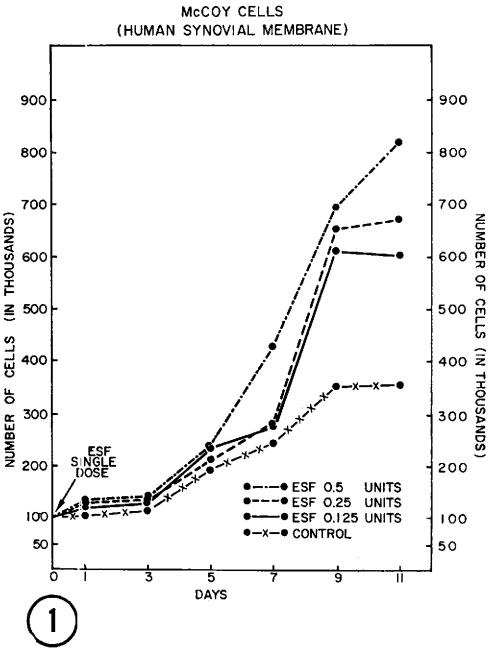

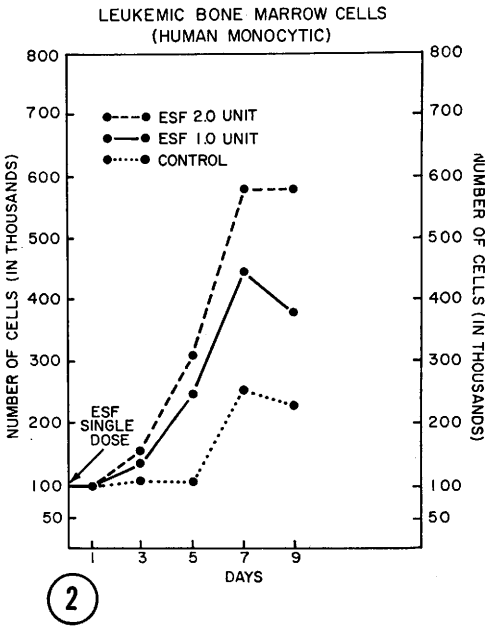

FIG. 1. Effects of administration of erythropoietin (ESF) to cultures of human synovial membrane cells (McCoy cells). ESF—single dose of erythropoietin. Values are mean values for 4 culture tubes, 2 counts per tube.

FIG. 2. Effects of administration of erythropoietin (ESF) to cultures of leukemic bone marrow cells (human monocytic). ESF—single dose of erythropoietin. Values are mean values for 4 culture tubes, 2 counts per tube.

level of the initial population proceeded optimally.

Effects of ESF on cell culture. In the first experiment, using McCoy cell cultures, very high cell counts were recorded for both control and treated cells. In these experiments, ESF in doses from 0.0125 unit to 1.0 unit demonstrated no significant increase in cell count. In some experiments using McCoy cells in which the cell population was spontaneously reduced by 75 to 80% within 24 hours, an indication of an inverse dose response relationship was observed. A slower growth rate was produced by culturing cells in a medium in which human serum was replaced with horse serum at the time of administration of ESF. Fig. 1 & 2 illustrate the responses to ESF added when the cell population returned to initial levels (day 0). In all instances values for peak effects differ significantly from other treatments and groups which were not treated with ESF ($p<0.05$). Treatment with ESF which had been deactivated by "storage inactivation" did not result in culture growth significantly different from untreated groups.

TABLE I. Survival of Cultures of McCoy Cells Under Varying Conditions.

Initial cell population	Medium (ml)	No. of cells surviving day 1	day 3	day 5
100,000	1.0	56,000	63,000	116,000
100,000	.5	30,000	57,000	86,000
50,000	1.0	15,000	28,000	38,000
50,000	.5	20,000	31,000	42,000

Discussion. The ability of erythropoietin (ESF) to stimulate growth of cultures of human synovial membrane and human monocytic leukemia cells was demonstrated in our experiments. Finding that ESF was a stimulant of cells of other than bone marrow origin *in vitro* paralleled earlier work(9).

From the data, we concluded that ESF was apparently more effective as a stimulant in slower growing cultures. Because ESF has been reported to stimulate non-erythroid cell multiplication both *in vivo* and *in vitro*, this material should more properly be called a

nonspecific growth factor (NGF).

The inverse dose-response relationship noted in some experiments in which (McCoy) cells were used could possibly have resulted from the presence of an inhibitor as well as a stimulant substance in these extracts. This concept is consistent with the isolation of substances such as the growth promoting substance "promine" and the inhibitory substance "retine" reported by Hegyeli *et al*(13) and Szent-Gyorgyi *et al*(14). It is postulated that these substances are either identical with erythropoietin (NGF) or, more likely, they are members of a closely related family of nonspecific growth controlling substances. A reexamination of ESF (NGF) in this light could yield valuable information concerning control of cell division and tissue growth in normal and malignant tissue.

Summary. Cultures of 2 cell lines have been tested for their response to administration of erythropoietin (ESF). The ability of ESF to stimulate growth of cultures of human synovial membrane cells and human monocytic leukemia cells was demonstrated. ESF is ineffective in stimulating cell culture multiplication in very rapidly growing cultures. Suggestion was made that ESF be called "Nonspecific Growth Factor" (NGF).

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Enhanced Efficacy of Plasma After Aging in Treatment of Tourniquet Shock.* (28998)

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In previously reported studies from this laboratory concerned with infusion therapy of rat tourniquet trauma, reconstituted lyophilized human plasma was shown to be the most effective infusion agent when compared with saline, dextran in saline and dextran in glucose-in-water(1). The initial choice of the human material was made because of the convenience of preparation of large quantities of sterile plasma using this commercially available material. When in practice it proved to be highly effective, its use was continued.

In later experiments, designed to investi-

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