amount. Thus, that warming rate was plotted at a value for the ratio of diffuse to normal-incidence solar radiation of 0.085—the same value as for 11 January, when the incoming solar radiation was reduced by only a very small amount—with probably less error of location than that associated with the points for 22 February and 15 April.

Finally, the study by Idso (*3*) yielded our last three points. It dealt with a several-hours-long dust storm on 3 February 1971, during which radiation measurements had been made every 10 minutes. Throughout this event an upright Eppley pyranometer had been alternately shaded and unshaded with a small blackened paddle, so both diffuse and direct solar radiation were determined. Thus, on the basis of geometrical considerations, the normal-incidence solar radiation was calculated and the diffuse/normal-incidence ratio obtained. The results for all ratios between 0.2 and 0.3 were averaged together, as were results for all ratios between 0.3 and 0.4. Then, three peak ratios above 0.7 were averaged to yield the last of three points.

Our resultant eight data points of Fig. 2 define two essentially linear trends, which we have connected and extrapolated somewhat with a dashed line. The first data set shows an increased warming influence with increasing dust loading of the atmosphere, while the second data set shows a decrease in the warming influence. Apparently, when small amounts of dust are injected into the atmosphere, the net income of solar radiation to the planet's surface is only slightly reduced (11 January 1977), if at all (15 May 1973). However, this minor addition of particulates seems to have a great effect on the net thermal radiation balance, acting, so to speak, to significantly "close" the atmospheric "window" that otherwise allows a good deal of the terrestrial thermal radiation of the earth to escape to space. Thus, a peak warming influence is manifest at a critical dust concentration characterized by a relatively low diffuse/normal-incidence ratio, on the order of 0.1. In addition, the curve turns downward from that point as dust loading increases, and, assuming a linear extrapolation, at a second critical dust concentration characterized by a diffuse/normal-incidence ratio on the order of 0.9, a cooling influence begins. Beyond this second point, solar radiation reductions are hypothesized to continue, but no additional heating influence caused by thermal blanketing is expected. The atmosphere has long since become a virtual blackbody in the thermal infrared region of the electromagnetic spectrum—that is, the atmospheric window has been shut, and it can be shut no tighter. Idso (*4*) described an experimental situation in which this actually happened. It could not be plotted as a point on Fig. 2, however, since diffuse and normal-incidence solar radiation were not measured on that day, and solar radiation was somewhat reduced.

What do these results portend for the earth's future climate? Since our data only apply to aerosols contained within the lower troposphere, we are not yet in a position to comment on the greatest potential source of atmospheric particulates—volcanoes—since volcanism generally injects large amounts of particulates into the stratosphere. However, we can comment on the climatological consequences of man's industrial pollution of the atmosphere—that is, the "human volcano," postulated by Bryson (*5*) to have a great cooling influence. If, and when, this source of tropospheric particulates ever becomes climatologically significant, our findings imply that the resultant surface temperature trend will definitely be one of warming, not cooling. Thus, whereas many groups assigned to assess the problem have looked on this aspect of intensified industrialization as acting as a "brake" on the warming influence of increased carbon dioxide production, just the opposite is actually the case—the two phenomena complement each other. It is time that we face this fact and consider its many ramifications.

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Simultaneous Effects of Erythropoietin and Colony-Stimulating Factor on Bone Marrow Cells

Abstract. *Erythropoietin or colony-stimulating factor, or both, were added to rat or mouse marrow cell cultures, and the responses to each inducer were measured. Colony-stimulating factor caused the suppression of erythropoietin-stimulated hemoglobin synthesis, and erythropoietin caused the suppression of the granulocyte-macrophage colony formation that is dependent on colony-stimulating factor. The extent of suppression by each inducer was dose-dependent. Marrow cells from plethoric rats were more sensitive to suppression of erythropoietin action by colony-stimulating factor than were normal marrow cells. These findings suggest that either (i) the receptors for erythropoietin and for colony-stimulating factor have overlapping specificities and that the "wrong" inducer may bind without having an inductive effect, or (ii) the target cells for erythropoietin and colony-stimulating factor are very closely related or are the same.*

Evidence suggests that competitive demands can influence erythroid and granulocytic lines of hemopoietic differentiation (*1, 2*). Normally, erythropoiesis and granulopoiesis occur simultaneously, and modest stimulation of one line of differentiation does not affect the other. Under conditions of a greatly increased requirement for red cells, however, erythroid differentiation is increased, and this increase is accompanied by a decline in granulopoiesis (*2*). The stage in the developmental pathway of the blood cells at which this competition occurs is not known. A current model of hemopoietic cell differentiation postulates a developmental level of unipotent cells which are derived from pluripotent stem cells but, unlike them, are sensitive to specific inducers such as erythropoietin and a putative granulopoietin (colony-stimulating factor, CSF) (*3*). In an alternative view, specific inducers act on the pluripotent stem cell itself to regulate differentiation (*4, 5*). Much of the evidence supporting the former model comes from studies in vivo where the complexity of whole animals makes unequivocal interpretation difficult. Appropriate assays in vitro for erythropoietin and CSF now exist (*6*), and the hypothesis that the presence of one inducer influences the response by bone marrow cells to the other factor can be examined.

We tested the action of CSF under conditions where erythropoietin added to marrow cells from normal rats caused

Table 1. Effect of CSF on hemoglobin synthesis by normal rat marrow cells in vitro. Marrow cells aspirated from tibiae and femora of male Long-Evans rats (250 to 300 g) were cultured as described (*6*) at a nucleated cell concentration of 15×10^6 per milliliter in a medium at *p*H 6.9 consisting of 65 percent NCTC-109 (Microbiological Associates) with 30 m*M* morpholinopropane sulfonic acid (Sigma) to maintain the *p*H, 30 percent calf serum (International Scientific Industries), 5 percent rat serum containing iron (4.1 μg/ml) as ferric nitrate, and 0.05 mg/ml of gentamicin (0.05 mg/ml) (Schering). All serums were heat-treated (30 minutes at 56°C) prior to use. Portions (0.2 ml) were pipetted into the wells of Disposo trays (Linbro); six replicates were used per group. The wells were sealed with the sterile plastic sheets provided and were incubated at 37°C for 24 hours, after which 20 μl of rat serum labeled with ^{59}Fe (0.2 μc) were added and incubation was allowed to proceed for five more hours. The cells were transferred to tubes and washed with phosphate-buffered saline (PBS) (0.14*M* NaCl, 0.01*M* phosphate, *p*H 7.2), then twice with 5 percent trichloroacetic acid. Heme was extracted into cyclohexanone, and radioactivity was determined in a portion of this extract as described (*6*). In this system, essentially all of the heme is derived from hemoglobin, and this method can be used to measure the synthesis of hemoglobin. Erythropoietin with a specific activity of 200 units per milligram of protein, prepared in this laboratory from anemic sheep plasma and diluted in 0.1 percent bovine serum albumin (Pentex Fraction V, Miles Laboratories) in PBS, was added to cultures in a volume of 3 μl. The CSF, with a specific activity of 7×10^5 units per milligram of protein, prepared from human urine and diluted in 0.01 percent polyethylene glycol (molecular weight 6000 (J. T. Baker Chemical) was added to cultures in volumes of 2 μl. The erythropoietin or CSF were added to the wells immediately after the cell suspension.

Addition	Radioactivity			Suppression (%)
	^{59}Fe* (count/min)	Increase (count/min)	Percentage of control	
None (control)	22 ± 6		100	
Erythropoietin (48 mU)	164 ± 8	142	745	
CSF (1800 units)	24 ± 6		109	
Erythropoietin plus CSF	88 ± 6†	64	400	55

*Mean ± standard deviation. †Significantly different ($P < .001$) from the erythropoietin group.

Table 2. Effect of heat-inactivated CSF on hemoglobin synthesis by normal rat marrow cells in vitro. Experimental procedures were the same as those in Table 1. The CSF had been heat-treated at 100°C for 30 minutes.

Addition	Radioactivity			Suppression (%)
	^{59}Fe* (count/min)	Increase (count/min)	Percentage of control	
None (control)	40 ± 10			
Erythropoietin (12 mU)	268 ± 8	228	670	
CSF (900 units)	48 ± 8		120	
Erythropoietin plus CSF	210 ± 24	162	525	29
CSF (heat-treated)	52 ± 8		130	
Erythropoietin plus CSF (heat-treated)	264 ± 12†	212	660	7

*Mean ± standard deviation. †The erythropoietin plus CSF group was significantly lower ($P < .001$) than the erythropoietin group, whereas the erythropoietin and CSF (heat-treated) group was not significantly different.

Table 3. Effect of erythropoietin on CSF-mediated colony formation by mouse marrow cells in vitro. The method of Metcalf (*6*) was used for assay of CSF activity. Equal volumes of 0.6 percent freshly prepared agar and modified Eagle's medium were combined with marrow cells to give a final concentration of 1×10^5 nucleated cells per milliliter. Portions (1 ml) were plated into 35-mm Lux Standard dishes (Lux Scientific) and incubated at 37°C in a fully humidified atmosphere containing 10 percent CO_2 in air for 7 days (five dishes per group). All colonies of more than 50 cells were counted under a dissecting microscope at ×25 by a person who did not know the key to the experimental design. Marrow cells were obtained from the femora and tibiae of 10- to 12-week-old female BDF_1 mice. Sheep plasma erythropoietin, with a specific activity of 5000 units per milligram of protein (56 percent pure) (*10*), was diluted as described (*6*) and added to cultures in microliter volumes (2 to 6 μl). The CSF, prepared from L cell culture medium, with a specific activity of 8×10^7 units per milligram of protein (approximately 50 percent pure) (*9*), was diluted in 0.01 percent polyethylene glycol and added to cultures in a volume of 3 μl.

Addition	Colonies*	Suppression (%)
None (control)	0	
Erythropoietin (300 mU)	0	
CSF (260 units)	439 ± 26	
CSF plus 100 mU of erythropoietin	370 ± 22	16
CSF plus 200 mU of erythropoietin	364 ± 33	17
CSF plus 300 mU of erythropoietin	294 ± 16	33

*Mean ± standard deviation.

a sevenfold increase in the rate of hemoglobin synthesis (Table 1). When added alone, CSF had no effect on hemoglobin synthesis, but when added simultaneously with erythropoietin, CSF suppressed stimulation of hemoglobin synthesis by 55 percent. Exposure of CSF to 100°C for 30 minutes, a treatment that is known to abolish its capacity to stimulate colony formation in vitro (*7*), also abolished its suppressive effect on erythropoietin action (Table 2). The suppression, by CSF, of induced hemoglobin synthesis shown in Table 2 is considerably smaller than that in the experiment illustrated in Table 1. In the two experiments, different amounts of both CSF and erythropoietin were used; therefore variation was to be expected.

Since erythropoietin has at least two different responsive cell populations, namely, progenitor cells that have no distinguishable erythroid characteristics and already differentiated, late erythroid cells (*8*), we tested the effect of CSF on the first of these by using marrow from plethoric animals. Bone marrow from rats housed in a hypobaric chamber for 3 weeks and then kept for 6 days at ambient pressure is almost completely devoid of hemoglobin-containing red cell precursors. Marrow from these rats was much more sensitive than marrow from normal animals to suppression of erythropoietin-stimulated hemoglobin synthesis. Colony-stimulating factor (110 units) suppressed 60 percent of the effect of 48 mU of erythropoietin, and 440 units of CSF completely prevented erythropoietin action. In contrast, the response by normal marrow cells to the same amount of erythropoietin was decreased by only 55 percent by 1800 units of CSF (Table 1). The increased sensitivity of marrow from plethoric rats suggests that CSF does not compete with erythropoietin for the more differentiated erythroid cells. Conversely, these data suggest that CSF and erythropoietin may compete for cells in a more primitive, less differentiated subpopulation.

If erythropoietin and CSF act on the same population of marrow cells, not only should CSF suppress the response to erythropoietin, but erythropoietin should suppress the response to CSF. This is demonstrated in Table 3. Qualitatively similar results to those in Table 3 were obtained when rat marrow cells were used (data not shown). The CSF derived from L cells had a specific activity of 8×10^7 units per milligram of protein (approximately 50 percent pure) (*9*); the human urinary preparation used in all other experiments reported here had a

specific activity of 7×10^5 units per milligram of protein. In addition, the sheep plasma erythropoietin preparation used for the experiment shown in Table 3 had a specific activity of 5000 units per milligram of protein (56 percent pure) (*10*), compared with 200 units per milligram of protein for the preparation used in all other experiments reported here.

Several observations make it unlikely that the suppressions of erythropoietin-stimulated hemoglobin synthesis and CSF-mediated colony formation were due to nonspecific actions of the impure preparations of CSF and erythropoietin: (i) CSF suppressed the action of erythropoietin, and erythropoietin that of CSF, at physiologic concentrations (approximately $10^{-11}M$); (ii) heat inactivation of the CSF preparation completely abolished its capacity to suppress erythropoietin action (Table 2); and (iii) CSF alone had no effect on baseline hemoglobin synthesis by marrow cells.

In contrast to our findings, Mitchell and Adamson (*11*) observed no reduction in the response by rat marrow cells to erythropoietin after incubation of the cells with CSF. A possible explanation for this difference may be found in the source and purity of the CSF. Mitchell and Adamson used a relatively crude material prepared from rat leukocyte culture medium. We have found that unfractionated serum from rats and mice, injected with endotoxin 3 hours prior to bleeding, is a potent stimulator of granulocyte-macrophage colony formation, but does not suppress the response by marrow cells to erythropoietin. In contrast, both human urinary CSF and CSF prepared from L cell culture medium are effective suppressors of erythropoietin action.

The results of our studies in vitro provide support for what has been found repeatedly in vivo; namely, that competing demands affect erythroid and granulocytic differentiation (*1, 2*). Our results, however, are not compatible with a "three-tier" stem cell model (*3*) in which inducers act only on unipotent stem cells, unless these committed stem cells can again become pluripotent, in which case "commitment" has an altered meaning. Our findings do fit an alternative model in which pluripotent stem cells, once they start cycling, become sensitive to inducers through the transient appearance of specific receptors for the inducer during the cell cycle (*4*). In addition, these data confirm our previous results suggesting that erythropoietin can act at the pluripotent stem cell level by predisposing spleen colony-forming cells to erythroid colony formation at the expense of nonerythroid spleen colonies (*12*).

An alternative explanation of these results suggests that the committed, unipotent cells have receptors with specificities that overlap, so that the erythropoietin receptor can bind CSF and the CSF receptor can bind erythropoietin. The binding of the "wrong" inducer will not result in the initiation of the developmental process, but will prevent the binding of the "right" inducer. These two different models could, in principle, be distinguished by study of the binding of labeled inducer by means of autoradiography.

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Liposome Accumulation in Regions of Experimental Myocardial Infarction

Abstract. *The uptake of liposomes bearing positive, negative, or no net charge on their membrane and containing a radioactive tracer, [^{99m}Tc]diethylenetriamine pentaacetic acid, was studied in 12 intact dogs 24 hours after the induction of myocardial infarction, and compared to the relative regional myocardial blood flow determined from radioactive microspheres. Positively charged and neutral liposomes concentrate in infarcted regions against a flow gradient, while negative liposomes are passively distributed according to regional blood flow. Because positively charged and neutral liposomes concentrate in infarct areas and have the ability to incorporate pharmacologic agents in their aqueous or lipid phase, they may serve as vehicles for drug delivery to infarct zones of low flow.*

Liposomes have been proposed as vehicles for delivery of therapeutic agents into cells. Other investigators have discussed the capacity of these lipid spheres to provide an enhanced mode of intracellular entry for metabolically and pharmacologically active substances such as enzymes, antineoplastic drugs, chelating agents, and hormones (*1*). In most studies, cell suspensions in vitro or tissue cultures were used, and attention was focused on mechanisms of liposome-cell interactions and characterization of elicited intracellular responses. Other workers have investigated the characteristics of the distribution of liposomes in normal and abnormal tissues in vivo. Recently, liposomes were found to be concentrated preferentially in certain tumors, a situation with major therapeutic implications. In fact, the administration of liposomes containing actinomycin D to tumor-bearing mice prolonged their survival compared to mice treated with the free chemotherapeutic agent alone (*2*).

Acute myocardial infarction secondary to coronary artery occlusion is a condition that might benefit from the enhanced delivery of pharmacologic agents. The work of Maroko and Braunwald (*3*) suggests that the degree of ischemic injury and subsequent necrosis resulting from myocardial infarction is not constant, but rather is subject to therapeutic modification. The selective concentration of appropriate therapeutic agents, particularly in infarct regions of markedly reduced regional blood flow,