PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 542–549 (1978)

Inhibition of Diffusion Chamber (DC) Granulopoiesis by Anti-CSF Serum[1] (40242)

RICHARD K. SHADDUCK, ARLAND L. CARSTEN, GUNDABHAKTHA CHIKKAPPA, EUGENE P. CRONKITE, AND EMILIE GERARD

Montefiore Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213 and Medical Department, Brookhaven National Laboratory, Upton, New York 11973

Diffusion chamber culture of bone marrow cells has proven a useful technique for the *in vivo* study of hemopoietic cell differentiation. After implantation of such chambers into the peritoneal cavity of recipient animals, there is an initial decline in recoverable leukocytic cells which is followed by a subsequent wave of granulocytic differentiation (1, 2). Granulocyte maturation is greatly augmented in animals made neutropenic either by irradiation or administration of cyclophosphamide (3, 4). This suggests that diffusion chamber granulopoiesis may be stimulated by a humoral granulopoietic factor which appears in the serum of neutropenic hosts.

In vitro granulocyte differentiation can be stimulated by a glycoprotein termed colony stimulating factor (CSF). When this factor is added to bone marrow cells immobilized in semisolid media, it leads to the development of discrete colonies of granulocytes and macrophages (5). Based on these observations, and the findings of increased CSF in the sera of neutropenic animals (6, 7) it is believed that CSF may represent an *in vivo* granulopoietin.

In vitro, the effects of murine CSF may be neutralized by an antiserum (8) to this glycoprotein. To determine whether CSF is responsible for diffusion chamber granulopoiesis, mice were irradiated, implanted with chambers containing normal mouse bone marrow cells and repetitively injected with anti-CSF serum. The results show that antibody treatment markedly inhibits diffusion chamber granulopoiesis. This provides substantial evidence suggesting that CSF is an active granulopoietic substance *in vivo*.

Materials and methods. Groups of 8- to 10-week old female Hale-Stoner-Brookhaven (HSB) mice were used as bone marrow donors and chamber recipients. Three hours before implantation, recipients received whole body X-irradiation, (700 Rads) from a 250 KVp X-ray machine, at a dose rate of 120 Rads per min. Diffusion chambers were constructed with Nuclepore filter walls as previously described (9). Murine bone marrow cells were obtained from HSB mice and passed through cotton wool fibers to remove adherent cells. Each chamber was innoculated with 5×10^5 nonadherent cells and sealed with nylon plugs. Two chambers were inserted into the perintoneal cavity of each recipient mouse. Animals were then randomized to receive intraperitoneal injections (0.5 ml) every 12 hr of saline, normal rabbit serum or anti-CSF serum for a maximum of eight doses.

Animals were killed by cervical dislocation on days 2, 4, and 7, chambers removed and shaken in 0.5% pronase solution for 1 hr. Chambers were then opened and rinsed of their contents with McCoy's 5A. Cells from chambers of each treatment group were pooled and cell counts obtained. After centrifugation, smears were prepared for Wright-Giemsa staining and 200 cell differential counts. In some cases, CFU_c and CFU_s assays were performed on the harvested cells. Chambers which had been inadvertently punctured by the multiple injections were excluded from analysis.

In a single experiment, the effect of antiserum treatment on granulopoiesis was evaluated in non-irradiated CF_{1s} mice. Groups of 15 mice each were injected ip with 0.5 ml of saline, control serum or antiserum every 12 hr. Five mice per group were killed on days 2, 4 and 6 for assessment of peripheral blood neutrophils, quantitative marrow counts and CFU_c assays.

Anti-CSF serum was prepared as previously described (8). Serum free L-cell CSF

[1] This work was supported in part by NIH Grant No. R01CA15237-03 and the U.S. Department of Energy.

0037-9727/78/1584-0542$01.00/0

was concentrated 100-fold by ultrafiltration and partially purified by Sephadex G150 chromatography. Active fractions were pooled, emulsified in complete Freund's adjuvant and injected into rabbits in multiple subcutaneous sites. After four weekly injections, antiserum was obtained and tested for anti-CSF activity using the in vitro agar-gel assay (8). Pooled antisera from three rabbits with a 50% neutralization titer in excess of 1:128 was used in these studies.

Prior to *in vivo* use, both normal rabbit serum and anti-CSF serum was adsorbed against HSB mouse red cells to remove cross-species agglutinins. Red cells were obtained by cardiac puncture, anticoagulated with Alsever's solution and washed in this solution to remove the plasma. The rabbit sera were adsorbed 4 times for 30 min at 37° with 15% packed RBC's (volume/volume); after adsorption both gross and microscopic agglutinins had been removed. Sera for a second study were, in addition, adsorbed against mouse liver powder (Pentex) for 4 hr at 37° and overnight at 4° using 1 mg of liver powder per ml of serum.

Sera were tested for possible cytotoxicity by both *in vivo* and *in vitro* techniques. For *in vivo* studies mice were injected with 0.5 ml of adsorbed serum (control or anti-CSF) every 12 hr and killed at 4, 24, and 48 hr after the first injection. Femoral marrow was obtained by "wash-out" technique, cell counts performed and the marrow evaluated for CFU_c and CFU_s content. *In vitro* cytotoxicity was assessed by incubating normal marrow cells in McCoy's medium supplemented with 10% fetal calf serum (FCS) with or without 10% control or anti-CSF serum. After overnight incubation at 37°, the cells were counted, washed in McCoy's-FCS and plated for CFU_c assay. In addition, the adsorbed sera were tested against mouse marrow cells by chromium release assay. In this technique, 10^8 mouse marrow cells were suspended in Hank's solution containing 10% FCS and nucleated cells were separated by Ficoll-Hypaque density gradient centrifugation. Cells from the upper layer were washed with Hank's 10% FCS and diluted to 5×10^6 cells per ml in 0.03 *M* Tris PBS buffer, pH 7.4. One ml of cell suspension was incubated with 100 μCi of ^{51}Cr (Na chromate) for 30 min at 37°. Unbound radioactivity was removed by washing the cells with Eagle's MEM containing 10% FCS. Labeled cells (2.5×10^4) in 0.1 ml volume were incubated with 0.15 ml of varying dilutions of control or anti-CSF serum for 1 hr at 37°. After addition of 10 μl of fresh guinea pig serum as a source of complement, each tube was incubated for an additional 60 minutes. The contents of all tubes were washed with 1.75 ml of PBS, spun at 1400 G for 10 min and 1 ml of supernatant counted for radioactivity. Assays, in addition, contained serum free tubes as a measure of spontaneous release and freeze-thawed cells to provide an estimate of total chromium release.

Further specificity of the CSF antibody was determined by testing the effect on erythropoietin-dependent erythroid colonies (CFU_e) (10). Culture medium consisted of alpha medium, 0.8% methyl cellulose, 30% fetal calf serum and 1% deionized bovine serum albumin. Each culture dish was prepared with 2×10^5 mouse marrow cells in 1 ml of medium to which one unit of erythropoietin was added. Culture plates were incubated in a 5% CO_2 incubator at 37° for 42–48 hr. All benzidine positive clusters containing eight or more cells were scored as erythroid colonies. The effect of control serum and anti-CSF serum was evaluated by addition of 0.04 ml of each serum per culture.

The *in vitro* agar gel assay was performed as previously described (6). In brief, each culture plate contained 10^5 mouse marrow cells immobilized in McCoy's-agar with 5% L-cell conditioned medium as a source of CSF. Cultures were incubated in a humidified 7.5% CO_2 atmosphere (37°) for seven days. Colonies were scored as all aggregates containing 50 or more cells; clusters were defined as 17–49 cells. Antibody titers were determined by mixing 0.1 ml of anti-CSF serum with each 1 ml culture. The titer was defined as that dilution of antiserum which caused 50% reduction in colony growth. CFU_c content of diffusion chamber cells was determined by the agar gel technique using the combined colony and cluster count. Each culture contained 10^5 such cells and 5% L-cell conditioned medium as a source of CSF. Three to five culture plates were used for each point.

CFU_s content of chambers from each treatment group was estimated by the spleen col-

ony technique (11). HSB mice were given 750 Rads total body X-ray exposure and injected intravenously with 1.5×10^5 chamber cells. Seven days after the injection, animals were killed and their spleens evaluated for macroscopic colony formation. Fifteen to twenty animals were used for the cell pool from each treatment point.

Results. Several tests for potential cytotoxicity showed little effect by control serum and no toxicity with anti-CSF serum. As shown in Table I, the administration of erythrocyte-adsorbed antiserum to normal mice had no deleterious effects on hemopoietic precursor cells. After 1 to 4 doses of antiserum, the numbers of bone marrow CFU_c and CFU_s were similar to those in mice injected with saline. Control serum recipients had a modest decrease in CFU_c while CFU_s were unchanged from values found in saline controls. The in vitro effect of antiserum was evaluated by incubation with marrow cells in liquid culture for 24 hr prior to CFU_c assay, Table II. In these experiments, sera were adsorbed against both mouse erythrocytes and liver powder. Neither the control serum nor the antiserum reduced the number of *in vitro* CFU_c irrespective of whether the samples had been adsorbed prior to assay.

Further experiments evaluated the effect of anti-CSF serum on erythroid progenitor cells. Incorporation of 4% antiserum into erythropoietin-dependent marrow cultures did not diminish CFU_e colony growth; 426 ± 48 and 541 ± 45 colonies were observed in control and antiserum cultures respectively.

Although the foregoing studies did not indicate a cytotoxic effect on various hemopoietic progenitor cells, further experiments evaluated this problem with a sensitive chromium release assay. Mouse marrow cells were separated by Ficoll-Hypaque density gradient centrifugation and the mononuclear cell layer was labeled with ^{51}Cr. Both unadsorbed control and anti-CSF serum caused appreciable chromium release, 68 and 40% respectively, Table III. After adsorption with mouse erythrocytes and liver powder, moderate reactivity was detected in the control serum whereas the anti-CSF showed no apparent cytotoxicity.

TABLE II. EFFECT OF ANTI-CSF SERUM ON *in Vitro* CFU_c.[a]

Culture medium	Total CFU_c recovered
McCoy's Medium	5074 ± 1570
Control Serum	7764 ± 1282
Adsorbed Control Serum	10235 ± 1437
Anti-CSF Serum	8004 ± 2299
Adsorbed Anti-CSF	8907 ± 1354

[a] 20×10^6 bone marrow cells were incubated in 5 ml supplemented McCoy's medium at 37° for 24 hr. Control serum and antiserum were evaluated by addition of 10% vol/vol to the supplemented McCoy's medium. Adsorbed sera were adsorbed against mouse erythrocytes and liver powder prior to assay. Shown are the total CFU_c recoveries 24 hr after incubation. Values are means ± 1 SE from four experiments. Only the CFU_c incubated in adsorbed control serum differed from the number of CFU_c incubated in supplemented McCoy's medium ($P < 0.05$).

TABLE I. EFFECT OF ANTI-CSF SERUM ON (*in Vivo*) CFU_c & CFU_s.[a]

	CFU_c/femur		
Treatment	4 hr	24 hr	48 hr
Saline	29784 ± 1904	28163 ± 2473	29478 ± 1971
Control Serum	26078 ± 1584	23160 ± 1195*	23542 ± 1977*
Antiserum	30725 ± 2195	29344 ± 3227	25943 ± 2200
	CFU_s/femur		
	4 hr	24 hr	48 hr
Saline	2833 ± 413	4066 ± 1355	3285 ± 514
Control Serum	1994 ± 680	2154 ± 407	3255 ± 498
Antiserum	3423 ± 480	3345 ± 230	4038 ± 426

[a] Values depict total CFU_c and CFU_s/femur at the indicated times following 1,2 or 4 doses (0.5 ml ip) of saline, anti-CSF serum or control serum. Both sera were repeatedly adsorbed with mouse erythrocytes to remove cross-species agglutinins. Three mice per group were injected. CFU_c values were calculated using 10^5 marrow cells (5 plates each). CFU_s were assayed in 6–9 irradiated recipients using 7.5×10^4 cells/animal.

* ($P < 0.05$).

The results of a four day course of saline, control serum or antiserum injections on diffusion chamber cell growth are shown in Fig. 1. The first experiment used erythrocyte adsorbed sera; in the second study the sera were adsorbed against both erythrocytes and liver powder. As seen, total chamber cellularity was low on day 2, rose by day 4 and reached high values on day 7. In both studies, cell recovery was 6 to 7 times cell input in the saline treated hosts, six to eightfold increased with control serum treatment but showed virtually no increment in chambers from the antiserum treated hosts.

Changes in chamber granulopoiesis are depicted in Figs. 2 and 3. Control serum appeared to have a mild suppressive effect in the first experiment. After liver powder adsorption, chambers from control serum recipients showed no inhibition of granulocytic differentiation. In the two studies, proliferative granulocytes increased 9- to 14-fold in saline treated hosts and 7- to 18-fold in control serum recipients seven days after implantation (Fig. 2). In contrast, only a one to twofold increase in these myeloid cells was observed in chambers from mice receiving the antiserum. The alterations in proliferative cells were further reflected by changes in the non-proliferative chamber granulocytes at day 7 (Fig. 3). These cells increased 10- to 12-fold in saline treated and 8- to 10-fold in control treated hosts. Only minimal granulocytic differentiation occurred in chambers from animals treated with the antiserum.

TABLE III. CHROMIUM RELEASE ASSAY OF ANTI-CSF SERUM USING MARROW TARGET CELLS.

	Serum dilution			
Type of serum	Undiluted	10^{-1}	10^{-2}	10^{-3}
Control serum	68%*	56%	18%	15%
Control serum (adsorbed)	36%	33%	10%	12%
Anti-CSF serum	40%	21%	17%	13%
Anti-CSF serum (adsorbed)	10%	10%	11%	11%
Spontaneous Cr release	11%			

* Values denoted % Cr release using the indicated serum with Ficoll-Hypaque separated mouse marrow cells.

To determine whether the sera had an effect on hemopoietic progenitor cells, the numbers of chamber CFU_c and CFU_s were evaluated on days 4 and 7. As shown in Table IV, a marked reduction in recoverable CFU_c was noted in the antiserum recipients at both time points. Control serum had no effect on CFU_c at day 4. However, as compared to chambers from saline recipients, a moderate decrease in these granulocytic stem cells was observed three days after discontinuance of injections (day 7). No decrease in chamber CFU_s content occurred in the control serum treated group; however, a 55% reduction in CFU_s was detected in the day 4 antiserum recipients.

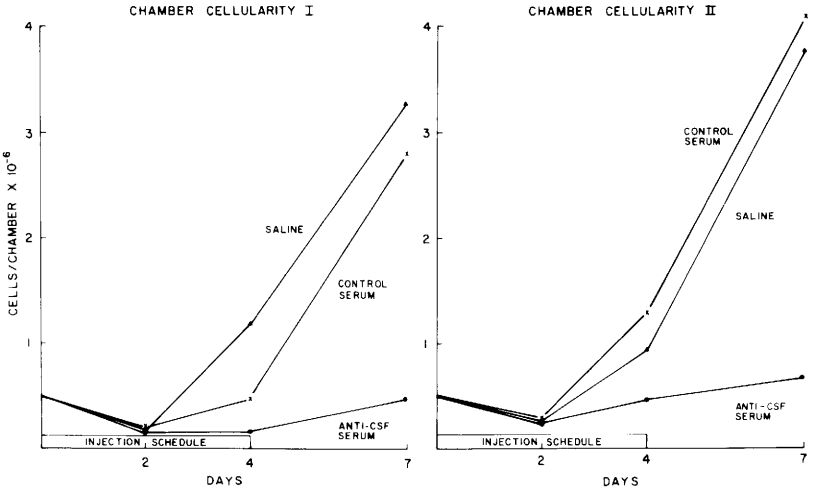

FIG. 1. Diffusion chamber total cell recovery. Values are mean cell counts obtained from five to nine chambers at the indicated time points.

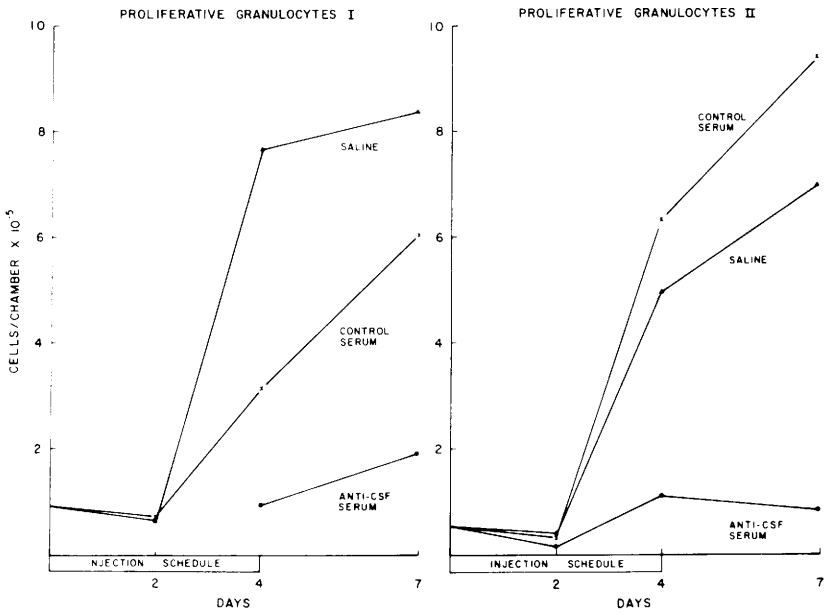

FIG. 2. Chamber granulopoiesis. Values depict total numbers of proliferative granulocytes (myeloblasts, promyelocytes and myelocytes) at the indicated times after the first injection.

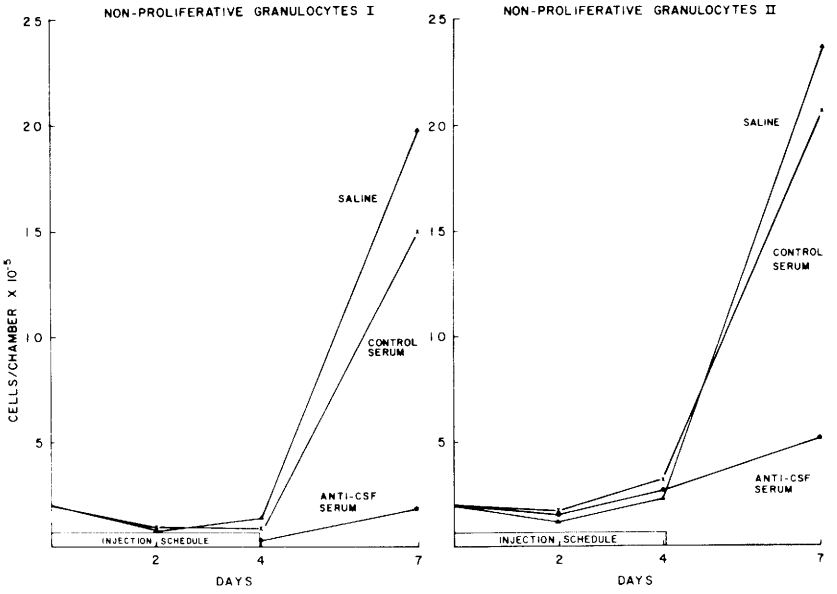

FIG. 3. Chamber granulopoiesis. Points indicate the total numbers of non-proliferative granulocytes (metamyelocytes, bands and segmented cells) at the indicated times after the first injection.

Although the CSF antiserum was markedly inhibitory to diffusion chamber granulopoiesis, no suppressive effects were noted in nonirradiated CF_{1s} mice. No decrease in peripheral blood neutrophils, marrow granulocytic precursors or marrow CFU_c were noted after 2 to 6 days of antiserum treatment.

Discussion. Several observations suggest that CSF may be an *in vivo* granulopoietin. Firstly, it is well established that addition of this factor to *in vitro* cultures leads to extensive granulocytic differentiation (5). Sec-

TABLE IV. EFFECT OF ANTI-CSF SERUM ON CHAMBER CFU_c AND CFU_s.[a]

Treatment	CFU_c/DC Day 4	CFU_c/DC Day 7
Saline	524 ± 44.6	1038.5 ± 132
Control serum	526.5 ± 67.9	587.5 ± 59.5
Antiserum	47.8 ± 8.0	18.4 ± 8.3
	CFU_s/DC	
Saline	103.4 ± 3.9	259.7 ± 15.7
Control serum	165.5 ± 5.8	255.6 ± 18.3
Antiserum	45.5 ± 1.7	n.d.

[a] Values indicate means ± 1 SE for chamber CFU_c and CFU_s content. Insufficient cells were available from chambers of antiserum recipients for day 7 CFU_s assays.

ondly, high levels of CSF activity are noted in the serum of animals made neutropenic by a variety of experimental manipulations (6, 7). In addition, Metcalf and Stanley have noted an *in vivo* neutrophilic response, in mice, to repetitive injections of partially purified human urinary CSF (12). However, owing to the lability of the neutrophil system, it has been difficult to conclusively demonstrate *in vivo* granulopoietic activity. Injection of many foreign proteins, including bacterial endotoxins, leads to an immediate neutrophil release from the marrow (13). Repeated injections of such substances causes a mild neutrophilia (14) which may reflect depletion of the marrow storage pool rather than a primary stimulus for proliferation and differentiation.

In order to circumvent the problems accruing from nonspecific neutrophil release or other foreign protein effects, the present studies were aimed at suppressing rather than stimulating granulopoiesis. As foreign sera are not known to diminish granulocyte production, these experiments examined the ability of anti-CSF serum to block granulocytic differentiation *in vivo*. Injection of antiserum into intact mice for intervals up to 6 days caused no suppression of bone marrow differentiation. In contrast, diffusion chamber granulopoiesis was markedly inhibited. The differences between these two studies may, in part, result from higher concentrations of antibody which are achieved around the chambers following intraperitoneal injection. The dilution and rapid clearance of rabbit IgG in mice may markedly reduce the amount of antibody available for systemic neutralization of CSF. In unpublished studies in which 50 mg of purified rabbit IgG was injected IP into mice in two injections 12 hr apart, only 5%–8% of the rabbit IgG was detected in the serum 12 hr after the second dose. Moreover, following a loading dose of 100 mg of rabbit IgG and daily administration of 20 mg IP only minimal increments in circulating IgG were observed at 10 days.

Alternatively, granulocytic differentiation may be controlled, to a major extent, by locally produced CSF in the marrow. Chan and Metcalf (15) have shown high levels of CSF activity in medium conditioned by the growth of marrow stroma from which the majority of hemopoietic cells were removed. If this local mechanism is normally operative in granulocytic differentiation, antibody neutralization of CSF in intact animals may require much larger doses of antiserum or suitably purified CSF antibody (16). In the present diffusion chamber studies, the marrow cells were removed from their stromal microenvironment and further depleted of possible CSF producing cells by an adherent procedure. Therefore the diffusion chamber studies were designed in such a way as to reduce local CSF production, thereby providing optimal conditions for demonstrating the effect of antiserum.

Neither the anti-CSF serum nor control serum appeared cytotoxic to hemopoietic precursor cells. No decrease was noted in granulocytic stem cells (CFU_c) or erythroid progenitors (CFU_e) by various *in vitro* and *in vivo* tests. Despite this, control serum appeared to have a mild suppressive effect in the first *in vivo* study as judged by a moderate restriction of chamber granulopoiesis. For this reason the sera were further adsorbed against mouse liver powder to remove presumed anti-H2 or other naturally occurring cell directed antibodies. After adsorption, anti-CSF serum showed no activity and control serum had reduced activity against murine marrow as judged by a sensitive chromium release assay.

Anti-CSF serum injections caused near complete inhibition of diffusion chamber granulopoiesis in both studies. This effect was noted with the multiply adsorbed antiserum which showed no *in vitro* or *in vivo* cytotoxicity. Control serum had a mild suppressive

effect prior to liver powder adsorption; however, after adsorption (Study 2), this inhibitory activity was removed. This strongly suggests that the antiserum-induced suppression of granulopoiesis was due to neutralization of CSF activity rather than a non-specific cytotoxic effect of rabbit serum on diffusion chamber granulopoiesis.

Control serum showed no effect on chamber granulocytic stem cells (CFU_c) on the fourth day and only a modest effect on the seventh day after implantation. In contrast, treatment with anti-CSF serum caused a marked reduction in chamber CFU_c to less than 10% of the 4-day control group. This decrease in myeloid progenitor cells suggests that CSF may have a dual role: namely, to stimulate expansion of the percursor cell pool (CFU_c) and to induce differentiation of these elements into recognizable granulocyte precursors.

Pluripotential stem cells, as measured by the spleen colony technique, were moderately depressed in antibody recipients and increased in chambers from control serum animals. The magnitude of these changes did not approach the observed reduction in CFU_c. These changes may in part, result from CFU_s depletion by accelerated differentiation into the markedly reduced CFU_c compartment. Similar degrees of CFU_s depletion have been observed in animals treated with ^{55}Fe. In this experimental model, the ^{55}Fe markedly reduces the differentiated erythropoiesis and within 24 hr a secondary decrease in pluripotent cells is observed (17). Alternatively, the reduced number of CFU_s may be explained by incomplete cell recovery from chambers with minimal cellularity. One hour postinoculation cell retrieval from diffusion chambers approximates 50% with high cell concentrations but may fall as low as 20–25% in low cell density chambers (18). A similar phenomenon may effect the recovery of hemopoietic stem cells.

Although the majority of studies, including those reported herein, strongly suggest that CSF is an *in vivo* granulopoietin, certain observations do not appear to support this hypothesis. Increased serum CSF activity is noted in irradiated mice (19), however, irradiated germfree animals do not show this change (20). Despite these differences in CSF response, germ free animals do show a modest, albeit limited, recovery of granulopoiesis (21). Perhaps these discrepancies between the apparent levels of CSF and degrees of granulocyte differentiation may be resolved by the use of a more sensitive radioimmunoassay for CSF (22) or by study of the CSF produced locally in the marrow microenvironment.

Summary. Diffusion chamber granulopoiesis was studied in irradiated mice injected with anti-CSF serum. Total cellularity, proliferative and nonproliferative granulocytes were markedly suppressed by 4 days injection of antiserum. Moreover, granulocytic stem cells were markedly decreased. This suggests that CSF is necessary for maintaining the integrity of the granulocytic stem cell and for inducing differentiating granulopoiesis.

The authors express their thanks to James E. Bullis, Jr., Lawrence M. Cook, and Mrs. Louise M. Honikel of Brookhaven National Laboratory and Mrs. Florence Boegel and Mrs. Francine Pope of Montefiore Hospital, University of Pittsburgh School of Medicine, for their invaluable technical assistance. We further appreciate the help of Dr. Adolfo Porcellini of Ospedali Riuniti, Pesars, Italy for his involvement in the *in vivo* studies of granulopoiesis.

1. Benestad, H. B., Scand. J. Haematol. **7**, 279 (1970).
2. Bøyum, A., and Borgstrom, R., Scand. J. Haematol. **7**, 294 (1970).
3. Bøyum, A., Boecker, W., Carsten, A. L., and Cronkite, E. P., Blood **40**, 163 (1972).
4. Tyler, W. S., Niskanen, E., Stohlman, F., Jr., Keane, J., and Howard, D., Blood **40**, 634 (1972).
5. Metcalf, D., and Bradley, T. R., *in* "Regulation of Hematopoiesis" (A.S. Gordon, ed.) Vol. I, p187. Appleton-Century-Crofts, New York (1970).
6. Shadduck, R. K., and Nagabhushanam, N. G., Blood **38**, 559 (1971).
7. Shadduck, R. K., and Nunna, N. G., Proc. Soc. Exp. Biol. Med. **137**, 1479 (1971).
8. Shadduck, R. K., and Metcalf, D., J. Cell. Physiol. **86**, 247 (1975).
9. Carsten, A. L., Chanana, A. D., Chikkappa, G., Cronkite, E. P., and Ohl, S., Proc. Soc. Exp. Biol. Med. **150**, 107 (1975).
10. Iscove, N. N., Sieber, F., and Winterhalter, K. H., J. Cell. Physiol. **83**, 309 (1974).
11. Till, J. E., and McCulloch, E. A., Rad. Res. **14**, 213 (1961).
12. Metcalf, D., and Stanley, E. R., Brit. J. Haematol. **21**, 481 (1971).
13. Boggs, D. R., Sem. Hematol. **4**, 359 (1967).

14. Quesenberry, P., Halperin, J., Ryan, M., and Stohlman, F., Jr. Blood **45,** 789 (1975).
15. Chan, S. H., and Metcalf, D., Blood **40,** 646 (1972).
16. Shadduck, R. K., and Waheed, A., Blood 50, Suppl. **1,** 159, (1977).
17. Reincke, U., Burlington, H., Cronkite, E. P., Hillman, M., and Laissue, J. Blood **45,** 801 (1975).
18. Cronkite, E. P., Carsten, A. L., Chikkappa, G., Laissue, J. A., and Ohl, S., *in* "Advances in the Biosciences" (T. M. Fliedner and S. Perry, eds.), p273. Pergamon Press, New York (1974).
19. Morley, A. A., Rickard, K. A., Howard, D., and Stohlman, F., Jr., Blood **37,** 14 (1971).
20. Morley, A., Quesenberry, P., Bealmear, P., Stohlman, F., Jr., and Wilson, R., Proc. Soc. Exp. Biol. Med. **140,** 478 (1972).
21. Heit, H., Fliedner, T. M., and Fache, I., Radiat. Res. **51,** 72 (1972).
22. Shadduck, R. K., and Waheed, A., Clin. Res., in press (1978).

Received December 1, 1977. P.S.E.B.M. 1978, Vol. 158.