Co.) as an ethanolic solution. The final ethanol concentration did not exceed 0.5 µl/ml and ethanol controls were also set up.

Results and discussion. As shown in figure 1, histamine did not inhibit MAgF production, over a wide concentration range. Indeed, there was a strong tendency for histamine to enhance MAgF production; although this was only marginally significant at 10^{-6}M ($p < 0.05$). By contrast, as shown in figure 2, both PGE_2 and hydrocortisone gave a dose-dependant inhibition. Previously, PGE_2 (0.1–1.0 µg/ml) has been shown to inhibit MIF production[9,10] although it enhances the production of 2 other lymphokines, skin reactive factor[10] and osteoclast activating factor[11]. Thus, PGE_2 may either enhance or depress the production of lymphokines, depending on which activity is measured. HC has been shown to either inhibit[12] or not inhibit[13] guinea-pig MIF production. Additionally, HC has been claimed to inhibit MAgF production[14], although a subjective assay system was used in this study. Histamine, unlike HC and PGE_2 which act directly on lymphokine-producing lymphocytes, inhibits MIF production indirectly through H_2 containing supressor lymphocytes. These cells are present in immunized and non-immunized guinea-pig spleen cell populations[5] and so the lack of inhibitory effect found in the present study should not be due to lack of this cell population. In conclusion, the inhibitory effects of histamine on lymphokine production have, to date, been shown only for migration inhibitory activities. The results presented here suggest that this is not generally applicable to all lymphokines.

1 R.E. Rocklin, J. clin. Invest. *57*, 1051 (1976).
2 P.W. Askenase, A. Schwartz, J.N. Siegel, R.K. Gershon, Int. Archs Allergy appl. Immun. *66*, suppl. 1, 225 (1981).
3 R.E. Rocklin, J. Immun. *118*, 1734 (1977).
4 D. Rigal, J.C. Monier and G. Souweine, Cell Immun. *46*, 360 (1979).
5 R.E. Rocklin, D.K. Greineder and K.L. Melmon, Cell Immun. *44*, 404 (1979).
6 P. Badenoch-Jones, Immunology *46*, 624 (1982).
7 S. Lolekha, S. Dray and S.P. Gotoff, J. Immun. *104*, 296 (1970).
8 P. Badenoch-Jones, Aust. J. exp. Biol. med. Sci. *59*, 575 (1981).
9 D. Gordin, M.A. Bray and J. Morley, Nature, Lond. *262*, 401 (1976).
10 M.A. Bray, D. Gordon and J. Morley, Agents Actions *6*, 171 (1976).
11 T. Yoneda and G.R. Mundy, J. exp. Med. *149*, 279 (1979).
12 S.M. Wahl, L.C. Altman and D.L. Rosenstreich, J. Immun. *115*, 476 (1975).
13 J.E. Balow and A.S. Rosenthal, J. exp. Med. *137*, 1031 (1973).
14 W.L. Weston, H.N. Claman and G.G. Krueger, J. Immun. *110*, 880 (1973).

Enhancement of macrophage colony-stimulating factor in mice by carbon particles[1]

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Summary. Carbon particles enhance hemopoiesis in irradiated mice. Serum from carbon-treated mice stimulated macrophage colony formation, and inhibited granulocyte colony formation. The finding suggests that carbon-treatment modulates the hemopoietic environment through the monocyte-macrophage system.

Colony-stimulating factor (CSF) stimulates the formation of granulocyte-macrophage colonies from granulocyte-macrophage progenitors (GM-CFC)[2,3]. An elevated serum CSF level was reported in response to neutropenia[4]. Injection of bacterial lipopolysaccharide (LPS) into mice induces increases in serum CSF levels[5], granulopoiesis[6], and in the survival of irradiated animals[7].

Blockade of the reticuloendothelial system (RES) by particulate substances such as carbon and latex particles also stimulates the recovery of hemopoiesis in irradiated mice[8,9]. Since the major source of CSF appears to be the monocyte-macrophage system in RES[10], the enhancement of hemopoietic recovery by RES-blockade in irradiated mice may also be due to the elevated serum CSF levels.

We found that the injection of carbon particles into mice enhanced serum CSF, thereby stimulating macrophage production.

Materials and methods. Mice of strain DDY were used. Serum was collected from the mice 3 and 24 h after the injection of carbon particles (Pelikan ink, Germany). Bone marrow cells were incubated in a petri dish (10^5 cells/dish) containing 0.3% agar, 25% horse serum in Fischer's medium and 15% mouse lung- and heart-conditioned medium as a source of standard GM-CSF[3]. Test serum for CSF assay was added to the soft-agar culture instead of CSF. After 7 days incubation at 37 °C in 5% CO_2 in air, colonies consisting of 50 or more cells were counted, and CSF level was expressed as the number of colonies per 10^5 bone marrow cells.

Results and discussion. Injection of more than 1 mg carbon particles protected mice from the lethal effects of irradiation, but 0.1 mg carbon particles did not protect the mice effectively (table 1). All doses of carbon particles tested, however, increased serum CSF levels 3 h after injection. The activity disappeared within 24 h after carbon treatment. Thus, there was a discrepancy between the dose of carbon particles for enhancement of serum CSF level and that for radioprotection.

All the colonies consisted of macrophages, and no granulocyte or granulocyte-macrophage (mixed) colonies were found in the culture. To determine whether this is due to the low concentration of serum tested, soft-agar culture was performed with various concentrations of the serum. The number of colonies per 10^5 marrow cells increased, depending on the amount of test serum. However, all the colonies thus induced were again macrophage colonies, wereas serum from LPS-treated mice induced all types of colonies (table 2). To determine whether or not the serum from carbon-treated mice actually inhibits the granulocyte colony formation, serum from carbon-treated mice was added to the bone marrow cell cultures that were stimulated with GM-CSF. Addition of 5% carbon-serum did not affect the ratio of colony types (data not shown), but the addition of 20% carbon-serum inhibited granulocyte colony formation completely (table 2).

It has been convincingly shown that murine CSF preparations contain different CSFs: one that primarily stimulates macrophage production and another that simulates granu-

Table 1. CSF levels in serum in mice after injection of carbon particles, and survival of mice after irradiation

Dose of carbon injection	Colonies/10^5 bone marrow cells 3 h	24 h	Survival after 675 rad of X-rays
0	0	0	3/10
0.1 mg	75±4	0	2/10
1.0 mg	97±11	0	8/10
8.0 mg	135±7	0	10/10
Standard CSF	118±4		-

Mice were injected with various doses of carbon particles i.v., and serum was collected 3 and 24 h after injection. Test serum was added to the culture at the final concentration of 10%. Mouse lung- and heart-conditioned medium (15%) was used as standard CSF. 30-day survival of mice after 675 rad of X-irradiation is shown for comparison.

Table 2. Type of colonies produced by CSF in serum from carbon-treated mice

Serum or CSF		Colonies/10^5 cells	Colonies consisted of Granulocyte	Mixed	Macrophage
LPS-serum	5%	96±10	42.2%	43.2%	14.6%
Carbon-serum	5%	35±11	0	0	100
	10%	61± 9	0	0	100
	20%	131±12	0	0	100
GM-CSF	15%	116± 8	21.7	45.5	32.8
GM-CSF+ carbon-serum	20%	127±16	0	0	100

Bone marrow cells were incubated with serum from carbon-treated mice, from LPS-treated mice or with mouse lung- and heart conditioned medium (GM-CSF), and the colonies were cytologically studied after staining with May-Giemsa. LPS-serum was obtained from mice 3 h after i.v. injection of 5 µg LPS.

locyte production[11,12]. Our results show that serum from carbon-treated mice contains not only the CSF that stimulates macrophage production but the inhibitory factor(s) of granulopoiesis. It is, therefore, unlikely that carbon-treatment acts as a direct stimulator of hemopoiesis.

We have already reported that RES-blockade results in the increased survival of the pluripotent stem cells (CFUs), and enhanced recovery of functional blood cells after irradiation[9,13]. Based on the finding that the bone marrow cells, irradiated in vitro, form more colonies in the spleen of the carbon-treated recipient mice than in control mice, we have proposed that RES-blockade offers a favourable microenvironment for the proliferation of stem cells[14]. Seki reported that macrophage layers formed on acetate cellulose membranes, supported granulopoiesis[15]. Carbon-treatment enhances granulocytic colony formation on such layers as well[16].

Together with these observations, present results suggest that carbon-treatment modulates the monocyte-macrophage system in RES, which consequently supports the increased survival and maturation of hemopoietic stem cells.

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2 Ichikawa, Y., Pluznik, D.H., and Sachs, L., Proc. natl Acad. Sci. USA *56* (1966) 488.
3 Bradely, T.R., and Metcalf, D., Aust. J. exp. Biol. med. Sci. *44* (1966) 28.
4 Morley, A., Rickard, K.A., Howard, D., and Stohlman, F.J., Blood *37* (1971) 14.
5 Metcalf, D., Immunology *21* (1970) 427.
6 Mcneill, T.A., Immunology *18* (1970) 61.
7 Smith, W.W., Alderman, I.M., and Gillespie, R.E., Am. J. Physiol. *191* (1957) 124.
8 Mori, K.J., and Nakamura, S., Experientia *26* (1970) 1386.
9 Mori, K.J., Seto, A., and Ito, Y., Experientia *31* (1975) 112.
10 Chervenick, P.A., and LoBuglio, A.F., Science *178* (1972) 164.
11 Horiuchi, M., and Ichikawa, Y., Exp. Cell Res. *110* (1977) 79.
12 Stanley E.R., Proc. natl Acad. Sci. USA *76* (1979) 2969.
13 Mori, K.J., Radiat. Res. *56* (1973) 494.
14 Mori, K.J., and Ito, Y., Radiat. Res. *69* (1977) 134.
15 Seki, M., Transplantation *16* (1973) 3.
16 Mori, K.J., Seto, A., and Ito, Y., Experientia *30* (1974) 1467.

Glucan-induced enhancement of hemopoietic recovery in gamma-irradiated mice

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Summary. A single pre- or postirradiation application of β-(1→3)-D-glucan, a potent reticuloendothelial stimulant, enhances hemopoietic recovery in sublethally gamma-irradiated mice. Pretreatment of mice with glucan significantly reduces lethal radiation effects.

Glucan (β-(1→3)-D-glucan), a component isolated from the cell wall of *Saccharomyces cerevisiae,* is a potent reticuloendothelial stimulant as well as a modulator of cellular and humoral immunity[1,2]. The administration of glucan to mice results in increased macrophage and granulocyte production, the enhanced leukopoiesis probably being mediated via an augmented release of colony-stimulating activity from macrophages[3,4]. The increased functional status of RES induced by glucan has been shown to increase nonspecific host resistance to infection[5]. Both hemopoiesis and the anti-infection defence-enhancing effects of glucan could be valuable in prevention and therapy of radiation damage to the organism. The current experiments were undertaken to evaluate these possibilities, and the effects of pre- and postirradiational application of glucan to mice were investigated.

Material and methods. Male mice of the inbred strain C57Bl/10, 12-14 weeks old, weighing 25-30 g, and kept in cages in groups of 20, were used. Standard stock diet and drinking water were given ad libitum. Control and experimental procedures were carried out concurrently in groups of mice from the same cage.