fibrinolytic activity of different groups of veins was compared by the Wilcoxon rank sum test[8].

The results are given in the Table. In the rats, irradiated with 1000 rads and killed 1 month later (group B), the fibrinolytic activity in their vessel walls tended to be decreased. In the group C, killed 2 months after irradiation, the decrease was highly significant ($p < 0.001$).

The findings indicate a decrease in the activity of the cells producing the plasminogen activator enzyme. This is in agreement with findings by SVANBERG et al.[9] of a decreased fibrinolytic activity in a case of ovarian tumour examined before and after irradiation. The decreased activity is probably a manifestation of a damage to the vascular endothelium. It is known that this is followed later by fibrotic changes in the vessel walls, which may explain why the decrease in the fibrinolytic activity was larger after 2 months than after 1.

Tumours possess fibrinolytic activity which can be related to their vascularity[9]. The fibrinolytic activity in the vessel walls is of importance for the patency of vessels. The present experimental findings may help to explain the complex effect of irradiation on tumour growth.

Zusammenfassung. Bei bestrahlten Ratten wurde die fibrinolytische Aktivität verschiedener Gefässe histochemisch untersucht und mit denen einer Kontrollgruppe verglichen. Es wurde eine Herabsetzung der fibrinolytischen Aktivität der bestrahlten Gefässe gefunden, welche durch Beschädigung der Gefässendothelzellen erklärt werden könnte.

B. ÅSTEDT, S.-E. BERGENTZ and L. SVANBERG[10]

Coagulation Laboratory, the Department of Gynaecology and the Department of Surgery, Malmö, Allmänna Sjukhus, University of Lund, S-21401 Malmö (Sweden), 27 August 1974.

[8] W. J. DIXON and F. J. MASSEY, *Introduction to Statistical Analysis* (McGraw-Hill Inc., New York 1957), p. 290.

[9] L. SVANBERG, F. LINELL, M. PANDOLFI and B. ÅSTEDT, Acta path. microbiol. scand., in press (1974).

[10] This investigation was supported by grants from the Swedish Medical Research Council No. B75-17X-759-10A, the Medical Faculty, University of Lund and Tore Nilsons's Medical Research Fund.

Enhancement of Hemopoietic Colony-Formation in the Mouse Peritoneal Cavity by the Treatment with Carbon Particles

Accumulated data suggests that stromal elements play an important role in hemopoietic regeneration[1-6]. In the previous study we have also been able to demonstrate that hemopoietic stem cells which had been irradiated in vitro, recovered more effectively in the RES-blockaded host mice than in the control hosts[7] and have concluded that this phenomenon could be responsible for the increased survival and enhanced recovery of hemopoietic system of the irradiated mice by the blockade of RES with carbon particles[8-11]. These findings strongly suggest that the RES-blockaded mice provide a favorable microenvironment for the slightly damaged hemopoietic stem cells to recover. Concerning the possible control mechanism through some humoral factor, METCALF et al.[12,13] reported that the release of the colony-stimulating factor was greatly stimulated by the treatment of animals with bacterial endotoxin. It may be possible that carbon particles have a similar effect on the release of such factor(s), since both carbon particles and bacterial endotoxin are known to be most effective radioprotectants and stimulate hemopoietic recovery after irradiation[14,15].

In order to clarify whether the above-mentioned favorable effect of carbon-treatment of host mice on the recovery of irradiated colony-forming cells is due to humoral factor(s), extramedullary hemopoietic colony-formation was studied in the host mice either treated with carbon particles or with bacterial endotoxin.

Materials and methods. Details of the method for colony-formation on macrophage layer were reported by SEKI[16]. A cellulose acetate (CA) membrane (20 × 20 mm) was inserted into the peritoneal cavity of 9-week-old male mice of DDD strain. 4 days later, they were injected i.v. either with 10 mg of carbon particles (Pelikan India ink C11/1431 a, Germany) or with 20 µg bacterial endotoxin (lipopolysaccharide B from *E. coli* O 111:B4, Difco, USA). 24 h after the treatment, the animals were irradiated with 680 R of X-rays, and then injected i.p. with freshly harvested bone marrow cells[10]. 7 days after the injection of the bone marrow cells, the CA membranes were taken out and colonies formed on the macrophage layer were counted after the visualization procedure by the peroxidase reaction. In order to investigate the effect of host microenvironment on the recovery of irradiated hemopoietic stem cells, other groups of host mice, into which the CA membrane was also inserted, were exposed to 480 R of X-rays, and then injected with the bone marrow cells. 1 h later they were irradiated again with 200 R of X-rays. Thus, the host mice received 680 R of X-rays, while the grafted bone marrow cells were exposed only to the second dose of 200 R in situ. Other procedures were the same as in those for colony-formation with the

[1] N. S. WOLF and J. J. TRENTIN, J. Cell Physiol. *75*, 225 (1970).
[2] M. BERAN, Strahlentherapie *144*, 719 (1972).
[3] J. H. HENDRY and L. G. LAJTHA, Radiat. Res. *52*, 309 (1972).
[4] W. FRIED, W. CHAMBERLIN, W. H. KNOSPE, S. HUSSEINI and F. E. TROBAUGH JR., Br. J. Haemat. *24*, 643 (1973).
[5] A. MANIATIS, M. TAVASSOLI and W. H. CROSBY, Blood *38*, 569 (1971).
[6] R. S. MCCUSKEY, H. A. MEINEKE and S. F. TOWNSEND, Blood *39*, 697 (1972).
[7] K. J. MORI and Y. ITO, submitted to Radiat. Res.
[8] K. J. MORI and S. NAKAMURA, Experientia *26*, 1386 (1970).
[9] S. NAKAMURA, Radiat. Res. *52*, 130 (1972).
[10] K. J. MORI, Radiat. Res. *56*, 494 (1973).
[11] S. NAKAMURA and K. J. MORI, J. Radiat. Res. *15*, 14 (1974).
[12] S. H. CHAN and D. METCALF, Blood *40*, 646 (1972).
[13] D. METCALF, Immunology *21*, 427 (1971).
[14] W. W. SMITH, I. M. ALDERMAN and R. E. GILLESPIE, Am. J. Physiol. *191*, 124 (1957).
[15] W. W. SMITH, G. BRECHER, S. FRED and R. A. BUDD, Radiat. Res. *27*, 710 (1966).
[16] M. SEKI, Transplantation *16*, 3 (1973).

Effect of the treatment of host mice with carbon particles on the hemopoietic colony-formation on macrophage layer formed in the peritoneal cavity of mice

	No. of colonies/4 $cm^2/10^6$ bone marrow (BM) cells					
	Normal BM cells in the host			200 R-irradiated BM cells in the host		
	N[a]	C	ET	N	C	ET
Experiment 1	16.3 ± 4.4	31.2 ± 11.5	23.3 ± 4.4	4.9 ± 1.9	8.8 ± 2.1	6.2 ± 1.1
Ratio	1	2.0	1.5	0.29	0.54	0.38
				1	1.8	1.3
Experiment 2	18.7 ± 3.4	18.8 ± 3.8	NT[b]	5.3 ± 2.1	10.9 ± 3.1	6.9 ± 2.6
Ratio	1	1.0	—	0.29	0.58	0.37
				1	2.0	1.3

[a] N, normal host mice; C, carbon-treated host mice; ET, endotoxin-treated host mice. [b] NT, not tested.

normal bone marrow cells. All mice were given 275 mg/l of chlortetracycline (Takeda Co., Japan) dissolved in drinking water from 1 week before the start of experiments until sacrifice.

Results and discussion. SEKI[16] has successfully achieved the formation of hemopoietic colonies on macrophage layer which covered CA membrane inserted in the peritoneal cavity of mice. This extramedullary colony-formation has made it possible to examine the carbon-induced changes at the level of humoral factor which possibly control the proliferation of the colony-forming cells. Results are summarized in the Table.

Although the fluctuation of the data is great, it seems plausible to assume that survival of the irradiated bone marrow cells was approximately twice as high in the carbon-treated host mice as that in the control hosts. Such increase in the survival was also greater than that in the hosts treated with bacterial endotoxin.

In the case of non-irradiated, normal bone marrow cells, irreproducibility of the data prevented us from drawing a conclusion, but our findings that the serum from carbon-treated mice enhanced the endogenous spleen colony-formation in irradiated mice (to be published elsewhere), and the data of the other workers that bacterial endotoxin stimulates the production of colony-stimulating factor[13], seem to support the similar possibility as those with the irradiated bone marrow cells.

Before interpreting these data, two factors should be taken into account. Firstly, more than 90% of the colonies were myeloid cell type[16] and, accordingly, only the myeloid cell colonies were counted in the present experiments. In the case of spleen colonies, however, erythroid colonies were shown to be more dominant[1]. Our previous discussion was also focused essentially on the regeneration of erythroid colony-forming cells rather than myeloid cells[7,10]. Since carbon particles are known to cause a hyperplasia of the lymphoreticular tissues, possibly as some sort of inflammatory response[10,17,18], the present results may not be surprizing if the colony formation with normal bone marrow cells are also augmented. Secondly, the colonies were formed on the macrophage layer which may act as a feeder layer. Although there was no detectable uptake of i.v. injected carbon particles by these macrophages, the possibility cannot be excluded that the macrophages which were activated by the carbon-treatment provided a favorable effect by the direct contact with the colony-forming stem cells.

Regardless of these limitations, present data together with the other findings[7,10] indicate that the blockade of RES with carbon particles induces an enhanced release or production of some humoral factor(s), as endotoxin does, which consequently is at least partly responsible for increasing the survival or proliferation of irradiated hemopoietic stem cells[19]. A possibility, however, should not be excluded that there may be concomitant pyrogenic substance such as endotoxin in the suspension of carbon particles we used. If so, it may well explain the similarity of the effect of carbon particles and that of the endotoxin.

Zusammenfassung. Nach Vorbehandlung mit Kohlepartikeln wird über die erhöhte Anzahl der in die Bauchhöhle injizierten und in vivo bestrahlten hämatopoietischen Kolonien bei Mäusen berichtet. Dieser Effekt kommt offenbar nicht durch Steigerung der Strahlenresistenz der Zellen selbst, sondern durch irgendeinen humoralen Mechanismus, durch welchen die Kohlepartikelbehandlung die Regeneration von koloniebildenden Zellverbänden verbessert, zustande.

K. J. MORI, A. SETO and Y. ITO

Department of Microbiology, Faculty of Medicine, Kyoto University, Kyoto 606 (Japan), 11 June 1974.

[17] D. STRAUCH, H. S. STENDER and H. WINTER, J. Immun. *82*, 298 (1959).

[18] K. J. MORI and S. NAKAMURA, Proc. Osaka Prefect. Inst. Publ. Hlth Indust. Hlth *6*, 10 (1968).

[19] This work was supported in part by US Public Health Service Contract No. 1-CP-33290 within the Virus Cancer Program of the National Cancer Institute. We are also grateful to Dr. M. SEKI, National Institute of Radiological Sciences, for demonstrating the technique of colony-formation on CA membrane.