parts. The opposite was observed by Lanyi with *Halobacterium cutirrubrum*[3], in accordance with our results at low detergent concentrations. The varying susceptibility of coupled mitochondria, high above 2×10^{-4} M Triton X-100 and low below 2×10^{-4} M detergent, is difficult to explain at the moment.

It is known that conformational changes of the mitochondrial inner membrane accompany its fluctuations in functional state[8], and that these ultrastructural changes determine also changes in molecular conformation, as indicated by studies of accessibility to membrane components[12,17]. These transitions could be invoked in order to explain the different susceptibilities towards Triton X-100; however, no simple explanation is available. State 3 respiring and azide-inhibited mitochondria are indistinguishable from the ultrastructural point of view, both being in the 'condensed' conformation[8]; however, they behave differently towards detergent solubilization. Also, as Triton X-100 is an uncharged molecule, the differences cannot be explained by changes in surface potential. As it is known that there is a selective affinity of Triton X-100 towards certain lipid and protein classes[4,8,9-12], we would like to suggest that the proportion of accessible molecules with high detergent affinity varies with the physiological state of mitochondria, and this determines the qualitative and quantitative differences observed.

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Effect of carbon particles on the recovery of bone marrow stem cells after irradiation in LPS-resistant C3H/HeJ mice[1]

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Summary. Effect of RES-blockade on bone marrow cells was studied serially after irradiation in LPS-resistant mice. Injection of carbon particles reduced damage and accelerated recovery of marrow hemopoietic stem cells, indicating that LPS-resistant mice can react normally to RES-blockade.

Blockade of the reticuloendothelial system (RES) with particulate materials, such as carbon particles (CP) is known to result in increased survival of the irradiated animals[2,3], and this effect has been attributed, at least partly, to its provision of a favorable microenvironment for hemopoietic recovery[4]. Although a similar radioprotective effect is observed in mice pretreated with bacterial lipopolysaccharide (LPS), some differences in the mechanism have been suggested between these two agents. Simultaneous injection of CP and LPS decreases the survival of the irradiated mice[5]. Pretreatment with CP protects the C3H/HeJ mice, which are genetically resistant to most known effects of LPS[6], from radiation lethality, whereas LPS has virtually no effect[7]. We have further demonstrated the difference between the hematological effect of CP and that of LPS on this strain of mice[8]. Since our results also suggested the importance of hemopoietic stem cells in the bone marrow, rather than those in the spleen, in the mechanism of radioprotection, we describe here the recovery of bone marrow stem cells serially after sublethal irradiation in CP-treated C3H/HeJ mice.

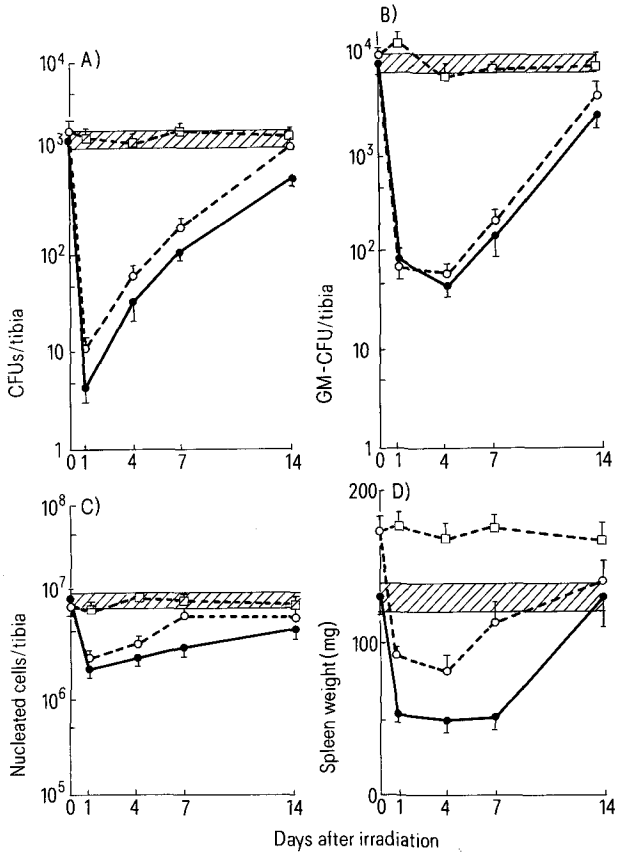
Materials and methods. C3H/HeJ mice (Jackson Laboratory, Bar Harbor, USA) were used at 9-10 weeks of age. They were injected i.v. either with 8 mg of CP (Pelican India ink, Gunther-Wagner, Germany) or with pyrogen-free saline 24 h before whole-body irradiation of 450 rad. Irradiation was carried out using a 180 kVp-20 mA X-ray unit with a filter of 1.0 mm Al + 0.5 mm Cu at a dose rate of 50 rad/min. Bone marrow cells were flushed from 4-6 tibias into Fischer's medium, and the total numbers of nucleated cells were counted. The number of granulocyte/macrophage progenitors (GM-CFC) was determined on soft agar by the modified technique of Bradley and Metcalf as previously described[8,9]. Pluripotent stem cells (CFUs) were assayed by the spleen colony method of Till and McCulloch[10]. Briefly, samples of marrow cell suspensions, each from at least 4 mice, were injected into lethally irradiated syngeneic recipients (8 mice per point). 8 days later, spleens were excised under ether anesthesia, fixed in Bouin's solution and the colonies were counted under a dissecting microscope.

Mice used as bone marrow donors were injected i.v. with 5 μg LPS or with 8 mg CP, and marrow cells were harvested 24 h later and injected i.v. into lethally irradiated recipients (1×10^5 cells/mouse). Spleen colonies were counted on day 8 and on day 12 (10 mice/point)

	Number of colonies/spleen	
	On day 8	On day 12
Normal	22.3 ± 1.8	21.5 ± 2.7
LPS*	25.1 ± 2.9	23.4 ± 2.8
CP**	20.8 ± 3.1	22.7 ± 4.2

Results and discussion. As shown in figure A, the number of CFUs decreased less and recovered earlier in CP-pretreated mice than in the irradiated controls. In 14 days the number recovered to 69% and 31% of those on day 0 (just before irradiation) in CP-pretreated and in control mice, respectively. The favorable effect of CP, however, was not so marked on GM-CFC as on CFUs (fig. B). The number of GM-CFC did not begin to rise until day 7 in either CP-pretreated or the control mice. This might be due to rapid differentiation of GM-CFC into more mature cells and/or increased self-renewal of CFUs. The response of the marrow cellularity to irradiation and CP-treatment was essentially similar to that of CFUs (fig. C). The recovery was more rapid and almost complete on day 7 in CP-pretreated mice, whereas the number was still 48% of the unirradiated control on day 14 in the control mice.

Injection of CP caused splenomegaly on day 0 (fig. D). This has already been reported by Nakamura[11]. The spleen weight recovered to almost normal values (130 mg) by day 7 postirradiation in CP-pretreated mice, whereas that in irradiated controls remained at a nadir until day 7. LPS injection is known to cause mobilization of hemopoietic stem cells from the marrow to the spleen[12,13]. Whether the increased splenic hemopoiesis observed in the CP-pretreated mice is a result of similar migration of cells from the marrow to the spleen or stimulation of in-situ proliferation remains to be clarified. We have reported that the increase in the survival of irradiated C3H/HeJ mice by CP-pretreatment was accompanied by induction of serum colony stimulating factor (CSF), increased numbers of the peripheral platelet and hemopoietic stem cells in the bone marrow and the spleen[8]. In that experiment, however, we examined the number of stem cells only at one point (day 6). In the present experiment, we examined the recovery serially after irradiation and confirmed an enhanced repopulation of bone marrow cells in CP-pretreated mice.



Effects of carbon particles (8 mg) given 24 h before irradiation of 450 rad on recovery of CFUs (A), GM-CFC (B) and total nucleated cell count (C) in the tibia, and of the spleen weight (D) in C3H/HeJ mice. Open squares represent the values in CP-treated control mice, solid circles and open circles represent the values in irradiated control and in mice irradiated after CP-treatment, respectively. Shadowed lines represent the ranges for normal controls. Mean ± SE (for at least 12 mice in 3 replicate experiments).

Spleen colony assays are usually performed between 8 and 12 days after irradiation of mice. Magli et al. reported the transient nature of 8-day spleen colony forming cells and suggested that the CFUs assay should be done on a later day[14]. We therefore assayed the possible difference in the number of spleen colonies between day 8 and day 12. No significant differences were observed in the numbers of spleen colonies in any recipient mice that had been injected with normal or treated bone marrow cells (table).

If a pluripotent stem cell is stimulated into differentiation, there will be no self-renewal. Self-renewal of a stem cell can be assessed only when a part of the daughter cells undergoes differentiation. A low number or a lack of CFUs in a colony, therefore, may not necessarily mean that the colony is derived from a committed cell. Our present data also indicate that the assay of spleen colonies on day 8 can represent the relative number of CFUs, and that the present results are acceptable as reflecting the changes in the actual numbers of CFUs.

Pretreatment of mice with LPS has been reported to result in increased hemopoiesis in the spleen of irradiated C3H/HeJ mice with no favorable effect on post-irradiation marrow CFUs recovery nor on the survival of the mice[6,8]. A discrepancy between the dose of CP necessary for enhancement of serum CSF level and that for radioprotection has been observed[15]. Accordingly, the increased survival of hemopoietic stem cells in the bone marrow with their differentiation into functional blood cells seems to be essential for the protection of the irradiated mice.

The present data are consistent with the notion that CP are engulfed non-specifically and affect favorably the hemopoietic microenvironment, resulting in an overall stimulation of the hemopoietic recovery after irradiation.

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