ally pinocytosed. Patching occurs at low temperatures, but cap formation requires metabolic activity of the cells. It is not clear whether a metal such as Be, Fe, Cu and Al inhibits the metabolic activity of the cells or whether it prevents binding of anti-Ig to the membrane Igs. However, it is obvious from the present study that such metals inhibit the free diffusion of Ig receptors induced by anti-Ig. The inhibitory mechanism of metals on cap formation is now being studied. The following conclusions may be drawn from the study: 1. At 200 μM each metal tested inhibits cap formation to about 60%; and 2. beryllium, even at a concentration of only 0.5 μM, has a strong inhibitory effect on cap formation.

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Survival and endogenous spleen colonies of irradiated mice after skin wounding and hydroxyurea treatment[1,2]

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Summary. Wound trauma-induced survival from radiation may be related to increased mitosis in hematopoietic cells. This is supported by the cell cycle-dependent drug hydroxyurea, which 1. blocked survival of wounded mice injected 2 or 3 days after 900 rad and 2. reduced the number of endogenous CFU-s in wounded mice injected shortly before 700 rad.

Survival of mice after lethal irradiation is made possible by skin wounding at 24 h before lethal whole-body ^{60}Co irradiation[3,4]. Incidence of survival is reduced when the wound trauma follows irradiation[5,6]. Wounding before 700 rad seems to induce the appearance of colony-forming units-spleen (CFU-s) and granulocyte-macrophage colony-forming cells (GM-CFC) in the bone marrow and spleen before they appear in irradiated controls[3]. The percentage increases of exogenously derived CFU-s and GM-CFC were greater in the spleens of wounded animals than in their marrow compartments[3]. Even though the splenic values of the hematopoietic proliferative cells are greater than those of the marrow, the survival of skin-wounded, irradiated mice is independent of extramedullary splenic myelocytopoiesis[4]. While wound trauma may enhance the number of exogenous CFU-s in irradiated assay mice, such cells may not be responsible for the survival of individuals wounded before radiation[7]. This was suggested by exogenous and endogenous CFU-s studies using cell cycle-dependent drugs in irradiated mice[7]. This report, then, provides data from 2 experimental protocols using hydroxyurea, and those data support the idea that survival from lethal irradiation induced by prior skin wounding is related to the proliferation of endogenous (E) CFU-s.

Materials and methods. Female, 5-week-old (C57BL/6 X CBA) F1 Cum/BR mice from Cumberland View Farms, Clinton, TN, were quarantined for 2 weeks in groups of 15. The animals used were those from groups found to be free of *Pseudomonas* sp. and histologic lesions of common murine diseases. Experimental mice were housed 4 per sanitized cage, given Wayne Lab-Blox diet and chlorinated (10 ppm) water, and kept in controlled-environment rooms. Wounding and irradiation were performed on 14- to 16-week-old mice. Methyoxyflurane-anesthetized mice were wounded in the anterior dorsal skin fold and underlying panniculus carnosus muscle with a steel punch repeatedly cleaned by immersion in 70% ethanol. The wound was 2.0-2.5 cm^2, which was 4% of the total skin surface. Wounding was done 24 h before exposure to ^{60}Co, between 10.00 h and 14.00 h. The wounds were left untreated and open to the environment. Irradiated, non-wounded control mice were anesthetized before irradiation. All animals were exposed to whole-body radiation at a rate of 40 rad/min from bilateral ^{60}Co sources. In the 1st series of experiments, a total of 351 mice were used in survival studies (900 rad) and were observed for 30 days. The cell cycle-dependent drug hydroxyurea (HU) (Sigma Chemical, St. Louis, MO) was dissolved in sterile distilled water (50 mg/ml) and injected into mice i.p. (1 mg/g b.wt) at selected times before or after irradiation. 8 days after irradiation, mice were cervically dislocated and the number of splenic E-CFU-s counted. In preliminary studies we determined that 1. no 8-day E-CFU-s were detectable in mice given only 900 rad and 2. wounding before 900 rad resulted in only 1-2 E-CFU-s per spleen. While that number of E-CFU-s was sufficient to promote survival from 900 rad, we used 700 rad to better quantify E-CFU-s responses in combined injured animals treated with HU. Thus, in a 2nd series of experiments, E-CFU-s were determined in 400 HU-treated, skin-wounded mice 8 days after 700 rad. 3 replicate tests were done for each experimental protocol over a 6-month period. Since the responses in all replicates were similar, the data are combined in the figures.

Results. In the 1st series of experiments, 7 treatment groups were established. The 30-day survival fractions of 3 of these groups are shown in figure 1. HU injected into either nonwounded-irradiated or wounded-irradiated mice 16-h before irradiation resulted in 100% survival. However, injection of HU into nonwounded-irradiated mice shortly after exposure or at daily intervals for 5 days thereafter was associated with nearly 100% mortality. In mice wounded before irradiation, 40-95% of the animals died after injection with HU. These mortality rates are compared to the 10% mortality found in wounded-irradiated mice not given HU. Survival (after radiation) induced by skin-wound trauma was maximally blocked by treatment with HU on either day 2 or day 3 postirradiation. In the remaining four groups of control mice, all animals given 900 rad died, while mice either wounded only, injected with HU only, or injected with HU after wounding lived.

The 2nd set of experiments also contained 7 experimental groups. Figure 2 shows the number of E-CFU-s detected in 4 groups of animals. These are mouse groups given a) 700 rad alone, b) wounding + 700 rad, c) HU + 700 rad, or d) wounded-irradiated mice injected with HU. In irradiated mice, wounding resulted in 9 nodules/spleen whereas less than 1 nodule/spleen was found in irradiated-nonwounded animals. Treatment of wounded animals with HU at selected times before irradiation caused an increase in splenic nodules almost double the increase in wounded-irradiated mice without the drug. When wounded mice were injected at either 4 h or 10 min before irradiation, the numbers of colonies were approximately the same numbers found in irradiated-nonwounded control animals. No E-CFU-s were found in mice either a) wounded alone, b) injected with HU, or c) injected with HU after wounding.

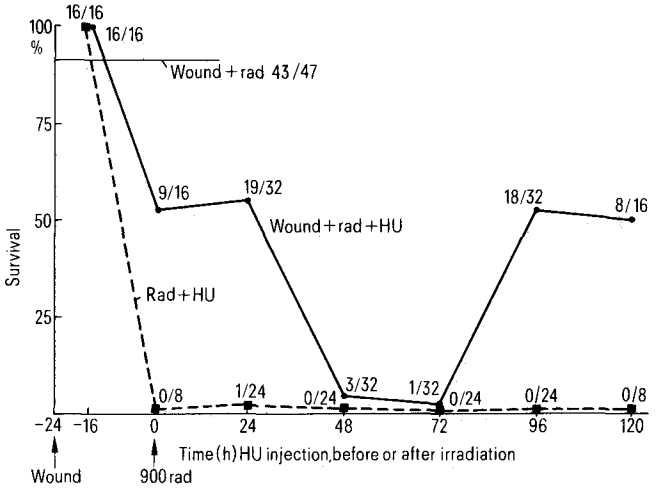

Figure 1. 30-day survival fractions of skin wounded-irradiated mice treated with hydroxyurea (HU). Mice were wounded 24 h before irradiation with 900 rad ^{60}Co. HU (1 mg/g) was injected into mice either 16 h before irradiation, 10 min after irradiation, or at 24-h intervals for a 5-day period after irradiation. The fraction at each point is the number of survivors/number of treated mice. ●—●, Wound + rad + HU. ■---■, Rad + HU. Survival fraction of control wounded and irradiated mice was 43/47. All mice (48/48) given only 900 rad died, and all mice (16/16) only wounded survived.

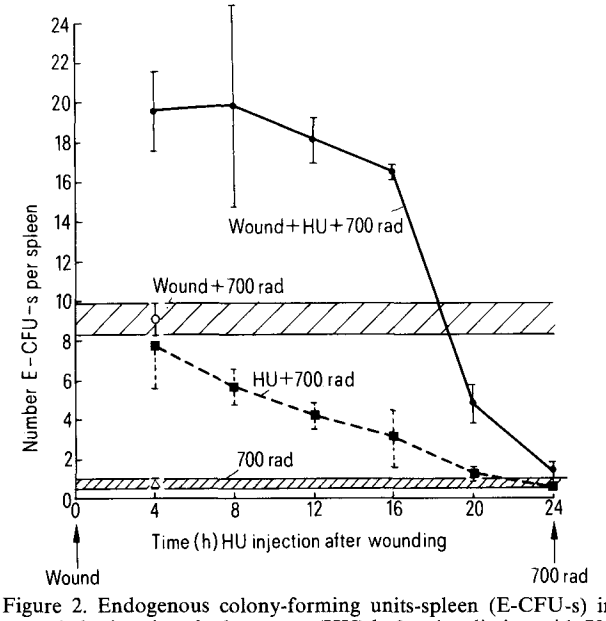

Figure 2. Endogenous colony-forming units-spleen (E-CFU-s) in wounded mice given hydroxyurea (HU) before irradiation with 700 rad ^{60}CO. Mice were wounded at time 0, and HU (1 mg/g) was injected into animal groups at 4-h intervals, commencing at 4 h after wounding and ending at 10 min before irradiation. ●—●, Wound + HU + 700 rad. ■---■, HU + 700 rad. ○, Wound + 700 rad. △, 700 rad only.

Discussion. The present data from 2 experimental protocols support the hypothesis that survival induced by skin-wound trauma before lethal whole-body irradiation depends on the presence of endogenous proliferating hematopoietic cells. In survival experiments, we observed that 1. treatment with HU at selected times after both wounding and 900 rad prevented the expected increases in survival number, and 2. in endogenous spleen colony assays, at selected times before 700 rad but after wounding, treatment with HU reduced the number of E-CFU-s to those found in control-irradiated animals.

HU kills hematopoietic stem cells while they are in the DNA-synthesizing (DNA-S) phase of the cell cycle and prevents other stem cells in other stages of the cell cycle from entering into S-phase[7,8]. This results in the buildup of cells at the end of the G_1 phase. Evidence from experiments[9] with bone marrow transplantation and from E-CFU-s studies with radiation and cytotoxic drugs[10] have established that myeloproliferative elements are necessary to support survival after radiation. Thus, from the mortality data in figure 1, it may be inferred that wounding stimulates synthesis of hematopoietic cell DNA in the irradiated animal. Preceded by cellular incorporation of HU, the maximum incidence of 30-day mortality is seen when mice are treated with this cytotoxic agent at 3 and 4 days after wound trauma.

Additional support that wound trauma stimulates resting hematopoietic stem cells into DNA synthesis is seen in the near eradication of spleen colonies (fig. 2) by HU when given shortly before irradiation. However, HU given at 4–16 h after wounding does not result in decreased numbers of E-CFU-s. Since E-CFU-s did not decrease after incorporation of HU, it may be that these cells are not in DNA-S at 4-16 h after wounding.

Our data suggesting that wound trauma induces the increased synthesis of DNA in hematopoietic tissues are supported by the work of Johnell[11], who found an approximate 20% increase in mitotic cells of the bone marrow and thymus of rats at 24 h after skin-wound trauma. Finally, it was reported that mouse bone marrow stem cells resist damage from radiation when they are irradiated in late DNA-S[10,12]. Our data support the idea that wounding stimulates stem cells to be in DNA-S 24 h later. Together with the increased radioresistance of cells in DNA-S, this may explain the survival after wounding at 24 h before irradiation.

The data presented in this report are based upon the accepted procedures routinely used in the in vivo assay system to delineate the presence of multipotential stem cells (CFU-s and E-CFU-s). However, it is essential to acknowledge the recent work of Magli et al.[13] who critically evaluated the in vivo spleen colony assay. They noted that most splenic surface modules that appeared 8 days after transplantation, not only did not contain the full spectrum of differentiated cellular elements, but disappeared by day 11. Nonetheless, the data presented in this report reflect upon a cell compartment that is necessary for survival from the combined trauma of radiation injury and wounding, and which is depleted by the appropriately timed treatment with HU.

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2 Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Research, National Research Council.
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Production of the lymphokine, macrophage aggregating factor, is not inhibited by histamine

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Summary. Unlike the previously reported inhibitory effect of histamine on the production of the lymphokine, migration inhibitory factor, histamine did not inhibit the production of macrophage aggregating factor (MAgF). By contrast, both prostaglandin E_2 and hydrocortisone inhibited MAgF production, in a dose-dependant manner.

Histamine has previously been shown to inhibit delayed hypersensitivity reactions in vivo[1,2], and lymphocyte proliferation and production of migration inhibitory lymphokines in vitro[3,4]. Inhibition of migration inhibitory factor (MIF) production has been shown to be mediated through a histamine-induced suppresor factor of mol.wt 23,000-40,000, produced by lymphocytes with H_2 receptors[3,5]. It has thus been postulated that histamine acts as a negative modulator of cell-mediated immune reactions. MAgF is a lymphokine activity which rapidly increases the adherence of macrophages to each other and to inert surfaces[6]; its relationship to MIF is not known. The production of MAgF has been shown to be immunologically specific and to be well correlated with the state of delayed hypersensitivity, in the guinea-pig[7]. It is shown here that MAgF production is not inhibited, indeed is slightly enhanced, by histamine. By contrast, 2 other agents reportedly inhibiting the production of MIF activity, prostaglandin E_2 (PGE_2) and hydrocortisone (HC), also inhibited MAgF production.

Methods. MAgF was produced by culturing 10×10^6 viable spleen cells/ml, from guinea-pigs immunized 14 days previously with 2 mg BCG (Bacillus Calmette Guerin) vaccine, with 50 µg/ml PPD (purified protein derivative) for 24 h in serum-free Eagles medium, as previously described[8]. Control cultures were reconstituted with PPD at the end of the culture period. Culture supernatants were dialyzed extensively against tap water and insoluble protein removed by centrifugation. After reconstitution of supernatants with 0.1 vol. of 10×Eagles medium, samples were assayed for MAgF activity. Oil-induced guinea-pig peritoneal exudate cells (containing > 80% macrophages) were prepared and their aggregation quantitatively measured, as previously described[8]. Histamine and hydrocortisone Na-succinate (both from Sigma Chemical Co.) were added to spleen cell cultures as saline solutions, and PGE_2 (Sigma Chemical

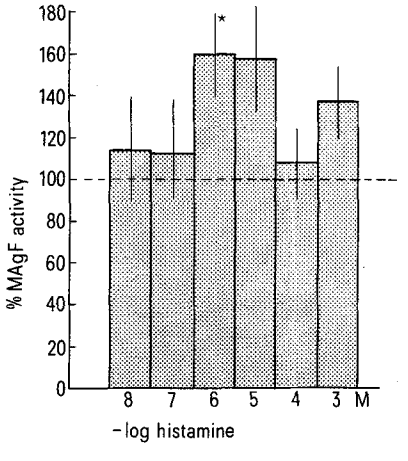

Figure 1. The effect of added histamine on the production of macrophage aggregating factor. Spleen cells, from BCG immunized guinea-pigs, were cultured with PPD antigen in the presence and absence of histamine. Dialyzed culture supernatants were then assayed for aggregating activity. Results are mean ± SEM for 3-6 separate cultures. * $p < 0.05$.

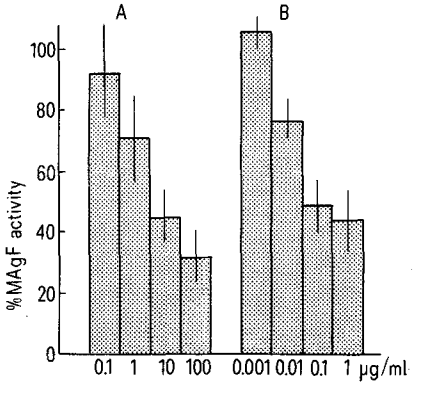

Figure 2. The effects of added hydrocortisone 21-succinate (A) and prostaglandin E_2 (B) on production of macrophage aggregating factor by guinea-pig spleen cells.