"Spleen Protection" in Gamma- and Neutron-Irradiated Mice.* (23237)

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Several investigators have confirmed the protective effects of spleen-shielding, injections of spleen homogenates, or infusions of bone marrow in X-irradiated mammals. Since there is evidence that fission neutrons and Co^{60} gamma rays may kill mice through different modes of mortality(1), it has been suggested that the earlier neutron death is largely attributable to effects on the intestine while the later X- or gamma-ray mortality peak is primarily associated with destruction of the hematopoietic system. It seemed of interest, therefore, to determine whether or not implanted spleens would protect neutron-irradiated mice. Since it was impracticable to attempt to shield the spleen from fission neutrons, attempts were made to utilize some of the other technics that have been developed. In preliminary experiments, several attempts were made to use Cole's technics(2) to protect neutron-irradiated mice with homogenates of spleens, but no protection was afforded. The experiments reported here were suggested by Jacobson's earlier work(3), in which spleens from newborn mice were implanted into the abdominal cavities of irradiated mice.

Methods. Four spleens from CF No. 1 mice ($<$ 2 weeks old) were implanted into an ether-anesthetized, neutron-irradiated mouse, within 2 to 3 hours after irradiation. All exposures were carried out in the gamma-neutron radiation chamber(4) using fission neutrons at the CP-3' and CP-5 Reactors at Argonne National Laboratory. Neutron exposures were for 2 hours or less; dose rates varied from 3-10 rads/min. The irradiated mice were CF No. 1 females 6-8 weeks of age. Half of each series of irradiated animals received spleens and half served as sham-operated controls, in which the body cavity was opened, a forceps inserted momentarily, and the incision closed. A third group of animals was randomly selected as unirradiated controls. There were 20 to 30 mice in each of these 3 groups.

Results. In Fig. 1, the results of 3 neutron experiments are summarized. In each case, the upper figure compares the average body weights of the 3 groups of mice, while the lower graph compares the cumulative mortality of the mice receiving spleens after irradiation with that of the sham-operated, neutron-irradiated group in the same experiment. There was no significant difference in body weight loss between the spleen-implanted and sham-operated groups. The cumulative mortality of the spleen-implanted groups was almost identical with that of the sham-operated groups at 30 days, whether the neutron dose was sufficiently high to kill 25%, 60%, or 80% of the mice. These data suggest that *spleen implantation gave no protection to the mice irradiated with fission neutrons.*

In all cases of death, autopsies were performed. Several implanted spleens were usually recovered from the body cavities of the irradiated mice. They were found in all stages from apparent normality to complete destruction, as judged by gross inspection. The implants were usually firmly attached to peritoneal surfaces.

To test the method, 2 similar experiments were carried out following 940-950 r Co^{60} gamma irradiation (exposure approximately 1 hour, dose rate $\sim$ 16 r/min.) Both weight loss and mortality appeared much later (end of the second week) after gamma irradiation than after comparable fission neutron exposures (Fig. 2). In both of these gamma ray experiments, the 30-day mortality data indicate that the spleen implants definitely conferred some protection. This is also evident in the lack of weight loss from the 10th to the 15th days in the spleen-implanted mice.

In view of the fact that the protection after

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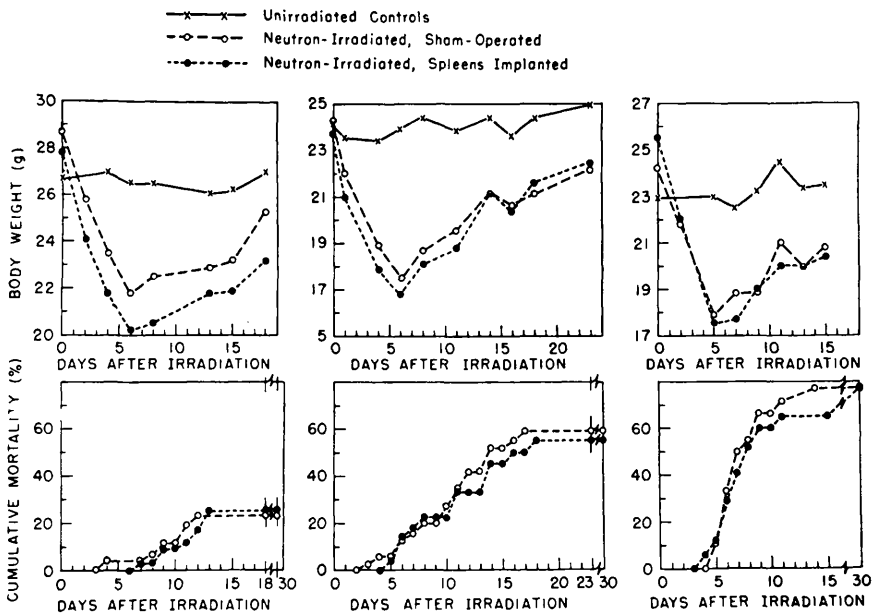

FIG. 1. Effects of spleen implantation on body weight and cumulative mortality of neutron-irradiated mice.

gamma irradiation was most evident at the end of the second week after exposure, it is perhaps not surprising that implantation of spleens did not protect neutron-irradiated mice during the early period, when mortality was high. By this time most of the neutron-irradiated mice were dead, presumably from a different mechanism of mortality. It is conceivable that the use of an antibiotic such as streptomycin, which can protect neutron-irradiated mice for at least 10 or 11 days after exposure(5), would keep a sufficient number

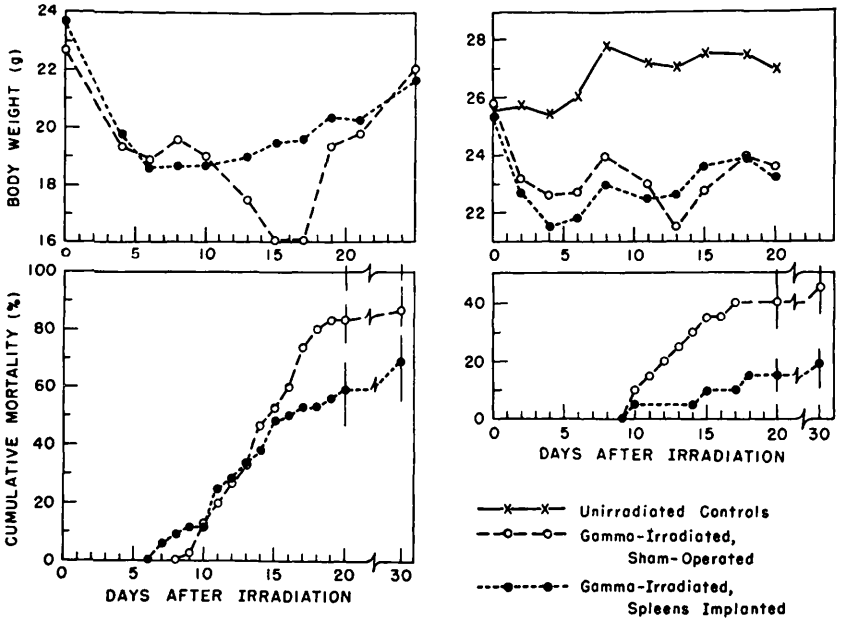

FIG. 2. Effects of spleen implantation on body weight and cumulative mortality of gamma-irradiated mice.

of mice alive so that the spleen implants could then help prevent the later death, associated with destruction of the blood-forming elements. Such an experiment is now being carried out.

Randolph and Congdon(6) have exposed RF male mice to total-body irradiation with 14 Mev neutrons generated by a Cockcroft-Walton linear accelerator. Preliminary results indicate that post-irradiation treatment with isologous bone marrow cells (intravenous injection) increased the 30-day survival. These mice were exposed to 650 rads of 14 Mev neutrons. Only 20% of the irradiated animals died during the first 8 days after exposure. Following a comparable dose (250 rads) of fission neutrons (mean energy, ~ 1 Mev), 86% of the irradiated mice (67/78) died from days 3 through 8 (7). These results indicate that fast neutrons of different energies may produce different modes of mortality, and that the early 4 to 10 day death characteristic of fission neutrons(7) is not so evident following 14 Mev neutrons.

Conger(8) has recently shown that for *Tradescantia* chromosomal aberrations there is a consistent increase in relative biological effectiveness (RBE) with linear energy transfer (LET), which is almost linear from the X-ray to the neutron energy range *and within the neutron energy range.* Since the linear energy transfer (Kev lost per micron) is approximately 3 times higher for 1 Mev than for 14 Mev neutrons, it seems probable that differences may occur in biological effects from neutrons of such different energy ranges.

It is significant that bone marrow treatment of the mice did not prevent death during the first week after irradiation, the period of high mortality following fission neutrons. It seems probable from these data that radiation with fast neutrons of different energies may give different results after treatment with blood-forming cells. This hypothesis is further substantiated by some recent work by Cole and Ellis(9) on the effect of bone marrow or spleen injections in LAF_1 mice after fast neutron irradiation. Mice were exposed to neutrons (2 Mev and 8 Mev) at the Berkeley 60-inch cyclotron. They received a single postirradiation intravenous injection of fresh isologous bone marrow (10^6 nucleated cells) or a single i.p. injection of isologous infant spleen homogenate (45 mg/mouse). Little or no protection of the neutron-irradiated mice (425-510 rep) was reported either for 30-day survival or for mean survival time. These data are in marked contrast to results observed with lethally X-irradiated mice.

Cole's work with intravenous bone marrow and our results with spleen implants confirm the lack of protection of mice exposed to fast neutrons, at least from 1 to 8 Mev. They add to the increasing evidence that the early death following fission neutrons is not a hematological death.

Summary. 1. Young adult female CF No. 1 mice were irradiated with single whole-body exposures of fission neutrons with dose range sufficient to kill 20 to 90% in 30 days. Four spleens from 2-week-old mice were implanted into body cavities of half this group; the remaining mice served as sham-operated controls. 2. Spleens gave no obvious protection to these neutron-irradiated mice: There was no significant difference in either body weight loss or in 30-day mortality between spleen-implanted and sham-operated groups. 3. Implanted spleens provided some protection to similar mice exposed to single doses of Co^{60} γ-rays in the 30-day lethal range. 4. Failure of implanted spleens to protect neutron-irradiated mice may be correlated with early (4-10 day) mode of mortality following single doses of fission neutrons. 5. It seems probable that radiation with fast neutrons of different energies may give different results after treatment with blood-forming cells.

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