Effect of Chemical Protection and Bone Marrow Treatment on Radiation Injury in Mice

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Doherty and Burnett synthesized S,2-aminoethylisothiuronium Br Hbr (AET). They found 70 and 88 per cent 28-day survival in male mice exposed to lethal total-body x-radiations of 950 and 800 r when this compound was administered intraperitoneally 10 minutes before irradiation. Doses of 450 mg./Kg. and 250 mg./Kg. of compound were used. Chemical studies revealed that at neutral pH, AET rearranges to 2-mercaptoethylguanidine HBr (MEG). Preparing and injection of MEG prepared by neutralization of at least 8.8 mg. of AET resulted in 95 per cent 30-day survival of 150 C3H mice exposed to 800 r of total-body x-radiation.

Early deaths resulting from lethal irradiation are attributed in large part to the injury suffered by the hematopoietic tissues. To aid in further evaluating the manner in which MEG is capable of reducing the effects of irradiation, its effect on the response of the hematopoietic system to lethal and sublethal irradiation in mice was studied. The object of this investigation, therefore, was a determination of the quantitative changes in the response of the bone marrow, peripheral blood leukocytes, hematocrit, spleen weight, thymus weight, and body weight, and the histologic appearance of the hematopoietic organs in mice treated with MEG and then x-irradiated. The effects on the same parameters by combining MEG given before irradiation with isologous bone marrow injected after irradiation were also studied. This type of treatment increases the LD50/30-day exposure from 700 to 1800 r in mice.

Materials and Methods

Mice. Ninety- to 100-day-old male (20–25 Gm.) and female (18–22 Gm.) (BALB/c × AF1) mice were used as the experimental animals. They are usually referred to as CAF1 mice. They were housed 5 to 10 in a cage and had free access to food and water.

Irradiation. The x-ray source was either a G. E. Maxitron 250 or a Phillips Constant Potential machine. Radiation factors for the G. E. Maxitron 250 were: 250 kvp. at 30 ma.; 3 mm. of Al added filtration; h.v.l., 0.55 mm. of Cu; target-object distance, 80 cm.; dose rate in air, ~80–90 r/min. The radiation factors for the Phillips Constant Potential machine were: 250 kvp. at 15 ma.; 1.0 mm. of Al added filtration; h.v.l., 0.5 mm. of Cu; target-object distance, 60 cm.; dose rate in air, ~150 r/min. The mice were exposed in groups of 10 or 12 in a partitioned, revolving, lucite cage.

MEG and bone marrow treatment. Different groups of male and female mice received the following: 900 r; MEG and 900 r; 450 r; MEG, 900 r, and isologous bone marrow; and 900 r and isologous bone marrow. Each type of experiment was repeated 2 or 3 times, the manner in which MEG and bone marrow were given being kept standardized. MEG was prepared by dissolving AET in distilled water and neutralizing with alkali to pH 7.2. Final concentration was 9 mg. dissolved AET per 0.3 ml. of neutralized water. The fresh preparations of MEG were injected in 0.3-ml. amounts intraperitoneally 8–12 minutes...
before irradiation. Within 2 days, this high dose of compound was lethal to 50 per cent of the females and to 10 per cent of the males. These animals were not considered in the survival data. The methods of preparation and injection of bone marrow have been described by Urso and Congdon. The dose of bone marrow used in these experiments was estimated to be about 10–12 × 10^6 nucleated cells (one-femur dose) by the method of Urso and Congdon.

**Measurements.** Mice from each group were killed serially at 1- to 5-day intervals beginning the first day after treatment. Determinations of all the parameters except body weight were carried out on the mice killed in the first replication. When the treatments were repeated, determinations were made of the bone marrow counts only or of the leukocytes, spleen weights, thymus weights, or hematocrits. Each point for the parameters thus represents 1 to 6 determinations. For determination of body weight, mice were weighed serially in groups of 10 for each type of treatment used. The techniques used in measuring the response of the bone marrow, peripheral blood leukocytes, spleen weight, thymus weight, and the histology of the hematopoietic organs were the same as reported earlier. The percentage of red blood cell mass (Hematocrit) was measured in blood drawn into small, oxalated capillary tubes 18 mm. long, inside diameter 0.5–0.8 mm., directly from the tail vein and centrifuged for 15 minutes at 12,000 r.p.m. Differential leukocyte counts were made on a drop of blood from the tail vein smeared on a clean glass slide and stained with either Wright's or May–Grünwald Giemsa stain. Mice of each group were usually set aside for survival studies. Similar data on normal mice were obtained by the same methods.

**RESULTS**

**Effect of MEG on nonirradiated mice.** Twenty-five normal CAF1 mice, 10 males and 15 females, were given the same dose of MEG and not exposed to x-radiation. Seven of the females and none of the males died within 2 days. The remainder of the animals were killed on days 1, 2, 3, 4, 5, 6, 8, 11, 15, and 20 after injection. MEG had no effect on the bone marrow count, leukocyte count, hematocrit, spleen, thymus, and body weights in these non-irradiated mice. The histology of the reticular tissues was not altered by the single injection of MEG.

**Survival.** None of the 58 irradiated mice receiving MEG or combined treatment died. Some mice (not included in the mortality calculations) died 1 to 2 days after treatment, and these deaths were attributed to the toxic effects of MEG, since death from 900 r alone occurred only between 5 to 13 days. One out of 20 mice exposed to 450 r died on the twenty-eighth day. In 80 irradiated mice injected with bone marrow only, 28 per cent did not survive the 60-day period, but all animals exposed to 900 r alone died within 13 days. Four per cent of the lethally irradiated mice injected with bone marrow alone died 30 to 60 days after injection.

**Quantitative bone marrow response.** MEG modified the quantitative response of the bone marrow to lethal and sublethal irradiation as early as the second day after treatment (fig. 1A). At both exposures, there was a less severe depression and an early recovery in the bone marrow count of the preinjected mice. A striking similarity between the counts obtained with 450 r alone and those obtained with MEG plus 900 r was observed.

The response of the bone marrow count to 900 r in mice receiving combined treatment (MEG and bone marrow) was the same as for MEG alone for the first 3 days and paralleled the response obtained for the bone marrow alone
TREATMENT OF RADIATION INJURY

Fig. 1.—Effect of MEG and isologous bone marrow (IBM) injections on the bone marrow cell response of the right femur shaft of x-irradiated mice. A, MEG injected intraperitoneally before irradiation; B, MEG injected (IP) before and IBM injected intravenously after irradiation.

on the fourth to the seventh days when recovery occurred (fig. 1B). On the whole, the bone marrow count appeared to respond best to combined treatment. With combined treatment, depression of the bone marrow count was less severe and recovery occurred earlier than for bone marrow alone. Recovery was faster with combined treatment than with MEG alone. Addition of the counts on days 4, 5, and 6 for MEG treatment alone with those for the bone marrow treatment alone, gives an over-all result less than the counts obtained for combined treatment.

Peripheral blood leukocyte response. Data obtained on leukocyte response to lethal and sublethal irradiation demonstrates that MEG is effective in modifying leukopenia at both exposure levels (fig. 2A). Recovery is more rapid in pretreated, lethally irradiated mice than in sublethally irradiated mice.

A study of the combined effect of chemical and bone marrow treatment on the leukocyte response to lethal irradiation revealed that recovery of leukocyte count was the same in time and in amount as the recovery for bone marrow alone (fig. 2B). The results also showed that MEG did not play a significant role in increasing leukocyte count when given in conjunction with bone marrow.

Granulocyte, agranulocyte, and hematocrit responses. Chemical pretreatment resulted in the protection of granulocytes and agranulocytes from the effects of lethal and sublethal x-irradiation (figs. 3A,3B). The granulocyte count includes heterophils and eosinophils, and the agranulocyte count includes lymphocytes and monocytes. The granulocytic elements responded in a manner similar to that of the bone marrow count to the effects of irradiation after chemical pretreatment with the exception of a delay in recovery by a few days. Counts somewhat above normal were recorded for
the granulocytes after recovery occurred. Modification of the effect of lethal irradiation on the agranulocytes by MEG was in the form of a delayed, slow recovery. The early destructive effects of 450 r on the agranulocytes were slightly modified by the chemical, and there was also an earlier recovery. The response of the packed red blood cell volume to x-irradiation was similar to
that of the granulocytes after chemical pretreatment. At 900 r there was no initial increase in hematocrit as was observed for the x-ray controls.

The effect of combined treatment on the granulocytic and agranulocytic response to lethal x-irradiation demonstrated that for both end points the response was the same as that which occurred with bone marrow alone (figs. 4A,4B). The recovered granulocyte count remained above normal, especially in the animals receiving bone marrow alone. For the agranulocytes, recovery was not observed until the third week after combined treatment. As recovery occurred, many of the observed agranulocytic cells were extremely large with irregular-shaped nuclei. This condition was more pronounced in the mice receiving bone marrow alone. The hematocrit response of 900-r mice receiving combined treatment showed no change from normal values. After following x-ray control levels for the first 6 days, the hematocrit values of the 900-r bone marrow mice were between those of the mice receiving MEG alone and those receiving combined treatment.

Spleen weight response. Generally, spleen weight of irradiated mice responded in the same way to chemical pretreatment as did the leukocyte count. In the preinjected, lethally irradiated mice, spleen weight began to recover on the eighth day and reached normal on the thirteenth day. On the second day, spleen weight loss at 450 r was modified by a factor of two with MEG, but recovery did not occur until the second week after treatment.

In lethally irradiated mice, recovery of spleen weight with combined treat-

Fig. 3. (Top)—Effect of MEG (injected intraperitoneally before irradiation) on the peripheral blood granulocyte and agranulocyte responses of x-irradiated mice. A, granulocyte response; B, agranulocyte response.

Fig. 4.—Effect of MEG (injected intraperitoneally before irradiation) and isologous bone marrow (IBM) (injected intravenously after irradiation) on the peripheral blood granulocyte and agranulocyte responses of x-irradiated mice. A, granulocyte response; B, agranulocyte response.
ment occurred in much the same way as leukocyte recovery with some differences. For example, the weight recovered in five days reached more than twice its normal weight on the sixth day and remained slightly above normal to the sixteenth day.

**Thymus weight response.** Chemical pretreatment resulted in a modification of the effect of lethal and sublethal irradiation on the thymus weight response after the fifth day, with a proportionally greater modification of the effect at the higher exposure (fig. 5A). A biphasic thymus weight reaction was observed in the mice exposed to 450 r alone. There was a temporary recovery, followed by a secondary weight loss and another recovery. The biphasic reaction was quantitatively modified in the mice exposed to MEG-450 r. In the mice exposed to MEG-900 r, this biphasic reaction also took place.

Combined treatment of lethally irradiated mice produced an early increase in thymus weight that temporarily resembled that noted with MEG and a very late recovery (twenty-fifth day) to and above normal levels (fig. 5B). The biphasic response was not clearly seen in combined treatment or treatment with bone marrow alone. With bone marrow treatment alone, however, thymus weight response is highly erratic.

**Body weight response, appearance, and behavior.** MEG reduced the effect of lethal and sublethal irradiation on the body weight response considerably. With MEG at 900 r, recovery of body weight was similar to that at 450 r alone with one exception. In the preinjected, lethally irradiated mice, there was a temporary recovery (fifteenth day), followed by a slight loss in weight and recovery (thirty-sixth day). It is not clear whether this biphasic weight response is related to the biphasic weight response observed in the thymus.

With MEG-450 r, body weight remained normal.

Body weight in lethally irradiated mice receiving MEG and bone marrow treatment recovered at about the same rate as those receiving MEG-900 r, but did not undergo the secondary weight loss. The appearance and behavior of all chemically protected mice was normal; the hair was shiny and smooth, and there was normal activity. In CAF1 mice receiving bone marrow alone at this radiation exposure level, appearance and behavior was abnormal in most animals; the hair was somewhat ruffled and activity was reduced. This is reflected in the subnormal body weight and the few deaths which occurred after 30 days with bone marrow treatment alone.

**Histology.** Histologic observation of the hematopoietic tissues in sublethally and lethally x-irradiated mice revealed that there was less destruction and early regeneration and recovery at both exposures in those treated with MEG as compared to those receiving x-rays alone for the bone marrow, red and white pulp of the spleen, and thymus. However, the same degree of initial destruction was observed in these tissues between the treated and untreated mice at both exposures, with the greatest destruction at the lethal dose. These tissues appeared normal earlier in the MEG-450 r mice than in the 450-r mice. In the MEG-900 r mice, the tissues appeared normal on the eleventh day, except for the white pulp of the spleen which remained atrophic until the fifteenth day. On the whole, the appearance of the bone marrow, spleen, and
Fig. 5.—Effect of MEG and isologous bone marrow (IBM) injection on the thymus weight response of x-irradiated mice. A, MEG injected intraperitoneally before irradiation; B, MEG injected IP before and IBM injected intravenously after irradiation.

The thymus in MEG-900 r mice was similar to that of 450-r mice. In the MEG-900 r mice and 450-r mice, moderate to mild hyperplasia was observed in the red pulp of the spleen after the twelfth day, although in the MEG-450 r mice, this was observed after the fourth day. During the secondary weight loss of the thymus, normal architecture was observed except for a reduction in size of the cortical region. Thereafter, as the thymus increased in weight
to normal levels, many large, immature lymphoid cells were observed in the cortex. The lymph nodes of the MEG-450 r mice showed regeneration slightly earlier than in the 450-r mice. Except for reactive changes and germinal center formation, this tissue did not become normal until the twenty-fifth day in the MEG-450 r mice and until the thirtieth day in the 450-r mice. In the MEG-900 r mice, the lymph nodes remained atrophic until the fifteenth day, after which there appeared many germinal centers and reactive changes.

Microscopic findings on the bone marrow and spleen of lethally x-irradiated mice receiving combined treatment revealed a mild destruction comparable to MEG-900 r mice and an early regeneration and recovery (on about the eighth or ninth day) comparable to the 900-r bone marrow mice. In the spleen, there was early hyperplasia of the red pulp (ninth day), which lasted until the thirty-third day and a slight delay in recovery of the white pulp. This was also observed in the 900-r bone marrow-injected mice. The thymus showed moderate destruction and early regeneration of architecture equal to that observed for MEG alone up to 21 days. After this time, the organ seemed normal for the mice with combined treatment. A simple atrophy was observed in many thymuses of the 900-r bone marrow mice. These animals usually had lung infections and liver necrosis. The lymph nodes of irradiated mice receiving combined treatment and bone marrow were abnormal until the thirty-third day, although before then, partial regeneration and germinal center formation was observed beginning on the seventh to twelfth days.

**Discussion**

The results reported in this paper indicate that an intraperitoneal injection of MEG (prepared from 9 mg. of AET) significantly decreases the radiosensitive response of the bone marrow, peripheral blood leukocytes, spleen weight, thymus weight, RBC volume of the blood, body weight, and histology of the hematopoietic organs by an early modification of the radiation-induced changes on these parameters. This modification subsequently results in their early recovery. In addition, the protective effect of MEG appears to be greater for the granulocytes than for the agranulocytes.

Previous to these studies, the most widely tested protective agents (glutathione, cysteine, and cysteamine) have been shown to modify the effects of x-irradiation on several of the same parameters reported here. Cronkite et al. observed that with glutathione injections, there occurred: a quicker recovery of leukocyte count, RBC count, thymus and spleen weights; an acceleration of myelopoiesis in the bone marrow and spleen; and only a slightly faster regeneration of lymphoid tissue in x-irradiated (825 r) mice than in untreated x-irradiated mice. These workers also reported no differences in the response of these end points for the first five days between the treated and untreated x-irradiated mice. Cysteine also caused a quicker recovery in the blood elements, especially in the heterophil count of rats exposed to 800 r after no change from x-ray control values for the first four days. A 31 to 56 per cent reduction of the effects of sublethal (400 r, 500 r) x-radiation on the blood elements and spleen involution in mice was observed with cysteine on the third day after treatment. Hartweg recently observed...
a distinct acceleration of regeneration in the bone marrow elements of cysteine-treated rats exposed to whole-body or local bone marrow irradiation (500 r) with a faster regeneration after whole-body exposure. Observations on the protective action of cysteamine in irradiated (700 r) C57BL mice revealed an early recovery in body weight and leukocytes after these end points temporarily followed x-ray control levels. At this dose, Gerebtzoff and Bacq observed an intensive regeneration in the spleen, but no regeneration in the thymus of cysteamine-injected mice killed on the fourth day. At 450 r, recovery of the leukocytes in cysteamine-injected mice did not begin until after the fifth day. Maisin et al. observed a secondary body weight loss in lethally irradiated rats injected with cysteamine that does not take place in irradiated (700 r) C57BL mice, but that was observed in our experiments with MEG at 900 r. Our results with MEG are basically similar to those reported for glutathione, cysteine, and cysteamine, with some differences. We observed a very early reduction of the effect of x-rays on the bone marrow. A quicker regeneration and an earlier reduction of the effect of sublethal and lethal x-irradiation on the hematopoietic tissues occurred with MEG, except for the thymus and agranulocytes, than was obtained with glutathione in mice exposed to 825 r by Cronkite et al. As compared to cysteamine, the results with MEG show earlier regeneration of the leukocytes and of the thymus.

The data obtained with 450 r and with MEG + 900 r show that MEG protection gives a dose-reduction factor (DRF) of approximately two for all the parameters studied except the agranulocyte count and histologic appearance of the lymph nodes. Burnett et al. found a DRF similar to that reported here for MEG on the LD50/30-day x-ray exposure for two strains of mice. Makinodan et al. also observed a comparable DRF on the rate of recovery of the immune mechanism of mice exposed to 950 r and pretreated with MEG. Thus the consistent DRF obtained for several of the radio-biologically sensitive parameters points to a uniform protective action of MEG on the hematopoietic system. Doherty and co-workers believe that MEG acts as a free radical trap for the toxic radiation products of water. This interpretation of the mechanism of action of MEG fits in with the protective effect of this compound on several of the end points reported in this study, especially since injection of S35-labeled AET reveals that this compound is temporarily concentrated in large amounts in the bone marrow, spleen, thymus, and plasma. However, the tissue in the lymph nodes of the irradiated mice did not respond as well to the effects of MEG as did the other parameters studied. The significance of this finding is not clear.

An interesting development arising from the results of this study was the biphasic thymic weight response to sublethal irradiation. Since the dose-reducing effect of MEG at 900 r produces a thymic response similar to that at 450 r alone, it may be concluded that thymic response results from the amount of effective radiation that reaches the gland. The thymus response may contribute a great deal to the subnormal recovery of the agranulocytic elements of the blood. Its significance, however, cannot actually be stated at the present time. Kaplan and Brown independently observed a similar
thymic weight response in C57BL mice exposed to 476 r of x-rays. In addition, they often noted large immature lymphoid cells in the cortex of the gland during the second weight increase. They call attention to the resemblance of these large immature cells to those found in early lymphoid tumors.

Combined treatment of lethally irradiated mice with AET, bone marrow, and streptomycin was used by Burnett and Doherty. They reported 100 per cent survival of mice exposed to 2000 r of γ radiation. As would be expected, treatment with MEG and bone marrow gave 100 per cent 30- and 60-day survival with a 900-r exposure. Our results also indicate that the effects produced by MEG and bone marrow, when given together, are largely independent of each other in the over-all response of several parameters. Thus bone marrow treatment seems to be ineffective in modifying the early radiation injury to the bone marrow, but a quick recovery of the bone marrow takes place with this treatment. With MEG treatment, early radiation injury is reduced to some degree, and recovery is slower. However, the evidence on the recovery of the bone marrow count with combined treatment might suggest a synergism between the bone marrow and MEG during the recovery period. Also, there appears to be an additive effect of the bone marrow and MEG on the hematocrit response to lethal irradiation and on late recovery of thymus weight. Maisin et al. reported a synergistic action between cysteamine injected before irradiation and bone marrow given after irradiation on survival and body weight in rats exposed to 800 r of x-rays.

Combined treatment showed that the quick recovery of the peripheral blood leukocytes and spleen was caused by the effect of bone marrow injection; whereas recovery of the thymus and body weight occurred faster, primarily because of the effect of MEG. This was confirmed histologically for the bone marrow, spleen, and thymus. In addition, prevention of the secondary thymic weight loss by combined treatment reflects the added influence of the bone marrow injection in causing complete recovery of this organ. The subnormal thymus weight of the bone marrow-treated mice can be explained by atrophy caused by secondary infections of many of the glands. These infections, however, were not observed in mice receiving combined treatment.

True recovery of the agranulocytic cells of the peripheral blood does not actually occur since a large percentage of these in mice receiving bone marrow and combined treatment are large abnormal-looking mononuclear cells. The significance of the appearance of these cells, however, is not immediately evident. The high granulocytic values observed with combined treatment and bone marrow probably represent a compensatory reaction owing to the lack of normal levels of agranulocytes.

**Summary**

MEG (prepared from 9.0 mg. of AET) significantly modified the response of the bone marrow, peripheral blood leukocytes, spleen, thymus, body weight, hematocrit, and histology of the hematopoietic organs to lethal (900 r) and sublethal (450 r) x-irradiation in CAF_1_ mice.

MEG reduced the effect of 900 r on the bone marrow, granulocytes of
the blood, hematocrit, spleen, thymus, and body weight by a factor of approximately two.

Combined treatment (MEG and isologous bone marrow) of mice exposed to 900 r of x-rays demonstrated that MEG is primarily responsible for preventing the early destruction of the bone marrow, but bone marrow injection was primarily responsible for causing a more rapid recovery of the bone marrow.

In mice receiving combined treatment, recovery of the leukocytes and spleen was primarily influenced by the bone marrow injection; whereas recovery of the thymus and body weight was primarily influenced by MEG. The hematocrit values were normal after combined treatment.

**SUMMARIO IN INTERLINGUA**

2-mercaptoethylguanidina·HBr (MEG), preparate ab 9,0 mg de S,2-aminoethylisothiurionium·Br·HBr (AET), modificava significativemente le responsas del medulla ossee, del leucocytos de sanguine peripheric, del splen, del thymo, del peso corporee, del hematocrite, e del histologia del organos hematopoietic a roentgenoradiationes letal (900 r) e subletal (450 r) in muses CAF1.

MEG reduceva le effecto de 900 r super le medulla ossee, le granulocytos del sanguine, le hematocrite, le splen, le thymo, e le peso corporee per un factor de circa duo.

Le tractamento combinate con MEG e isologe medulla ossee in muses exponite a 900 r de radios X demonstrava que MEG es primarimente responsable pro prevenir le precoce destruction del medulla ossee, sed le injection de medulla ossee esseva primarimente responsabile pro le acceleration del restablimento in le medulla ossee.

In muses que recipeva le tractamento combinate, le restablimento del leucocytos e del splen esseva effectuate primarimente per le injection de medulla ossee, durante que le restablimento del thymo e del peso corporee esseva primarimente influentiate per MEG. Le valores del hematocrite esseva normal post le tractamento combinate.

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