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# MERCAPTOETHYLAMINE AND LIVER SHIELDING IN IRRADIATED RATS; STUDY OF ERYTHROPOIESIS WITH IRON-59

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## ABSTRACT

1. The authors observed the protective effect, in Long-Evans rats, at 850 r, of the simultaneous injection of MEA before irradiation, and of the shielding of the liver region during irradiation. Possible reasons for the protection afforded in relation to the major postirradiation syndromes--e.g., intestinal and marrow aplasia, expressed chiefly by anemia--are discussed. It seems that it is the marrow which is the most vital tissue involved in protection when MEA is injected before irradiation; MEA also appears to play a role similar to intestinal shielding. On the other hand, the injection of MEA after irradiation, combined with the shielding of the liver region during irradiation, has no therapeutic action in Long-Evans rats.

2. Finally, $Fe^{59}$ was used to study the effect of shielding of the liver region during irradiation, of injection of MEA before irradiation, and of these two procedures combined, on erythropoietic activity of Long-Evans rats. From these experiments it seems that, at 500 r, MEA injected before irradiation has a stimulating effect on erythropoiesis. This action is delayed, however, and starts between the eighth and the thirteenth day after irradiation. The recovery of erythropoietic activity in rats with their liver region lead-shielded begins earlier, or between the fourth and the seventh day; the authors conclude that it is the shielded area of marrow which plays the important role in this case.

# MERCAPTOETHYLAMINE AND LIVER SHIELDING IN IRRADIATED RATS: STUDY OF ERYTHROPOIESIS WITH IRON-59

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## INTRODUCTION

Jacobson[1] was the first to report radiation protection by the shielding of the exteriorized spleen of mice during irradiation. Protection of rats by shielding of the spleen was less effective. It has been reported by J. Maisin et al.[2] and Gershon-Cohen et al.[3] that at a dose level of 500r to 600r shielding any region of the body protects to approximately the same extent provided the area contains bone. Shielding the liver region of rats seems to be more important at higher doses, e.g. 700r (J. Maisin, et al.[4])

Other investigators preferred to try to afford protection by injection of chemical agents before irradiation. Herve and Bacq[5] showed the influence of NaCN in mice; Chapman and Cronkite that of glutathione[6] in mice; Patt et al.[7,8] that of cysteine in rats and mice. Finally Bacq, Herve, et al.[9,10] observed good protection provided by mercaptoethylamine in mice. J. Maisin et al.[11] confirmed this finding in rats.

In the first part of the experiments presented here we studied the effect of simultaneous use of mercaptoethylamine, MEA,** injected before or after irradiation, in combination with shielding of the liver region during irradiation. In the second part the influence on erythropoiesis of MEA injection before irradiation, as well as the influence of shielding the liver region, and finally the influence of the combination of the two preceding procedures, have been studied by use of $Fe^{59}$. Huff et al.[12] had already used $Fe^{59}$ to explore erythropoiesis in Curtis-Dunning rats irradiated while their spleens were shielded in a lead box.

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** The mercaptoethylamine (MEA) used was Becaptan Labaz, kindly supplied by the Squibb Co. in New York.

## PART I

### Methods

Long-Evans strain rats of 160 to 200 g weight were used. The unanesthetized animals were irradiated while tied to a rack ventral side up under a lucite shelf. The shelf served to support the lead shield over the liver region in some of the experiments. This lead shield, which is 3.5 by 5 cm and 0.5 cm thick, was placed over the liver region so that the edge (5 cm long) was aligned at the edge of the last rib extending posteriorly, with its inner (3.5 cm) side just covering the spine. Thus a region which contains the largest portion of the liver, several portions of ribs, a few vertebrae, and several loops of the small intestine is shielded. A Westinghouse Maximar x-ray machine was used to deliver 180-kv x-rays at 15 ma at a distance of 55 cm from the target. The dose rate, measured by a Victoreen condenser r meter, was 90 r per minute, and the only filtration in addition to the inherent filtering of the machine was that of the 0.5-cm-thick lucite shelf. The animals were irradiated on a turntable at 2 rpm to compensate for inhomogeneous irradiation field conditions. The MEA was administered intraperitoneally before or after irradiation. Animals were fed the so-called green diet* and water ad lib, except during the time of the irradiation. At least 20 animals were used in each experimental condition. The number of dead was recorded every day, and the weight of the rats noted every other day.

### Results

MEA is an effective protective agent when administered before irradiation. Furthermore, the preirradiation protective influence of MEA is considerably enhanced by liver shielding during the irradiation. Control animals receiving 850 r of x-ray without liver shielding or MEA administration all die within 5 days after irradiation. Animals receiving MEA before irradiation, without lead shielding of the liver region, survive to the extent of 10% after 28 days. The rats that had only their liver region shielded during irradiation without MEA have a survival of 45%. For animals receiving both MEA and lead shielding of the liver region, the 28-day survival is 80%. The survival curves of these experiments are recorded in Fig. 1; the weight curves in Fig. 2. Disease in the animal colony prevented the extension of the studies to other dose values.

MEA was observed to be toxic when administered after irradiation. Of the animals given 10 mg MEA directly after irradiation with the liver region shielded, 25% survive 28 days. Survival of rats irradiated without MEA and with liver shielded is approximately 45%. Survival is approximately the same, respectively 20% and 30%, whether the MEA dose is 5 or 2.5 mg per animal. If 10 mg of MEA is injected 3 hours after irradiation, survival is even less (20%). The influence of MEA after irradiation without shielding was not studied, since J. Maisin et al.[18,19] have observed that the compound was inactive under these conditions. Toxicity of MEA administered after

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* Simonsen Farm, Gilroy, California

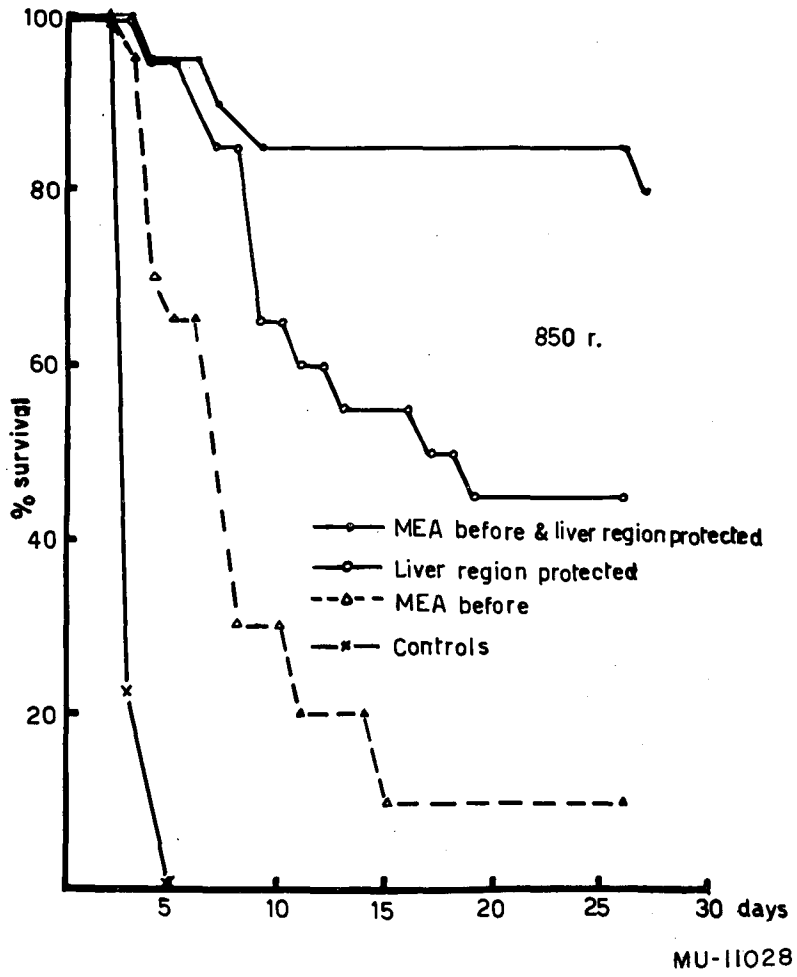


Fig. 1. Survival curves: β mercaptoethylamine and liver region shielding in irradiated rats – study of their erythropoiesis with Fe 59.

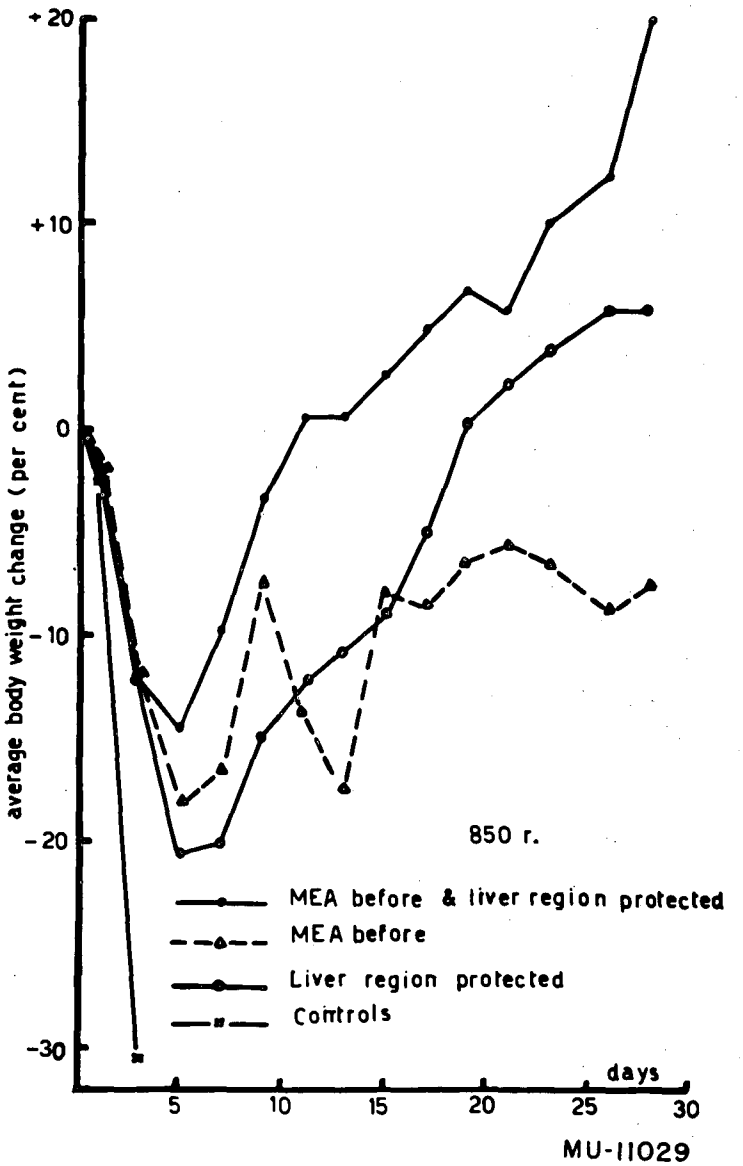


Fig. 2. Weight-change curves: β mercaptoethylamine and liver region shielding in irradiated rats – study of their erythropoiesis with Fe 59.

irradiation disappears upon dilution; the 28-day survival of 48 liver-shielded animals injected with 10 mg of MEA diluted in 1 cc of distilled water was about 45%. Survival of animals injected with diluted MEA after irradiation is the same as that of uninjected animals similarly treated.

## Discussion

The protection afforded by shielding the liver region during irradiation is considerably enhanced by injection of MEA immediately before irradiation. The protective effects of liver shielding and MEA administration are cumulative. The work of J. Maisin et al.[13, 14, 15] has shown that at a dose of 700 r the protection provided by shielding the liver region is not primarily the result of the shielding afforded by the protection of the liver, but that it is related to the protection of two other tissues shielded simultaneously (the intestine and the marrow). According to these authors, the two tissues must both be protected simultaneously, for shielding one of those tissues alone affords almost no protection. Furthermore, they have shown that shielding of the intestinal region prevents death, chiefly during the first days after irradiation; marrow protection prevents delayed death resulting from anemia occurring after the ninth day. Maisin et al.[13, 15] have also shown that protection of the intestinal region reduces the initial loss of weight of irradiated rats, and that the protection of marrow[15] diminishes the second loss of weight observed in animals with only the intestinal region protected. In other words, these authors differentiate between two major syndromes after irradiation: the first is an intestinal, syndrome, and the second the consequence of marrow aplasia, evidenced chiefly by anemia. Furthermore, it is known from similar experiments[15, 16] that the most vital tissue to be protected is the bone marrow. The same authors find that MEA always seems to play a role in intestinal protection, because MEA almost suppresses diarrhea, a major symptom of the intestinal syndrome, as does lead shielding of the intestine. Moreover, histological studies by H. Maisin and C. Fievez,[17] comparing intestinal mucosal lesions in control animals with MEA-treated animals, have shown that MEA-treated tissues regenerate much faster, as demonstrated by high mitotic activity. Thus the result of MEA treatment resembles that observed when intestinal tissue is protected by lead shielding. The tissue regeneration in lead-shielded intestine, however, starts 24 hours earlier than it does with MEA only.

Differences in weight loss were noted in comparison of liver-shielded irradiated rats with and without MEA administration. MEA-treated animals lost less weight. On the other hand, animals injected with MEA before irradiation without lead shielding seemed to have two successive losses of weight. H. Maisin, in collaboration with J. Maisin and Dunjic,[15, 16] after repeating such experiments with white rats, observed this in another laboratory. Moreover, it was shown--concerning the loss of weight--that MEA injected before irradiation renders good intestinal protection by diminishing the initial loss of weight following irradiation. After regaining some weight, such animlas undergo a second loss of weight, as do animals which had only the intestinal region protected. H. Maisin et al.[15] showed that, to suppress this second loss of weight (resulting chiefly from anemia), an area of bone marrow must be shielded, or a suspension of bone marrow injected.

In Long-Evans rats the therapeutic effect of MEA administered after irradiation to animals x-rayed with the liver region shielded could not be shown. In Belgium H. Maisin, in collaboration with J. Maisin and A. Dunjic, repeated these experiments, using white rats. The marked differences, reported by J. Maisin et al.,[18, 19] between such animals and animals that had only their liver region shielded were not observed. Indeed, J. Maisin et al.[20] were able to find differences only of the order of 10% in favor of a therapeutic action. They are convinced that the marked therapeutic effect observed earlier resulted firstly from the fact that the different groups of animals utilized in the previous experiments were not in the same condition of nutrition, and secondly from the fact that the lead shield was not always at exactly the same site over the liver region. Fasting produces differences in the mortality of the order of at least 10% in favor of fasting animals, and variation of the shield position allows protection of more or less intestine or marrow.

## PART II

### Methods

The experiments with $Fe^{59}$ were done dusing animals irradiated with 500 r. The following groups of animals were used: a group injected with MEA before irradiation, a second group with the liver region shielded during irradiation, a third group which received a combination of the two preceding treatments, and a fourth group of irradiated controls. $Fe^{59}$ was not given to animals that received MEA after irradiation. Finally, unirradiated animals were given $Fe^{59}$. All four groups of irradiated rats received $Fe^{59}$ three days after irradiation. $Fe^{59}$ was given intravenously under ether anesthesia. Each rat received approximately 1/3 μc of $Fe^{59}$, citrated and bound to plasma. The volume of the injected solution was 0.2 cc and its pH was 7. In each group, 3 to 5 animals were killed at 15 min, 1 hr, 3 hr, 6 hr, 12 hr, 24 hr, 3 days, 5 days, and 10 days after the injection of $Fe^{59}$. From each animal blood was withdrawn from the inferior vena cava in a heparinized syringe. After incision of both jugular veins, the animals were perfused through the inferior vena cava with 60 cc of 0.9% NaCl. The liver, the spleen, a piece of muscle, and the marrow of one posterior leg of each animal were then removed and weighed. The blood was centrifuged for 30 minutes, the plasma was separated and the red cells washed three times with saline. Radioactivity was assayed* in 1 cc of plasma, in 1 cc of red cells, in the marrow removed from the femur, and in samples of the liver, spleen, and muscle, with a vial scintillation counter.[21]

### Results

The activity obtained in red cells, liver, spleen, and marrow are shown in Figs. 3, 4, 5, and 6. The points on the graphs represent the mean of the results obtained with each group of sacrificed rats. The results were computed per 100,000 counts injected into each animal. The activity in the red cells is expressed per minute and per milliliter. The activity plotted for

* We wish to thank J. Garcia for his advice on the method of undertaking these procedures.

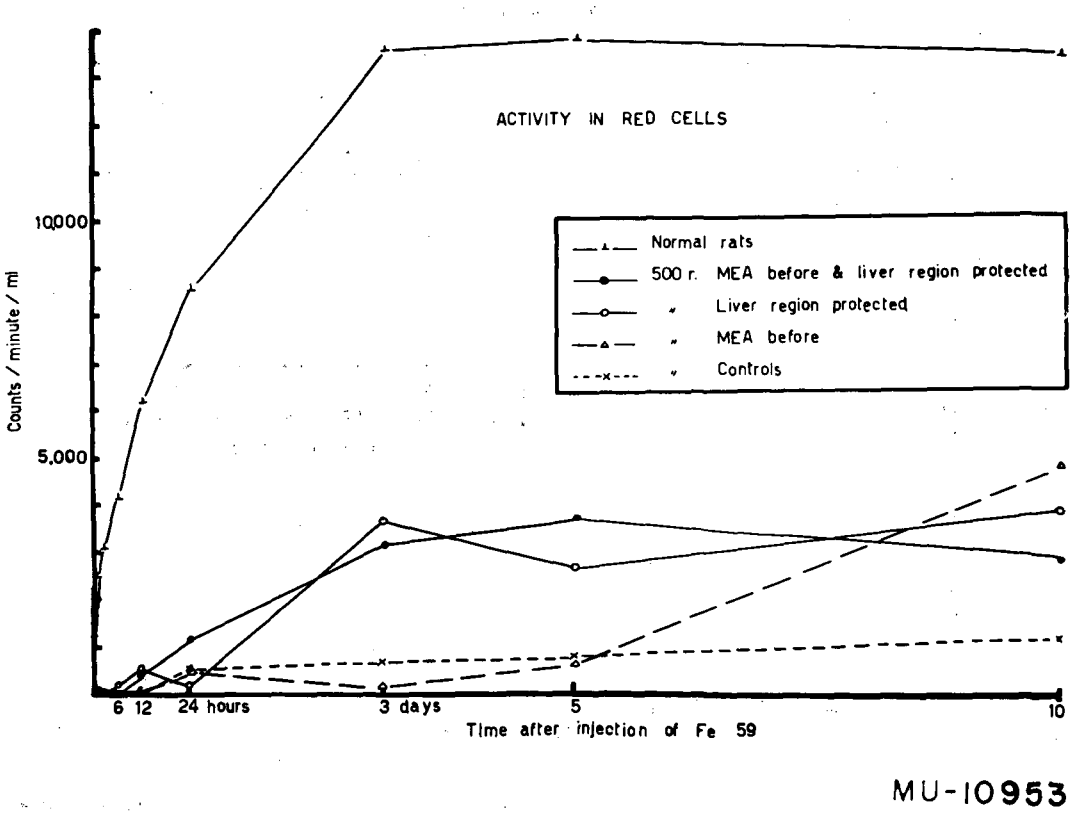


Fig. 3. Activity in red cells.

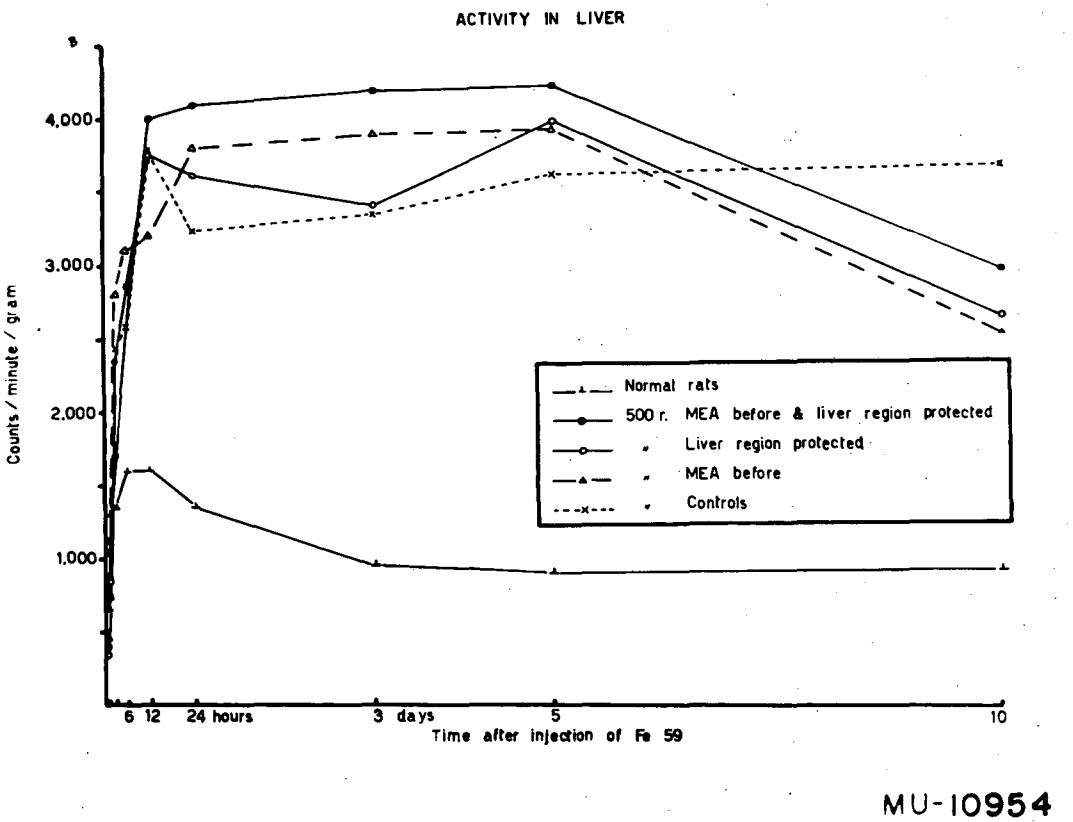


Fig. 4. Activity in liver.

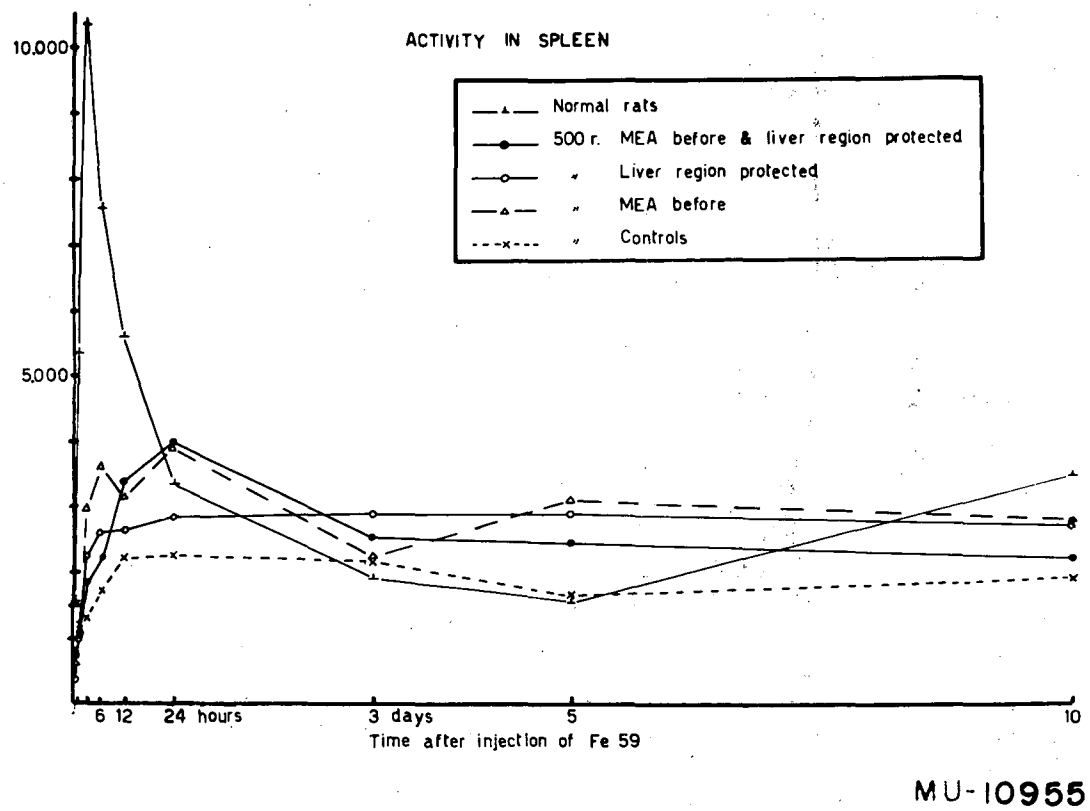


Fig. 5. Activity in spleen.

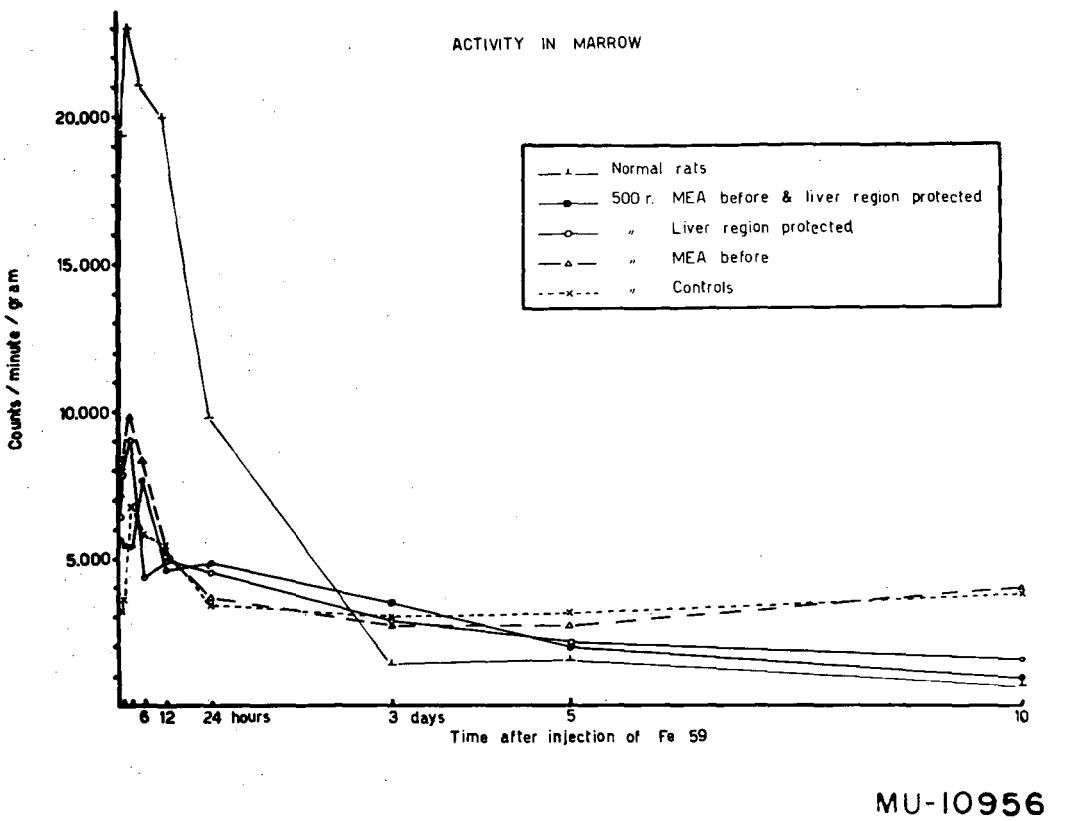


Fig. 6. Activity in marrow.

liver, spleen, and marrow is the activity per minute per gram of tissue. The activity in the muscle was determined to ascertain the perfection of the perfusion; it was of the same order of magnitude in all groups of rats.

The activity in the red cells was approximately the same whether or not the rats with the liver region protected were injected with MEA. At 10 days, the red cell activity of rats injected with MEA before irradiation was higher than that observed in unshielded irradiated controls. However, the iron uptake of the red cells of MEA-treated animals started only on the third day after injection, or on the sixth day after irradiation. With the two liver-shielded groups, the activity was considerably higher on the first day after injection or on the fourth day after irradiation. The activity of the red cells of these four groups (irradiated control, MEA-treated, liver-shielded, and combined MEA with liver shielding) remained far below that of the group of unirradiated rats. The iron uptake in the red cells of the controls at no time exceeded slightly more than 1000 counts per minute per ml. The half time of $Fe^{59}$ in plasma was 45 min in the normal rats, and always about 2.45 hours in all the four different groups of irradiated rats. The total activity in the liver of all the irradiated groups was of the same order of magnitude although much higher than in normal rats. The activity of the liver decreased after the fifth day in all irradiated groups, except in the controls, although it remained higher than that observed in unirradiated animals.

The activity in the spleen of the normal rats showed a very high peak at the third hour after injection of iron. Later this activity became much lower, but rose again to a certain extent, at about the tenth day. However, the irradiated animals did not show this initial high uptake of iron in the spleen; the rats that were injected with MEA showed slightly higher spleen activity during the first day after injection than did the others.

A very high uptake of iron was observed in the marrow of normal animals from the third to the twelfth hour after injection of $Fe^{59}$. The marrow of all the irradiated rats presented comparatively depressed uptake during the same period; this uptake was lowest in the irradiated controls.

## Discussion

From our experiments with $Fe^{59}$ it seems that at 500 r, with Long-Evans rats, MEA without liver shielding is able to induce an important delayed uptake of iron in the red cells, or, in other words, a regeneration of the red cells. The recovery of the erythropoiesis starts later than in animals in which an area of bone marrow or liver region was protected (see Fig. 3). This regeneration seems to be quantitatively significant. The action of MEA appears to be significant only for dosages not exceeding 500 r, for if it were effective at the 850 r dose, there would have been more than 10% of the rats surviving after 28 days. J. Maisin et al.[15,16] have shown that with a dose of 850 r, MEA does not influence marrow regeneration. They have further shown that the lack of marrow regeneration, as characterized by anemia, can be compensated for by lead shielding of a small volume of marrow, or by the injection of marrow.

This erythropoietic stimulation by MEA injected before irradiation is comparable with the histological regeneration that H. Maisin and C. Fievez[17] found at 700 r in intestinal mucosa of rats injected with MEA before irradiation. Of the two phenomena, the regeneration after physical shielding precedes that following MEA injection.

Whereas the increase of the erythropoietic activity in lead-shielded rats or in rats injected with MEA before irradiation is easy to establish when the red-cell iron uptake is read, the increase cannot be explained by marrow or spleen activity. It should be pointed out that in the lead-shielded rats the activity of the marrow in the shielded area was not analyzed. It is perhaps pertinent to remember that the iron uptake of the liver of all irradiated rats, except the controls, decreases after the fifth day.

Hennessy,[22] working with $Fe^{59}$ on glutathione and cysteine injected in rats prior to irradiation with 300 r, failed to observe a protective influence on erythropoiesis. Other workers, however, had already pointed out the protective action of sulhydryl compounds on hematopoiesis. Cronkite et al. have shown this action[23] of glutathione in mice; Rosenthal et al.[24] of cysteine in rats.

The curves of the $Fe^{59}$ uptake in liver, marrow, and red cells of the Long-Evans unirradiated animals resemble those by Huff et al.,[12] who used Curtiss-Dunning rats; however, in contradiction to the latter, we have observed a very large iron uptake in the spleen of our unirradiated rats three hours after injection. Huff et al. did notice such a high activity peak, but only in spleen-protected animals. The spleen of the Long-Evans rats thus appears to be highly erythropoietic. Indeed, histologically they are rich in megakaryocytes. More work should be done in order to explain the higher uptake of $Fe^{59}$ in the spleen of normal rats on the tenth day after its injection. In the irradiated controls curves were obtained similar to those by Huff et al.

Finally, it should be pointed out that the total iron value of samples of all the livers of our animals injected with $Fe^{59}$ was determined. The samples were wet ashed with nitric acid and hydrogen peroxide. The residue was taken up in 1 ml 6N hydrochloric acid, its pH adjusted to between 4 and 5; the sample was then diluted to 10 ml with distilled water and the iron determined with the Coleman spectrophotometer on 1 ml of the sample, according to the Ramsay method.[25] The mean values were 39.9 $\gamma$ of iron per gram of tissue in unirradiated animals, and ranged from 78 $\gamma$ to 84.8 $\gamma$ of iron per gram of tissue in the four groups of irradiated rats. However, the mean values of the liver weights of the unirradiated and irradiated rats were practically the same. The total iron per liver (and the iron per gram of liver) was greatly increased in irradiated animals. This is in agreement with the observed increase in the liver uptake of $Fe^{59}$ in the irradiated rats. The liver appears to play a role in the storage of iron disengaged, for example, by red cell destruction, or of unutilized iron in irradiated rats. The mean value of the plasma iron determination in each group of rats was approximately the same.

## SUMMARY

1. MEA administered before x-irradiation has a protective effect, as evidenced in 28-day survival of rats.

2. The protective effects of MEA and that afforded by shielding the liver and part of the bone marrow are additive.

3. Administration of MEA after x-irradiation with the liver region shielded has no significant protective effect in Long-Evans rats.

4. The influence of MEA and of lead shielding of the liver region in modifying effects of x-irradiation on erythropoiesis was studied by use of $Fe^{59}$.

5. Preirradiation treatment with MEA stimulates delayed erythropoiesis.

6. Erythropoietic activity, is partially preserved, when the liver region is lead-shielded, and this level of erythropoietic activity is not significantly altered by concomitant MEA treatment.

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## REFERENCES

1. L. O. Jacobsen, E. K. Marks, M. J. Robson, E. Gaston, and R. E. Zirkle, J. Lab. Clin. Med. 34, 1538 (1949).

2. J. Maisin, M. Mandart, and G. Lambert, J. belge radiol. 35, 337 (1952).

3. J. Gershon-Cohen, M. B. Hermel, and J. Q. Griffith, Jr., Science 114, 157 (1951).

4. M. Mandart, G. Lambert, H. Maisin, and J. Maisin, Compt. rend. soc. biol. 146, 1647 (1952).

5. A. Herve and Z. M. Bacq, ibid. 143, 881 (1949).

6. W. H. Chapman, and E. P. Cronkite, Proc. Soc. Exptl. Biol. Med. 75, 318 (1950).

7. H. M. Patt, E. B. Tyree, R. L. Straube, and D. E. Smith, Science 110, 213 (1949).

8. H. M. Patt, D. E. Smith, E. B. Tyree and R. L. Straube, Proc. Soc. Exptl. Biol. Med. 73, 18 (1950).

9. Z. M. Bacq, A. Herve, J. Lecomte, P. Ficher, and J. Blavier, Arch. intern. physiol. 59, 442 (1951).

10. Z. M. Bacq, and A. Herve, Bull. acad. roy. med. belg. 17, 13 (1952).

11. G. Lambert, J. Maisin, and M. Mandart, Compt. rend. soc. biol. 146, 1434 (1952).

12. R. L. Huff, W. F. Bethard, J. F. Garcia, B. M. Roberts, L. O. Jacobson, and J. H. Lawrence, J. Lab. Clin. Med. 36, 40 (1950).

13. J. Maisin, A. Dunjic, and H. Maisin, Compt. rend. soc. biol. 148, 611 (1954).

14. J. Maisin, H. Maisin, and A. Dunjic, ibid. 148, 743 (1954).

15. J. Maisin, H. Maisin, and A. Dunjic, Radiobiology Symposium, Liege, 1954, London, Butterworths, in press.

16. J. Maisin, H. Maisin, and A. Dunjic, Compt. rend. soc. biol. 148, 1293 (1954).

17. H. Maisin, and C. Fievez, Radiobiology Symposium, Liege, 1954, London, Butterworths, in press.

18. J. Maisin, M. Mandart, G. Lambert, and H. Maisin, Compt. rend. soc. biol. 147, 362 (1953).

19. J. Maisin, G. Lambert, M. Mandart, and H. Maisin, Nature 171, 971 (1953).

20. J. Maisin, A. Dunjic, G. Lambert, and L. Passeau, Compt. rend. Soc. biol. 147, 1517 (1953).

21. H. O. Anger, Rev. Sci. Instr. 22, 912 (1951).

22. T. G. Hennessy, F. B. Folsom, and John B. Glover, Naval Radiological Defense Lab. Report No. AD-267b, 1950.

23. E. P. Cronkite, G. Brecher, and W. H. Chapman, Proc. Soc. Exptl. Biol. Med. 76, 396 (1951).

24. R. L. Rosenthal, L. Goldschmidt, and B. I. Pickering, Am. J. Physiol. 166, 15 (1951).

25. W. N. M. Ramsay, Biochem. J. (London) 53, 227 (1953).