Post-Radiation Parabiosis and Survival in Rats.* (18754)

G. Brecher and E. P. Cronkite.

From the National Institutes of Health and the Naval Medical Research Institute, Bethesda, Md.

A variety of measures employed before or during whole body exposure to X-ray has been shown to afford partial protection against the lethal effects of irradiation. These procedures include administration of estradiol 10 days prior to irradiation(1,2), administration of sulfhydryl compounds immediately before exposure(3,4), pre-irradiation parabiosis(5), shielding of the spleen or other organs(6) and anoxia(7) during irradiation. Measures employed after irradiation have been less successful. Antibiotics(8) exchange transfusion(9) and DOCA(10) have been reported to reduce mortality, but no published data are available on the efficacy of these 3 therapeutic measures after doses of radiation which would be uniformly fatal in untreated animals. The present experiments were undertaken to determine whether a post-irradiation procedure can be of benefit in animals exposed to a uniformly fatal dose of whole body irradiation.

Methods. Radiation was delivered by a Picker therapy X-ray machine operating at 200 KVP (25 ma. 1.0 mm Al, 0.25 mm Cu, TSD 50 cm, field size 20 x 20 cm, HVL 0.8 Cu, dose rate 44 r in air per minute). Half of each total dose was delivered to the dorsal and half to the ventral surface of the rats in order to make irradiation more uniform throughout the body.

Female Sprague-Dawley rats weighing 80-120 g were used in all experiments. Parabiosis was accomplished under nembutal anesthesia. Silk was used in the first experiment and monofilament nylon No. 0 in the 2 subsequent experiments to hold the scapulae and femurs of the parabions together. The skin was joined with skin clips.

Results. In the first experiment, 8 female parabions joined 6 weeks before irradiation, were used. One partner was irradiated with 700 r while the other was shielded with 4 mm of lead. By measurement, the shielded rat received less than 1% of the incident radiation. Three pairs died on the 5th, 14th, and 33rd days respectively after irradiation. The 5 surviving pairs were separated 34 days after irradiation and are alive 5½ months later. At the time of irradiation the partners were essentially the same weight. At the present time the irradiated members weigh less, but in other respects appear normal. Fertility has not been tested. The 10 single control animals for this experiment all died from the effects of the 700 r dose, 9 of them by the 10th day and the 10th on the 19th day.

In the second experiment 14 rats were irradiated with 700 r and joined to non-irradiated litter mates within a few hours after exposure to X-ray. The parabions and single irradiated controls were treated with 15,000 units of penicillin, 20 mg streptomycin, and 2 cc intraperitoneal saline daily for the first 3 days after irradiation in an effort to avoid deaths from dehydration associated with early diarrhea, from wound infection, and from shock. Eight of the 14 pairs died at 1, 2, 4, 6, 8, 9, 11, and 26 days after irradiation. The surviving 6 pairs were separated on the 28th day. Of these, 1 irradiated animal died on the

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1. Treadwell, A. deG., *et al., Endocrinology,* 1943, v32, 161.

2. Patt, H. M., *et al., Am. J. Physiol.,* 1949, v159, 269.

3. Patt, H. M., Tyree, E. B., and Straube, R. L., *Science,* 1949, v110, 213.

4. Cronkite, E. P., and Chapman, W. H., *Fed. Proc.,* 1950, v9, 329.

5. Woenkhaus, E., *Arch, f. exp. Path. and Pharm.,* 1930, v150, 183.

6. Jacobson, L. O., *et al., J. Lab. Clin. Med.,* 1950, v35, 746.

7. Dowdy, A. H., *et al.,* U. of Cal., *Atomic Energy Project Report,* UCLA 55, 1950.

8. Miller, C. P., *et al., Science,* 1950, v111, 719.

9. Salisbury, D. F., *et al., Science,* 1950, v113, 6.

10. Ellinger, F. M., Proc. Soc. Exp. Biol. and Med., 1947, v64, 31.

45th day after irradiation. The remaining 5 are alive and gaining weight 3½ months later. The irradiated animals weigh less than their litter mates, but appear otherwise normal. The 10 single controls had all died by the 14th day.

In the third experiment, designed to provide tissues for histologic examination, 12 irradiated animals were joined to non-irradiated litter mates within a few hours after irradiation. Twenty-four litter mates of these pairs served as controls. Antibiotics and saline were given to all animals as in the preceding experiment. Nine of the 12 parabiotic pairs and 11 of the 24 single controls were alive on the 10th day after irradiation. Five parabions and 5 single animals were killed on the 10th day, and the remaining 4 parabiotic and 6 single rats on the 11th day. At autopsy the mesenteric and cervical lymph nodes of all single rats were hemorrhagic. No hemorrhages were noted in any of the irradiated parabions. On histologic examination, the femoral bone marrows of the single animals were largely atrophic and showed only scattered centers of myelopoiesis of varying size. In contrast, regeneration of the bone marrow in irradiated parabiotics was usually diffuse, and the overall cellularity often approached that of normal femoral marrow. In 2 of the parabiotic animals the cellularity of the bone marrow was within the range of normal.

In addition to the 20 animals used as controls in the first 2 experiments, 84 female rats of comparable age and weight were exposed to 700 r. Thirty of these rats were treated for 3 days with saline, penicillin, and streptomycin as in our 2nd and 3rd experiments. None of these animals survived beyond the 22nd day. Statistical analysis showed that there is only one chance in 20 that the true mortality of a 700 r dose for these rats is less than 97% and only 1 chance in 100 that it is below 95%. An additional 45 animals were exposed to doses of radiation from 325-625 r. The dose-mortality data are presented in Table I. It may be concluded that 700 r is either uniformly lethal for this age group of the Sprague-Dawley strain or approaches the uniformly lethal dose very closely.

TABLE I. Dose-Mortality Data for Rats Exposed to 325-700 r Whole Body X-radiation.

Dose in r	No. of rats exposed	No. dead at 30 days
325	9	0
400	9	2
475	9	4
550	9	8
625	9	8
700	104	104

Mortality in probits $= -1.0508 + .01266$ r. S. E. of slope $= .00198$. Estimated $LD_{50} = 478$ r $\pm$ 18 r. Estimated $LD_{99} = 662$ r $\pm$ 29 r.

Discussion. The above experiments confirm earlier findings that parabiosis performed some time before irradiation results in survival of the unprotected parabion after exposure to an otherwise fatal dose of X-ray(5). This protective effect of pre-irradiation parabiosis might be considered as similar to the shielding of part of an individual animal. However, as demonstrated here, parabiosis performed after irradiation is still of benefit in spite of the additional stresses of anesthesia and trauma. Post-radiation parabiosis must therefore be considered as akin to a therapeutic procedure.

Death following the doses of ionizing radiation used in these experiments is primarily though not solely, due to the sequelae of pancytopenia. It appears reasonable to assume that the survival of the parabiotic rats is linked to the accelerated regeneration of their bone marrow. This accelerated regeneration of hemopoiesis may be due to one or more of 3 mechanisms: transfer of some material necessary for the orderly differentiation of primitive mesenchymal cells, transplantation of blood-forming cells from the non-irradiated partner, or "detoxification" of a hypothetical substance produced by radiation in the exposed parabion. Since a "toxin" has never been satisfactorily demonstrated as playing a major role in radiation injury, the last possibility appears remote.

Summary and conclusions. Rats can survive an otherwise fatal dose of radiation if joined after radiation to non-irradiated litter mates. Such rats show accelerated bone marrow regeneration when compared with single controls. It is suggested that post-radiation

parabiosis is primarily of benefit because it accelerates recovery of myelopoiesis by the transfer of some cellular or non-cellular material from the non-irradiated partner.

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Assay of Plasma Antihemophilic Activity in Normal, Heterozygous (Hemophilia) and Prothrombinopenic Dogs.* (18755)

JOHN B. GRAHAM,† D. L. COLLINS, JR., I. D. GODWIN, AND K. M. BRINKHOUS.
From the Department of Pathology, University of North Carolina, Chapel Hill

The corrective action of normal plasma on the delayed clotting of hemophilic blood is attributed to its content of antihemophilic factor (AHF). It has been shown(1-3) that the amount of prothrombin consumed in whole hemophilic blood is directly proportional to the amount of AHF added. On the basis of this relationship, an assay procedure for AHF (2,3) was recently developed. In this paper, a series of studies on AHF are reported in which an improved assay procedure was used. The new method is more sensitive than the previous one, and it permits expression of results in per cent of normal control plasma. Studies have been made on the AHF level in normal dogs and in female dogs heterozygous for hemophilia, as well as in animals with hypoprothrombinemia due to liver injury and to dicumarol administration.

Assay of antihemophilic activity. The plasmas being tested for AHF were prepared from citrated whole blood (9 parts blood and 1 part 3.2% sodium citrate) in an angle centrifuge at about 3000 g for 20 minutes, and then dialyzed ("Visking" cellulose casing) with continuous agitation against 0.032% sodium citrate in 0.9% NaCl solution for 2 hours at 4°C. The normal control and test plasmas were diluted varying amounts (1-2 to 1-50) with saline. To 0.1 ml of each dilution of each plasma were added 0.15 ml imidazole buffer pH 7.25, and 1 ml freshly drawn hemophilic dog blood. A single hemophilic dog, either male or female(4), was used for each assay. After a reaction period of 20 minutes (28°C), 0.15 ml 3.2% sodium citrate was added to each tube, and the serum (or plasma) expressed by immediate centrifugation. Residual prothrombin was determined by the two-stage method(5), with added Factor V(6) prepared from dog plasma. The results were expressed in per cent (citrated plasma from the hemophilic dog = 100%). The prothrombin is plotted against the calculated amount of native test or control plasma added to 1 ml native hemophilic plasma. Fig. 1 illustrates the method used for comparing AHF in the test plasma with

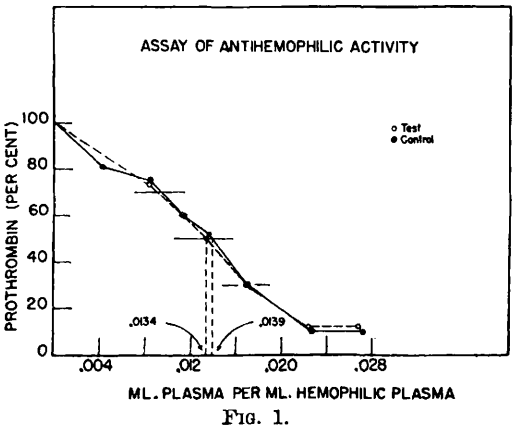

FIG. 1.

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† Markle Scholar in Medical Science.

1. Graham, J. B., Buckwalter, J. A., Hartley, L. J., and Brinkhous, K. M., *J. Exp. Med.*, 1949, v90, 97.

2. Graham, J. B., Penick, G. D., and Brinkhous, K. M., *Fed. Proc.*, 1950, v9, 330.

3. Graham, J. B., Penick, G. D., and Brinkhous, K. M., *Am. J. Physiol.*, 1951, v164, 710.

4. Brinkhous, K. M., and Graham, J. B., *Science*, 1950, v111, 723.

5. Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, v66, 801.

6. Owren, P. A., *The Coagulation of Blood*, J. Chr. Gundersen, Oslo, 1947, p. 82 ff.