AUG 5 1966

6 July 1966

MASTER

BNL 10385

CFSH PRICES

H.C. \$ 1.00; MN .50

BONE MARROW STEM CELL SURVIVAL IN THE
NONUNIFORMLY EXPOSED MAMMAL

V.P. Bond and C.V. Robinson

Brookhaven National Laboratory

Medical Research Center

Upton, L.I., New York

RELEASED FOR ANNOUNCEMENT
IN NUCLEAR SCIENCE ABSTRACTS

Research supported by the U.S. Atomic Energy Commission

Presented at the IAEA Panel on Effects of Radiation on the Bone Marrow,
Vienna, 17-20 May, 1966

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor, prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

For comparison of the effectiveness of non-uniform vs uniform irradiations in causing hematological death in mammals, a model of the irradiated hemopoietic system has been proposed (1). The essential features of this model are: 1) that different parts of the hemopoietic system have numbers of stem cells which are proportioned to the amounts of active marrow in those parts as measured by Fe^{59} uptake, 2) that stem cells in the different parts are subject to the same dose-survival relationship, and 3) that survival of the animal depends on survival of a critical fraction of the total number of stem cells independent of their distribution among the parts of the total marrow mass. In order to apply this model one needs to know a) the relative Fe^{59} uptakes of the different parts of the hemopoietic system, b) the doses delivered to those parts by each of the exposures to be compared, and c) the dose-survival curve applicable to the stem cells. From these one can calculate the fraction of stem cells surviving each exposure.

In a preliminary communication (1), the applicability of the model was investigated using data obtained entirely from the literature. Additional data, particularly on bone marrow distribution, have since been obtained and are included herewith. The primary object of the present paper is to test further the validity of the above "stem cell survival model". Data on bilateral (essentially uniform) versus unilateral and non-uniform rotational exposures in mammals are examined with respect to the surviving fraction of stem cells at the $\text{LD}_{50/30}$ day dose level. Although an adequate test is not presently possible for lack of a full set of data in any one species, a partial test indicates compatibility with data for dogs and rats. Other possible mortality determinants such as doses or exposures at entrance, midline or exit; or the gram-rads or average dose to the marrow, appear to be less useful than the critical stem-cell survival fraction.

Data Used

Two sets of data were used primarily - one in which the lethal effects of bilateral versus unilateral exposure of dogs to 1000 KVP x-rays were compared (2), and one in which rats were exposed nonuniformly to Co^{60} gamma radiation (3). These energies are sufficiently high so that the average absorbed dose to the bone marrow closely approximates that to other adjacent soft tissue. In all studies, dose distribution determinations were made allowing accurate estimates of local doses to tissues throughout the animal. Data on swine were considered previously (1).

Distribution of active marrow in the dog was determined by injecting either radioactive iron or colloidal Tc^{99m} and determining the relative amount of activity in each bone (4). The distributions of the two compounds were remarkably similar in the dog. Marrow distribution in the rat was determined by injecting colloidal Tc^{99m} , after which the animals were eviscerated, the abdominal and thoracic cavities filled and molded with melted paraffin, and the rat frozen in the approximately cylindrical configuration (4.4cm diameter) in which other rats had been exposed for mortality rate determinations (3). The frozen assembly was placed in a lathe and the carcass was turned down by successive 1 mm cuts. The relative activity in each cut yielded the relative amount of active marrow as a function of depth from the animal's surface, in the configuration used for mortality determinations.

$\text{LD}_{50/30}$ day values obtained are shown in Table I. For unilateral and bilateral irradiation, exposures are given in terms of that in air at the midline of the exposure volume (phantom absent). Values are also given for the entrance, midline and exit positions in tissue, as derived from depth-dose data provided by the authors. LD_{50} values for rats are given in terms of absorbed dose at entrance.

The distribution of active marrow in the dog (4) is shown in Table II. With lateral exposure, all marrow can be considered as in either the proximal, middle or distal thirds of the body thickness relative to the source. Vertebral marrow

only (42.6%) was taken as centrally located. With bilateral exposure, the remaining marrow is equally divided yielding values of approximately 29, 43 and 29 for the proximal, middle and distal thirds, respectively. With unilateral exposure, the skull and sternum must be considered to be in the proximal third, yielding values of 30.5, 42.6 and 26.7 per cent for the proximal, middle and distal thirds respectively.

No dose-survival curve is available for dog or rat bone marrow, and a curve for mouse bone marrow has been used. Since high-energy radiations were used in the large animal studies, a mouse stem cell curve for Co^{60} exposure was selected (5). This curve has a D_0 of 95 rads and an extrapolation number 1.5. In a comparison among a number of stem cell survival curves for the mouse (6,7), this curve, obtained following in vivo exposure of the donor, was considered representative.

Method of Evaluation

The validity of the basic hypothesis that survival with uniform and nonuniform exposure depends on the same fraction of total stem cells remaining intact is most easily examined by calculating the relative number of stem cells surviving at the $\text{LD}_{50/30}$ day exposure level, for uniform versus nonuniform exposure. This was done for the dog data (Table I) as follows:

The calculations for the dog exposed unilaterally are given in Table II. In column one is given the approximate average absorbed dose to the proximal, middle and distal thirds at the $\text{LD}_{50/30}$ day exposure level. In column two is given the relative amount of active marrow (stem cells) in each third. In column three is given the surviving fraction of stem cells for each third, obtained from the mouse stem cell survival curve (5). The product of the surviving fraction and the relative amount of marrow in that third yields the number of surviving cells in that third (given in column four), and the sum of the values for the

three thirds yields the number surviving per 100 cells of the entire marrow, or the surviving percentage of stem cells. The value obtained is 8.0%.

The surviving percentage of stem cells for bilateral irradiation can be easily approximated as follows: The dose to all bone marrow is approximately the same, and equal approximately to 280 rads. From Ref. 2, this yields a surviving fraction of the total bone marrow stem cells of 7.1%. A more refined calculation using the approach outlined in Table III together with the appropriate values yields a surviving fraction of 7.9%.

The above values were obtained using superficial entrance and exit doses in the calculation. If the doses taken at a depth of 1 or 1.5 cm in from the skin are taken as being more representative of that to the more superficial marrow, the values for unilateral and bilateral are still in quite good agreement. If 2.5 cm depth is used, the agreement is appreciably worse.

Similar calculations were made for the rat, using the depth-dose data reported (3) and the bone marrow distribution shown in Table III. The surviving fraction of stem cells for uniform exposure is approximately 0.02%. The calculated value for nonuniform exposure is approximately three times this value.

Discussion

The data available, while incomplete, are consistent with the thesis that survival in the mammal after exposure in the lethal (marrow death) range is determined by the surviving fraction of total marrow stem cells, independent of location in the body. Additional detailed information on the distribution of active marrow in the body, as well as refined depth-dose data are required to establish definitely the validity of the stem cell survival model. In particular, the specific dose-effect curves for the stem cells of the dog and rat are required. If the values for D_0 or extrapolation number for these species are markedly different from those used in the calculations, the conclusions drawn would of course be altered.

As discussed previously (5), the model serves to clarify what dose parameters may or may not be useful in "normalizing" biological effectiveness (lethality) for a variety of radiations of different qualities, or a single radiation delivered with different depth-dose distributions. The conclusion was drawn (1) that no single physical dose parameter is suitable to express the lethal capacity of radiations delivered nonhomogeneously. The situation is similar to that involving exposure to radiations of different qualities. Here a relative biological effectiveness (RBE) factor is introduced, which when multiplied by the dose delivered yields the "dose equivalent" in "rem". Similarly, when a given radiation is delivered with different body distributions, an "efficiency of the distribution function" or "distribution effectiveness factor" (DEF) may be introduced to arrive at an "effective dose" or dose equivalent. The model provides a basis for defining such a distribution factor, as discussed previously (1).

The model would, of course, be expected to apply only under circumstances in which the biological effect scored (mortality in example used) is related to marrow stem cell survival, and can be checked only under circumstances in which the dose to the bone marrow is either known or can be closely approximated by the dose to adjacent muscle or other soft tissue.

The fraction of surviving stem cells in exposed rats was higher following non-uniform than that following uniform exposure. This probably is related to gut damage in the nonuniformly exposed animals. Some basis for this explanation is seen in the shortened survival time noted in some animals exposed (nonuniformly) (3). Groups of animals dying with a shortened survival time showed a higher percentage of surviving stem cells than did uniformly exposed animals, indicating that marrow failure was not the primary cause of death.

The results have pertinence with respect to possible reasons for the difference in LD_{50} values between the dog (and most other large species (8,9)).

and the mouse (and other small species). If the dose-effect curve for marrow stem cells of the mouse is applicable to the dog as assumed above, then the per cents of total surviving stem cells at the LD_{50/30} day level apparently are quite different (about 7% for the dog; and 0.2% for the mouse).

TABLE I

LD_{50/30} day Values for Dogs and Rats Exposed Uniformly and Non-Uniformly
to Megavoltage Radiation

Species, Exposure Type	LD _{50/30} days			
	Midline Air	Entrance Tissue	Midline Tissue	Exit Tissue
Dog, bilateral (uniform)	319*	266**	280**	206**
Dog, unilateral (non-uniform)	384*	530**	337**	168**
Rats, uniform		846**		
Rats, nonuniform (rotational)		2522**		

* Exposure in Roentgens

** Absorbed dose in rads

TABLE II

Calculation of the Surviving Fraction of Stem Cells in the DogExposed Unilaterally to 1000 KVP X-radiation

Body Region	Dose (rads)	Relative No. of Stem Cells	Surviving Per Cent (from Fig. 1)	Relative No. of Surviving Stem Cells
Proximal third	530	30.5	0.5	0.2
Middle third	337	42.6	4	1.7
Distal third	168	26.7	23	6.1
Total		99.8		8.0

TABLE III

Distribution of Active Bone Marrow of the Rat, as a
Function of Distance from the Body Surface*

Distance from Surface, Millimeters	Active Marrow (% of Total)
0-1	1.1
1-2	1.9
2-3	4.4
3-4	5.0
4-5	5.6
5-6	7.0
6-7	9.8
7-8	10.4
8-9	10.1
9-10	9.9
10-11	7.6
11-12	5.5
12-13	4.8
13-14	4.6
14-15	3.6
15-16	2.1
16-17	3.3
17 to center **	1.5
	<hr/> 98.2

* See text for methods employed

** Radius of rat, 2.2 cm

References

- 1) V.P. Bond and C.V. Robinson, A mortality determinant in non-uniform exposures of the mammal. Presented at the Workshop Conference on Space Radiation Biology, Berkeley, Calif., 7-10 Sept., 1965.
- 2) E.J. Ainsworth, G.F. Leong, K. Kendall and E.L. Alpen, Comparative lethality responses of neutron and x-irradiated dogs: influence of dose rate and exposure aspect. U.S. Navy Radiological Defense Lab. Report. USNRDL-TR-787, 6 Oct., 1964.
- 3) K.L. Jackson, The lethal effectiveness of a solar flare-type dose distribution delivered to the rat. Proc. of Symposium on Protection Against Radiation Hazards in Space, Gatlinburg, Tenn., Nov., 1962. TID-7652, Book 1.
- 4) M.L. Greenberg, H.A. Atkins and L.M. Schiffer, Distribution of functional erythropoietic and reticuloendothelial compartments in bones of adult dogs. Science 152:526 (1966).
- 5) E.A. McCulloch and J.E. Till, The sensitivity of cells from normal mouse bone marrow to gamma radiation in vitro and in vivo. Rad. Res. 16:822-32 (1962)
- 6) L.G. Lajtha, Response of bone marrow stem cells to ionizing radiations, In: Current Topics in Radiation Research, M. Ebert and A. Howard, Eds., North-Holland Publishing Co., Amsterdam, 1965.
- 7) G.F. Whitmore and J.E. Till. Quantitation of cellular radiobiological responses. Ann. Rev. Nuclear Sciences, 14:347-374 (1964)
- 8) V.P. Bond and J.S. Robertson, Comparison of the mortality response of different mammalian species to x-rays and fast neutrons. Presented IAEA Symposium "Biological Effects of Neutron and Proton Irradiations", Brookhaven Nat'l Lab., Upton, L.I., N.Y., USA, 7-11 Oct., 1963. Report Vol. II, pp. 365-77 (1964).
- 9) V.P. Bond, T.M. Fliedner and J.S. Archambeau, Mammalian radiation lethality: a disturbance in cellular kinetics. Academic Press, New York, 1965.