Bone Marrow Treatment of Mice Lethally Irradiated with Gamma-Rays under High Dose Rate. (IV)

Supplementary Studies

Morihisa YAMAGISHI, Kazushige ADACHI, Shigeru SHIRAKAWA, Michiaki YAMABA and Haruto UCHINO*

(First Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University)

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Mice were irradiated with gamma-rays under high dose rate (42 r per second) and were treated with isologous, homologous, and heterologous bone marrow in various experimental conditions not reported in the previous study. By increasing the dose of isologous bone marrow from 10×10^6 to 20×10^6 nucleated cells, a fairly good survival rate was obtained even when isologous bone marrow (IBM) was injected at as late as 6 days after irradiation. Even with this dose IBM injection at 7 days was not effective in obtaining surviving mice. The IBM dose as small as 0.1×10^6 nucleated cells was effective in obtaining fairly good survival rate when IBM was given immediately after irradiation. However, larger IBM dose is desirable because of quicker regeneration of hematopoietic organs in mice receiving a larger dose. The quick recovery of hematopoietic organs in mice lethally gamma-irradiated and treated with IBM demonstrated previously by hematological and histological study was also proved chemically by the early incorporation of formate-C^{14} into the bone marrow and spleen after irradiation. Iron metabolism in irradiated and IBM treated mice was also studied. The delayed treatment with homologous bone marrow (HBM) from one to four days post-irradiation seemed effective in obtaining a good initial 14 day survival rate. Heterologous rat bone marrow (RBM) has so far been ineffective even by changing experimental conditions variously.

As one of the means of facilitating take of foreign bone marrow and of preventing secondary disease, combined use of AET protection and bone marrow treatment was tried. When homologous donor mice were given AET followed by 500 r (or 100 r) irradiation, neither initial nor subsequent survival of lethally irradiated recipient was good. When recipient mice were given AET followed by an otherwise supralethal dose of irradiation (1500 r) good initial survival was obtained.

Bone marrow dose not seem to modify radiation damage to tissues other than those of the hematopoietic system as shown by retarded growth and reduced life span. Furthermore, evidences of late radiation effect such as nephrosclerosis and calcification of myocardium were observed in gamma-irradiated and IBM treated mice.

INTRODUCTION

In this atomic era one may be exposed any time to a large dose of radiation instantaneously. The very potent Co^{60} irradiation facility available to us can irradiate mice lethally within half a minute. Previous studies on bone marrow

* 山岸司久，安達百成，白川　茂，山羽庚昭，内野治人

treatment in mice lethally gamma-irradiated under high dose rate has been reported[1,2,3]. Experimental conditions in bone marrow treatment were changed variously to observe their effect on the survival of mice. Further, experiments were directed toward finding out measures of preventing so-called socondary disease which is a major limiting factor of successful bone marrow transplantation. The results of preliminary studies obtained to the present time is to be reported here.*

MATERIALS AND METHODS

Dd/s strain mice supplied from the Kyoto University Inbred Animal Center were used in IBM treatment study and as recipients in HBM and RBM treatment study. Other animals used were na 2 and CF#1 mice and Wistar and Sprague-Dawley rats. These mice and rats were 2 to 3 months old unless specified. The method of obtaining mouse bone marrow suspensious and the conditions of irradiation were described elsewhere[1]. All mice were irradiated under the dose rate of approximately 42 r per second at place A of the Co^{60} gamma-irradiation facility[1,16] "Lethal" irradiation in this study means approximately 900 r irradiation which is 100% lethal to mice within 30 days. In order to obtain rat marrow suspensions, bilateral femurs and tibias were removed aseptically and cut longitudinally, and marrow was scraped off and suspended in Tyrode's solution. The suspension was stirred up, gently centrifuged to remove supernating fat, and filtered through several layers of gauze. Bone marrow suspensions were injected intravenously within several hours after irradiation unless otherwise indicated.

RESULTS AND DISCUSSION

I. Delayed IBM Treatment (Table 1)

When the dose of IBM given was approximately 10×10^6, IBM given at 5 days after irradiation resulted in poor 30 day survival rate[3]. In this study the dose was doubled to 20×10^6 nucleated cells. The survival rate was good at 5

Table 1. Survival rate of $900r+20\times10^6$ IBM mice at various post irradiation days.

Days after irradiation	No. of Survival/No. of Irradiated (at days)						% Survival	
	7	14	21	30	60	90	30	90
5	6/6	6/6	6/6	6/6	6/6	6/6	100	100
6	7/10	5/10	4/10	4/10	3/10	3/10	40	30
7	9/10	0/10	—	—	—	—	0	0

* The results reported in this paper was presented before the symposium on bone marrow transplantation held by the Japan Hematological Society in November, 1961 in Tokyo[15].

days and fairly good at 6 days. IBM treatment at 7 days with this double dose was not effective, however. Recovery of body weight seemed to be retarded when the interval between irradiation and bone marrow treatment was longer (Fig. 1).

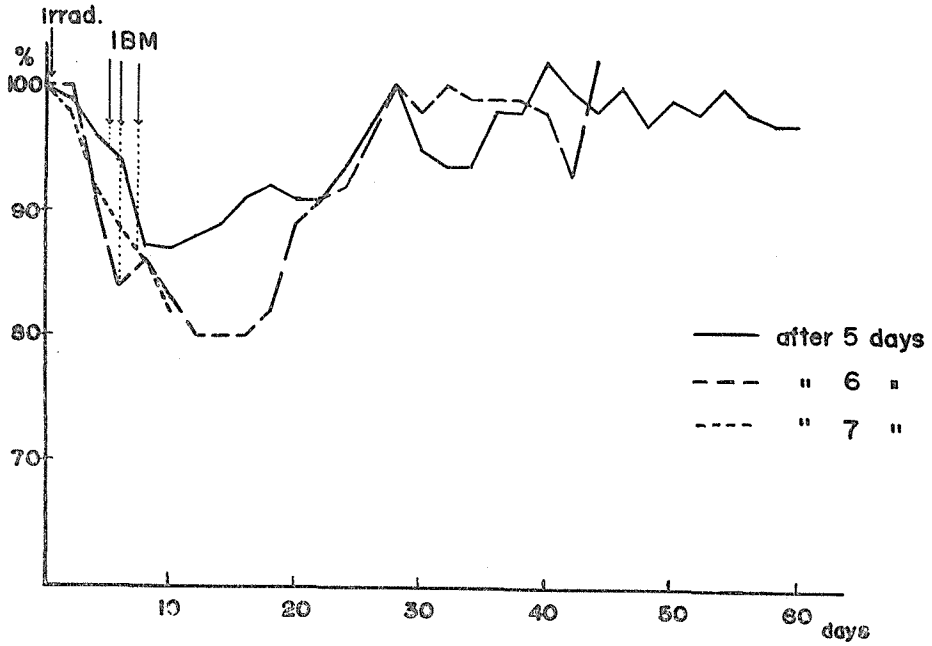

Fig. 1. Body weight change of 900 r+20×10⁶ IBM mice at various post irradiation days.

II. The Effect of IBM Dose on 30 Day Survival (Table 2)

When the standard dose of IBM (5 to 10×10⁶ nucleated cells) is given, 40 to 100% 30 day survival is expected to be obtained. How is the survival rate when the dose is changed? In this study the dose was changed from 20×10⁶ to zero. One million cells gave good survival. It was rather a surprise to note that 0.1×10⁶ cells still gave fairly good 30 day survival. When all cells were removed by filtering bone marrow suspensions through Seitz filter, there was no survival and accelerated recovery of hematopoietic organs was not observed either histologically or hematologically. Although survival rate in the group receiving rela-

Table 2. Survival rate of lethally irradiated mice treated with various amount of IBM.

	Amount of IBM (Nucl. cell count)	No. of Survival/No. of Irradiated (at days)						% Survival	
		7	14	21	30	60	90	30	90
1	20×10⁶	11/11	11/11	11/11	10/11	9/11	9/11	91	82
2	1×10⁶	11/11	11/11	9/11	9/11	9/11	9/11	91	82
3	0.1×10⁶	11/11	7/11	5/11	5/11	5/11	4/11	45	36
4	0*	7/7	0/7					0	

* Filtrate through Seitz filter of IBM suspension.

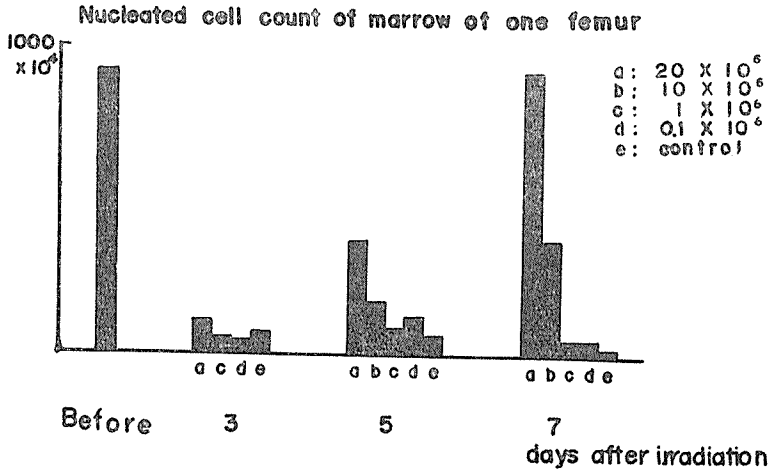

Fig. 2. Nucleated cell count of femoral marrow of lethally irradiated mice treated with various amount of IBM.

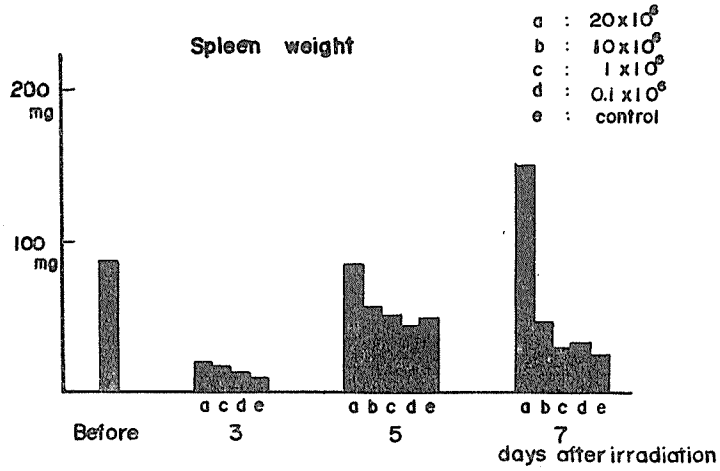

Fig. 3. Spleen weight of lethally irradiated mice treated with various amount of IBM.

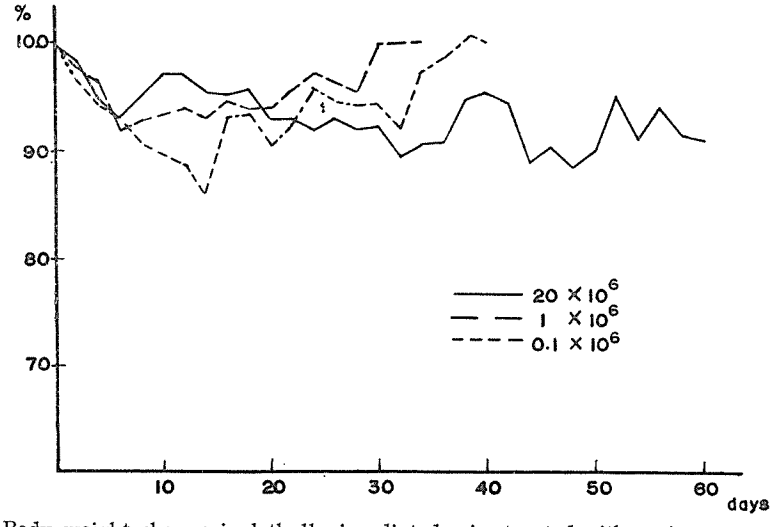

Fig. 4. Body weight change in lethally irradiated mice treated with various amount of IBM.

tively small dose of IBM was fairly good, recovery of nucleated cell count in the femoral marrow, of spleen weight and of body weight was faster in the group receiving larger dose (Figs. 2, 3 & 4).

III. Nucleic Acid Metabolism in Lethally Gamma-Irradiated Mice Treated with Isologous Bone Marrow

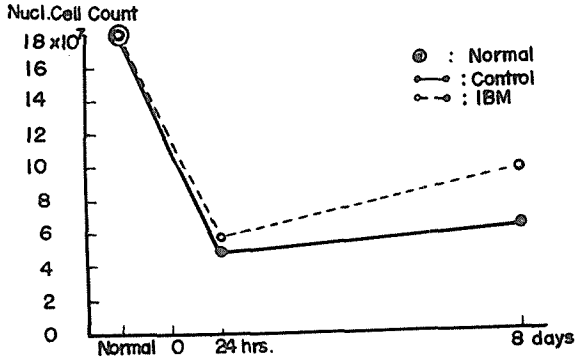

Fig. 5. Chonges of bone marrow nucleated cell counts.

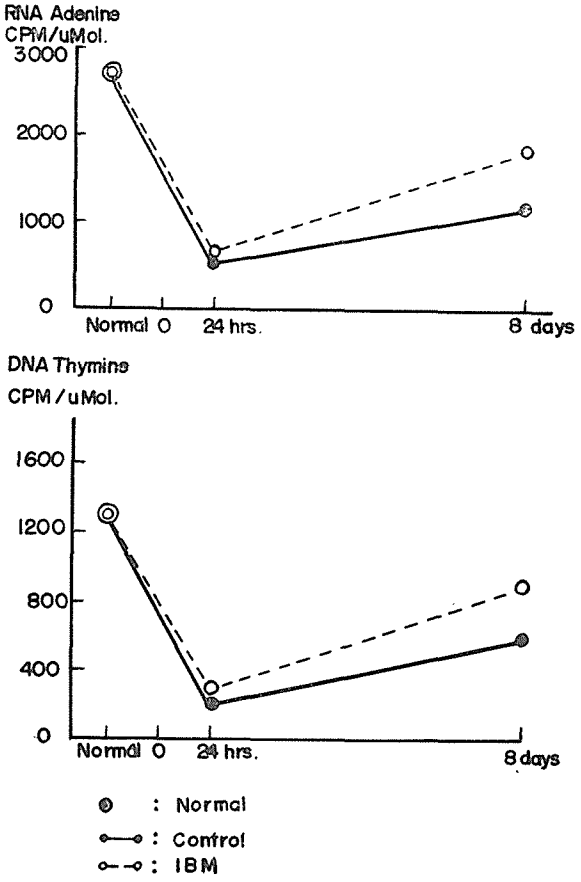

Fig. 6. Formate-C^{14} incorporation into uncleic acid bases in bone marrow.

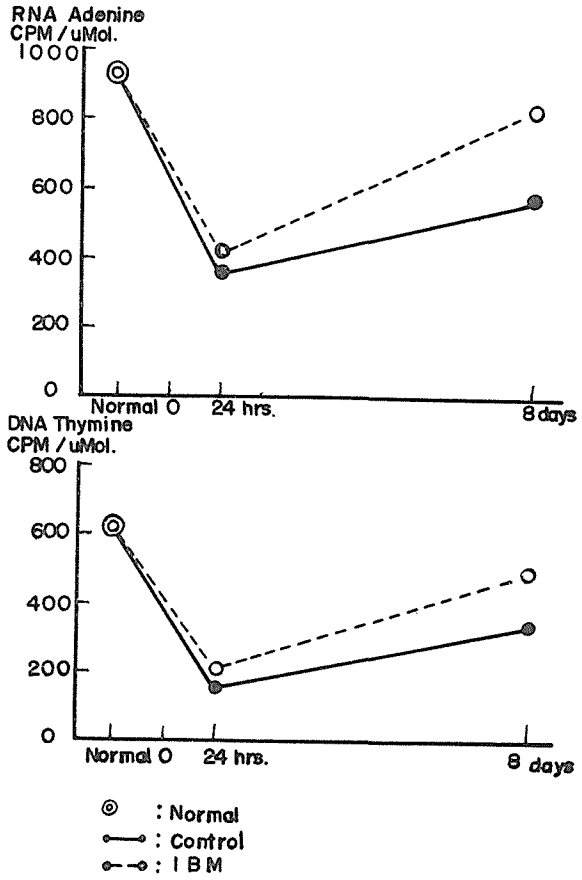

Fig. 7. Formate-C^{14} incorporation into nucleic acid bases in spleen.

To demonstrate chemically the early regeneration of hematopoietic organs in irradiated and IBM treated mice, incorpoation of formate-C^{14} into nucleic acid bases of bone marrow and spleen in them was studied. Four hours after intraperitoneal injection of 2.5 μC of formate-C^{14} solution, bone marrow suspensions and spleen homogenate were made for uptake study. The incorporation of formate-C^{14} into DNA thymine and RNA adenine in bone marrow and and spleen was slightly higher in IBM treated mice than the irradiated control already at 24 hours after irradiation. The difference was apparent at 8 days (Figs. 5, 6 & 7). The tendency that the recovery of formate-C^{14} incorporation in IBM treated mice was faster than that of nucleated cell counts suggests that recovery of nucleated cells was mainly due to an increase of immature blood cells.

IV. Iron Metabolism in Lethally Gamma-Irradiated and IBM treated Mice

Fe^{59} incorporation in red cells of lethally irradiated and IBM treated mice was studied (Fig. 8). One μC of Fe^{59} per mouse was injected intravenously from one to 24 days after gamma-irradiation and IBM treatment, followed by serial collection of blood for observing Fe^{59} incorporation in red cells. The incorpora-

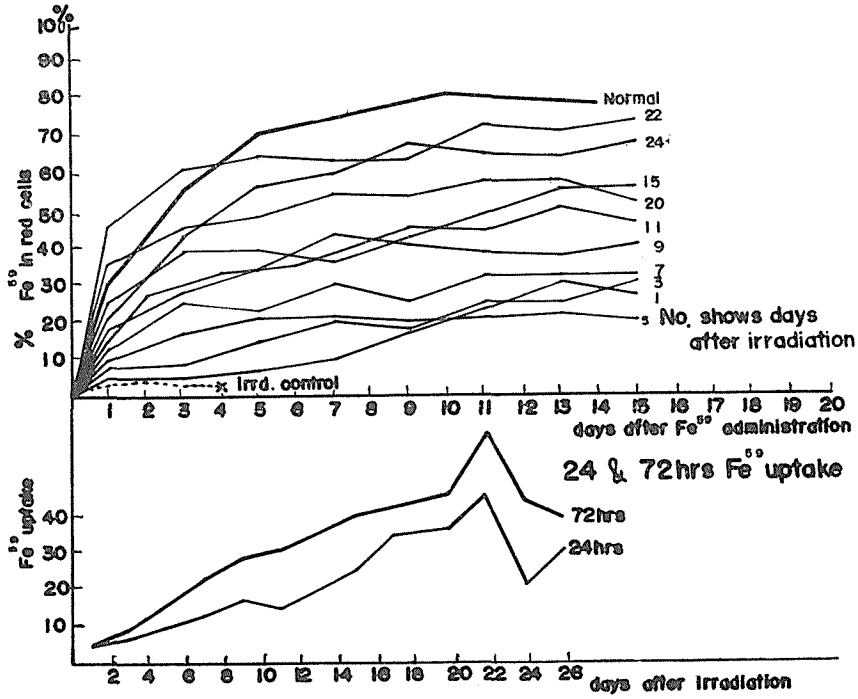

Fig. 8. Fe^{59} incorporation in red cells of lethally irradiated IBM treated mice.

tion was considerably depressed within several days after irradiation followed by gradual recovery, and was almost normal at 22 days.

V. Delayed Homologous Bone Marrow Treatment (Table 3).

Homologous bone marrow was injected one to for days after lethal gamma-irradiation to know if there was any beneficial effect on survival. Serum antibody formation as manifested by hemolysin formation has been reported to be minimum at 24 to 48 hours after irradiation[11] and, furthermore, acellular or hypocellular marrow cavity could be better mechanically for donor marrow cells to seed and repopulate there than normo-celluar marrow. Immediate treatment

Table 3. Survival rate of lethally gamma irradiated mice treated with 10×10^6 HBM at various post irradiation days.

Days after irradiation	No. of Survival/No. of Irradiated (at days)							% Survival	
	7	14	21	30	60	90	120	21	90
1	7/9	7/9	3/9	3/9	3/9	3/9	3/9	33	33
2	8/9	6/9	2/9	2/9	2/9	2/9	0/9	22	22
3	7/10	4/9	3/9	2/9	0/9			33	0
4	7/10	6/10	3/10	2/10	2/10	1/10	1/10	30	10
control	3/6	1/6	0/6					0	

with HBM after 900 r gamma-irradiation usually gives, in our study[2], less than 50 or 60% 14 day survival. As seen from Table 3, 14 day survival was good when HBM was given one, two, and four days after irradiation. However, subsequent surival rate was low as in the case of immediate treatment. HBM treatment at 5 days after lethal irradiation in the pigeon was reported to have elongated mean survival time[12]. Therefore further delaying bone marrow treatment would be worthwhile trying.

VI. Heterologous (Rat) Bone Marrow (RBM) Treatment

RBM treatment has, so far, been ineffective even by changing experimental conditions variously (Table 4). In experiment 2, 3, 4, 5, 6, 7 and 8, the dose of

Table 4. Survival rate of gamma-irradiated mice treated with heterologous bone marrow.

	Donor	Recipient	Radiation dose (r)	No. of cell injected ($\times 10^6$)	No. of Survival/No. of Irradiated (at days)					
					7	14	21	30	60	90
1	2 Mos Wist. Rat	Na_2	900	14~25	T : 12/14 C : 9/9	9/14 0/9	0/14			
2	2 Mos Wist. Rat	Na_2	900	42	T : 14/20 C : 8/10	0/20 0/10				
3	1 Mos Wist. Rat	dd/s	900	15	T : 9/12 C : 4/8	0/12 0/8				
4	2 Mos Wist. Rat	dd/s	780	18	T : 6/14 C : 6/8	0/14 1/8	0/8			
5	2 Mos Wist. Rat	dd/s	1100	37	T : 0/14* C : 0/11					
6	2 Mos Wist. Rat	dd/s	AET + 1300	70	T : 3/8 C : 5/9	1/8 5/9	1/8 2/9	1/8 2/9	1/8 2/9	1/8 1/9
7	2 Mos Sp-Dow.	dd/s	900	200	T : 3/3	0/3				
8	2 Mos Wist. Rat	CF_1 Na_2	900	200	T : 3/3	3/3	1/3	0/3		

* 0.25 mg/mouse of hydrocortisone daily for 3 days.

RBM was increased to 42×10^6, young rats were used, relatively smaller dose of irradiation was given, irradiation dose was increased to 1100 r, AET was given 10 minutes prior to 1300 r irradiation, a large dose of bone marrow (20×10^6) was used, and recipient mice were changed from dd/s to CF#1 and na2 together with administation of 20×10^6 cells, respectively all were without effect. In any mice surviving at 2 weeks after irradiation and RBM treatment, alkaline phosphatase positive neutrophils were not found in the peripheral blood indicating no evidence of take of rat marrow in the mice. In regard to many papers reporting successful transplantation of RBM into mice[4~10], further study is needed to find out the cause of this failure in the experimental conditions described here.

VII. A Few Trials to Facilitate Implantation of Bone Marrow and to Preclude Secondary Disease

Delaying bone marrow treatment, use of steroids and changing bone marrow dose as part of the means to facililate implantation of homologous (heterologous) bone marrow and to preclude so-called secondery disease have been described. In a study of giving a double dose (20×10^6 cells) of HBM, no better 14 day survival was obtained.

AET (S, 2-aminoethylisothiourea dihydrobromide) has been known to be effective in protecting animals from radiation injury. AET is also deduced that it protects predominantly non-antibody forming cells[13]. Although this deduction is based on the experiment dealing with circulating serum antibody formation and not with cellular antibody formation which plays an important role in transplantation immunity, combined use of chemical protection and bone marrow treatment would be worthwhile to try. The results of a preliminary study in regard to this combined use are to be reported as follows:

Table 5. Survival rate of lethally (900 r) gamma-irradiated mice treated with homologous bone marrow (or spleen) from the mice given AET (10.0 mg) and 500 r (or 100 r).

			Survival rate (at days)					
			7	14	21	30	60	90
1	AET+500 r → Na_2	900 r → dd/s	HBM 9/12 H.spl 7/8	4/12 0/8	4/12	4/12	1/12	1/12
2	AET+100 r → Na_2	900 r → dd/s	HBM 8/10 H.spl 5/8	3/10 2/8	3/10 2/8	1/10 1/8	1/10 1/8	0/10
3	500 r → Na_2	900 r → dd/s	HBM 3/5 H.spl 4/6	0/5 2/6	2/6	2/6	1/6	
4	100 r → Na_2	900 r → dd/s	HBM 6/6 H.spl 5/6	1/6 3/6	0/6 1/6	1/6	1/6	

a) Homologous donor mice are injected with AET (10 mg. per mouse) followed by 500 r (or 100 r) of gamma-irradiation 10 minutes later. By this procedure antibody forming cells of the donor is incapacitated leaving relatively intact nonantibody forming cells. Therefore, the graft to host reaction, if it occurs, in secondary disease may be prevented to develope. The recipient mice were irradiated with 900 r of gamma-rays followed by the intravenous injection of bone marrow of the donor mice treated as described. The result is shown in Table 5. Neither initial 14 day nor subsequent 30 or 60 day survival was good. Irradiation dose to the donor mice may be too large an amount killing or incapacitating non-antibody forming cells as well as antibody forming cells.

b) Recipient mice are injected with AET (6.5 mg. per mouse) followed by a large dose of gamma-irradiation. The dose of irradiation is so large that irradiated mice may die within a few days after irradiation due to intestinal damage unless protected with AET. By this procedure, graft rejection by the

Table 6. Survival rate of AET protected, gamma-irradiated and HBM treated mice.

	No. of survival/No. of irradiated (at days)					% survival 21 day
	7	14	21	30	60	
AET (6.5 mg)+1300 r ↓ dd/s←——Na_2	T : 11/14	6/14	2/14	0/14		16
	C : 5/9	5/9	2/9	2/9	1/9	22
AET (6.5 mg)+1500 r ↓ dd/s←——Na_2	T : 12/12	12/12	12/12	7/12	1/12	100
	C : 3/5	3/5	2/5	1/5		40
AET (6.5 mg)+1300 r ↓ dd/s←— Wistar Rat	T : 3/8	1/8	1/8	1/8	1/8	12
	C : 5/9	5/9	2/9	2/9	2/9	22

T : AET+gamma rays+HBM or RBM.
C : AET+gamma rays.

host may be prevented to develope due to severer damage of antibody forming cells of the host. The result is shown in Table 6. Although AET plus 1300 r may not be sufficient to depress immunological competence, AET plus 1500 r seems sufficient as shown by 100% 21 day survival. However, subsequent 30 or 60 day survival was low as that in secondary disease. Appropriate administration of AET to both the donor and recipient in the same experiment would give good initial 14 day and subsequent 30 or 60 day survival and experiments based on this assumption is under way.

VIII. Long Term Observation of Mice Lethally Irradiated with Gamma-Rays under high Dose Rate and Treated with IBM

Bone marrow treatment dose not seem to modify radiation damage to tissues

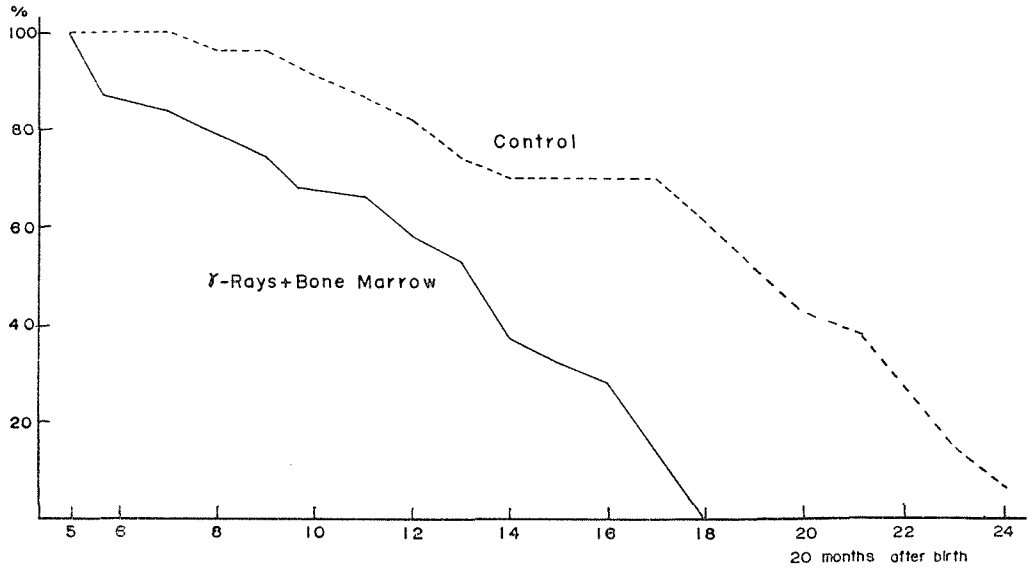

Fig. 9. Survival rate of 900 r+IBM mice (long term observation).

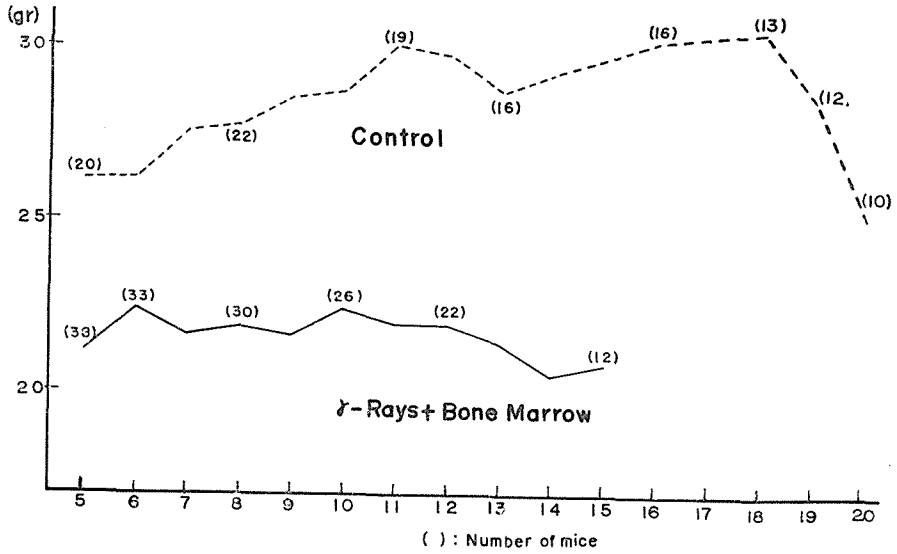

Fig. 10. Body weight changes of 900 r+IBM mice (long term observation).

Table 7. Main autopsy findings in 900 r+IBM mice (long term observation).

	No. of r-rays+IBM/34	No. of control/21
1) Neoplasma		
Breast	0	4
Lung	3	1
Ovary	2	0
Hematopoietic organ (Leukemia)	1	0
Stomach	0	1
Orbit	0	1
2) Infection		
Lung	10	4
Liver	2	2
Kidney (Pyelonephritis)	1	0
Ovary	1	0
Generalized abscess	1	0
3) Others		
Nephrosclerosis	8	0
Marked pulm. congestion	1	0
Splenic bleeding & rupture	1	0
Pulm. vein thrombosis	1	0
Calcification of heart	5	0

other than those of the hematopoietic system. The bodily growth is retarded and survival time is shorter in the gamma-irradiated and IBM treated female mice than those in non-irradiated and non-IBM treated control mice (Figs. 9 and 10). Histologically, increase in incidence of nephrosclerosis and myocardial calcification were found in irradiated and IBM treated mice (Table 7). Another kind of

control mice, namely, non-irradiated but IBM injected mice, and the mice, irradiated twice each followed by IBM treatment, are now under observation and final results in regard to late radiation effects will be reported[14].

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