various intervals after parathyroidectomy and sham operation. In young rats studied one day after parathyroidectomy there was a marked decrease in plasma calcium and a small decrease in the "6-hour exchangeable calcium." One week after parathyroidectomy there was no further decrease in plasma calcium but a significant further decrease in 6-hour exchangeable calcium. In old rats studied one week after parathyroidectomy there was a somewhat smaller decrease in plasma calcium concentration and no change in 6-hour exchangeable calcium. It is concluded that removal of the parathyroids does not affect the amount of bone calcium available for exchange directly, but may act indirectly in the following sequence: decreased parathyroid hormone → decreased bone resorption → decreased new bone formation → decreased exchangeable calcium.

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Persistence of Heterologous Bone Marrow in Mice as Function of X-Ray Dose. (24646)

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Previous studies on recovery rate of mouse's immune mechanism after irradiation and treatment with AET† or bone marrow(1-4) led to formulation of the following hypothesis: There are many antibody-producing cells, or an antibody-producing cell, that can recognize degrees of antigenicity (homologous, closely related heterologous, and distantly related heterologous antigens); *i.e.*, degree of injury to the recognition mechanism by X-irradiation would increase in the order of mention. Makinodan and Gengozian(5,6) tested and confirmed this hypothesis on the basis of mouse's ability to produce titratable, circulating antibodies. A subsequent test would then be a biological one; *i.e.*, persistence and proliferation of foreign tissue in X-irradiated recipients as a function of genetic relation between donor and host. Our findings on such an investigation are reported here.

Materials and methods. Approximately 1100 (C3H x 101) F_1 male and female mice 12 weeks old were used as recipients. Bone marrow donors were normal male and female Sprague-Dawley rats (150-250 g), 12-week-old golden hamsters, weanling guinea pigs, and albino rabbits (1.5-2 kg). A constant-potential Phillips X-ray machine was used under following conditions: 250 kv; 15 ma; 160-170 r/min at 60 cm; inherent filtration, 1 mm of Al; added filtration, 1 mm of Al; and hvl, 0.5 mm of Cu. Mice were irradiated in circular perforated lucite container attached to re-

* Operated by Union Carbide Corp. for U. S. Atomic Energy Comm.

† AET = S, 2-aminoethylisothiuronium bromide hydrobromide (fomerly called S,β-aminoethylisothiuronium bromide hydrobromide).

volving turntable. Generally, within 4 hr after a single exposure to 0, 300, 400, 450, 500, 600, 700, 800, 950, 1150, 1300, 1500, or 2000 r, mice in groups of 20 to 40 were given no treatment or bone marrow of rat (RBM), hamster (HBM), guinea pig (GPBM), or rabbit (RbBM). Intravenous bone marrow dose/mouse was ~ 140 x 10^6 nucleated cells from femora, tibiae, and humeri suspended in 1 ml of Tyrode's solution. After treatment, 2-10 mice/group were sacrificed on following intervals, with some exceptions: 1, 2, 3, 4, 5, 6, 16, 24 hr; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days. Usually, 2 blood and bone marrow smears and 15 spleen imprints from each mouse were fixed in acetone and stained for alkaline phosphatase-positive cells by Gomori technic(7). Imprints were always obtained from 3 representative areas of the spleen.

Results. Granulocytic cells of normal and X-rayed mice were alkaline phosphatase negative; those of donors were positive. To express relative concentration of donor cells in treated mice, we rated stained preparations 1+, 2+, 3+, and 4+ (Fig. 1). On this basis, concentration of donor cells followed essentially 3 patterns during first 10 days after injection: (a) it decreased; (b) decreased, then increased, then decreased again; and (c) decreased and then increased. Fig. 2 illustrates these patterns, which we interpreted as follows: Patterns A and A′ show elimination of HBM and RBM cells, respectively, by the unirradiated host. Presence of donor cells in

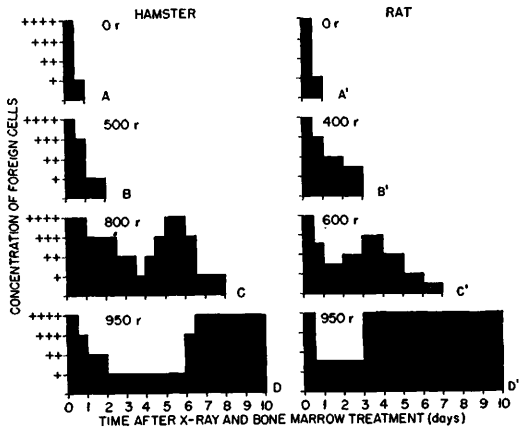

FIG. 1. Alkaline phosphatase-positive cells in spleen of mice treated with foreign bone marrow. A, 1+; B, 2+; C, 3+; D, 4+ reactions.

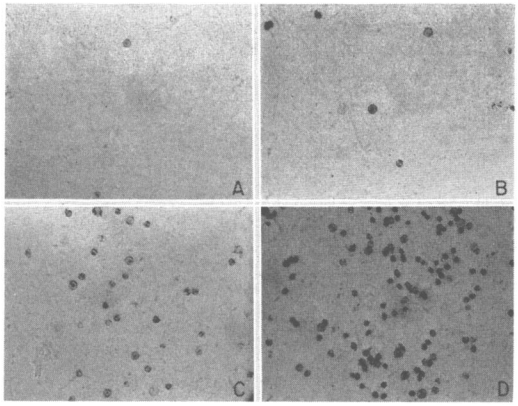

FIG. 2. Persistence, temporary and prolonged transplantation of hamster and rat bone marrow cells in mouse spleen after X-radiation and bone marrow treatment. A and A′, control: 0 r–HBM, 0 r–RBM; B and B′, persistence: 500 r–HBM, 400 r–RBM; C and C′, temporary transplantation: 800 r–HBM, 600 r–RBM; D and D′, prolonged transplantation: 950 r–HBM, 950 r–RBM.

an irradiated host for significantly longer time than in normal host indicates *persistence* (patterns B and B′). A secondary rise in concentration of donor cells in an irradiated host indicates proliferation or transplantation; patterns C and C′ are examples of *temporary* proliferation or transplantation of HBM and RBM cells, respectively, and patterns D and D′ are examples of *prolonged* proliferation or transplantation.

In normal and irradiated mice treated with foreign bone marrow, large patches of intercellular alkaline phosphatase-positive material appeared in blood and bone marrow smears 2 or 3 days after treatment and persisted several days after positive cells were no longer distinguishable in these tissues. Since these patches hampered evaluation of smears, spleen imprints were generally used for determining whether positive cells were present.

Rat bone marrow injection (Table I, column 2). Donor cells were detected in blood, bone marrow, and spleen of normal mice 1 hr after injection of RBM but none were seen in blood 4 hr after treatment. Very few, if any, were found in bone marrow and spleen 1 day after treatment. With increase in X-ray dose, RBM cells persisted longer in the host—2, 3, 4, and 5 days after 300, 400, 500, and 600 r, respectively. Donor cells were present among the very few surviving 710 r-RBM mice 10

TABLE I. Persistence of Donor Cells in Mouse Spleen after Various X-ray Doses and Bone Marrow Treatment.

X-ray dose, r	Persistence time, days — Donor bone marrow — Rat	Hamster	Guinea pig	Rabbit
0	1	1	<1	<1
300	2			
400	3	1		
500	4*	1		
600	5*	5*		
700		6*		
710	>10†			
800	" ‡	8*	<1	
950	" ‡	>10‡	"	<1
1150	" ‡	> 5†	3	"
1300	> 6†	> 4†	4	1
1500	> 4†	> 3†		4
2000	> 3†			"

* Temporary transplantation.
† Short-term survivors.
‡ Prolonged transplantation.

days after treatment. The minimum X-ray dose that would permit temporary transplantation of donor cells in mouse spleen was 500 r; prolonged transplantation was evident among RBM-treated mice receiving 800 r or more.

Hamster bone marrow injection (Table I, column 3). HBM cells met the same fate as RBM in normal mice; *i.e.*, no donor cells could be detected in blood of mice 4 hr after HBM treatment or in bone marrow and spleen 1 day later. Persistence time of HBM cells among the 400- and 500-r mice was comparable to that of unirradiated mice, but was 5, 6, and 8 days after 600, 700, and 800 r, respectively. Minimum X-ray dose that permitted temporary transplantation of donor cells in mouse spleen was 600 r. Prolonged transplantation was evident among the HBM-treated mice receiving 950 r or more.

Guinea pig bone marrow injection (Table I, column 4). In normal, unirradiated mice, blood, bone marrow, and spleen contained donor cells 1 hr after injection of GPBM; donor cells were eliminated from blood in 4 hr, and from bone marrow and spleen within 18 hr. Similar elimination was observed after 800 and 950 r. However, donor cells persisted for 3 and 4 days among 1150 and 1300 r-GPBM mice, respectively. There was no evidence for even a temporary transplantation among these mice. X-ray doses higher than 1300 r were not used.

Rabbit bone marrow injection (Table I, column 5). Donor cells were rapidly eliminated from normal mice after RbBM injection; *i.e.*, blood of host became negative in 1 hr, bone marrow in 3 hr, and spleen in 6 hr. In these mice, as early as 1 hr after treatment, the spleen became congested and its weight markedly increased. Donor cells were detected in spleens of 1300 r-RbBM mice 1 day after treatment but not thereafter. However, a significantly prolonged persistence time of 4 days was seen among the 1500 and 2000 r-RbBM mice. As with the GPBM-treated mice, even a temporary transplantation was not evident among these mice.

Discussion. Of 4 heterologous bone marrows tested, RbBM was eliminated by normal adult mice slightly faster than GPBM, HBM, or RBM. Regardless of genetic relation between host and donor, however, these unirradiated adult mice eliminated foreign bone marrow within 1 day after injection. Consequently, we assumed that persistence of foreign bone marrow for 3 or more days after injection indicated an impaired immune mechanism of the host when dose, route, and injection time of bone marrow were kept constant. Increasingly large doses of X-rays (400, 600, 1150, and 1500 r, respectively) were required to permit persistence of rat, hamster, guinea pig, and rabbit cells in the mice. A higher X-ray dose was required for transplantation of HBM than RBM in the mouse. The immunogenetic experiments of Odell and Caldwell(8) showed that the minimum X-ray dose that would permit prolonged transplantation of homologous RBM in the rat was 300 r. Thus the degree of X-ray injury to the host's immune mechanism depends on the genetic relation between the host and donor; *i.e.*, a higher X-ray dose is required to impair the immune mechanism of the host against a more distantly related antigen.

GPBM may have failed to transplant in irradiated mice (950-1300 r) because the concentration of cells used (130-140 x 10^6) was insufficient; it is known that the optimum dose of RBM that will protect lethally irradiated mice is ~ 10 times that of isologous bone mar-

row(9-11). Lorenz and Congdon(12) and van Bekkum and Vos(11) succeeded in prolonging the survival time of a few lethally irradiated mice treated with GPBM. This would indicate at least temporary transplantation of donor cells, if it can be assumed that transplantation of donor cells is necessary for even an extension of survival time of lethally irradiated mice.

We found that (a), during maximum depression period after varying X-ray doses, mice responded beter to sheep than to rat antigens(5) and (b), recovery rate was faster for sheep antigens than for rat antigens(1,3). Published results and findings here reported substantiate our hypothesis: antibody-producing cells of normal adult mice can differentiate between and respond to closely and distantly related antigens with equal effect. The degree of X-ray injury to the recognition and response mechanisms decreases with the foreignness of the antigen.

Summary. Bone marrow from species closely and distantly related to the mouse was injected after various X-ray doses to the total body. Rat, hamster, guinea pig, and rabbit bone marrow persisted in normal, nonirradiated mice for not more than 1 day. The minimum X-ray dose that would permit persistence of rat, hamster, guinea pig, and rabbit bone marrow in mice for more than 3 days was 400, 600, 1150, and 1500 r, respectively. The minimum X-ray dose that permitted (a) temporary transplantation of rat and hamster bone marrow in mice was 500 and 600 r, respectively, and (b) prolonged transplantation of rat and hamster bone marrow in mice was 800 and 950 r, respectively. The significance of these findings is discussed.

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Effects of Immunized Parental Strain Bone Marrow on Lethally Irradiated F_1 Hybrid Mice. (24647)

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Nonirradiated bone marrow cells inoculated into irradiated recipients transplant and prevent death from hemopoietic failure. However, a syndrome called "foreign bone marrow reaction" occurs in many animals receiving antigenically foreign marrow cells after whole-body irradiation(1). Mice so afflicted seem to recover from radiation-induced injury but soon lapse into progressively wasting state, and die 30-90 days after treatment. Since implantation of adult parental spleen cells in irradiated F_1 mice hastens death if donor is pre-sensitized to recipient(2), this experiment was undertaken to determine effects of pre-sensitized parental bone marrow on survival of irradiated F_1 hybrids.

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